D.Sc.

GENETICAL AND PHYSIOLOGICAL STUDIES OF THE DOMESTIC FOWL.

by

F. B. HUTT

Thesis for the Degree of D. Sc.

1949

PUBLICATIONS OF F. B. HUTT

Arranged according to subject matter and chronologically within each subject. Where the author is not specified, F. B. Hutt is the sole author; in cases of joint authorship, names are given in the order in which they appear on the paper.

A. GENETICS OF THE FOWL

In the series so designated:

1930 I The inheritance of frizzled plumage. Journ. Genet. 22: 109-127.

1933 II A four-gene autosomal linkage group. Genetics 18: 82-94.

1934 III Congenital tremor in young chicks. Journ. Hered. 25: 341-350. (Hutt and Child)

1936 IV Linkage relations of crest, dominant white and frizzling in the fowl. Amer. Nat. 70: 379-394. (Warren and Hutt)

V The modified frizzle. Journ. Genet. 32: 277-285.

VI A tentative chromosome map. Neue Forschungen in Tierzucht und Abstammungslehre (Duerst Festschrift): 105-112.

1938 VII Breed differences in susceptibility to extreme heat. Poultry Science 17: 454-462.

1939 VIII Breed differences in resistance to a deficiency of vitamin B_1 in the fowl. Journ. Agric. Res. 58: 305-316. (Lamoreux and Hutt)

1938 IX Naked, a new sex-linked mutation. Journ. Hered. 29: 370-379. (Hutt and Sturkie)

X A relation between breed characteristics and poor reproduction in White Wyandotte fowls. Amer. Nat. (In press)

Not in the regular series:

1929 A note on Lambert's mosaic in the fowl. Journ. Hered. 20: 323-324.

Sex dimorphism and variability in the appendicular skeleton of the Leghorn fowl. Poultry Science 8: 202-218.

1932 Eight new mutations in the domestic fowl. Proc. Sixth Internat. Congress of Genetics, Ithaca, New York, 1932. Vol. 2: 96-97.

B. MAMMALIAN GENETICS

1930 Bovine quadruplets including twins apparently monozygotic. Journ. Hered. 21: 339-348.

1932 Congenital taillessness in the rat. Journ. Hered. 23: 363-367. (Hutt and Mydland)

1934 A hereditary lethal muscle contracture in cattle. Journ. Hered. 25: 41-46.

C. HUMAN GENETICS

1934 Sex differences in the expression of autosomal genes affecting human dentition. A Decade of Progress in Eugenics, Scientific Papers of the 3rd International Congress of Eugenics, 1932: 447-452.

1935 An earlier record of the toothless men of Sind. Journ. Hered. 26: 65-66.

D. EMBRYONIC MORTALITY IN THE FOWL

In the series so designated:

1929 I The frequencies of various malpositions of the chick embryo and their significance. Proc. Roy Soc. Edin. 49, Pt. 2, No. 10: 118-130.

II Chondrodystrophy in the chick. Proc. Roy. Soc. Edin. 49, Pt. 2, No. 11: 131-144. (Hutt and Greenwood)

III Chick monsters in relation to embryonic mortality. Proc. Roy. Soc. Edin. 49, Pt. 2, No. 12: 145-155. (Hutt and Greenwood)

1930 IV Comparative rates of mortality in eggs laid at different periods of the day and their bearing on theories of the origin of monsters. Poultry Science 9: 194-203. (Hutt and Pilkey)

1934 V Relationships between positions of the egg and frequencies of malpositions. Poultry Science 13: 3-13. (Hutt and Pilkey)

VI The relation between abnormal orientation of the 4-day embryo and position of the chick at hatching. Journ. Agric. Res. 48: 517-531. (Cavers and Hutt)

1938 VII On the relation of malpositions to the size and shape of eggs. Poultry Science 17: 345-352.

Not in the regular series:

1930 On the origin, common types and economic significance of teratological monsters in embryos of the domestic fowl. Proc. 4th World's Poultry Congress, London: 195-202.

E. AVIAN PHYSIOLOGY (mostly endocrinology and physiology of reproduction)

1928 Further experiments in feeding thyroid to fowls. Poultry Science 7: 60-66. (Cole and Hutt)

Potentially fatal fatigue of the cervical muscles of the fowl resulting from an excessively large comb. Vet. Journ. 84: 579-584.

1929 On the relation of fertility in fowls to the amount of testicular material and density of sperm suspension. Proc. Roy. Soc. Edin. 49, Pt. 2, No. 9: 102-117.

1930 A note on the effects of different doses of thyroid on the fowl. Journ. Exper. Biol. 7: 1-6.

1933 On the fecundity of partially ovariotomized fowls. Journ. Exp. Zool. 65: 199-214. (Hutt and Grussendorf)

1935 Idiopathic hypoparathyroidism and tetany in the fowl. Endocrinology 19: 398-492. (Hutt and Boyd)

1938 The influence of estrogens in egg yolk upon avian blood calcium. Endocrinology 23: 793-799. (Altmann and Hutt)

1939 Variability of body temperature in the normal chick. Poultry Science 18: 70-75. (Lamoreux and Hutt)

An intrafollicular ovum laid by a fowl. Poultry Science (In press)

F. ORNITHOLOGY

1932 Birds observed from shipboard in crossing the North Atlantic. The Auk 49: 184-190.

1938 Number of feathers and body size in passerine birds. The Auk 55: 651-657. (Hutt and Ball)

G. REVIEWS AND GENERAL

1932 Paradoxical terminology in genetics. Amer. Nat. 66: 274-277.

1933 Research with a hen. Science 78: 449-452.

1934 Inherited lethal characters in domestic animals. The Cornell Veterinarian 24: 1-25.

1938 The geneticist's objectives in poultry improvement. Amer. Nat. 72: 268-284.

A. GENETICS OF THE FOWL

THE GENETICS OF THE FOWL
I. THE INHERITANCE OF FRIZZLED PLUMAGE

BY

F. B. HUTT

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THE GENETICS OF THE FOWL.
I. THE INHERITANCE OF FRIZZLED PLUMAGE.

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(With Two Plates and Two Text-figures.)

CONTENTS.

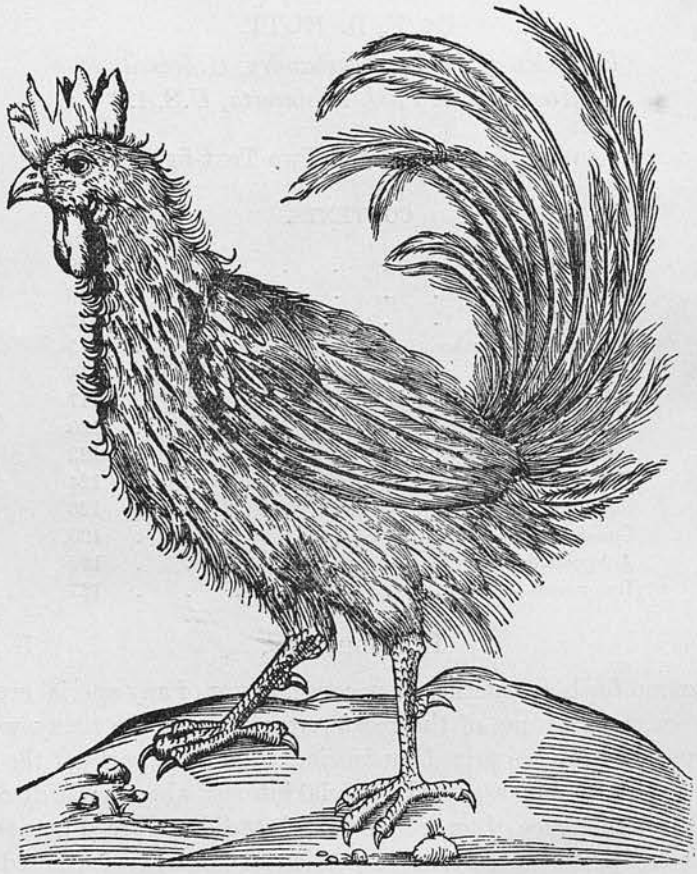
Introduction.

Frizzled fowls are neither very common nor of any special economic importance, but, as one of the most remarkable of the many varieties of the species, they are prized by fanciers in various parts of the world. For the geneticist they assumed a special interest when it was discovered that, in the experience of some breeders, they did not breed true to type, but always "threw" a certain proportion of normally feathered birds. Arising from this observation, the theory has become current that the homozygous Frizzle is non-viable and presumably dies at an early stage of embryonic development. The investigation herein reported was undertaken to substantiate or to disprove this theory and to determine the mode of inheritance of the character.

[1] The greater part of this investigation was conducted at the Animal Breeding Research Department, University of Edinburgh, in 1928, but the work was completed in 1929 at the University of Minnesota. This report is published with the approval of the Director as Paper No. 904 of the *Journal Series of the Minnesota Agricultural Experiment Station.*

HISTORICAL NOTES.

A frizzled fowl is illustrated (Text-fig. 1) in the classical work of Aldrovandus (1600), to whom a drawing and description of such a bird had been sent from Parma. His note concerning it, kindly translated for



Text-fig. 1. The frizzled fowl from Aldrovandus' *Ornithologiae* (1600).

the writer by Prof. J. B. Pike of the Department of Latin of this university, is as follows:

Pompilio Tagliaferro, the distinguished physician of Parma, has written to me with regard to this fowl as follows:

"I am sending you a drawing of a strange fowl; the artist, however, has by no means satisfied me with his representation. However, I wish you to note two noteworthy points discovered in regard to this fowl—things not seen in our cocks and hens. First

and most important, the feathers of the wings lie in a manner quite contrary to those in others, for the lower surface of the feathers, which ordinarily in other fowls turns inward, in this one curves out, with the result that the whole wing seems reversed. The second peculiarity worthy of note is that the feathers of the neck turn toward the head, like curls; the whole tail curves up in the same direction."

Such is his description. Neither the picture sent to me nor my own drawing brings out satisfactorily the peculiarities which he mentions. His words indicate that this was owing to the artist's lack of skill.

The variety was also known to Willughby, who, in his *Ornithology*, published in 1676, refers to its occurrence in England and, according to Tegetmeier (1867), states that it was at that time known as the Frisland hen

not because it was first brought to us out in Frisland, but because the feathers of the body are curled or frizzled; by which epithet I believe this bird was first called, the word being afterwards, by the mistake of the vulgar, corrupted into Frisland of like sound. For knowing it to be an outlandish hen, they thought it could not be more fitly denominated than from its country and thereupon imagined it to be called a Frisland hen, instead of a frizzled hen. Nor did they want a probable argument to induce them to think it to be of a Frisland breed or original, *viz.* the curling of the feathers which one would be apt to attribute to the horror of cold.

Linné (1758) calls the frizzled fowl *Gallus crispus* in the tenth edition of *Systema Naturae*, but in a later edition refers to it as *Gallus pennis revolutis*.

The frizzled fowl was known as "le coq frisée" to Buffon (1799) who states that it is to be found in Java, Japan and southern Asia. This range agrees with the statement of Temminck (*ca.* 1813, quoted by Tegetmeier, *loc. cit.*) that the "coq à plumes frisées" is kept under domestication in Java, Sumatra, southern Asia and the Philippines, the prevailing colour being white. Dürigen (1923) quotes the German ornithologist, Bechstein, as giving in 1793 a similar range with the addition of Surinam for its domestication, and states that it had also been reported by Captain Tollemache for Mauritius and Mozambique as the Hurricane Fowl.

The only published data concerning the inheritance of frizzling are those of Davenport (1906) who raised only one generation from a Silky × Frizzle cross and found that the plumage of the latter variety was dominant. Of the 10 chicks raised, 6 were frizzled while 4 were normally feathered. Such a result would be expected if frizzling were caused by a single factor, and if some or all of the four Frizzles tested were heterozygous, but since the birds were not individually pedigreed no conclusions can be drawn other than that the character is dominant over normal plumage.

Description.

The peculiarity of the frizzled fowl is that the contour feathers, instead of conforming to the shape of the body, have their shafts so recurved that the outer surface of each feather becomes concave. The resulting general appearance is as if the feathers had been rubbed the wrong way (Plate III, fig. 2). In good specimens a prominent ruff is formed in the neck region. In the original flock used in this investigation the shafts of the rectrices and remiges were recurved only in the slender region at the tip of the feather, but in several parts of the vane in these large feathers small groups of the barbs, numbering from three to ten, were so twisted that they appeared to curl round the rachis (Text-fig. 2). In most cases these barbs were eventually worn off so that there remained only the shaft of the feather with small portions of the vane attached to it. This was particularly evident in the primaries. Because of this handicap the birds were unable to use their wings even as little as do other heavy domestic fowls, and had to be given low roosts to which they could easily hop. It is doubtful whether the mutation could persist long in a state of nature.

All of the 22 mature fowls with which the investigation was begun were fairly uniform in appearance, and conformed closely to the description given above. In the progeny of these birds, however, there appeared a distinctly different type of frizzling which will be described below.

Tegetmeier (*loc. cit.*) found in examining Darwin's collection of domesticated birds from all over the world that some specimens exhibited frizzling in all parts of the body, while in others it was confined to the neck region. The character is also found in the pigeon, where it has given rise to a variety known as the Frillback.

Frizzled fowls are found in various colours—black, white, buff and blue (dilute black), the chief preference of the fancier being for a bird all one colour and for uniformity in exhibition pairs, trios and pens. Those used in the present study were all red (*i.e.* as in the Rhode Island Red), excepting four bantams of which the cock was blue and the hens black, buff and blue.

There is some evidence that the frizzled fowls were originally black-skinned like the true Silkies of to-day. Marsden (1784), in his account of the island of Sumatra, refers to the domestic fowls, "some with black bones and some of the sort we call Friezland (*cf.* Willughby) *or negro fowls.*" Darwin (1875) describes the Frizzled or Caffre fowls as being

"not uncommon in India, with the feathers curling backwards, and with the primary feathers of the wing and tail imperfect; *periosteum of bones black.*" Dürigen (*loc. cit.*) gives the colour of the skin as one of the peculiarities of the variety, stating that it was originally dark red or purple, but that as a result of crossing with other fowls it has become light. This character has not been observed in any of the present writer's material, and there is no experimental evidence that it is linked with frizzling.

The writer was unable to detect any satisfactory indication of this type of feathering in the newly hatched chick, but frizzled birds could usually be positively identified as soon as the wing feathers had grown to half an inch in length or even less. In one case the classification of 47 chicks hatched at 6 days of age agreed exactly with a second classification at 14 days. Another lot of 66 chicks sorted out at 9 days was subsequently found when re-examined at 2 weeks to have been accurately classified on the first occasion. In some cases of extremely slow-feathering individuals the type of plumage could not be accurately decided upon till the chicks were over 2 weeks of age. In the data given below, only those chicks are included in which determination of the frizzled or normal type of feathering was definite. With the exception of birds dying early, all descriptions were re-checked at 2 weeks or later.

MATERIAL AND METHODS.

In the first year of the present study there were used 29 mature birds, of which 7 were normal and 22 were frizzled. All of the latter came directly or indirectly from the flock of Major G. S. Williams, Tredrea, Perranwell, Cornwall. Some of these had been purchased in 1927; a few were raised at Edinburgh that year, and 10 were loaned for the breeding season of 1928.

Matings in 1928.

In order to get sufficient numbers of chicks, incubation was begun early in February 1928, and chicks were hatched up to 26 July. Matings were made as follows:

Series A. Frizzle × Normal.

1. Eight frizzled females were mated with ♂ 283, a normally feathered cockerel extracted in 1927 from a Frizzle × Frizzle mating. One of these eight, ♀ 129, and another Frizzle, ♀ 132, had also been mated earlier in the season to a Brown Leghorn male, no. 142.

2. Two normal females, nos. 272 and 300, hatched in 1927 from a Frizzle × Frizzle mating, were bred in separate pens to the frizzled cockerels P. 101 and M. 102 respectively. Both of these males had been hatched at Edinburgh in 1927 from a Frizzle × Frizzle mating.

3. Three normal Bantam females were mated to a blue frizzled Bantam cock, B. 18.

Series B. Normal × Normal.

1*a*. Two normally feathered females, nos. 272 and 300, were mated concurrently with Series A 1 to the normal male, no. 283. All three of these birds had been extracted from matings of Frizzle × Frizzle.

Series C. Frizzle × Frizzle.

1. Sixteen frizzled females were mated with the two frizzled cockerels, P. 101 and M. 102, each male having eight females in a separate pen. These matings were made in the same pens as those referred to in Series A 2 and concurrently with them. Ten of these females had previously been used in Series A 1, but care was taken to ensure that the influence of the normal male was lost before their eggs were included in Series C 1.

2. Three frizzled Bantam females were mated with the frizzled Bantam cock, B. 18, these birds being in the same pen as the normal Bantam females mentioned under Series A 3.

Incubation.

All eggs were incubated as nearly as possible in the same manner. The majority of them were started in a gas-heated Phipps incubator and moved to Hearson electric incubators a week before hatching. All hens were trap-nested, each egg was marked with the number of the hen laying it, and the chicks were hatched in pedigree bags, so that the ancestry of each chick was definitely known. The eggs were candled for infertility and for embryonic mortality at least twice during each hatch. Every dead embryo was examined and the estimated period of its death recorded, as well as any evidence of abnormality. Since there was a possibility that a lethal factor (if present) might be operative at an early stage of development of the zygote, all eggs which appeared infertile when candled were broken for more accurate observation, and when necessary an examination was made under the dissecting microscope.

Results.

The results obtained in the three series of matings listed above are presented in Tables I, II and III. Since evidence of a lethal factor may

TABLE I.

Matings of Frizzle × Normal, 1928.

Series	Parents ♂	Parents ♀	Eggs set	Infertile	Embryonic mortality in 2-day periods 1–2	3–4	5–6	7–8	9–10	11–12	13–14	15–16	17–18	19–21	Hatched No.	Hatched %	Chicks Frizzle	Chicks Normal
A 1	Normal	Frizzle																
	283	50	15	—	—	1	—	—	—	—	—	—	—	5	9	60·0	6	3
	,,	83	17	1	—	—	—	—	1	1	—	—	—	—	14	87·5	3	6
	,,	128	10	—	—	1	—	—	—	—	1	—	—	1	7	70·0	2	3
	,,	129	14	—	—	—	—	—	1	—	—	—	—	1	12	85·7	4	8
	,,	134	10	—	—	—	—	—	—	—	—	—	—	—	10	100·0	7	3
	,,	293	15	—	—	—	—	—	—	—	—	—	—	2	13	86·7	4	7
	,,	301	16	2	1	5	2	—	—	—	—	—	1	4	1	7·1	1	0
	,,	398	19	1	—	—	1	—	—	—	—	—	—	1	16	88·8	5	11
	142	129	28	2	—	—	—	—	—	—	1	—	—	1	24	92·3	9	12
	,,	132	12	2	—	1	—	—	—	—	—	—	—	1	8	—	4	4
A 2	Frizzle	Normal																
	P. 101	272	17	—	—	1	—	1	—	—	—	—	—	3	12	70·6	5	6
	M. 102	300	16	1	—	1	—	1	—	—	—	—	—	4	9	60·0	6	2
A 3	B. 18	B. 10	13	1	—	1	—	—	—	—	1	—	—	1	9	75·0	3	4
	,,	B. 63	10	—	—	—	—	—	—	—	1	—	1	—	8	80·0	3	4
	,,	B. 394	9	1	1	—	—	—	—	—	—	—	—	—	7	87·5	2	3
		Totals	221	11	2	11	3	2	2	1	4	—	2	24	159	—	64	76

TABLE II.

Matings inter se *of normally feathered birds extracted from Frizzle × Frizzle matings*, 1928.

Series	Parents ♂	Parents ♀	Eggs set	Infertile	Embryonic mortality 1–10 days	11–18 days	19–21 days	Hatched Number	Hatched %	Chicks Frizzle	Chicks Normal
B 1	283	272	19	0	—	1	5	13	68·4	0	13
	283	300	19	0	—	—	2	17	89·5	0	14
	Totals		—	—	38	0	—	1	7	30	—	0	27

TABLE III.

Matings of Frizzle × Frizzle, 1928.

Series	Parents ♂	Parents ♀	Eggs set	Infertile	Embryonic mortality in 2-day periods 1–2	3–4	5–6	7–8	9–10	11–12	13–14	15–16	17–18	19–21	Hatched No.	Hatched %	Chicks Frizzle	Chicks Normal
C 1	M. 102	83	46	—	—	—	—	—	1	1	1	—	—	5	38	82·6	24	12
	,,	128	20	—	1	—	1	—	—	—	—	—	—	2	16	80·0	10	4
	,,	129	32	3	2	2	1	1	1	2	2	—	—	4	14	48·3	8	5
	,,	132	28	1	2	1	—	—	—	—	—	—	—	4	20	74·1	15	4
	,,	134	29	—	—	—	—	—	1	—	—	—	—	3	25	86·2	19	5
	,,	286	30	—	—	—	—	1	—	—	—	—	—	1	28	93·3	16	7
	,,	301	9	1	—	1	1	—	1	—	—	—	—	—	5	62·5	4	1
	,,	398	13	1	1	1	—	—	—	—	—	—	—	—	10	83·3	9	1
	P. 101	50	17	2	—	1	1	—	1	—	1	—	—	1	10	66·6	5	2
	,,	293	10	—	—	1	—	—	—	—	—	—	—	3	6	60·0	4	1
	,,	341	37	1	1	—	1	—	1	—	2	1	—	6	24	66·7	12	11
	,,	342	46	1	1	1	1	—	—	—	1	—	—	7	34	75·5	22	10
	,,	343	13	5	—	3	—	1	—	—	1	—	—	1	2	25·0	2	0
	,,	344	33	—	1	—	—	2	—	—	1	—	—	4	25	75·7	14	9
	,,	345	41	—	—	—	—	—	—	—	—	—	—	7	34	82·9	25	5
	,,	346	27	1	3	—	—	—	—	—	1	—	—	2	20	76·9	12	4
C 2	B. 18	Black	26	—	—	—	—	1	—	1	—	—	—	7	17	73·1	9	7
	,,	Blue	20	—	—	—	—	1	—	—	—	—	—	3	16	80·0	9	4
	,,	Buff	22	1	1	—	1	—	—	—	—	—	—	2	17	80·9	11	4
		Totals	499	17	13	11	7	7	6	4	10	1	0	62	361	—	230	96

be sought in the rates of embryonic mortality, as well as in the ratios of the two types of feathering in the progeny from each hen, the incubation records and the classifications of progenies are given in detail for each hen in each mating. Any differences between the numbers of chicks hatched and of those classified from the same individual indicate chicks dying too early to be classified. The percentage hatch is calculated for the fertile eggs only.

INTERPRETATION OF 1928 DATA.

A. *Matings of Frizzle × Normal.*

On reference to Table I it is seen that in Series A every one of the 13 frizzled birds tested, whether male or female, produced both frizzled and normal offspring in approximately equal numbers when mated to normal fowls. (♀ 301 had only 1 chick in this series but was subsequently shown in Series C to be heterozygous.) The total numbers in the two classes were 64 Frizzles to 76 Normals. The deviation from the 1 : 1 ratio expected, if all the Frizzles tested were heterozygous in the pair of factors affecting frizzling (Table IV), is only 1·47 times the probable error of the ratio and may therefore be considered insignificant.

The results in Series A may therefore be construed as indicating:

(1) That frizzling is a dominant character dependent for its expression upon a single pair of factors.

(2) That the 9 females and 3 males tested were all heterozygous with respect to that pair of factors.

(3) That no sex-linked genes are involved, since the crossing of frizzled males with normal females gave results similar to those in the reciprocal cross.

B. *Matings* inter se *of extracted normals.*

In conformity with the results obtained in Series A it was found (Table II) that breeding together normally feathered birds extracted from Frizzle × Frizzle matings produced only normals and hence that these were true simple recessives.

C. *Matings of Frizzle × Frizzle.*

In this series (Table III) normally feathered chicks were obtained from every one of the 19 hens, 10 of which had not previously been tested. Since all of these Frizzles were thus shown to be heterozygous, the expectation in their progeny (considering the findings in Series A) was a

simple 3 : 1 ratio, provided no lethal factors are involved. While the deviation of 14·5 from expectation (Table IV) seems fairly large for a population of 326 individuals, it is only 2·75 times the probable error of the ratio, and is therefore well within the limits of fluctuations due to sampling.

TABLE IV.

Ratios obtained in Series A, B and C compared with those expected on the basis of frizzling being a unifactorial dominant character either lethal or non-lethal in the homozygous condition.

Series		Frizzle	Normal	Deviation	Probable error	Deviation P.E.
A	Observed	64	76	6	—	1·47
A	*Expected* (1 : 1)	*70*	*70*	—	±4·08	—
B	Observed	230	96	14·5	—	2·75
B	*Expected if* **FF** *were non-lethal* (3 : 1)	*244·5*	*81·5*	—	±5·27	—
B	*Expected if* **FF** *were lethal* (2 : 1)	*217·3*	*108·7*	12·7	±6·08	2·09
C	Observed	0	27	—	—	—
C	*Expected*	*0*	*27*	—	—	—

At the same time it should be pointed out that in both Series A and Series C there is a deficiency of frizzled chicks. In fact the ratio in the latter series fits a 2 : 1 expectation equally as well as one of 3 : 1 (Table IV).

The deficiency of Frizzles in Series C results from an extreme deviation from a 3 : 1 ratio in the progeny of certain females mated to ♂ P. 101 and ♂ B. 18. (The progenies from ♂ M. 102 make up an almost perfect 3 : 1 ratio, there being 105 Frizzles to 39 Normals where the expectation is 108 : 36.) Of the hens mated to ♂ P. 101, nos. 341, 342 and 344 gave deviations from the expected ratio so marked that they might be considered as indicating a differential production of gametes by ♂ 101, were it not for the fact that by the same sire ♀ 345 produced an excess of frizzled chicks and ♀ 346 an exact 3 : 1 ratio. Moreover, it had been shown in Series A 2 (Table I) that ♂ P. 101 was producing the two classes of gametes in equal porportions.

The use of the χ^2 test for goodness of fit of Mendelian ratios was formerly confined to polyhybrid ratios, but Kirk and Immer (1928) have recently shown that it may be applied equally well to a monohybrid ratio, where several progenies are under consideration. By its use the deviations from the expected ratio in each progeny are considered, and one obtains a more accurate conception of the validity of an observed ratio than is possible by the use of the probable error, when the latter is applied to the ratio obtained by the summation of the class frequencies in all progenies.

When the χ^2 test, using the tables of Fisher (1928), is applied to the data in Table III, it is found that $\chi^2 = 19{\cdot}345$, $n = 19$, $P = 0{\cdot}44$.

In other words, a deviation from an expectation of 3 : 1 as great as that observed in the ratio of 230 Frizzles : 96 Normals would be expected in about 44 per cent. of similar trials. It is, therefore, quite insignificant and the hypothesis that the homozygous Frizzle is viable is supported by the ratio obtained.

Embryonic mortality.

It was more difficult to explain why each of the 22 frizzled birds tested in this investigation and some, at least, of the four used by Davenport (*loc. cit.*) should have been heterozygous. Since one would expect that in an unselected population of this size considerably more than one-third would be homozygous, there was every reason to suggest that a lethal factor is involved.

Such a lethal might be either gametic or zygotic. The possibility of a gametic lethal is eliminated by the results in Series A of this experiment (Table I), which showed that 10 females and 3 males were producing viable gametes carrying the factor for frizzling in the same proportion as gametes carrying the recessive factor.

If a zygotic lethal were involved, it might be operative either during embryonic development or at any time after hatching. Consideration of the time of effect of lethals in other animals, and of the few known in fowls, leads one to suspect such a factor to be effective during the period of incubation. The percentage mortality in each four-day period of incubation, for all eggs set in Series A and C, is shown in Table V.

TABLE V.

Comparison of embryonic mortality in Series A and C.

Series	Mating	Fertile eggs	% mortality in 4-day periods					Total mortality	Hatch %
			1–4	5–8	9–12	13–16	17–21		
A	Frizzle × Normal	210	6·19	2·38	1·43	1·90	12·38	24·28	75·71
C	Frizzle × Frizzle	482	4·98	2·90	2·07	2·28	12·86	25·10	74·89

If homozygosity for frizzling were lethal to the embryo, a peak of mortality would be expected at some stage of incubation for the eggs set from matings of Frizzle × Frizzle. In addition one would expect approximately 25 per cent. higher mortality in this series than in eggs from matings of Frizzle × Normal. Neither of these conditions was

present. The death rate in one series ran closely parallel to that in the other. The peak of the mortality during the last five days is quite usual, and to a considerable extent is due to fully formed chicks dying from being in positions which make it impossible or extremely difficult for them to hatch.

Embryos representing various types of teratological monsters, chondrodystrophic chicks and abnormalities in position were found, but in no greater numbers in one series than in the other, and in no greater proportion altogether than in a large number of eggs from other sources.

In view of these findings it seems reasonable to conclude that homozygosity for frizzling is not lethal to the embryo.

Reason to doubt the validity of this conclusion is found (Table III) in the fact that from the 8 females with less than 75 per cent. hatch the proportion of frizzled to normal chicks was only 59 : 31 (*i.e.* 2 : 1), while from the 11 fowls with more than 75 per cent. hatch the proportion was 171 Frizzles : 65 Normals, a fairly close fit to the expectation of 3 : 1. At first sight this association of a deficiency of Frizzles with the poorer hatches certainly suggests that a lethal factor is involved. However, further examination shows that the 2 : 1 ratio in the progeny of the first class arose from the inclusion therein of ♀ 341 and ♀ B. Black, both of which gave extreme deviations from a 3 : 1 ratio and whose offspring made up 39 of the 91 chicks in this class. The other 6 hens of this class all had ratios closely fitting the 3 : 1 expected, but not enough chicks to offset the influence of the two exceptional progenies on the totals.

Similar fluctuations are found in the group of hens with hatches over 75 per cent., where ♀ 83 had an exact 2 : 1 ratio (out of an 82 per cent. hatch) and ♀ 344 produced only 14 Frizzles to 9 Normals. In this class, however, there were enough large-sized progenies nearer to the expected ratio to make the totals for the whole class fit fairly close to a 3 : 1 ratio. The difference between the proportions of frizzled to normal chicks in the group with hatches under 75 per cent. and in those over 75 per cent. may therefore be considered a coincidence and not owing to the action of some lethal factor linked with the gene for frizzling.

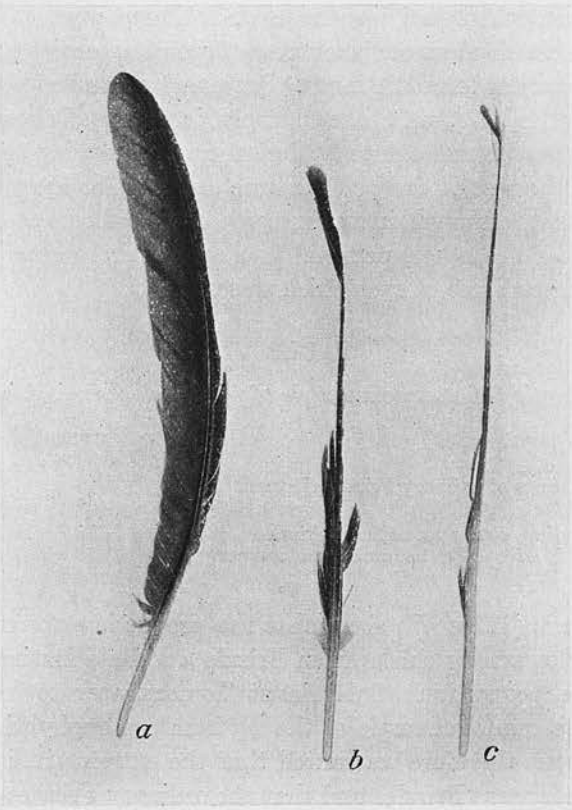
The possibility that the homozygous individual may be killed off at some time after hatching was entertained but was later discarded for reasons given below.

"Extreme" frizzling.

A clue to the whereabouts of the lost homozygous Frizzle appeared when the chicks hatched in 1928 began to assume their definitive plumage.

It became evident that there was an extreme type of frizzling not present in any of the breeding stock from which the chicks had sprung.

In this extreme type the body feathers have not only the rachis extremely recurved but also the barbs so curled that not a feather on the body has the normal flat vane. As a result the general appearance of



Text-fig. 2. Primaries from (*a*) a Rhode Island Red, (*b*) a heterozygous Frizzle, and (*c*) a homozygous Frizzle. All are from the left wing and from females of comparable size.

the bird is woolly (Plate III, fig. 1) rather than frizzled. Microscopic examination has shown that the absence of the normal vane of the feather is not caused, as in the Silky, by the extreme reduction, or absence from the barbules, of the barbicels and hamuli which ordinarily lock the barbs together. It arises from the extreme curling of the barbs, and even of the barbules, so that only a few of the former can remain fastened together. The shafts of the rectrices and remiges are curled

more than in the other type of frizzling, and their barbs are so curled that very little normal vane is formed. The barbs are quickly worn off the primary feathers. A comparison of the feathers in the extreme type, in the ordinary frizzle and in the normally feathered fowl is shown in Text-fig. 2 and in Plate IV, fig. 3.

By the time this new type was recognisable a number of the chicks had died, and others had been marketed. Some were not sufficiently grown to be classified as of the extreme type or as ordinary Frizzles by the time the writer left Edinburgh. However, a classification was made on 16 September, 1928, of 70 frizzled chicks, including all those which were at that time mature enough to be described as belonging to one type or the other. Pedigrees of these chicks were unknown at the time of classification so that, although it was in some cases difficult to place a bird in one class or the other, personal bias was entirely eliminated. Subsequently the pedigree of each chick was traced.

TABLE VI.

Classification of a random sample of frizzled chicks raised in 1928.

	Extreme frizzling	Ordinary frizzling
From matings of ordinary Frizzle × Normal	0	4
Expected	*0*	*4*
From matings *inter se* of ordinary frizzles	25	41
Expected if extreme Frizzles are **FF** *and ordinary Frizzles* **Ff**	*22*	*44*

The records (Table VI) show that the proportion of extreme to ordinary Frizzles among chicks from Frizzle × Frizzle matings was quite close to the proportion of dominant homozygotes to heterozygotes expected in a random sample of the F_2 from a monohybrid cross. The assumption was therefore warranted that the extreme individuals were the lost homozygous Frizzles and that the ordinary Frizzles were always heterozygous.

This view was strengthened when Major Williams wrote that it was his policy "to discard in all cases the narrow feathered and unevenly feathered birds." He stated further that, with his Bantams, mating of plain cock with frizzled hens, or the reciprocal cross, produces frizzled birds having the quality of feather and curl desired, whereas mating frizzled birds together produces "perhaps eighty per cent. Frizzles and of these about three-quarters are narrow feathered and useless."

These facts fit perfectly with the hypothesis that the homozygous frizzled fowl is viable but is discarded by the breeder, with the exception

that one would expect about one-third rather than three-quarters of the last class mentioned to be undesirable. The difference probably indicates a selection for the desired show type so rigid that it excludes not only the homozygous individuals but some of the heterozygous ones as well.

In consideration of all these facts, it is reasonable to assume that the reason why no homozygous frizzled fowls were present among the 22 birds tested in the first year of this investigation was that these birds constituted not a random sample but a population of individuals selected according to a breeder's standard which, presumably, barred out the homozygous specimens.

MATINGS IN 1929.

Fourteen frizzled fowls, offspring of Frizzle × Frizzle matings, were brought to the University of Minnesota in December 1928. One of these was killed by a rat, one died of diphtheritic roup, and another was killed after a prolonged attack of paralysis. The remaining 11 fowls (all females) were mated in March 1929 to a White Leghorn cockerel. One of them (with ordinary frizzling) had no hatchable eggs among the 14 set, and died in April, apparently as a result of having become crop-bound.

Of the 10 Frizzles which produced chicks, 5 exhibited extreme frizzling and 5 were of the ordinary type. Their progenies, shown in Table VII, indicate clearly that, as was anticipated, the five of the extreme type were all homozygous (**FF**) while the ordinary type were all heterozygous (**Ff**).

TABLE VII.

Progenies from frizzled females × White Leghorn male (**ff**), 1929.

	Classification of progeny		
	Extreme frizzling	Ordinary frizzling	Normal feathering
Ordinary Frizzles (**Ff**)			
A 1	None	6	10
A 2	,,	6	3
A 7	,,	5	8
A 11	,,	14	6
A 14	,,	2	2
Total	None	33	29
Expected	*None*	*31*	*31*
Extreme Frizzles (**FF**)			
A 3	None	1	None
A 5	,,	15	,,
A 8	,,	16	,,
A 9	,,	12	,,
A 10	,,	4	,,
Total	None	48	None
Expected	*None*	*48*	*None*

Discussion.

The case of the Frizzle is thus shown to be parallel to that of the Blue Andalusian. In both varieties the phaenotype preferred by the fancier is heterozygous and so cannot breed true to type, but fowls of the kind desired for exhibition can be secured by mating together the two rejected phaenotypes.

The use of the White Leghorn male in the 1929 matings provided an opportunity of observing the effect of combining the sex-linked gene for rapid feathering, carried by that breed, with the autosomal gene for frizzling. The writer ventures no definite opinion concerning the manner of action of the gene for frizzling, but it was considered possible that it might cause one side of the feather follicle to grow more rapidly than the other—the same condition as that causing the curling of hair. If that were the case, the gene **s** for rapid feathering might be expected to produce upon frizzling an effect different from that of its allelomorph **S** for slow feathering. Both members of this pair obviously influence the rate of growth of feather follicles.

As was expected, the White Leghorn male proved to be of the constitution **ffss**. The females were of the following genotypes: 3 **FFs**-, 1 **Ffs**-, 2 **FFS**- and 4 **FfS**-.

Figs. 6 and 7 (Plate IV) show that in both rapid-feathering and slow-feathering frizzled chicks the tips of the feathers are recurved to about the same degree. However, 3 of the 15 chicks (all rapid feathering) from ♀ A. 5 exhibited only a slight degree of frizzling. In one case (Plate IV, fig. 5) there was doubt as to its correct classification till it was found that the barbs of the primaries were curled as in the more obvious specimens. Since only three of these appeared, it is likely that this phaenotype arose from interaction of the gene **F**—not with the gene **S** or its allelomorph—but with some other unknown factors influencing feather growth. The differences commonly observed between rates of feathering of individuals within a flock of which all members are slow-feathering indicate that such factors exist.

During the breeding season the feathers of the Frizzles become broken in most specimens so that the birds appear half naked. This is particularly the case with the homozygous fowls. In July, after the males had been with the flock four months, it was quite easy to sort out the five homozygous hens from the rest of the flock since the former were all nearly naked (Plate IV, fig. 4) while the others were in much better feather. This condition did not result from precipitate moulting but from the

breaking of the feathers, usually quite close to the follicles. This was not confined to the back and head, but also occurred to a lesser extent on the breast and on the outer surface of the neck. It was apparently not all due to the treading by the male. In some cases (Plate IV, fig. 4) only the barbs were broken so that the shafts remaining gave some of the pterylae an appearance of being spiny rather than feathered.

Breeders' opinions concerning the hardiness of frizzled fowls, as given by various poultry books, are controversial, but the majority of them rate the Frizzle as rather delicate and unsuited to cold climates. Buffon (*loc. cit.*) says "ce coq appartient plus particulièrement aux pays chauds, car les poussins de cette race sont extrêmement sensibles au froid et n'y résistent guère dans notre climat."

Sir Claude Alexander, Bart., writing of *Frizzles* in the *Feathered World* (London) of 12 April, 1929, says, "The first difficulty to a beginner lies in the three types of plumage, the correct frizzle, the overdone nearly naked curly and the smooth....Kill the 'curlies'; they cease to thrive as soon as they shed their down and cannot survive our winters."

The writer's observations are not in accord with those just given in these two quotations. The 4 mature frizzles which have died since their arrival in Minnesota were all of the ordinary (heterozygous) type. The remainder, half of which were homozygous (*i.e.* "curly") spent the winter in a well-built but unheated pen while the temperature outdoors ranged as low as 24° F. below zero, *i.e.* 56° below freezing. The mean temperatures (Fahr.) here for the months of January and February, 1929, kindly furnished by the United States Department of Agriculture Weather Bureau at St Paul, were as follows:

	Mean daily minimum	Mean daily maximum	Mean daily mean
January	−6·1°	9·9°	1·9°
February	2·1°	17·2°	9·6°

Under these circumstances the conclusion is justified that both types of Frizzles are quite viable and able to withstand exceedingly cold weather, at least when kept dry. Furthermore, the fact that homozygous and heterozygous fowls were found in the expected proportions in a random sample of the population at Edinburgh (Table VI) indicates that the survival rate of the homozygotes is quite as good as that of the heterozygotes.

Summary.

1. An investigation has been conducted to determine the mode of inheritance of frizzling in the domestic fowl and to substantiate or disprove the current theory that homozygosity for frizzling is lethal.

2. Two types of frizzling, ordinary and extreme, are differentiated.

3. Incubation records failed to show any evidence of a zygotic lethal factor being operative during embryonic development. It was found that no gametic lethal factors are involved.

4. Mating of ordinary Frizzles to normally feathered fowls produced the 1 : 1 ratio of parent phaenotypes expected in a back cross to a recessive. Offspring from matings *inter se* of normally feathered fowls extracted from matings of Frizzle × Frizzle were all normal.

5. The progeny from matings *inter se* of ordinary Frizzles gave a 3 : 1 ratio of Frizzles to Normals and in a random sample of this population the ratio of extreme to ordinary Frizzles was as 1 : 2.

6. Five extreme Frizzles were tested and proved to be homozygous for the character, producing, when mated with a normally feathered male, only chicks showing the ordinary type of frizzling.

7. Eighteen ordinary Frizzles were tested and found to be heterozygous.

8. Reciprocal crosses showed that the character is not sex-linked.

Conclusion.

It is concluded that frizzling is a dominant character, the expression of which is dependent upon a single autosomal pair of factors. The homozygous individual exhibits a more extreme type of frizzling than does the heterozygous one. The reputed inability of Frizzles to breed true arises from a preference by the fancier for the phaenotype exhibited by the heterozygous fowl, resulting in the exclusion from the breeding pen of all those that are homozygous for frizzling. Both genotypes are quite viable.

Acknowledgments.

The writer wishes to express his indebtedness to Major G. S. Williams, Tredrea, Perranwell, Cornwall, who not only supplied valuable data concerning his own flock but also, at his own suggestion, very generously loaned ten Frizzles for the entire breeding season. Thanks are also due to the Animal Breeding Research Department, Edinburgh, for birds and facilities placed at the writer's disposal, and to Prof. F. A. E. Crew for suggestions and advice.

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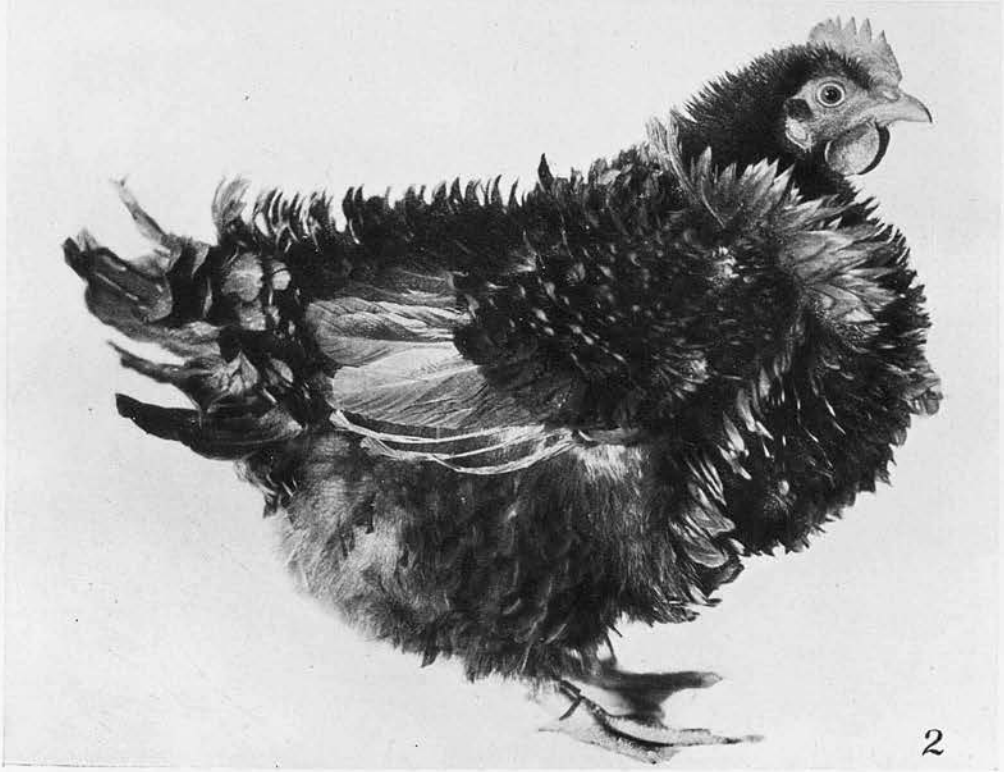

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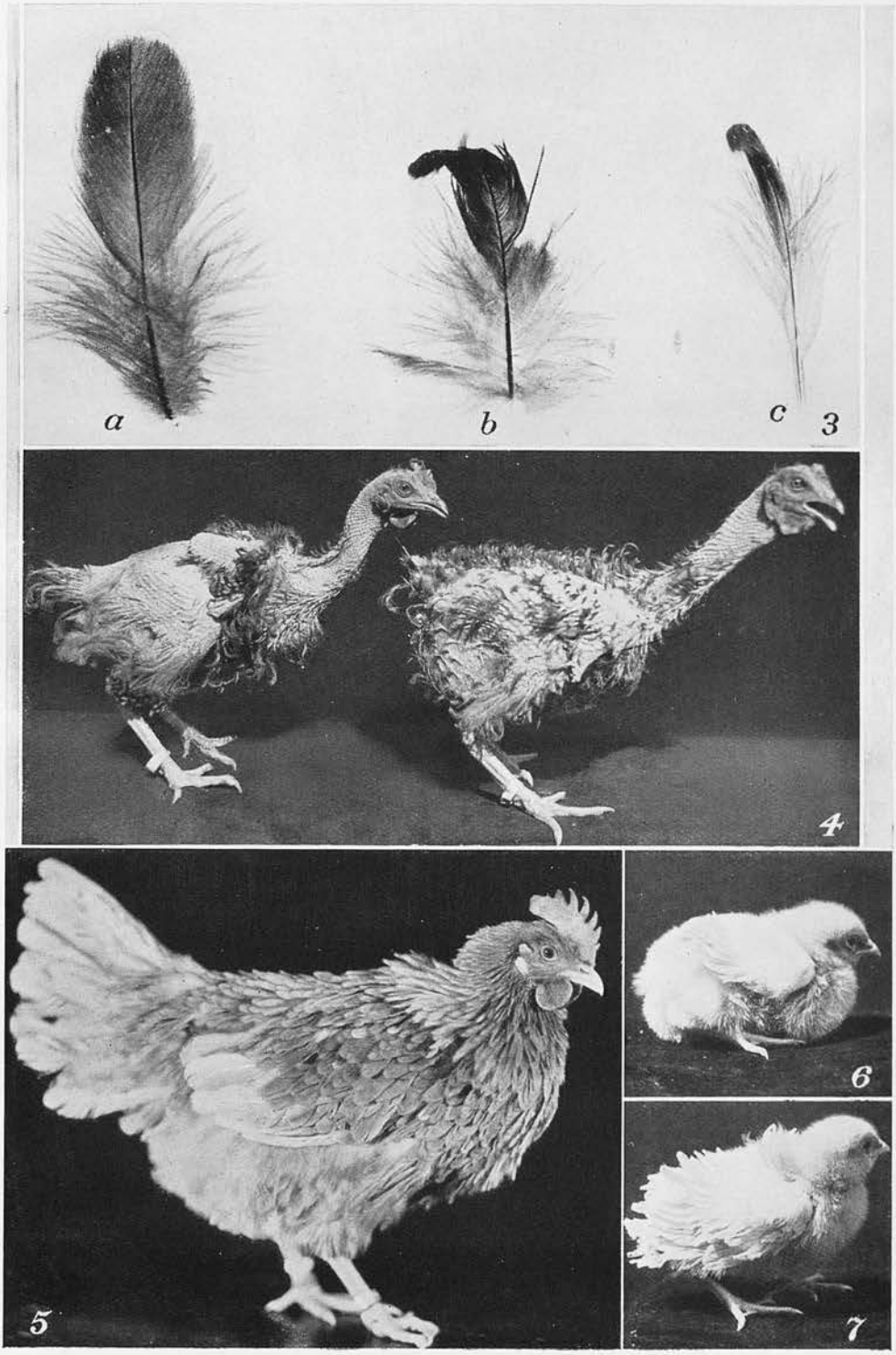
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EXPLANATION OF PLATES III, IV.

PLATE III.

Fig. 1. A Frizzle of the extreme or "curly" type, homozygous for the character.

Fig. 2. A Frizzle of the ordinary type preferred by the fancier, heterozygous for the character.

PLATE IV.

Fig. 3. Feathers from the wing bow of (*a*) a Rhode Island Red, (*b*) a heterozygous Frizzle, and (*c*) a homozygous Frizzle. All are from females in definitive plumage.

Fig. 4. Two homozygous female Frizzles photographed 18 July, 1929, at the end of the breeding season. The spiny appearance of the ventral pteryla in the lower neck region, caused by the feathers losing their barbs, is evident.

Fig. 5. One of three fowls, from a homozygous Frizzle ♀ × White Leghorn ♂, exhibiting only a slight degree of the character.

Fig. 6. A 14-day male chick heterozygous for frizzling and for slow feathering (**FfSs**).

Fig. 7. A 14-day rapid-feathering female chick heterozygous for frizzling (**Ffs-**).

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GENETICS OF THE FOWL. II. A FOUR-GENE AUTOSOMAL LINKAGE GROUP

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University of Minnesota, St. Paul, Minnesota

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GENETICS OF THE FOWL. II.
A FOUR-GENE AUTOSOMAL LINKAGE GROUP[1]

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Received August 5, 1932

While the study of sex-linked genes in the fowl has proceeded in a very satisfactory manner, geneticists have experienced some difficulty in discovering linkage groups among the autosomes of that species. In this paper there are reported certain data on a case of autosomal linkage which, when considered along with the findings of other workers, permit the definite assignment of four genes to one linkage group and give some idea of their arrangement in the chromosome.

The literature on autosomal linkage in the fowl is so scanty that it may be briefly reviewed. A start in this direction was made when DUNN and JULL (1927) reported some evidence for the linkage of dominant white, cerebral hernia and polydactyly. Additional data presented by DUNN and LANDAUER (1930) made it clear that there is close linkage between the genes for the first two of these characters and suggested that polydactyly might be in the same chromosome but comparatively loosely linked with the other two. More extensive data obtained by WARREN (1932) show independent segregation of dominant white and polydactyly in a backcross progeny of 310 fowls.

Meanwhile SEREBROVSKY and PETROV (1928, 1930) reported linkage of creeper and rose comb, with crossing over of 9.1 percent in 297 progeny from diheterozygous males. A much closer linkage of these two genes was decisively demonstrated by LANDAUER (1931) who found only 0.45 percent of crossing over between them in 4483 backcross progeny.

SEREBROVSKY and PETROV (1930) postulated that creeper also belonged in the linkage group containing dominant white, but their evidence for such an assumption was hardly adequate and the possibility is disproved by the evidence (see table 6) for the independence of dominant white and rose comb. These investigators also present evidence that the genes for blue plumage color and bare neck may be linked.

More recently SUTTLE and SIPE (1932) have reported the finding of about 28 percent of crossing over between frizzling and crest. The data presented below show that frizzling is linked with dominant white. It therefore follows that dominant white, cerebral hernia, frizzling and crest are in one linkage group.

[1] Paper No. 1132 of the Journal Series of the MINNESOTA AGRICULTURAL EXPERIMENT STATION.

It must be pointed out that while a considerable number of linkage trials not reviewed above have shown only the independence of the genes tested, the possibility remains that certain pairs might be in one chromosome but too far apart to show any linkage. Thus WARREN (1932) has presented linkage-test data which, taken with some from the work of SEREBROVSKY and PETROV (1930), cover 35 of the 36 possible combinations in pairs of the following nine autosomal characters: naked neck, rumpless, white skin, dominant white, leg feathering, rose comb, pea comb, crest and polydactyly. All of these 35 trials showed independence. Since the fowl has (probably) 17 pairs of autosomes, one would expect to find by chance two cases of linkage in 35 pairs tested, and the failure to do so does not mean that certain of the pairs of characters may not subsequently be found to belong in one linkage group.

SYMBOLS

The following symbols will be used to designate the pairs of allelomorphs considered in this paper:

I —dominant white	*i* —no inhibitor (of melanin)
h_e—cerebral hernia	H_e—normal skull
F —frizzled plumage	*f* —normal plumage
C_r—crest	c_r —no crest
W—white shanks	*w* —yellow shanks
R —rose comb	*r* —single comb
C —color (melanin)	*c* —recessive white

CROSSING OVER BETWEEN *F* AND *I*

In females. Backcross. Repulsion phase

It has long been known that the White Leghorn is a potentially colored bird in which pigmentation is inhibited by the dominant gene *I*. Fowls heterozygous for *I* are white but usually have a few feathers wholly or partially black. Frizzling has been shown by LANDAUER and DUNN (1930) and HUTT (1930) to be a unifactorial character dependent upon the dominant gene *F*. It is quite useful for linkage studies partly because heterozygotes are quite easily distinguished from both homozygotes and normal fowls, but also because the character is usually recognizable at two weeks or earlier.

In 1930, twelve females derived from a cross of colored frizzled females and White Leghorn male (*ff II CC*) were backcrossed to a White Orpington male. These twelve were thus of the constitution $\frac{Fi}{fI}$ *CC* and the Orping-

ton was $\frac{fi}{fi}$ *cc*. Since the females were homozygous for *C*, the recessive white introduced by the Orpington could not in any way complicate the 1:1 ratio of white to colored fowls expected from the segregation of *I* and *i*. Classifications were made at three weeks and in most cases were checked at four months. Both of the dominant characters are so distinct from their recessive allelomorphs that errors in classification are most unlikely. Red color was introduced by the twelve females, the dams of which were phenotypically frizzled Rhode Island Reds. Although this red is only partially inhibited by *I*, the presence or absence of the latter gene is easily recognized by the absence or presence, respectively, of black pigment in the wings and tail.

In the segregation of *F* and *I* in each of these twelve progenies (table 1) there is evident a distinct departure from the 1:1:1:1 ratio to be expected if the two genes were independent.

TABLE 1

Segregation of F and I in backcross, repulsion phase.

FEMALE	FRIZZLED		NORMAL	
	WHITE *FI*	COLORED *Fi*	WHITE *fI*	COLORED *fi*
K 451	3	6	10	3
K 452	2	10	3	1
K 453	1	7	3	1
K 454	3	5	7	0
K 455	0	5	4	1
K 458	0	4	6	0
K 459	1	4	2	1
K 460	1	6	6	1
K 461	3	5	9	2
K 462	0	3	3	1
K 463	0	4	1	0
K 475	2	2	5	2
Unpedigreed eggs*	2	2	4	0
Totals	18	63	63	13

* Included because all females in the pen were of the same genotype.

While there are 81 frizzled to 76 normal and 81 white to 76 colored, a close fit to the expected 1:1 ratio in each pair, there is a marked excess in the *Fi* and *fI* classes with a corresponding deficiency in the *FI* and *fi* groups. Since *F* and *I* entered the cross in the repulsion phase, the segregation observed is to be expected if these two genes are linked. The birds in all four classes may be grouped as follows:

Parental combinations (*Fi* and *fI*): 126

New combinations (*FI* and *fi*): 31

Crossing over in this case is thus 19.74 percent.

In females. Backcross. Coupling phase

The only data available for this type of cross are those obtained from a mating made in 1932 of females having the genetic constitution $\frac{FI}{fi}$ to an Ancona male having the genotype *ffii*. Unfortunately only three females produced viable chicks so that the data are quite meagre. Classifications were made at two weeks and doubtful cases were re-examined at later periods. The numbers in the four possible classes were as follows:

Frizzled white	*Frizzled colored*	*Normal white*	*Normal colored*
FI	*Fi*	*fI*	*fi*
15	2	4	12

Parental combinations, *FI* and *fi*: 27
New combinations, *Fi* and *fI* : 6

Considering that the numbers are small, the amount of crossing over —18.18 percent—is remarkably close to that found with larger numbers in the repulsion phase.

In the male. Backcross. Coupling phase

In 1932, A 1064, a white male proved to be heterozygous for frizzling and for dominant white, was backcrossed to twelve colored females having normal plumage. Subsequent segregation of *F* and *I* showed that these two genes were carried by the male in the coupling phase, so that the male was $\frac{FI}{fi}$ and the females *ffii*. The progeny were distributed as follows:

Frizzled white	*Frizzled colored*	*Normal white*	*Normal colored*
FI	*Fi*	*fI*	*fi*
39	5	8	30

Parental combinations (*FI* and *fi*): 69
New combinations (*Fi* and *fI*) : 13

The amount of crossing over in the gametes of this male was thus 15.85 percent.

Other evidence of linkage of *F* and *I* was obtained in 1931 from the mating of L 15, a male heterozygous for frizzling, dominant white and color, to five colored females which were heterozygous for frizzling and color. All of these birds were from dams *FfIiCC* in constitution and were sired by a White Orpington. L 15's genetic formula was therefore $\frac{FI}{fi}$ *Cc* and the five females were $\frac{Fi}{fi}$ *Cc*.

The occurrence of recessive whites (*cc*) among the progeny of this cross

would at first sight appear to complicate any reading of the segregation of the *I* and *i* genes, but the theoretical expectations can be easily calculated.

The data are not suitable for the computation of the amount of crossing over, but the observed distribution can be compared with that expected on the basis of the linkage found in other matings. Although crossing over between *F* and *I* in females was 19.74 and 18.18 percent in repulsion and coupling phases, respectively, there is a possibility that linkage may be stronger in males than in females, and accordingly it would seem safer to assume for L 15 an amount of crossing over approximately the same as that found in the gametes of ♂A1064, namely, 15.85 percent. Allowing for 16 percent crossing over between *F* and *I* and the independent segregation of *I* and *C* (BATESON and PUNNETT 1908, HADLEY 1914) the expected proportions of male and female gametes in this cross are as follows:

Male gametes		*Female gametes*	
FIC	21	*FiC*	25
FIc	21	*Fic*	25
fIC	4	*fiC*	25
fIc	4	*fic*	25
FiC	4	..	..
Fic	4	..	..
fiC	21	..	..
fic	21	..	..
	100		100

The theoretical distribution of phenotypes derived from these gametes and that distribution to be expected if there were no linkage are given in table 2 along with the actual frequencies in each class.

TABLE 2

Actual and theoretical distributions of the progeny of L15.

	FRIZZLED		NORMAL	
	WHITE*	COLORED	WHITE*	COLORED
Observed distribution	27	12	3	11
Expected (1) with 16 per cent crossing over between *F* and *I*.	28.2	11.5	4.9	8.4
Expected (2) with independent assortment.	24.8	14.9	8.3	5.0
For (1) $\chi^2 = 1.613$; $n=3$; $P=.66$				
For (2) $\chi^2 = 11.343$; $n=3$; $P=.01$				

* White fowls may be *IC*, *Ic* or *ic*.

By the application of the χ^2 test for goodness of fit it is found that the value of P for the fit to the expectation with independence is so small as to preclude the possibility of independent segregation of *F* and *I* in this cross. On the other hand the P value of 0.66 indicates a good fit to the expectation with 16 percent crossing over. It is recognized that this is only an approximate measure of the amount of crossing over which actually occurred, but the χ^2 tests do eliminate the hypothesis of independence and do indicate linkage.

From all the evidence for the linkage of *F* and *I*, summarized in table 3, it is clear that these two genes are in the same chromosome and that crossing over between them amounts to about 18 percent. The crossing over in a total of 272 measurable gametes is 18.38 percent; but in determining this figure the shortcomings of a result obtained by lumping together gametes of both sexes and having the genes in both the coupling and repulsion phases, are fully recognized.

TABLE 3

Summary of tests for linkage of F and I.

BIRDS TESTED	SEX	TYPE OF MATING	PHASE	GAMETES TESTED NUMBER	CROSSING OVER PERCENT
12 "K" females	♀	Backcross	Repulsion	157	19.74
3 other females	♀	Backcross	Coupling	33	18.18
A1064	♂	Backcross	Coupling	82	15.85
L15	♂	Partial backcross	Coupling	53	Approximately 16.00

ARRANGEMENT OF THE LINKED GENES

From the work of SUTTLE and SIPE (1932) it would appear that the genes for frizzling and crest are about 28 crossover units apart. So far as can be ascertained from their data, it is not clear that all the FfC_rc_r females used in their final crosses had the two dominant genes in the coupling phase. If some of these were $\frac{F\ c_r}{f\ C_r}$ and others $\frac{F\ C_r}{f\ c_r}$ (as seems possible from the fact that these birds were apparently two or more generations removed from the original frizzled, crested parent), the resultant distribution would not give a true measure of crossing over. However, since the percentage of crossing over in these females was almost the same as in a male which was evidently $\frac{F\ C_r}{f\ c_r}$, the data for the females are evidently valid, and it may be accepted that *F* and C_r are about 28 units apart.

This being the case, and the gene *I* being about 18 units from *F*, it follows that *I* and *Cr* should be either closely linked (about 10 units apart) or very loosely linked (depending upon whether *I* lies between *F* and C_r or to the left of both). In the latter case, the crossing over to be expected between *I* and C_r would be 46 percent, less whatever reduction would be caused by double crossing over. The only evidence so far available on this point is found in a small F_2 generation involving *I* and C_r reported by DUNN and JULL (1927) in which the segregation approached that to be expected if the genes were independent. The same authors mention (p. 32) a backcross of an IiC_rc_r male to Brown Leghorn females but report only the ratio of crested to non-crested among the offspring. If *I* and C_r were closely linked, the fact would surely have been noticed in this mating, whereas crossing over of more than 40 percent would have been almost indistinguishable from independent assortment except in larger numbers than they had. It therefore seems probable that *I* and C_r are very loosely linked and that *F* lies between them.

The data thus far available do not definitely establish the relationships between dominant white and cerebral hernia, but DUNN and LANDAUER (1930) have shown that the *I* and h_e are very closely linked. Unfortunately the expression of h_e is somewhat irregular in any but specially selected material so that it was impossible for these investigators to determine more exactly the linkage relations of the genes with which they worked. In one backcross in which the distribution indicates that h_e was fully expressed, crossing over between *I* and h_e was about 11 percent.

In consideration of the findings reviewed above and pending further data, it seems not unlikely that the four genes in this chromosome are arranged in the following order: *I*, h_e, *F* and C_r, and spaced somewhat as is suggested in figure 1. The gene h_e may be on either side of *I*.

It is desirable that some gene be located between *F* and C_r to permit working with shorter map distances in this region, and also that all the existing data be confirmed and extended. Experiments in these directions are in progress at this laboratory.

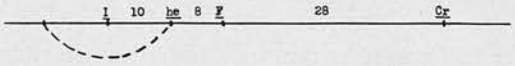

FIGURE 1.—Tentative arrangement of genes in the IFC_r chromosome.

GENES NOT IN THE IFC_r CHROMOSOME

To facilitate further progress in mapping the chromosomes of the fowl it is desirable to report all genes which have thus far been tested and found to show no linkage with those in the IFC_r chromosome. (Students of the genetics of maize have found it preferable to label the chromosomes

of that species by some distinctive gene or genes, rather than as chromosomes I, II, III, etc. For the present, their example seems well worth following.)

Rose comb (R)

In 46 progeny from two *FfRr* females backcrossed to an *ffrr* male the following distribution was found.

Frizzled rose	*Frizzled single*	*Normal rose*	*Normal single*
13	9	11	13

The numbers are small but the class frequencies suggest independence.

Better evidence that *R* is not in the IFC_r group was obtained indirectly by LANDAUER'S (1932) finding of no linkage between frizzling and creeper (table 6). Since creeper is very closely linked to *R* (LANDAUER 1931), the elimination of creeper should automatically eliminate *R* from this linkage group. It is therefore probable that the excess of parental combinations observed by DUNN and JULL (1927) in a backcross of rose, crested males to single, non-crested females (table 6) merely represented a chance deviation from the expected ratio. *R* and *I* were independent in their data.

White Shanks (W)

Evidence that *F* and *W* are independent was obtained in five matings involving these two genes (table 4).

Although the deviations from expectation in the totals for the backcrosses are fairly large, it seems likely that they arose from chance deviations in the progenies of the two males. In these some error may have been incurred by classifying some of the birds before maturity. The dis-

TABLE 4

Segregation of F and W in five matings.

TYPE OF MATING	FRIZZLED		NORMAL	
	WHITE SHANKS *FW*	YELLOW SHANKS *Fw*	WHITE SHANKS *fW*	YELLOW SHANKS *fw*
1. Backcross ♀♀ *FfWw* × ♂ *ffww*.	9	8	8	11
2. Backcross ♂ *FfWw* × ♀♀ *ffww*.	12	14	12	5
3. Backcross ♂ *FfWw* × ♀♀ *ffww*.	2	11	15	7
Totals	23	33	35	23
Expected with independence	*28.5*	*28.5*	*28.5*	*28.5*
4. F_2 observed	24	9	9	2
Expected with independence	*24.75*	*8.25*	*8.25*	*2.75*
5. Partial backcross, ♂ *FfWw* × ♀♀ *Ffww*	11	5	2	6
Expected with independence	*9*	*9*	*3*	*3*

tributions in matings 4 and 5 are based on adult classifications and fit the expectations fairly well. More data are desirable but so far these two genes appear to be independent.

This is confirmed by the independent segregation of dominant white and white shanks in reciprocal backcrosses and in a partial backcross (table 5).

TABLE 5

Segregation of I and W in three matings.

TYPE OF MATING	WHITE*		COLORED	
	WHITE SHANKS *W*	YELLOW SHANKS *w*	WHITE SHANKS *W*	YELLOW SHANKS *w*
1. Backcross ♂*IiWw* × ♀ ♀ *iiww*	17	11	7	8
2. Backcross ♀ ♀ *IiWw* × ♂*iiww*	10	3	6	4
Totals	27	14	13	12
Expected with independence	*16.5*	*16.5*	*16.5*	*16.5*
3. Partial backcross ♂*IiWwCc* × ♀ ♀ *iiWwCc*	17	6	14	5
Expected with independence	*19.7*	*6.6*	*11.8*	*3.9*

* In mating 3 white fowls may be *IC*, *Ic* or *ic*.

If the excess in the *IW* class of the backcross progeny were accompanied by a corresponding excess in the *iw* group, linkage would be indicated, but the deficiency in the latter class (table 5) suggests that the excess of *IW* birds can be ascribed to chance or, more likely, to errors in classification. The distribution in mating 3 is based upon descriptions of adults and fits the expectation remarkably closely.

From these two sets of data it is evident that *W* is either not in the IFC_r chromosome, or, if it be present, too far removed from both *I* and *F* to show linkage with either of them.

Other Genes

Evidence that fourteen characters are independent of the IFC_r group is summarized in table 6.

As is to be expected, characters showing no linkage with one member of the group have also shown independence with other members of the IFC_r chromosome. It may be noted that the possibility that polydactyly belongs to this linkage group, as was suggested by DUNN and JULL (1927), is not excluded by the data thus far available. The combined backcross ratio of parental to new combinations when polydactyly was tested with dominant white was 213:186 and the corresponding ratio in trials with crest was 281:243. The question probably will not be settled till some gene is located to the left of *I* in the chromosome, so that the possibility

TABLE 6

*Characters apparently independent of the IFC$_r$ group.**

LINKED GENE	TESTED WITH	INVESTIGATOR	EVIDENCE FOR INDEPENDENCE
I	Recessive white (or black)	BATESON and PUNNETT (1908)	Independence in 77 backcross and in 67 F_2
I	Recessive white (or black)	HADLEY (1914)	Independence in 167 F_2
I	Rumplessness	DUNN (1926)	No linkage in 231 progeny
I	Rumplessness	WARREN (1932)	Backcross 84:104
I	Rose comb	DUNN and JULL (1927)	Independence in 170 F_2 Backcross 31:28
I	Rose comb	WARREN (1932)	Backcross 88:91
I	Pea comb	WARREN (1932)	Backcross 285:262
I	Polydactyly	SEREBROVSKY and PETROV (1930)	Backcross 52:37
I	Polydactyly	WARREN (1932)	Backcross 161:149
I	Creeper	SEREBROVSKY and PETROV (1930)	Backcross 96:80
I	Silky	DUNN and JULL (1927)	Independence in 93 F_2
I	White shanks	HUTT (this paper)	See table 5
I	White shanks	WARREN (1932)	Backcross 122:107
I	Leg feathering	WARREN (1932)	Backcross 157:137
I	Naked neck	WARREN (1932)	Backcross 304:308
F	Black	SEREBROVSKY and PETROV (1930)	Backcross 63:50
F	Blue	SEREBROVSKY and PETROV (1930)	Backcross 79:88
F	Split comb	SEREBROVSKY and PETROV (1930)	Backcross 33:40
F	Split comb	LANDAUER (1932)	Backcross 84:91
F	Rose comb	SEREBROVSKY and PETROV (1930)	Backcross 24:24
F	Rose comb	HUTT (this paper)	See page 89
F	White shanks	HUTT (this paper)	See table 4
F	Creeper	LANDAUER (1932)	Backcross 97:96
F	Naked neck	LANDAUER (1932)	Backcross 116:117
F	Mesodermal pigment	SEREBROVSKY and PETROV (1930)	Backcross 39:35
C_r	Silky	DUNN and JULL (1927)	Fair fit to independence in 62 F_2
C_r	Split comb	SEREBROVSKY and PETROV (1930)	Backcross 225:203
C_r	Pea comb	SEREBROVSKY and PETROV (1930)	Backcross 177:173
C_r	Rose comb	DUNN and JULL (1927)	Backcross 110:75
C_r	Rose comb	WARREN (1932)	Backcross 80:92
C_r	Rose comb	SEREBROVSKY and PETROV (1930)	Backcross 336:282
C_r	Blue	SEREBROVSKY and PETROV (1930)	Backcross 120:119
C_r	Black	SEREBROVSKY and PETROV (1930)	Backcross 222:215
C_r	White shanks	SEREBROVSKY and PETROV (1930)	Backcross 87:73
C_r	White shanks	WARREN (1932)	Backcross 107:115
C_r	Muff and beard	SEREBROVSKY and PETROV (1930)	Backcross 388:328
C_r	Rumplessness	WARREN (1932)	Backcross 112:122
C_r	Naked neck	WARREN (1932)	Backcross 125:107
C_r	Leg feathering	WARREN (1932)	Backcross 115:119
C_r	Polydactyly	SEREBROVSKY and PETROV (1930)	Backcross 160:130
C_r	Polydactyly	WARREN (1932)	Backcross 121:113

* The ratio given in backcross data is that of parental combinations to new combinations.

of polydactyly being near the extreme left end may be examined, unless the character be first found linked to some other which is definitely known to be independent of this group.

SEREBROVSKY and PETROV (1930) have reported a considerable number of linkage trials involving *I*, *F*, or C_r, but some of them had numbers too few to indicate either linkage or independence and for that reason not all of their data are included in table 6. Additional evidence that genes for leg feathering and mesodermal pigment are not in the IFC_r group was provided by DUNN and JULL (1927), chiefly in F_2 data. These workers also found several characters independent of cerebral hernia, but since h_e was somewhat irregular in its appearance in their stocks, and since DUNN and LANDAUER (1930) found it closely linked with *I*, it seems unnecessary to present their data. Genes independent of *I* are bound to be independent of h_e.

The association between crest and the trifid or multiple-point condition of the rose comb is well known. In 545 rose-combed birds raised by JULL (1930) from silky crosses, there were only 12 exceptions to the rule that crested birds had multiple point combs and non-crested fowls had single-spike combs. JULL concluded that the twelve had been incorrectly classified and that there is not close linkage between crest and some factor for multiple points but rather that the rose comb is modified by the gene for crest.

THE INFLUENCE OF SEX ON CROSSING OVER

To the best of the writer's knowledge, the first definite evidence that crossing over occurs in the female fowl was found by DUNN and LANDAUER (1930) in a backcross of $\frac{I\ H_e}{i\ h_e}$ females to an $ii\ h_eh_e$ male. This was to be expected in view of the previous finding by CHRISTIE and WRIEDT (1923) that crossing over occurs in the autosomes of the pigeon, which suggested that in birds female digamety is not a bar to crossing over as it is in the silkworm. The numbers observed in either of these cases were hardly adequate for the measurement of the effect of sex on crossing over in birds.

The data thus far available are not sufficient to permit conclusions about sex differences in crossing over in the fowl but it is of interest to note that males thus far tested have displayed slightly less crossing over than females. The figures are:

	Genes	*Crossing over percentage*	
		in ♂♂	*in* ♀♀
LANDAUER (1931)	C_p* and *R*	0.19 (2136)	0.68 (2347)
SUTTLE and SIPE (1932)	*F* and C_r	27.16 (81)	29.87 (77)
HUTT (this paper)	*I* and *F*	15.85 (82)	19.47 (190)

* Creeper.

The numbers in parentheses give the number of gametes tested in each case. Pending further data it would appear that the effect of sex on crossing over is somewhat the same in the fowl as in the rat and the mouse. In these mammals crossing over is somewhat lower in the male than in the female.

If further data on crossing over in the two sexes of the fowl should confirm those given above, it would then be apparent that sex differences in crossing over do not depend upon heterogeneity of the sex chromosomes but upon other conditions associated with sex. In this connection it is of interest to point out that in the locust, Paratettix, and the crustacean, Gammarus, crossing over is also lower in males than in females, and that it is not found at all in the male of Drosophila. The absence of crossing over in the female silkworm is apparently at variance with the situation in other species thus far investigated.

SUMMARY

Crossing over between the genes for frizzling and for dominant white was found to be about 18 percent.

This finding along with those of other workers permits the tentative assignment to one linkage group of the genes for dominant white, cerebral hernia, frizzling and crest, and gives some idea of the arrangement of these genes in the IFC_r chromosome.

It was found that the genes for rose comb and for white shanks are not in this linkage group and the available evidence for the independence of other genes is presented.

Evidence that in the fowl crossing over is less in males than in females is considered and it is suggested that sex differences in crossing over may depend, not on heterogeneity of the sex chromosomes, but upon other conditions associated with sex.

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NILS HERMAN NILSSON-EHLE was born in 1873 at Skurup, Sweden. He obtained his scholastic training at Lund, and in 1909 received the doctor's degree from that institution. In 1910 he was appointed Professor of Botany at Lund, and in 1917, Professor of Genetics. His work has covered a wide field, but his most important contributions have been in the field of cereal genetics.

Contents of previous issues of GENETICS can be found by consulting the AGRICULTURAL INDEX

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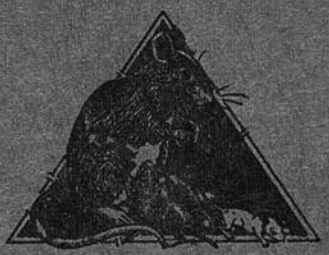

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CONGENITAL TREMOR IN YOUNG CHICKS

F. B. HUTT* and G. P. CHILD
University of Minnesota, St. Paul, Minn.†

IN THE hatching season of 1929 there appeared among some pedigreed S. C. White Leghorn chicks two from the same hen—H287—which were affected with a very evident tremor. These died within a week after hatching. The fact that H287 was mated to a closely related male suggested that there might be a genetic basis for the abnormality. This was confirmed in 1930 when four more chicks were obtained from H287 and related stock. Accordingly an attempt was made to determine the genes involved in its inheritance.

Description

Affected chicks exhibit all degrees of tremor from an agitation so extreme that the animal cannot stand, down to a barely perceptible tremor. In nearly every case, however, the tremor is evident only when the chicks are standing and is quite imperceptible when they squat. In a few of the more extreme cases some twitching was evident in the squatting bird but such instances were rare. This method of finding relief from their peculiar affliction was quickly learned by the chicks and those with tremor were usually quite easily detected in the brooder by their habit of squatting when not actually feeding or drinking.

Contrary to the situation in some cases of involuntary muscular movements in other species, the tremor observed in these chicks is not induced by sudden shock nor is it noticeably increased by excitement. When the chick is standing, the attack is not spasmodic, but continuous.

In some instances affected chicks were not recognized as such when removed from pedigree bags or baskets, but showed the abnormality distinctly after they were placed in the brooders. Apparently while they were closely confined or crowded, with little or no opportunity to use their legs, the symptoms had no opportunity to be manifested. In other cases some chicks which appeared to have a slight tremor when banded shortly after hatching showed no sign whatever of the condition when re-examined a day later. The majority of these had apparently merely been shivering from the temporary chilling suffered when they were removed from the brooder, but the possibility cannot be excluded that some of the birds genotypically "tremblers" were so slightly affected that they could not be recognized.

Some of the chicks affected with tremor have the toes curled underneath and apparently partially paralyzed. This led to the designation of the character in an earlier brief report of this condition (Hutt[3]) as "congenital palsy." It is now clear, however, that many of the less extremely affected birds have no sign of paralysis and accordingly the term tremor is the correct one.

In the few trembling chicks that survived longer than a week, the tremor became less and less noticeable. After eight weeks of age there was no sign of the tremor, although all but two of such chicks were markedly stunted. The anatomical or physiological defect responsible for the character has not yet been discovered.

Rate of Vibration

Some idea of the rapidity of movement in these chicks is indicated in Figure 4*A*. The five birds shown are

*Now at Cornell University, Ithaca, N. Y. This is the third of a series of papers by the senior author prepared under the general title: "Genetics of the Fowl."

†Paper No. 1284 of the Journal Series of the Minnesota Agricultural Experiment Station. The investigation was supported by a grant from the Fluid Research Funds of the Graduate School of the University of Minnesota.

all siblings of exactly the same age—three days. The time of the exposure, which was made with a flash bulb, was only one-fiftieth of a second. This was short enough to ensure sharp images of the four normal chicks at the right, but the one at the left was shaking so rapidly that only its toes present an unblurred image. The two separate images at the anterior end of this chick give some idea of the distance travelled by the head in one-fiftieth of a second.

The rate of vibration has been measured more accurately with a kymograph. A string was tied around the body of the chick to be tested, just behind the wings. This was fastened to one end of a recording pencil, the other end of which touched the revolving drum of the kymograph. To prevent chilling an electric light bulb was placed near the chicks.

Owing to the fact that chicks with tremor will not stand long, but either fall, squat or move about, it was difficult to record their movements, and only short records were obtained of standing chicks. Typical examples are shown in Figure 5, *A* and *B*. Figure 5*A* shows the movement of a two-day-old chick and Figure 5*B* shows that of the same chick at four days. In all the records shown, the smoked paper on the kymograph drum was revolving at the rate of 1.026 centimeters per second. The average number of complete (double) vibrations for this chick at two days and four days was 7.3 and 6.2 respectively per second.

Figure 5*C* shows the record of a day-old chick with tremor whose head was supported. The number of vibrations per second was 10, but, owing to the support provided, the amplitude of the vibration was comparatively small. Figure 5*D* shows the record of this same chick when squatting. The undulations represent breathing movements and there is no sign whatever of tremor.

In Figure 5*E* is shown the record of a fifteen-day-old chick standing, which at two days was shaking at the very rapid rate of 17 vibrations per second. Any vibration remaining is practically imperceptible. The record in Figure 5*F* shows a record of breathing for a normal control chick, standing, at two days of age.

The following data on the rate of vibration at different ages show the tendency for the rate of vibration to become less as the chicks get older:

Chick	Age	Complete Vibrations per second
C1709	1	10
D2310	1	13
C1655	2	16.5
I 29	2	10
D2167	2	7.3
D2310	4	8.5
D2315	4	8.5
D2167	4	6.2
C1655	15	0

Effects on Viability

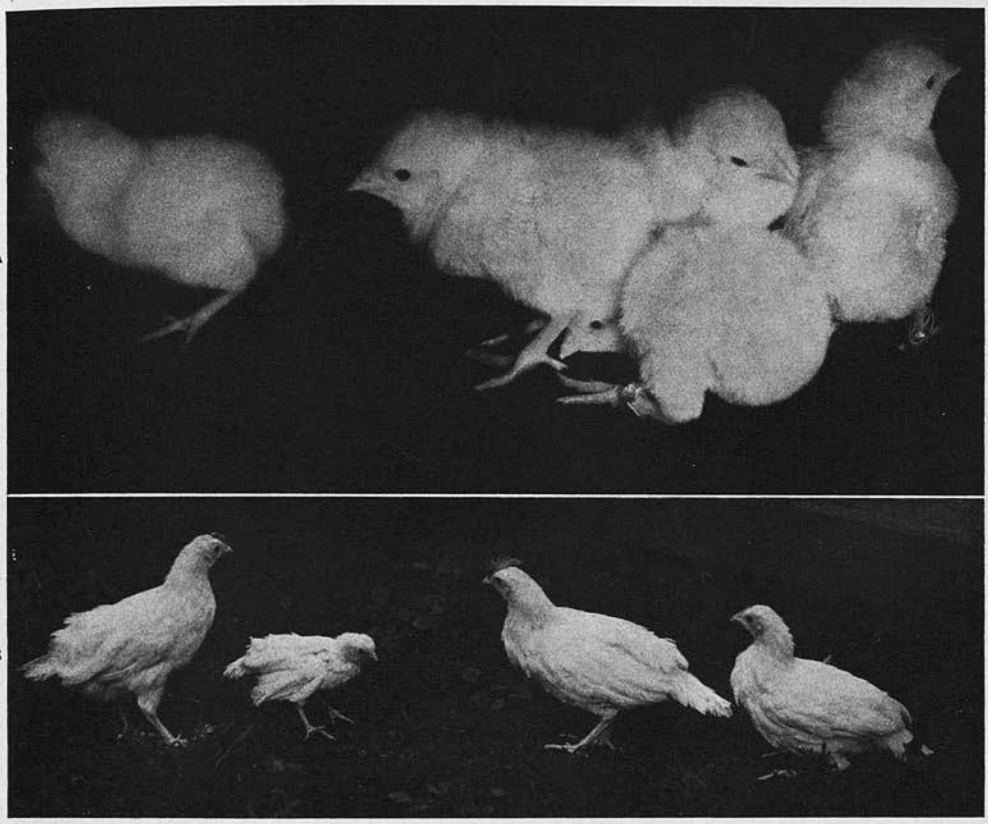
The majority of the chicks affected with the tremor die within a week after hatching. Among 35 such chicks for which exact data are available the ages at death were as follows:

1 to 7 days	24
7 to 30 days	7
30 to 90 days	2
277 days	1
Still alive at 14 months	1

These figures show that 68 per cent of the affected chicks died within a week and 88 per cent within a month after hatching. To some extent the early mortality resulted from the inability of extremely affected chicks to move about freely and to find feed and water. Others literally shook themselves away from the heat and got chilled. But even some which were kept in extremely favorable conditions, where it was difficult for them to get chilled and where feed was easily accessible, grew very slowly and soon died.

Effects on Growth

In Figure 6 the growth rates of four chicks with tremor are compared with those for their normal siblings. One of the four died at two weeks of age, but it is obvious that growth in the others was greatly retarded. Except for two birds with tremor which lived to over nine months of age, the three whose



A TREMBLING CHICK AND NORMAL SIBLINGS

Figure 4

Five siblings from a tremor-producing hen. The exposure, one-fiftieth of a second, was short enough to result in sharp images for the four normal chicks on the right, but not short enough to catch the chick on the left, which was affected with congenital tremor. In the lower photograph are shown four of the five chicks photographed again at 51 days. The chick which originally had tremor weighed, when this photograph was taken, about one-third as much as her normal sister on the right. Her retarded growth has resulted in an abnormal bulging of the cranium.

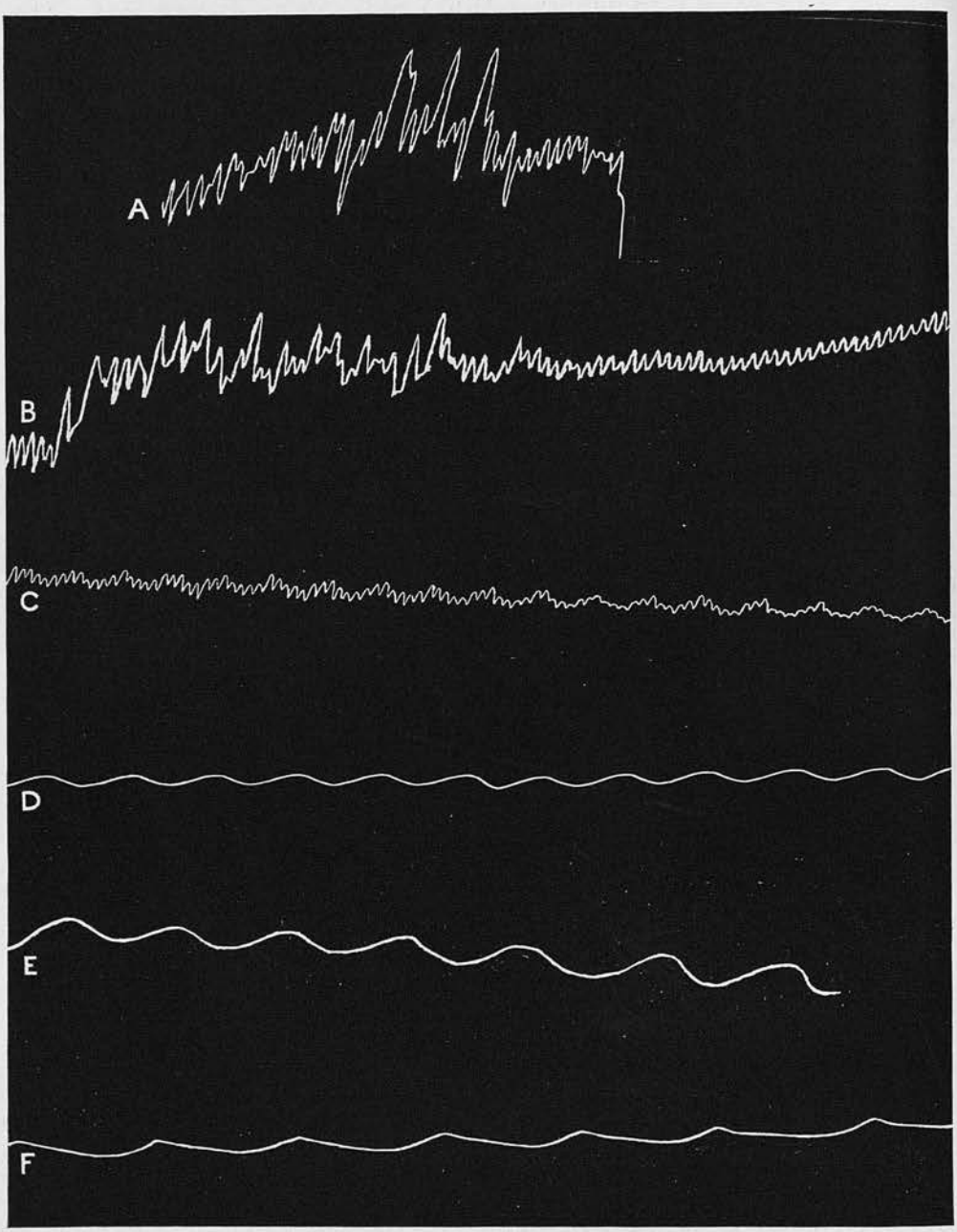
growth curves are shown were the most vigorous of all the chicks with tremor.

The effect of the character is more strikingly shown in Figure 4*B*. The four chickens illustrated were all hatched from the same hen on March 30, 1932. At the time of the photograph they were 51 days old. Their weights, reading from left to right, were as follows:

Bird	Weight in grams at 2 days	at 51 days
♂ B2104 (normal)	34	314
♀ B2101 (tremor)	31	103
♂ B2103 (normal)	33	387
♀ B2102 (normal)	32	299

The chick with tremor, second from the left, shows the bulging of the cranium so commonly seen in stunted and dwarf chicks. This malformation was evident in nearly every chick with tremor that survived beyond a month. It is attributed, not to any direct effect of the gene for tremor, but rather to the retardation of growth induced by that gene.

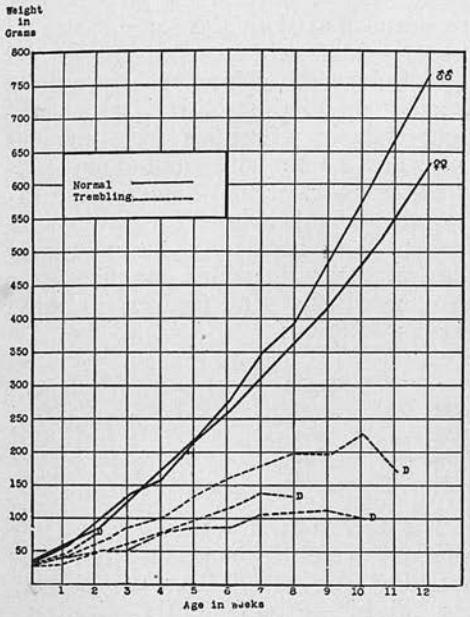
Of the two chicks with tremor which survived longer than three months, one attained a maximum weight of 1,029 grams at 30 weeks, the other grew to 1,375 grams at 40



GRAPHIC CONTRAST BETWEEN TREMBLING AND NORMAL CHICKS

Figure 5

Kymograph records of chicks with tremor (*A-E*), and of a normal chick (*F*). *A*—Record of a chick with tremor two days old, standing without support. *B*—Same chick as above four days, standing. *C*—Tremor, one day, partially supported, and *D*—same chick as in *C*, squatting. *E*—Chick with tremor fifteen days old, standing. *F*—Normal control two days old, standing. The tremor is most marked in the very young chicks and becomes less as the chicks grow older. The regular pulsations of the lower three records (*D-F*) are due to breathing, and are not the result of tremor. The disappearance of tremor does not restore the vigor of the "tremblers," for most of those that survive the first week die within a month.



GROWTH RATE OF TREMBLING CHICKS

Figure 6

Growth rate of four chicks with tremor as compared with their normal siblings. The chicks in this brood were among the most vigorous of the chicks showing this condition. Their extremely slow growth rate emphasizes the semi-lethal nature of this variation. Only two "tremblers" have reached maturity.

weeks. The latter figure is somewhat below the weight for normal Leghorn females of that age in the stock from which the bird was raised. The former weight is decidedly sub-normal. Since these two chicks were hatched on April 13th and May 4th, their failure to attain normal weight cannot be attributed to their having been raised out of the normal growing season.

Effect on Reproduction

The smaller of the two birds just mentioned died at 277 days, without ever having laid an egg. The larger one laid her first egg at 239 days of age. The average age at first egg for eight other White Leghorn pullets hatched on the same day as this bird, and, like her, from matings disregarding fecundity, was 191 days. Only one of these eight began laying at a later age—246 days.

Up to 413 days of age, this bird had laid only 35 eggs, but since a number of her less productive pen-mates had been disposed of, there are not adequate controls against which to compare her record. However from the fact that her monthly egg records from December to May were 9, 2, 5, 5, 4, and 10, it is obvious that she was far below normal with respect to fecundity. Of 22 eggs set between December 15th and May 15th, 15 were fertile and these yielded 7 chicks, a proportion which is not unusually low.

Genetics

The genetic analysis of this character has been extremely difficult to conduct. Since affected chicks (with one exception) did not attain sexual maturity, the usual methods—studying an F_1, F_2 and backcross—were out of the question. Any genetic analysis had therefore to be based solely on the observed ratios in matings yielding congenital tremor. These were as follows:

	Chicks	
A—Dams	Normal	Tremor
H 287	19	2
K 167	15	1
K 196	36	3
K 219	20	1
L 152	10	1
A 155	11	1
A 525	12	2
B 121	7	1
B2010	7	2
B2033	6	1
B2024	19	2
B2047	50	5
B2053	20	2
B2058	48	3
B2062	18	1
B2065	24	1
B2067	18	2
C2004	4	1
C2005	12	2
C2006	19	1
C2007	1	1
C2008	5	2
C2078	27	1
Total	408	39
Ratio	10.5 : 1	

B—Sires	(in matings with hens tremor)	that produced
L 2	33	2
L 7	65	6
A1021	6	1
A1024	6	1
B2232	28	7
B2233	107	9
B2241	1	1
B2243	13	2
B2244	85	6
C2221	60	3
C2222	4	1
	408	39

It should be clearly understood that these ratios are for matings in which both male and female were proven producers of congenital tremor. Fowls yielding chicks with this affliction are apparently normal in every respect and the character is therefore recessive in nature. The ratios given above, however, are far from the 3:1 expected for a unifactorial recessive. In some cases where a single chick with tremor was obtained in a progeny of eighteen chicks or more, it was at first believed that there must have been some error in marking the egg or in recording the chick. However, there are at least five such cases in the list of 23 females above. These occurred in different hatching seasons and in different matings. It seems improbable, therefore, that these aberrant ratios can be ascribed to errors in technique. Indeed, the rarity of the character would suggest that it is not genetic in origin were it not that (1) chicks with congenital tremor occurred originally in a mating of closely related stock (2) they have not been observed in other White Leghorns or in other breeds of the university poultry flocks during the six years in which the character has been observed, and (3) in twenty-five years' experience with poultry the senior author has never seen chicks elsewhere similarly afflicted. Moreover, other females in the same pens and mated to the same males as those yielding chicks with tremor did not produce the character in progenies ranging up to thirty. It is also pertinent that in progenies from matings in which both sire and dam produced tremor, or had done so, the ratio of normal chicks to those with tremor was remarkably constant in the four years in which complete data were available. The figures for these are as follows:

	Chicks		
Year	Normal	Tremor	Ratio
1931	80	7	11.4:1
1932	64	7	9.1:1
1933	164	16	10.2:1
1934	94	9	10.4:1

The fact that practically all of the affected chicks died shortly after hatching suggested that the character might be sufficiently disadvantageous to the embryo to cause death in enough of them to account for the observed deficiency of chicks with tremor. This possibility seemed all the more plausible because several chicks with tremor occurred among those which had to be assisted from the shell at hatching time. More extensive data have shown this hypothesis to be untenable. The figures given below compare the rates of embryonic mortality among embryos from *a,* matings in which both sire and dam were known to be tremor-producers, and *b,* matings in which the sire was a producer of tremor but the individual females did not yield the character in eight or more chicks.*

The difference between mortality rates in these two types of matings is neither statistically significant nor large enough to account for a deficiency of chicks with tremor as great as that observed. With the evidence established that embryonic

*Embryonic mortality rate.

	Fertile eggs	Mortality	
		Number	Per cent
(a) Matings yielding tremor	572	241	42.13 ± 2.06
(b) Females not producing tremor	773	295	38.16 ± 1.74
Difference			3.97 ± 2.69

mortality was not responsible for any aberrant ratios in hatched chicks, an explanation of the observed ratios of normal chicks to those with tremor may be attempted.

If the character were sex-linked as well as recessive, one would expect it to occur more often in females (the heterogametic sex) than in males. Since in 35 affected chicks of known sex the ratio was 19 ♂♂ : 16 ♀♀, it is obvious that the character is autosomal.

There are two interpretations of the available data on the genetic basis for this character which seem possible. It may be the result of duplicate factors, with affected chicks homozygous for the recessive allelomorph of each. If these genes be designated T and T_1, the expectation in the progeny from a mating of two diheterozygotes, $Tt\ T_1t_1$, is 15 normal chicks to 1 with tremor. The observed ratio in the general population, as given above, is 10.5 to 1. This, however, does not by itself invalidate the duplicate factor hypothesis because that population contains several females with affected chicks in progenies of much less than 16. Presumably there could be a corresponding number of undiscovered females producing no chicks with tremor in progenies of less than 16, but which would have produced the character in larger families above that figure. Examples of this type are afforded by females K219, B2062, B2065, C2006 and C2078, each of which yielded only one chick with tremor in progenies of 20, 18, 24, 19 and 27 respectively.

If one considers that progenies of at least 16 are necessary for the manifestation of a 15:1 ratio and selects only the thirteen hens with families of that size or over, the ratio of normal chicks to those with tremor is as follows:

	Normal	Tremor
In families of 16 or over	330	25
Expected (15:1)	332.8	22.2

This is a very close fit to expectation. A corollary to the duplicate factor hypothesis is that some of the birds in the population must be by chance of the constitution Ttt_1t_1 or ttT_1t_1 and that these when mated together would give a ratio of 3 normal: 1 tremor. If mated to a diheterozygote, TtT_1t_1, they should yield a ratio of 7:1. In the list of ratios given above it may be noted that the following progenies suggest a 3:1 ratio:

	Chicks	
Dam	Normal	Tremor
B2010	7	2
C2004	4	1
C2007	1	1
C2008	5	2
	17	6

There are also in the list several progenies suggesting 7:1 ratios more than anything else, but some of these may also really belong in the 15:1 class. Unfortunately no 3:1 ratios occur in large progenies, so that it cannot be definitely stated that any mating was yielding a 3:1 ratio.

The duplicate factor hypothesis could be substantiated or disproved if a large number of daughters from tremor-producing matings could be adequately tested. Such a test has been practically impossible in this case because the usual difficulty of getting large progenies from individual hens has been aggravated by the fact that the mutation originally appeared among fowls of rather low fecundity. Their naturally poor reproductive capacity has been anything but improved by the inbreeding resorted to in the attempt to study the character. Some of the tremor-producers died after producing only five or six chicks or less. Others were not detected until the second breeding season. The difficulty of testing either males or females when such large progenies are needed to prove the genotype is obvious.

Tests of genotype were attempted with 65 daughters of birds which had

produced tremor. Of this number 24 had a carrier of tremor in both parents and 41 had only one parent producing the character. Owing to mortality and low production more than half of these birds were not adequately tested, but the following data are of interest:

	No tremor in 6 or more chicks	Produced tremor
Birds with both parents carriers	7	7
Birds with one parent carrier	16	3

While 6 chicks are undoubtedly too few to constitute an adequate test, the limitations of that number should apply equally to the two groups compared. On this basis, when both sire and dam were carriers, 50 per cent of the progeny were also carriers, but when only one parent had produced the character, only 16 per cent of the progeny were carriers. The comparison illustrates the hereditary nature of the character even though it does not elucidate the genetic basis.

Another possibility is that congenital tremor may be a unifactorial recessive, but one so subject to the influence of modifying genes that the degree of its manifestation, or its "penetrance" (in the sense of Timoféeff-Ressovsky), is unusually low in most matings. This is suggested partly by the fact that in moderate cases the tremor becomes less extreme within a week of hatching and eventually disappears as the chick gets older. It is also suggested by the occasional chicks from tremor matings, which display a very slight shiver when taken from the incubator. These are difficult to classify, but, because they have not subsequently shown any signs of tremor, have been considered as merely chilled at banding time.

This hypothesis is supported by the fact that the one bird with tremor which produced offspring yielded in matings with males carrying congenital tremor no chicks with tremor among the seven which were hatched from her. Three of these seven, however, had their toes curled under and apparently slightly paralyzed or incapacitated when hatched. This peculiarity was not evident a day or two later, when the chicks appeared normal in every respect. It seems not improbable that the same defect, which causes the agitation when the chick is standing, but which is inoperative (except in extreme cases) when the chick squats, might be also manifested as a temporary partial paralysis of the extremities of the legs. As chicks so affected get older, both symptoms disappear. The latter interpretation appears somewhat more likely than that of duplicate factors only.

The observed number of chicks with tremor in the whole population of 447 was 39. The expected number for a simple recessive with complete penetrance was 111.75. The actual penetrance (*i. e.*, percentage of those homozygous for the character which actually manifest the phenotype) was therefore 35 per cent, although marked deviations from that figure were evident in individual matings. This is a rather low degree of penetrance, but that fact does not rule out the probability that the character is caused by one gene. Among several genes producing the character "abnormal abdomen" in *Drosophila funebris*, Timoféeff-Ressovsky[7] found some with a penetrance of 20 to 30 per cent and one with a penetrance of only 10 to 15 per cent.

Discussion

Congenital tremor in chicks is evidently a hereditary character undesirable both to the species and to the poultryman. Since it is so subject to the influence of modifying factors that it may appear as infrequently as once in 27 chicks from one mating, the usual methods of testing for carriers would not be efficient. Accordingly, there is no other method of weeding out the character than to

eliminate from the breeding pens all sires, dams, and siblings of affected chicks. Even this would not remove all heterozygotes, but it is doubtful if the loss from the few affected chicks would warrant any greater sacrifice of breeding stock.

The tremor described in this paper is apparently quite different from the hereditary ataxia observed in pigeons by Riddle,[6] nor is it at all comparable to the "congenital loco" studied by Knowlton,[5] a condition which is fairly common in chicks. In neither of these abnormalities does one find the consistent vibration of the whole body which was characteristic of the chicks affected with tremor. It is apparently quite similar in some respects to the hereditary tremor which occurs, though rarely, in man. The pedigree of this reported by Bergman[1] suggests dominance of the condition. Moreover, in his cases and others, the tremor was not apparent at birth, but, contrary to the condition in chicks, became progressively worse after onset at ages of 30 and over. Curschmann,[2] however, states that in some cases of hereditary tremor in man the symptoms may entirely disappear as the affected person gets older. In Bergman's cases the rates of vibration were very much like those measured in the writers' chicks and in both species the tremor disappeared when the afflicted subject was at rest.

Of all the cases of hereditary nervous disorders in other species with which the congenital tremor in chicks has been compared none resembles it as much as the recessive hereditary trembling in guinea pigs, briefly reported by Ibsen.[4] In both species there is a tremor right from birth and in both it becomes less extreme as the animals get older. He does not say if the symptoms are absent when the affected animal is at rest.

It seems not impossible that mutations such as the tremor described in this paper, though undesirable in most respects, might be of value to pathologists seeking to determine the anatomical or physiological bases for similar hereditary diseases in man. The aetiology of hereditary tremor (as distinguished from various ataxias, choreas and *paralysis agitans*) is apparently not known. If principles of human nutrition can be learned from studies with rats and pigeons, it seems equally feasible that genetic abnormalities in lower animals could be utilized to shed some light upon similar conditions in man.

Summary

Chicks affected with congenital tremor exhibit degrees of agitation varying from a shaking so extreme that they cannot stand, down to a barely perceptible tremor. In most cases this movement disappears when the chick squats at rest. The rate of tremor varies from 16.5 to 10 complete (double) vibrations per second in chicks one and two days old. The affliction is most extreme at hatching and gradually disappears in the few chicks which survive to a month or more.

Eighty-eight per cent of affected chicks died within a month of hatching and only one out of 35 was raised to sexual maturity.

Growth of chicks with tremor was greatly retarded and fecundity was unusually low in the one bird raised to laying age.

Twenty-three fowls producing the defect yielded in matings to carrier males a total of 408 normal chicks and 39 with tremor. The deficiency of affected chicks below the number expected for a simple recessive character is shown not to be caused by embryonic mortality in these matings.

Of 35 affected chicks 19 were males and 16 females.

A duplicate-factor genetic basis is shown to account for the observed ratios fairly well, but it is considered a more likely interpretation that the character is a simple autosomal

recessive character having an unusually low degree of penetrance because of the effects of modifying genes.

Other evidence is adduced to show that the defect is definitely hereditary.

The similarities and differences between hereditary tremor in the fowl and similar conditions in other species are briefly considered.

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CONTENTS OF RECENT ISSUES OF THE JOURNAL OF HEREDITY

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LINKAGE RELATIONS OF CREST, DOMINANT WHITE AND FRIZZLING IN THE FOWL[1]

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THIS paper presents data which establish through linkage relationships the arrangement in one chromosome of the three genes, *Cr, I* and *F,* representing the incompletely dominant characters, crest, dominant white and frizzling, respectively. Data are also adduced to show that the gene for cerebral hernia, previously reported to be linked with *I,* is probably identical with the gene for crest.

PREVIOUS STUDIES

Data presented by Dunn and Landauer (1930) made it clear that there is close linkage of the gene for dominant white with that for cerebral hernia, which they considered to be independent of the gene for crest. Subsequently, Hutt (1933) showed that dominant white, *I,* is closely linked with frizzling, *F,* there being about 18 per cent. crossing-over between the two. Meanwhile, Suttle and Sipe (1932) had reported *F* to show about 28 per cent. crossing-over with the gene for crest, *Cr.* It was evident, therefore, that *Cr, I, F* and the gene for cerebral hernia were all in one chromosome, but their arrangement was not definitely established because the linkage relationship of *I* and *Cr* had not been determined. If the arrangement were *I–F–Cr,* then *Cr* and *I* should show something less than 46 per cent. crossing-over, but, if their linear order were *Cr–I–F,* then the cross-over percentage should be approximately 11 or 12 per cent.

[1] Contribution No. 89 from the Department of Poultry Husbandry, Kansas State College, and No. 1362 of the Journal Series of the Minnesota Agricultural Experiment Station.

[2] Now at the Department of Poultry Husbandry, Cornell University, Ithaca, New York. This is the fourth paper in the series by the junior author entitled "Genetics of the Fowl."

LINKAGE DATA

The writers have independently made genetic tests to permit accurate mapping of this chromosome and, in order to present all the available data in one place and to avoid unnecessary expansion of the literature on genetics of the fowl, have collaborated in the presentation of results.

The three characters, crest, dominant white and frizzling, are all incompletely dominant unifactorial characters which have been adequately described in previous papers. Chicks can be classified for the presence or absence of crest with a high degree of accuracy as soon as hatched, but, in order to be more accurate, data concerning crest in this paper are restricted to adult classifications. Dominant white is easily recognized in heterozygotes at hatching. It was shown by Hutt (1930) that chicks can be classified accurately for the presence or absence of frizzling as early as 9 days after hatching, but, since extremely slow-feathering birds may present difficulties in classification, the data concerning frizzling are restricted in this paper to those chicks for which two consistent consecutive classifications were made after 9 days of age.

Cross-over data for the three possible combinations in pairs of *Cr, I* and *F* are presented in Tables 1, 2, and 3. In every case crossing-over was measured by back-crossing heterozygotes to double or triple recessives. Since the three genes all have been shown previously to be autosomal, the data for the back-cross progeny are not presented separately for each sex. When the progeny from any one bird under test were numerous enough to afford a reasonably accurate measure of crossing-over in that bird, they are presented separately. This is done to record the variation among the birds with respect to the amount of crossing-over in their germ cells.

Cr and I

In a previous paper, Warren (1933) presented data on tests for linkage in 35 of the 36 possible combinations in

TABLE 1

LINKAGE RELATIONS OF CREST AND DOMINANT WHITE (BACK-CROSS DATA)

Bird No.	Crossing-over in male—coupling						
	Non-cross-overs		Cross-overs		Total gametes tested	Total cross-overs	Per cent. crossing-over
	Cr I	cr i	Cr i	cr I			
Males							
Kans. 1212M	96	110	11	19	236	30	12.7
Kans. 1216M	119	103	10	12	244	22	9.0
Kans. 1259M	122	124	13	15	274	28	10.2
Total	337	337	34	46	754	80	10.6
	Crossing-over in male—repulsion						
	Cr i	cr I	Cr I	cr i			
Males							
Kans. 1289M	82	86	13	16	197	29	14.7
Kans. 1301M	33	37	3	4	77	7	9.1
Minn. B2237	61	67	8	10	146	18	12.3
Minn. B2240	67	47	9	7	130	16	12.3
Minn. C2223	15	22	5	6	48	11	22.9
Minn. 3 birds	19	26	4	6	55	10	18.2
Total	277	285	42	49	653	91	13.9
Total all males	614	622	76	95	1407	171	12.2
	Crossing-over in female—coupling						
	Cr I	cr i	Cr i	cr I			
Females							
Kans. 9007A	13	18	2	3	36	5	13.9
Kans. 9012A	19	18	3	2	42	5	11.9
Kans. 9014A	12	20	3	3	38	6	15.8
Kans. 9015A	13	12	2	2	29	4	13.8
Kans. 9016A	10	23		3	36	3	8.3
Kans. 9018A	16	15	2	3	36	5	13.9
Kans. 9019A	21	17		4	42	4	9.5
Kans. 9020A	16	18	6	4	44	10	22.7
Kans. 9115A	30	19	5	2	56	7	12.5
Kans. 9183A	33	25	1	2	61	3	4.9
Kans. 9184A	10	13	1	2	26	3	11.5
Kans. 9185A	10	7	4	4	25	8	32.0
Kans. 6 birds	32	27	7	5	71	12	16.9
Total	235	232	36	39	542	75	13.8
	Crossing-over in female—repulsion						
	Cr i	cr I	Cr I	cr i			
Females							
Minn. 3 birds	16	17	2	0	35	2	5.7
Total all females	251	249	38	39	577	77	13.3
Total both sexes					1984	248	12.5

pairs of 9 autosomal characters which were submitted to test. None of these showed any linkage. The only one of the 36 pairs not tested was *Cr* and *I*, and the only reason they were not tested was that the stock prepared for that purpose came to untimely deaths. That this pair of genes does show quite close linkage is now established by the data in Table 1.

The gene for crest used in the Minnesota tests and in the Kansas male 1289M was derived from Houdans, but in all other Kansas data the *Cr* gene originated from Silkies.

The following observed ratios, summarizing data in Table 1, show that a normal segregation was obtained for each pair of genes:

Dominant white: colored – 1003: 981
Crested: non-crested – 979: 1005

The percentage of crossing-over, based on a total of 1,984 gametes, is 12.5, but the variation from bird to bird is quite marked. Disregarding the smaller families, there is a range from 4.9 per cent. (in ♀ 9183A) to 32.0 per cent. (in ♀ 9185A). Much of this variation is probably due to the smallness of the sample, but some of it may be attributed to individual variability in amount of crossing-over.

I and F

Proof that these two genes were linked with about 18 per cent. crossing-over between them was given by Hutt (1933). The additional data presented in Table 2 are in quite close agreement with the previous findings.

The Frizzles used by both of us were descended from the same English stock as that used by the junior author in earlier work. Considering the new data in Table 2, the following ratios were observed in the backcross:

Frizzled: non-frizzled – 448: 385
Dominant white: colored – 429: 404

These two ratios indicate a segregation of the genes *F* and *I* reasonably close to the expected 1:1 ratio. Cross-

TABLE 2

LINKAGE RELATIONS OF DOMINANT WHITE AND FRIZZLING
(BACK-CROSS DATA)

Bird No.	Crossing-over in male—coupling						
	Non-cross-overs		Cross-overs		Total gametes tested	Total cross-overs	Per cent. crossing-over
	FI	fi	Fi	fI			
Males							
Kans. 1289M	156	143	34	28	361	62	17.2
Kans. 1301M	32	25	12	8	77	20	26.0
Minn. B2209	35	34	10	6	85	16	18.8
Minn. B2249	22	27	2	2	53	4	7.5
Minn. B2840	26	23	3	2	54	5	9.3
Minn. C2223	24	17	4	3	48	7	14.6
Minn. 6 birds	45	36	9	7	97	16	16.5
Total	340	305	74	56	775	130	16.8
	Crossing-over in female—coupling						
Females							
Minn. 13 females	29	20	5	4	58	9	15.5
	Previously published results						
Hutt—repulsion in female					157	31	19.7
—coupling in female					33	6	18.2
—coupling in male					82	13	15.9
Grand total in females					248	46	18.5
Grand total in males					857	143	16.7
Grand total in both sexes					1105	189	17.1

ing-over in 1,105 gametes, which comprise all the available data on this pair of genes, was 17.1 per cent. It is noteworthy that the amount of crossing-over varied in different males from 7.5 to 26 per cent.

Cr and F

The *Cr* gene used by both of us in measuring the linkage relationship of these two genes was derived from Houdans. The new data presented in Table 3 yield the following ratios:

Frizzled: non-frizzled – 200 : 161
Crested: non-crested – 162 : 199

While there is an excess of frizzled birds and of non-crested ones, the deviations from the expected ratio are not greater than might occur by chance. The amount of crossing-over in our data, 30.2 per cent. in 361 gametes, is remarkably close to the 28.5 per cent. found by Suttle and Sipe (1932) in the 316 gametes tested by them.

TABLE 3

LINKAGE RELATIONS OF CREST AND FRIZZLING (BACK-CROSS DATA)

Bird No.	Non-cross-overs		Cross-overs		Total gametes tested	Total cross-overs	Per cent. crossing-over
	F cr	f Cr	F Cr	f cr			
	Crossing-over in male—repulsion						
Males							
Kans. 1289M	66	55	26	22	169	48	28.4
Kans. 1301M	30	21	15	11	77	26	33.8
Minn. C2223	19	11	9	9	48	18	37.5
Minn. 2 birds	16	10	2	4	32	6	18.8
Total	131	97	52	46	326	98	30.1
	Crossing-over in female—repulsion						
Females							
Minn. 3 birds	14	10	3	8	35	11	31.4
	Previously published results						
Suttle and Sipe—							
Crossing-over in female					235	68	28.9
Crossing-over in male					81	22	27.2
Grand total in males					407	120	29.5
Grand total in females					270	79	29.3
Grand total in both sexes					677	199	29.4

MAP OF THE CHROMOSOME

The theoretical map distance *Cr* to *F* measured by the cross-over percentages for its two component parts is 12.5 + 17.1 = 29.6, and the actually observed distance as directly measured by crossing-over between *Cr* and *F* is 29.4. If double crossing-over should occur in the distance between *Cr* and *F*, it would reduce the cross-over percentage for this pair of factors. Double crossing-over should make the map distance between *Cr* and *F*, as

measured by the percentage of crossing-over between these two factors, less than that calculated from adding the distances *Cr* to *I* and *I* to *F*. The map distance between *Cr* and *F*, as calculated by the former method, is 29.4 and by the latter, 29.6. The difference between these two values is not statistically significant and furnishes no evidence for double crossing-over occurring in the map distance between these two factors.

Independent confirmation of this suggested map is found in data presented by Sungurov (1933) who observed 21 per cent. crossing-over between *I* and *F*, and 33.6 per cent. crossing-over between *Cr* and *F*. Data on the *Cr* and *I* relationship were apparently not secured. Unfortunately, this paper is known to us only through an abstract which does not give the actual numbers involved.

Double Crossing-over

In order to permit simultaneous measurement of crossing-over between *Cr* and *I*, *Cr* and *F*, and *I* and *F*, a triheterozygous stock of the genotype $\frac{F\ I\ cr}{f\ i\ Cr}$ was produced at Minnesota by crossing *F I* birds with Houdans which carried *Cr*. One of these birds was sent to the senior author and others were tested at Minnesota. The combined results are shown in Table 4.

Since the frequency of crossing-over between *Cr* and *I* is 12.5 per cent., and of that between *I* and *F*, 17.1 per cent., the probability of getting simultaneous crossing-over in the two regions is theoretically $12.5 \times 17.1 = 2.13$ per cent., provided that crossing-over in one region is unaffected by crossing-over in the other. In the observed population of 284 gametes the theoretical expectation would be 6.05 double cross-overs, where in fact none was found. The deficiency might be ascribed to chance, but it is more likely due to the phenomenon of interference whereby a cross-over in one region reduces the chance of a cross-over in an adjacent region. The data from these triheterozygous birds, therefore, show that the fowl con-

TABLE 4
THREE-POINT TEST FOR LINKAGE OF *F*, *I* AND *Cr*

	Non-cross-overs		Cross-overs between F & I		Cross-overs between I & cr		Double cross-overs between F & cr		Total gametes tested
	F I cr	f i Cr	F/i Cr	f/I cr	f i/cr	F I/Cr	F/i/cr	f/I/Cr	
Minn. ♂ C2213	8	7	1	1	2	0	0	0	19
Minn. ♂ C2216	6	3	1	0	1	0	0	0	11
Minn. ♂ C2223	19	11	4	3	6	5	0	0	48
Minn. 3 ♀ ♀	14	10	3	6	0	2	0	0	35
Kans. ♂ 1289M	67	58	13	10	12	11	0	0	171
Total	114	89	22	20	21	18	0	0	284

forms to the behavior observed in other species adequately investigated genetically in that it exhibits the phenomenon of interference. They also confirm the actual measurements of crossing-over, which, since the crossing-over between *Cr* and *F* agrees closely with the sum for the two component parts of that distance, indicate that in this region of the chromosome there is very little, if any, double crossing-over in a distance of approximately 29 cross-over units.

Cerebral Hernia

The literature on the genetic status of cerebral hernia is somewhat confusing. Krautwald (1910) concluded from his anatomical studies that crest is the outward expression of the underlying condition causing cerebral hernia, namely, an accumulation of fluid in the ventricles of the brain, which he described as *hydrocephalus internus*. He observed not only that cerebral hernia never appeared without crest, but also that the size of the crest was always directly proportional to the degree of cerebral hernia. The relationship between the extent of hernia and the size of crest was mentioned by earlier investigators and has been verified by the present writers. Moreover, as Krautwald demonstrated, in the somewhat similar condition in the duck, an encephalocoel occurring in the parieto-occipital region is invariably associated with crest in that region. In both these species, therefore, the crests, though occurring in the chicken in the frontal region of the cranium, and in the duck in the parieto-occipital region, are associated with underlying abnormalities in the brain. It seems unlikely that this would occur unless both crest and cerebral hernia resulted from one and the same underlying cause. In both species the character is hereditary.

The idea that crest might be due to one gene and cerebral hernia to another apparently originated with Davenport (1906), who concluded from his not-very-extensive data that the latter was recessive, the former dominant.

This conclusion was accepted by Dunn and Jull (1927) and by Dunn and Landauer (1930), who obtained some evidence that dominant white and cerebral hernia were linked, with about 11 per cent. crossing-over between them. It should be noted that their determination, though based on very small numbers, agrees closely with the cross-over percentage of 12.5 between *Cr* and *I* determined by the present writers.

Considering all the available data, the observations of Krautwald and especially the fact that no one has yet produced fowls with cerebral hernia and without crest or observed the segregation of such fowls from crested and herniated breeds, it seems fairly certain that one gene only is responsible for these two conditions. A similar view-point was recently expressed by Fisher (1934), who found about 25 per cent. of herniated fowls in an unstated number of offspring from a mating *inter se* of heterozygous crested birds.

If the above hypothesis be accepted, there is some difficulty in explaining the irregular incidence of cerebral hernia in matings involving crest. In Table 5 are pre-

TABLE 5
THE INCIDENCE OF CEREBRAL HERNIA IN MATINGS INVOLVING CREST

Type of mating	Crested-cerebral hernia	Crested non-cerebral hernia	Non-crested-non-cerebral hernia
Heterozygous crest by non-crest:			
1262M	4	4	3
1280M	9	30	38
1300M	6	31	42
Total	19	65	83
Heterozygous crest by homozygous crest-hernia:			
1289M	16	132	0
1287M	23	47	0
Total	39	179	0
Heterozygous crest by heterozygous crest:			
1287M	9	46	26

sented data secured at Kansas State College bearing on the relationship of these two conditions.

It will be noted that among fowls from the mating of *Cr cr* × *cr cr* birds, some with hernia appeared. The explanation might be offered that the supposedly non-crested birds had crests which were overlooked, since the crests of heterozygous birds sometimes are quite small, but the resulting approximately equal numbers of crested and non-crested progeny belie this explanation and indicate that the cross was really a back-cross of heterozygous to non-crested birds. All the non-crested breeders came from uncrested ancestors. This means that cerebral hernia appeared in birds heterozygous for crest. Numerous matings of this type have been observed in which no cerebral hernia appeared, but the matings are given in Table 5 to demonstrate that hernia does sometimes appear in birds not homozygous for crest.

From matings of *Cr cr* × *Cr Cr* herniated birds there was obtained a population all crested as expected but with a ratio of 39 herniated to 179 non-herniated, where equality of these two classes would be expected if the *Cr Cr* genotype were always manifested as hernia. It would appear either that many *Cr Cr* birds do not exhibit hernia, or else that a large percentage of the herniated birds fail to hatch.

Finally, from heterozygous crested birds mated together there were secured only 9 herniated birds in a population of 81, where about 20 *Cr Cr* birds, all herniated, would be expected if the character were fully expressed.

These apparently inconsistent data can be accounted for in either of two ways or in both together. It is suggested that cerebral hernia and crest result from the gene *Cr* and that manifestation of hernia (which is really not accurately measured by the method of palpation employed in all genetic studies to date) is subject to varying degrees of "penetrance" in the sense of Timoféeff-Ressovsky. Ordinarily the hernia is not expressed in hetero-

zygotes, but in exceptional cases it may be. Ordinarily it is manifested in homozygotes, but in some genotypes it is not present to a degree detectable by the comparatively inaccurate methods of palpation and visual inspection in the down-covered chick. Behavior of this irregular type was encountered in the *radius incompletus* and abnormal abdomen characters in *Drosophila funebris* by Timoféeff-Ressovsky (1927, 1932) and has also been found by the junior author in the character "down-defect" in fowls (Hutt, 1932) which may appear in some heterozygotes and not at all in some homozygotes. Such irregular penetrance may be attributed to the effects of modifying genes, which, though numerous, differ in number and effects in any but relatively homozygous lines.

Such behavior would explain the irregular ratios shown in Table 5. There may also be involved some mortality in the homozygotes from an abnormality resulting from a double dose of the *Cr* gene. The incubation records for the matings in Table 5 do not show excessive mortality in the mating of *Cr cr* × *Cr Cr* and of *Cr cr* × *Cr cr*, where it would be expected if anywhere, in comparison with the *Cr cr* × *cr cr* matings where it would not be expected.

In connection with this hypothesis, it should be remembered that Rüst (1932) obtained very fair evidence that crested ducks are heterozygous for a gene which is lethal to the majority, if not all, of the homozygotes. Moreover, this gene is apparently expressed in only about half of the heterozygotes, the other half being indistinguishable from ordinary uncrested ducks. Apparently a somewhat similar variability occurs in the crested fowls, except that homozygosity for *Cr*, if lethal at all, affects only a few of the embryos. The records of hatchability for the matings given in Table 5 were not exceptionally poor and would not account for the great shortage of herniated birds. Heterozygotes in the fowl manifest the crest, but may or may not manifest cerebral hernia. Similarly, some homozygotes do not manifest hernia.

This variability in the expression of cerebral hernia is quite in accord with the early history of that character. Darwin (1868) cites the statements of both Blumenbach and Bechstein that originally hernia was restricted to females, but that, by artificial selection in affected breeds, the males came to manifest the character. This merely indicates that it is much subject to modifying factors, as might be expected from its very irregular penetrance discussed above. A similar sex difference in penetrance was noted by Timoféeff-Ressovsky (1927) in *Drosophila funebris* and has been observed by the junior author (unpublished data) in the down-defect character mentioned above.

Genes Independent of the *Cr I F* Chromosome

In former papers (Warren, 1933; Hutt, 1933) there were presented data showing independent assortment of many genes which had been adequately tested for linkage with *Cr, I* or *F*. Since then the additional data presented in Table 6 have become available. Undated tests ascribed to the present authors represent data previously unpublished.

It is evident that no other genes have yet been shown to be linked with *Cr, I* or *F*. Landauer (1933) has published some data in support of his view that rumplessness is linked with frizzling. He himself has produced good evidence that rumplessness is independent of crest, and Warren has found it independent of both dominant white and crest. Since *Cr* and *I* belong to the same linkage group as *F*, it seems unlikely that the comparatively loose linkage reported will be substantiated when adequate data have been obtained.

Sungurov (1933) has suggested that the rose comb-creeper linkage group may belong to the same chromosome as *Cr, I* and *F*, but adequate support for this hypothesis has not yet been adduced. In fact, unpublished data secured at Kansas State College strongly support the view that rose comb segregates independently of *Cr*

TABLE 6

CHARACTERS APPARENTLY INDEPENDENT OF THE *Cr I F* GROUP

Linked gene	Tested with	Investigator	Back-cross ratio*
Cr	Muff	Hutt	202 – 185
Cr	Muff	Warren	105 – 98
Cr	Duplex comb	Warren	199 – 210
Cr	Duplex comb	Hutt	210 – 179
Cr	Flightless	Warren	202 – 200
Cr	Rumplessness	Landauer (1933)	246 – 256
I	Rumplessness	Warren	310 – 287
I	Muff	Hutt	199 – 191
I	Muff	Warren	207 – 209
I	Muff	Serebrovsky and Petrov (1930)	93 – 101
I	Duplex comb	Warren	230 – 242
I	Duplex comb	Hutt	240 – 213
I	Silkie	Warren	94 – 71
I	Flightless	Warren	135 – 135
F	Rumplessness	Landauer (1933)	259 – 190
F	White skin	Warren	85 – 82
F	Polydactyly	Warren	108 – 114
F	Polydactyly	Hutt	55 – 52
F	Muff	Warren	181 – 171
F	Muff	Hutt	45 – 46
F	Duplex comb	Warren	121 – 109
F	Duplex comb	Hutt	51 – 41

* The ratio given is that of parental combinations to new combinations.

and *I*. The data from back-cross matings are 377 parental combinations to 334 new combinations in the test with *I* and 374 to 355 in the test with *Cr*.

SEX DIFFERENCES IN AMOUNT OF CROSSING-OVER

There are recorded here sufficiently large numbers, and factors which show high enough percentage of crossing-over, to secure fair evidence for the relative amount of crossing-over in the two sexes. When the data in Tables 1, 2 and 3 are summarized for each sex, the following rates of crossing-over are found:

The figures in parentheses give the number of gametes tested in each case. These results indicate that the rates of crossing-over are very similar in the two sexes of the

Genes	Perecentage of crossing-over in ♂♂	in ♀♀
Cr and *I*	12.2 (1407)	13.3 (577)
I and *F*	16.7 (857)	18.5 (248)
Cr and *F*	29.5 (407)	29.3 (270)

fowl, but that there appears to be a slight tendency for a higher rate in the females. This is in agreement with the data of Landauer (1933), and of Taylor (1934) who, from large numbers for the closely linked genes for rose comb and creeper, concur in finding slightly more crossing-over in females than in males.

Summary

The data here presented definitely establish the fact that crest, dominant white and frizzling belong to the same linkage group.

It is shown that these genes have the arrangement in the chromosome of *Cr–I–F* with approximately 12 per cent. crossing-over between *Cr* and *I* and 17 per cent. between *I* and *F*. Since the percentage of crossing-over between *Cr* and *F* is 29, the distance in the chromosome between *Cr* and *F*, as measured by the summation of the segments *Cr* to *I* and *I* to *F*, is practically identical with that secured by directly measuring the percentage of crossing-over between *Cr* and *F*.

This indicates that little double crossing-over occurs in the approximate distance of 29 units on this chromosome. In segregation of these genes in birds heterozygous for all three, no double cross-overs were recorded in 284 gametes.

Evidence is adduced to support the view that cerebral hernia is ordinarily the homozygous expression of crest, but a number of irregularities were found in the manifestation of cerebral hernia. It may appear in heterozygotes or may be suppressed in homozygotes.

The rates of crossing-over were very similar in the two sexes, there being some evidence for a slightly higher amount in females.

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GENETICS OF THE FOWL

V. THE MODIFIED FRIZZLE

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(With Plate XV)

Introduction

In the first paper of this series it was shown (Hutt, 1930) that frizzling is caused by a single dominant autosomal gene, **F**, which produces in fowls homozygous for it a narrow, much-curled plumage, markedly different from that of the heterozygote. A similar conclusion was also reached by Landauer and Dunn (1930).

In all progeny raised by the writer from the original stock obtained from Major G. S. Williams, Tredrea, Perranwell, Cornwall, there were observed only these two types of frizzling, but, after outcrossing to entirely unrelated stock, there appeared a new type of plumage which was earlier briefly reported and designated as modified frizzling (Hutt, 1932). Investigations of that condition are reported in this paper.

Descriptions

Unmodified heterozygous Frizzles (Plate XV, figs. 1 and 2) have body feathers the shafts of which are recurved so that the feathers curl toward the head or have their apices pointed outward in planes roughly perpendicular to the surface of the body. The vane of the feather is usually intact, except in the outer remiges, where the barbs, in groups of three to ten or more, are twisted and partially curled around the rachis (Plate XV, fig. 2). This effect is least in evidence at the distal end of the rachis, which is also slightly recurved. It is most pronounced in the outer remiges, less so in the inner primaries, and much less so in the secondaries.

Unmodified homozygous Frizzles are characterised by a woolly appearance resulting from the fact that in all feathers both rachis and barbs are so curled that the normal flat vane is completely destroyed

[1] The greater part of this investigation was conducted at the University of Minnesota, but the work was completed at Cornell University.

(Plate XV, figs. 5 and 7). Shafts of the remiges and rectrices are markedly recurved. Barbs of these feathers are originally closely twisted around the shaft, but in most cases these barbs quickly wear off, leaving only a single spike which in turn frequently breaks off or is moulted. Wings of the homozygotes frequently have the outer remiges in several different stages of growth even at times other than that of the regular moult (Plate XV, fig. 7).

Modified heterozygous Frizzles sometimes exhibit so little frizzling as to be almost indistinguishable from normal fowls (Plate XV, fig. 4). There is only very slight recurving of the shafts of body feathers, and the normally somewhat convex outer surface of the remiges is often fully retained. The rectrices sometimes seem entirely unaffected, but, in any case of doubtful identity, the presence or absence of the frizzling gene is readily detected by examination of the outer remiges. In the subapical region of these feathers the vane on the inner side of the rachis is broken by varying degrees of curling in small clusters of barbs (Plate XV, fig. 3). This effect is usually localised, being less evident at the proximal end of the vane, and entirely lacking at the tip. It is most marked in the outermost remex and becomes progressively less extensive in each succeeding inner remex, being sometimes entirely absent in the inner two of the ten primary remiges. Secondaries of modified heterozygous Frizzles are usually almost normal, but in any case, the inner ones are least affected. Feathers of the neck usually stand out just enough to appear somewhat ruffled.

Most modified heterozygotes show somewhat more frizzling than the one illustrated, particularly in the feathers of the wing.

Modified homozygous Frizzles differ from those not modified in that, while the latter have a woolly appearance, the former seem to have a softer velvety covering almost as if the feathers constituted a deep pile on an enveloping rug. This general effect results from the fact that each feather is less extremely curled than in unmodified homozygotes and is somewhat wider, but, nevertheless, has all of its barbs curled, so that no feather has a normal flat vane (Plate XV, fig. 6). In most feathers the curling of the barbs begins farther from the rachis than in feathers of unmodified heterozygotes, and a narrow strip on each side of the rachis has thus a normal vane. The shafts of the rectrices and remiges are less extremely curled, and the outspread wings of the modified and unmodified homozygotes (Plate XV, figs. 6 and 7) present decidedly contrasting appearances.

Frizzled chicks homozygous for the modifier show less of its effects

than do adults. At from 1 to 5 weeks of age it is difficult to determine the exact type of frizzling present, but there is no difficulty in distinguishing frizzled chicks from normal ones. As the birds become older and feathered out, the effects of the modifier are much more evident.

GENETIC ANALYSIS

The results secured in several different types of crosses involving the frizzling gene, **F**, its normal allele, and the modifier, justify the conclusion that the modified types of frizzling just described arise from the action of a recessive autosomal gene. For it the symbol **mf** is proposed. In fowls heterozygous for the modifier there are no readily perceptible effects, but, when homozygous, it induces the modified plumage in all birds heterozygous for **F** and in some, but not all, of those homozygous for that gene.

The first modified frizzles appeared in 1929 in the progeny of a White Leghorn ♂ × **Ff** and **FF** ♀♀. Among 76 **Ff** birds raised to maturity there were only four with modified frizzling, and all of these, together with seven unmodified ones, were produced by a single **FF** hen, No. A 5. It was obvious, therefore, that the White Leghorn was either **mfmf** or **Mfmf**, and that the modifier exerted no effect in birds only heterozygous for it. Apparently only one of the females carried the modifier.

This mating also demonstrated that the gene **mf** is not sex-linked. If it were so and were effective in the hemizygous state, then modified frizzling should have been evident in either all or half of the White Leghorn's frizzled daughters, depending upon whether he were **mfmf** or **Mfmf**. This was not the case. If **mf** were sex-linked, but unexpressed except where homozygous, then modified frizzling when it appeared (in the progeny of any frizzled female also carrying **mf**, as did A 5) should have been manifested only in the sons, whereas actually two of the four modified birds were females. Obviously **mf** is autosomal.

Data from seven different types of matings involving **F** and **mf** are presented in Table I.

Mating 5 indicates clearly that heterozygous frizzles showing the modified type of frizzling must be homozygous for the modifier, since the 11 **Ff** offspring were all modified.

Matings 4 and 5 bring out the interesting point that in fowls homozygous for both **F** and **mf**, there is frequently no manifestation whatever of the modifier. In mating 4 the segregation of **F**—20 **FF** : 41 **Ff** : 26 **ff**—shows a close fit to the expected 1 : 2 : 1 ratio, but, whereas the expectation for the homozygotes is that half should also be homozygous

for the modifier, only 3 out of 20 had the modified phenotype. Considering matings 4 and 5 together, the modified plumage was manifested in only six (or 40 per cent.) of the 15 **FF** birds which should have shown it.

These same two matings also demonstrate that homozygosity for **mf** is manifested in 100 per cent. of the birds heterozygous for the frizzling gene. The expectations that half of the **Ff** progeny would be modified in mating 4, and all modified in mating 5, were both realised.

TABLE I

Matings involving **F, mf** *and their normal alleles*

Nature of mating	Frizzled offspring: Homozygous: Modified	Frizzled offspring: Homozygous: Not modified	Frizzled offspring: Heterozygous: Modified	Frizzled offspring: Heterozygous: Not modified	Offspring not frizzled
1. **Ff** unmod. ♀♀ × **ff** ♂♂ carrying **mf**	—	—	—	33	29
2. **FF** unmod. ♀♀ × **ff** ♂ carrying **mf**	—	—	—	32	—
3. **FF** unmod. ♀♀ carrying **mf** × **ff** ♂ carrying **mf**	—	—	4	7	—
4. **Ff** unmod. carrying **mf** × **Ff** mod.	3	17	23	18	26
5. **Ff** mod. × **Ff** mod.	3	2	11	—	7
6. **Ff** mod. × **ff** to be tested for **mf**:					
(*a*) × Ancona ♂	—	—	18	—	18
(*b*) × Barred Rock ♂	—	—	23	—	30
(*c*) × 18 Rhode Island Red and Barred Rock ♀♀	—	—	68	—	66
(*d*) × 4 Polish ♀♀	—	—	22	—	24
(*e*) × 1 Silkie ♀	—	—	1	—	3
7. **FF** mod. ♂ tested for homozygosity for **mf**:					
(*a*) × 2 Brown Leghorn ♀♀	—	—	18	—	—
(*b*) × 2 White Wyandotte ♀♀	—	—	4	—	—

In mating 7, a male with the type of plumage attributed to the genotype **FFmfmf**, with manifestation of the modifier (Plate XV, fig. 6), was tested by outcrossing to entirely unrelated Brown Leghorns and White Wyandottes. Since all the 22 progeny from this mating showed the characteristic modified plumage of **Ffmfmf** birds, it was evident that the phenotype of this male was dependent upon homozygosity for both **F** and **mf**.

Discussion

In its behaviour the modifier of frizzling resembles somewhat the recessive semiforked gene of *Drosophila* which Lancefield (1918) found to be a modifier of the sex-linked character, forked, but only in flies heterozygous for the forked gene. Homozygotes for forked were unaffected, whereas in Frizzles about 40 per cent. of the homozygotes are

affected by the modifier. In both cases the modifying gene exerts no perceptible effect except when the modified character is present.

Landauer (1933) has reported a recessive modifier of frizzling which is probably identical with **mf**, but, contrary to the finding of the present writer, states that in his material all fowls homozygous for the frizzling gene and for the modifier showed the modified phenotype.

Independence of **F** *and* **mf**. Different degrees in modification of a structure in one direction are frequently the results of multiple alleles. This is definitely not the case here, since some birds were proven to be of the genotype **FfMfmf**, which would be impossible if multiple alleles were responsible. From mating 4, with only two pairs of genes affecting frizzling, there emerged five different phenotypes, which could not have occurred even if four different members of a series of alleles were involved, but could arise from different combinations of two pairs of independent but interacting genes.

Other modification of frizzling. While the processes of development responsible for frizzled feathers have not yet been determined, it seems probable that in these, as in curly hair, there may be different rates of growth on opposite sides of the follicle. Such a difference would be accentuated in rapidly growing feathers and minimised in those growing slowly. On this basis it is not surprising that the modifier exerts its least effect on the plumage of the chick during the period of its most rapid growth, and its greatest effects upon the feathers acquired at or near maturity.

It is evident from the descriptions and illustrations of the wings of modified and unmodified frizzles that in the primary remiges there is a postero-anterior gradient of increasing manifestation of the gene for frizzling. This gradient is most conspicuous in **Ff** birds homozygous for the modifier (Plate XV, fig. 3). Its course is the same as the order of moulting of these feathers both when juvenile birds are assuming adult plumage and when adults are moulting. The first primary to be replaced is the inner, or most posterior one, after which the other primaries are replaced in order, the last to grow being the outer or most anterior of the ten primaries. If in modified birds, frizzling is least manifest in feathers with slow growth rates, the inner primaries must grow in at a slower rate than the outer ones, even though the former are acquired first, before the general rate of body growth has slowed down as much as when the outer primaries are grown. This is not as paradoxical as it may seem. The inner primaries are acquired before moult is general, the outer ones are replaced when many feathers are being acquired all

over the body and frequently two or more remiges are shed at once. Larionov *et al.* (1932) found in the pigeon that when many feathers were being acquired at once the rate of growth of individual remiges was more rapid than when few feathers were being replaced.

Within individual remiges, especially in the outer primaries, the manifestation of frizzling varies both in modified and unmodified heterozygotes. In general, the distal and proximal ends of the feather are least affected, while the intervening area shows most frizzling. This may possibly be associated with retarded growth in early development prior to expulsion of the antecedent feather, and again when the new feather is nearing completion.

The role of different growth rates in modifying the structure and pattern of feathers in different parts of the body has been demonstrated by Juhn, Faulkner and Gustavson (1931) and by Lillie and Juhn (1932). In frizzled fowls the action of the gene, **F**, is apparently conditioned by such regional differences as well as by the modifier, which exerts its effects on all parts of the plumage.

Significance of **mf** *in evolution.* There can be little doubt that in nature fowls carrying the frizzling gene, **F**, would be somewhat at a disadvantage in comparison with those having normal plumage. Homozygotes are frequently almost bare after the mating season. Benedict, Landauer and Fox (1932) found that homozygous Frizzles have a much higher rate of metabolism and greater food requirement than normal fowls, especially at low temperatures, and Landauer and Dunn (1930) state that in their experience "a high percentage of homozygous Frizzles of both sexes never reach maturity".

However, as Fisher (1930) points out, the persistence or elimination of an undesirable mutation depends almost entirely upon the degree of disadvantage which it brings to the heterozygote and very little upon the disadvantage to the homozygote, even if it were there fully lethal. There is little definite evidence that **Ff** birds are at a disadvantage under modern conditions of domestication. The writer's earlier data (Hutt, 1930) do not support the conclusions of Landauer and Dunn (1930) and of Landauer (1932) that eggs of unmodified heterozygous frizzled females are subject to greater embryonic mortality than are those of non-frizzled fowls. There can be no doubt, however, that in nature the heterozygote's chances of survival in competition with fowls of normal plumage would be considerably reduced. Their plumage provides less protection against extremely low temperature and against rain. The remiges and rectrices are so abnormal that flight is quite impossible, and,

since many of these birds are unable to reach roosts only three feet from the ground, they would be caught by predaceous enemies more easily than normal fowls.

The modifier, which not only improves the covering but also restores somewhat the ability to fly, is evidently a very desirable gene, having definite "survival value". The fact that it has no effect on more than half of the birds homozygous for **F** makes little or no difference in its value to the species, so long as it conveys a definite advantage to the heterozygotes which are much more numerous in the wild population than are the homozygotes.

Distribution of the modifier. Where man got his first Frizzles is unknown, but there can be little doubt that the mutation has occurred many times in the history of the species. In fact, since frizzling is also found in canaries and pigeons, the mutation has probably occurred even farther back in the ancestry of the domestic fowl. If it were an old mutation, one would expect to find the modifier well distributed in divers breeds of present-day poultry. With this in mind, a number of breeds and varieties have been tested for the presence or absence of the modifier.

In Table I, the various breeds used in matings 6 and 7 are listed to show that the modifier was present in at least seven different breeds. Moreover, while some of them were not adequately tested for homozygosity, the fact that all frizzled progeny were shown by their phenotypes to be homozygous for the modifier indicates that practically all of the 29 non-frizzled fowls tested must likewise have been homozygous for it. In addition to those listed the modifying gene was also found in Mottled Houdans, Silver Laced Wyandottes, White Leghorns and White Orpingtons, making a total of eleven breeds or varieties found to carry it. Since these breeds represent both Mediterranean and Asiatic ancestry such a distribution may be some indication that its "survival value" has resulted in its early inclusion in the germ plasm of the ancestors of the domestic fowl.

It would be of considerable interest to determine the distribution of the modifier in native stocks of poultry in various parts of the world. Evidence that frizzled fowls have a wide distribution in southern Asia, the East Indies, Surinam, Mauritius and Mozambique was summarised in the writer's earlier paper (1930). Prof. J. B. S. Haldane informs me that they also occur in Dahomey and Jamaica, and Rattray (1927) found them in Ashanti. Such a distribution suggests that in warm climates frizzling may be less of a handicap than in colder ones, but whether its

survival in these regions has been facilitated by the modifier or not is an interesting but unanswered problem. Some of the descriptions, and the fact that frizzled fowls are especially prized in some countries, suggest that unmodified frizzles are available through much of the range given above.

Modification of dominance. The interactions of **F**, **mf** and their normal alleles provide an excellent example of the modification of dominance in accordance with the theory of Fisher (1930). Without modification, **F** is an incompletely dominant gene, but the selective disadvantage which it conveys to the heterozygote results, with natural selection, in the accumulation of modifying genes which suppress its effects. The result is that the heterozygote becomes modified toward the phenotype of whichever homozygote has the greater chance against natural selection, in this case the wild (non-frizzled) type. In this way a character originally having marked, though incomplete, dominance becomes eventually almost or entirely recessive.

Preservation of the full effects of the frizzling gene is therefore dependent upon man's efforts to eliminate the modifier. The modern fancier does so because his show-room standards prefer the unmodified heterozygotes. Presumably his forerunners preserved them from curiosity, for sacrificial rites as in Ashanti (Rattray, 1927), or because of special properties believed associated with frizzled fowls such as their supposed ability in Malaya to ward off "the evil eye", to which Fisher (1935) refers.

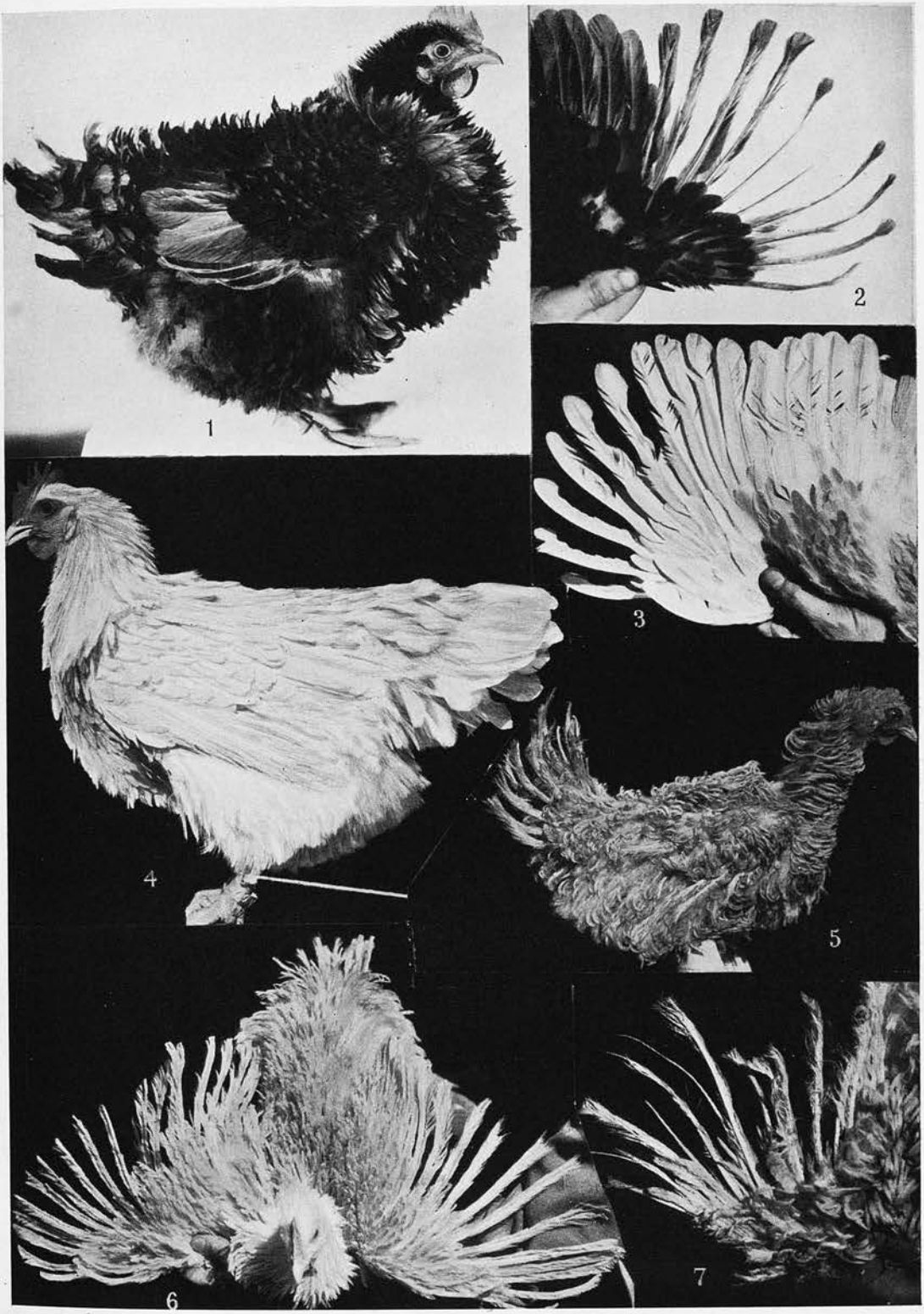
Summary

Partial suppression of the frizzled plumage of heterozygous Frizzles is induced by a recessive autosomal gene, **mf**, which is independent of the gene for frizzling.

This modifying gene is manifested when homozygous in all birds heterozygous for frizzling and in about 40 per cent. of those homozygous for frizzling.

Modified heterozygotes may show only a slight ruffling of the body feathers, but always have abnormal feathers in the primary remiges. Modified homozygotes have less curling of the feathers in all parts of the body.

The manifestation of frizzling is also apparently conditioned by growth rates of feathers, which vary with age, in different regions of the body and within certain feathers.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The modifying gene is widely distributed among domestic fowls, presumably because of its selective advantage in their ancestors.

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EXPLANATION OF PLATE XV

Fig. 1. Unmodified heterozygous Frizzle, **FfMfMf**.
Fig. 2. Outspread wing of the same.
Fig. 3. Outspread wing of a modified heterozygote.
Fig. 4. Modified heterozygous Frizzle, **Ffmfmf**.
Fig. 5. Unmodified homozygous Frizzle, **FFMfmf**.
Fig. 6. Modified homozygous Frizzle, **FFmfmf**.
Fig. 7. Outspread wing of the unmodified homozygote shown in Fig. 5.

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Genetics of the fowl.
VI. A tentative chromosome map.

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The assignment of the genes of the fowl to their proper chromosomes began when Spillman (1908) showed that the gene for barring is located in the sex-chromosome. This discovery was a landmark not only in avian genetics but also in genetics generally, for, along with the coincident report by Durham and Marryat of a sex-linked gene in canaries, it permitted the extension to vertebrates of the phenomenon of sex linkage which had been discovered shortly before by Doncaster in the moth, *Abraxas*.

Since Spillman's discovery, genetics has made no more important advances than those making possible the allocation of both sex-linked and autosomal genes to their proper linkage groups, and the construction of maps indicating the approximate locations and the relations to one another of the genes in each chromosome. In *Drosophila melanogaster* each of the four chromosomes is well mapped and in maize from two to ten genes have been located in each of the ten chromosomes. No vertebrate has as yet a linkage group for each chromosome, but linked genes have been found in three species of fish, in the pigeon, budgerigar and fowl, and in the rat, mouse and rabbit.

In the fowl the most extensive linkage group is in the sex-chromosome where seven genes can be located in their approximate positions. Altogether, 18 genes have been allocated to one or another of five linkage groups and this number will probably be increased when certain cases of apparent linkage have been further investigated.

The sex chromosome.

The following sex-linked genes have been discovered:

1. *B* – *Barring.* Recessives are non-barred.
2. S – *Silver.* Recessives have golden, or black and golden, plumage.
3. *Id* – *Inhibitor of dermal and mesodermal pigment.* Recessives have slate, blue, or willow shanks and, as in Silkies, may have black skin over the entire body.

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4. *K* – *Slow feathering.* Birds with the dominant allele lack tail feathers and have comparatively short wings at 14 days, while *k* – and *kk* birds of that age have tail feathers up to 2 cm. long, and well developed wing feathers. Most Leghorns are rapid feathering. Most heavy breeds are slow feathering.

5. *ko* – *Head spot.* Chicks homozygous for the recessive allele have in the down an irregular streak of dark pigment extending on the back of the head and extending down the neck. Those carrying the dominant allele lack a head streak. This head spot has been found in Golden Spangled Hamburgs and in Houdans, the inhibitor of it in Plymouth Rocks, Wyandottes and Orloffs (Hertwig, 1930).

6. *Li* – *Light down.* The dominant allele inhibits brown pigment in the down of chicks not black. Recessives have more or less brown down unless it be obscured by black (Hertwig and Rittershaus, 1929)

7. *Br* – *Light iris* (yellow orange to bay). Recessives have brownish black irides (MacArthur, 1933).

The first measurement of linkage in the fowl was reported by Haldane (1921) who found 34.6 per cent. crossing over between *B* and *S*, a figure somewhat lower than the 42 to 49 per cent. found by subsequent workers. The discovery of *K* by Serebrovsky (1922), *Li* by Hertwig and Rittershaus (1929), *ko* by Rittershaus (1930) and *Br* by MacArthur (1933) has greatly facilitated further study, and the linkage studies of these investigators and of Agar (1924), Serebrovsky and Wassina (1926), Warren (1928, 1934) and Hertwig (1933) now permit a reasonably accurate mapping of the sex-chromosome.

It is unnecessary to give in detail the results of each worker, since, where several determinations of any linkage relationship have been made, the results have been quite consistent. However, there have been different interpretations of the order of the genes.

At each end of the chromosome, there is a series of three genes and these groups are so far apart that any member of one series shows almost independent assortment with any member of the other. Hertwig (1933) proposed the order *B*, *Ko*, *K*, *S*, *Li*, an arrangement concurred in by MacArthur, whose new gene *Br* (light iris) was located between *Ko* and *K*. However, Serebrovsky and Wassina (1926) and Warren (1928) interpreted their data as showing that *S* lay between *B* and *K*, an opinion still held by Warren (1934) after further study of the problem.

Barring and the inhibitor of dermal pigment are so closely linked that only two doubtful crossovers between them have been

reported. This means that in mapping the chromosome these two can be considered as occupying almost the same locus. Measurement of their exact relationship is difficult because ordinarily barred fowls have genetically black plumage, and in such birds the black pigment is extended through the epidermal layer of the shanks in sufficient concentration to render difficult any accurate classification of shank colour in birds heterozygous for barring. It is certain, however, that two distinct genes are concerned, since non-barred birds may carry the inhibitor of mesodermal pigment, as in Black Leghorns, or may lack it, as do Hamburgs and Andalusians.

Because these two genes are so closely linked, the data of MacArthur on the relation of *Id* to other genes can be used equally well to indicate the relation of those other genes to *B*. On this basis the following cross-over determinations have to be reconciled in the correct map of the sex-chromosome.

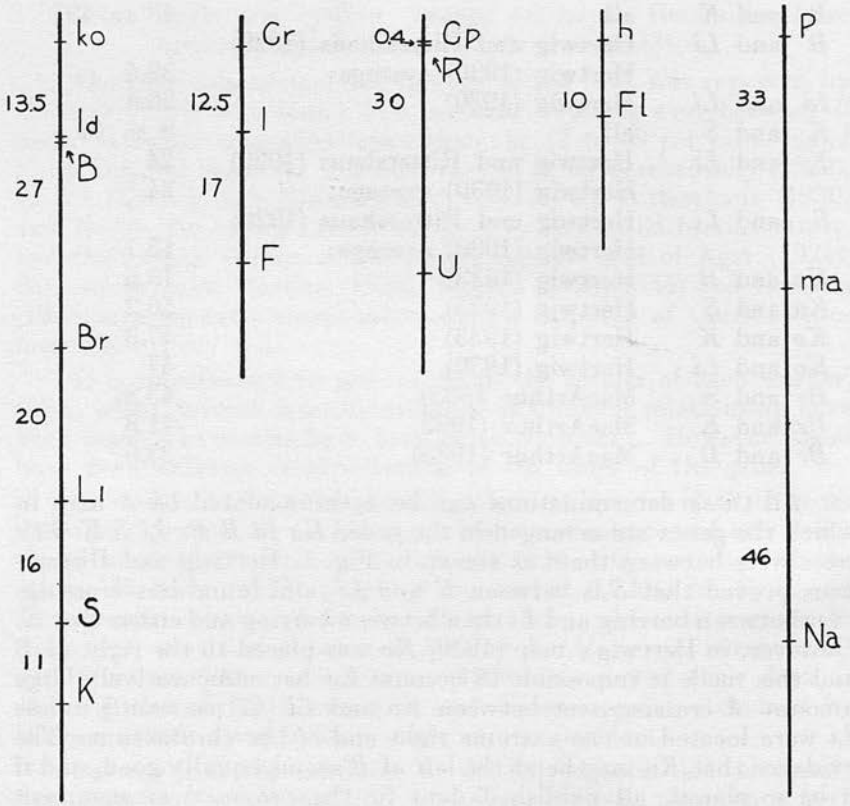
Genes tested	*Investigator*	*Crossing over per cent.*
B and *S*	all	42 to 49
B and *K*	all	43 to 49
B and *Li*	Hertwig and Rittershaus (1929)	
	Hertwig (1930) average:	39.4
Id and *Li*	Hertwig (1930)	30.5
K and *S*	all	8 to 14
K and *Li*	Hertwig and Rittershaus (1929)	24
	Hertwig (1930) average:	24
S and *Li*	Hertwig and Rittershaus (1929)	
	Hertwig (1930) average:	15.7
Ko and *B*	Hertwig (1933)	13.5
Ko and *S*	Hertwig (1933)	50.2
Ko and *K*	Hertwig (1933)	47.3
Ko and *Li*	Hertwig (1930)	47
Br and S	MacArthur 1933)	43.8
Br and *K*	MacArthur (1933)	41.8
Br and *D*	MacArthur (1933)	27.5

All these determinations can be accommodated by a map in which the genes are arranged in the order *Ko Id B Br Li S K* with crossovers between them as shown in Fig. 1. Hertwig and Rittershaus proved that *S* is between *K* and *Li*, and found less crossing-over between barring and *Li* than between barring and either *S* or *K*. However, in Hertwig's map (1930) *Ko* was placed to the right of *B* and this made it impossible to account for her comparatively large amount of crossing-over between *Ko* and *Li* (47 per cent.) unless *Li* were located at the extreme right end of the chromosome. The evidence that *Ko* may be at the left of *B* seems equally good, and if it be so placed, all published data fit the proposed arrangement (Fig. 1), provided allowance be made for double crossing-over in

long map distances and for variations in cross-over values arising from smaller numbers and from individual differences when few birds are tested. That *Li* might lie in the centre of the chromosome rather than at the extreme end was earlier recognized by MacArthur.

Final arrangement of these seven genes must be delayed pending further data on the relationship of other genes with *Br* and with *Li*, and the critical test of the linkage relationships of these two. If the proposed map be correct, one should expect from 16 to 20 per cent. crossing-over between them. In MacArthur's limited data on this relationship, the cross-over percentage was 23. The discovery of a new gene between *B* and *S* would greatly facilitate further study of this chromosome.

FIG. 1. TENTATIVE CHROMOSOME MAP FOR THE FOWL



The Cp R U Group.

The following genes are known in this group:

1. *Cp* — Creeper. Lethal to the homozygote. Recessives have normal limbs.
2. *R* — Rose comb. The recessive allele is single comb.
3. *U* — *Uropygial.* Heterozygotes have two uropygial papillae, UU birds usually have no uropygial gland or papillae, and *uu* birds have one normal papilla. This mutant was briefly reported by Hutt (1932) and a more detailed account of it is in preparation.

Autosomal linkage was first reported by Serebrovsky and Petrov (1928) who found creeper linked with rose comb. In later and more extensive studies by Landauer (1932) and Taylor (1934) the linkage was much closer than that first reported, Landauer finding only 0.36 per cent. crossing-over and Taylor an average of about 0.50 per cent.

The present writer has found linkage of *R* and *U*, with crossing-over of 29.6 per cent. in 348 gametes tested up to January 1, 1936 (Unpublished data). It cannot yet be said whether *R* is between *Cp.* and *U*, or whether *Cp* is between *R* and *U*.

The Cr I F Group.

The following genes are linked:

1. *Cr* — *Crest.* Homozygotes usually show cere bra hernia in addition to crest; heterozygotes may do so, but in them crest is usually suppressed. Recessives are non-crested, without hernia.
2. *I* — *Dominant white.* Recessives may show melanic pigment.
3. *F* — *Frizzled plumage.* Recessives have normal plumage.

Clarification of this linkage group was delayed gy tenporary confusion in the literature which indicated that crest and hernia were caused by two separate genes. This is not the case. Close linkage of dominant white with cerebral hernia was found by Dunn and Landauer (1930). Suttle and Sipe (1932) found about 28 per cent. crossing-over between *Cr* and *F*, and Hutt (1933) reported *F* to be linked with *I*, the two genes showing 18 per cent. crossing-over. This meant that *I* must be linked with *Cr.* Fisher's (1935) analysis of earlier data from Dunn and Jull indicated this to be the case and Warren and Hutt (1936) have recently reported extensive investigations of the relationship of these three genes showing that their arrangement is *Cr I F*, with cross-over values as shown in Fig. 1.

This group illustrates the ease with which a chromosome may be accurately mapped when the distances between genes are short.

Ample evidence has been accumulated to show that *Cr I F* genes show independent assortment with *R* and *Cp* and it therefore seems probable that these two linkage groups belong to separate chromosomes.

The h Fl Group.

The two genes thus far known in this group are:

1. *h* — *Silkie* (absence of hamuli and scrolls on the barbules). The dominant allele restores the normal feather.
2. *Fl* — *Flightless.* This dominant mutation, discovered by Warren (1932), causes the remiges and rectrices to break off easily.

Crossing over between these genes was found by Warren (1935) to by only 10 per cent. and his unpublished data indicate that these two genes are independent of those in the two autosomal linkage groups previously mentioned.

The P ma Na Group.

The genes in this group are:

1. *P* — *Pea comb.* Recessives have single combs.
2. *ma* — *Marbling* („Marmorierung"). This recessive gene produces a colour pattern in the down of chicks but details concerning it were not given by Hertwig who studied its linkage relations.
3. *Na* — *Naked neck.* Recessives have normal pterylae and hence normal plumage.

Hertwig (1933) found 32.8 per cent. crossing-over between *P* and *ma* and 45.6 per cent. between *ma* and *Na.* Since these determinations are based on populations of 789 and 1141 respectively, both may be considered quite accurate. The order of these three genes is shown by the fact that both Hertwig (1933) and Warren (1933) have reported independent segregation of *P* and *Na* in large populations. This means that *Na* is remote from *P.*

Serebrovsky and Petrov (1930) found evidence that blue plumage might be linked with *Na* but in Warren's tests (1933) these two genes were quite independent and accordingly blue is not included in this linkage group.

Discussion.

Several cases of suspected linkage have been reported in addition to those considered above and shown in Fig. 1, but these have been omitted from the tentative map pending further proof.

No attempt is made to assign the known genes to definite loci in the chromosomes. Since some of the longer map distances will be changed when genes closer together have been studied it seems wiser at the present time to measure distances only by the observed percentage of crossing-over between adjacent genes.

It is considered unwise as yet to designate these linkage groups as belonging to the first, second, third or any other chromosome. It may even be shown that what now appears as a separate linkage group really belongs on the end of one of the others, with which, because of the genes being far apart, no linkage can be measured

till some intervening gene be found. Furthermore, until the relative lengths of the linkage groups and the individuality of the chromosomes are better known, no attempt can be made to associate any linkage groups with any particular chromosome. If earlier cytological studies were correct, the sex chromosome would, as the largest, be No. 1, but from the work of Suzuki (1930) and of Sokolow and Trofimow (1933) it now seems more probable that the sex-chromosome is a smaller V-shaped one, fifth in size.

Reasons for the difficulties experienced in detecting linkage are found in the cytological studies of the forementioned workers and of White (1932) and Popoff (1933) which indicate that the haploid number of chromosomes in the fowl is between 33 and 37. It is to be expected that six relatively large linkage groups will be found to correspond to the six large chromosomes, but the detection of linkage in from 27 to 31 small chromosomes presents a much more difficult undertaking.

Summary.

A map is presented showing the tentative arrangement and approximate distances apart of 18 genes in 5 chromosomes of the fowl.

In the sex chromosome are head spot, inhibitor of dermal melanin, barring, brown iris, light down, silver and slow feathering, tentatively arranged in the order given.

Crest, dominant white and frizzling are in one chromosome in that order and the distances between them are accurately known.

Creeper, rose comb, and uropygial are linked, but their order is unknown. Silkie and flightless constitute another small linkage group.

Pea comb, marbling in down and naked neck are so arranged but loosely linked in a longer group than any but the sex-chromosome.

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GENETICS OF THE FOWL. VII. BREED DIFFERENCES IN SUSCEPTIBILITY TO EXTREME HEAT

BY

F. B. HUTT

Reprint from POULTRY SCIENCE: Vol. XVII, No. 6
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Genetics of the Fowl

VII. BREED DIFFERENCES IN SUSCEPTIBILITY TO EXTREME HEAT

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INTRODUCTION

THE various breeds and varieties of domestic fowls, like most of those in other domestic animals, have been differentiated at the time of their establishment by characteristics of structure, size, and color rather than by variation in the physiological characters and functions by which their economic use is now measured. Association of some morphological character, or group of such characters, with a physiological one, without artificial selection for the latter, is comparatively rare in domestic animals although a number of such cases have been demonstrated in plants. The present paper reports an association between breed characteristics and an interesting psysiological character, the ability to withstand extreme heat.

The unusual heat of July, 1936, in the United States caused abnormally high mortality in the human race, with deaths, for the week ending July 18, 64 percent higher than in 1935 for 86 cities with a population of 37 millions.[1] Corresponding figures for fowls are not available, but numerous reports to the author suggested that the mortality in that species was very much higher than in man. At Cornell University, where the maximum daily temperature for the period July 7-12 maintained a higher figure than for many years, the poultry population was sufficiently large and varied that differential susceptibilty of breeds could be measured and the data concerning the birds were adequate to reveal the extent to which other factors might affect susceptibility.

[1] Weekly Health Index, Bureau of the Census, United States Department of Commerce. Vol. 7, No. 29.

MATERIAL AND CONDITIONS

The following birds on hand on July 1, 1936, are considered in this study:

S. C. White Leghorns	1,156
Rhode Island Reds	119
Barred Plymouth Rocks	164
Crossbreds	98

Conditions of feeding and management were uniform for all of these except that the majority of the Leghorns were confined in large shed-roof houses, some of them partially insulated, whereas most of the other birds had access to grassy yards with shade from trees. In this respect, the heavy breeds had somewhat more favorable conditions than the Leghorns. During the hot days laying birds were released from trapnests about every half hour from 10 a.m. to 3 p.m. to avoid possible ill effects of confinement in close quarters, and very few of these showed symptoms of heat prostration when taken from the nests.

Fowls prostrated by heat are easily recognized by their acute semi-comatose condition and dyspnoea, and were so recorded. Such an ante-mortem diagnosis is more accurate than any that could be made post-mortem in fowls killed by the heat. All birds dying from other causes were diagnosed at the New York State Veterinary College, Ithaca, and it was therefore possible to differentiate between those in which the heat was primarily responsible for death and those in which neoplasms, kidney dis-

eases, internal laying, and other causes were primarily responsible, even though aggravation by the heat may have accelerated their fatal termination.

Maximum temperatures from July 1-6 did not exceed 82°F., but from July 7-12 the maximum temperature varied from 91 to 103 degrees, with official readings of 101, 103 and 102 degrees on July 8, 9, were only two other days on which the maximum temperature at Ithaca reached 100 degrees or higher. In this period mortality in the college flocks attributable to heat had been extremely light. Obviously the flocks had experienced little or no natural selection in this respect and were therefore suitable material in which to measure breed differences when the unusually severe heat did strike them in 1936.

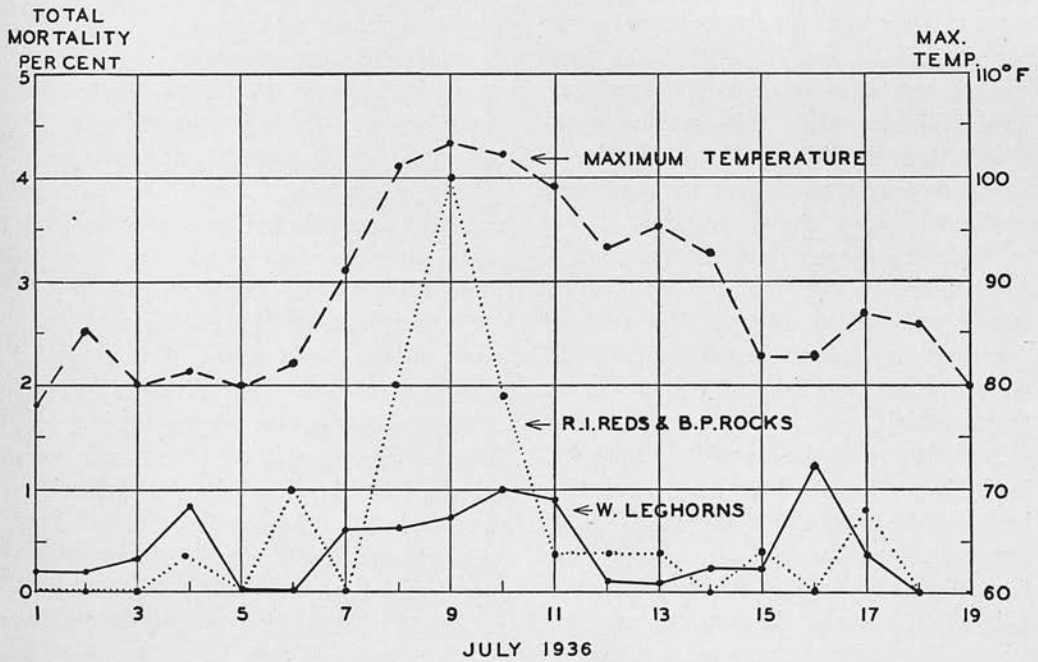

FIG. 1. Maximum daily temperature at Ithaca, July 1-18, 1936, and daily mortality for the same period in (a) White Leghorns and (b) Rhode Island Reds and Barred Rocks together. Mortality is expressed in percentages based on the number of birds on hand at the close of the previous day.

and 10. It was during this period that all deaths attributed directly to heat occurred. Temperatures in the poultry houses ranged from one to four degrees higher than in the shade outdoors except in one well insulated pen where the temperature was two degrees lower than outdoors.

The official records of the U. S. Weather Bureau show that except for the three successive days of July, 1936, on which maximum temperature exceeded 100°F., during the whole 11-year period 1927-1937 there

The mean relative humidity at Ithaca for July, 1936, was 63, 41, and 53 percent at 8 a.m., noon, and 8 p.m., respectively. These conditions undoubtedly made the temperatures experienced more severe than those same temperatures would be in drier areas.

BREED DIFFERENCES IN SUSCEPTIBILITY

Figure 1 shows the maximum daily temperature on the Cornell campus from July

1-18, and the daily mortality during the same period. The figures for temperature were taken by the local office of the United States Weather Bureau within a mile from the poultry houses. Subsequent analyses showed that the Rhode Island Reds and Barred Rocks did not differ significantly in their mortality rates during the heat wave and it is evident that these two breeds (together) did not differ greatly in that respect from White Leghorns during the periods July 1-6 and 13-18 when the maximum temperatures were only moderately high. However, while both groups experienced extremely high total mortality during the six days of extreme heat, the death rate in the two heavy breeds far exceeded that in White Leghorns. It reached a peak of 3.97 percent on July 9, when the maximum temperature was 103 degrees. This was over five times as high as the mortality of 0.7 percent experienced by the Leghorns on the same day.

Distribution of the mortality shows that in the heavy breeds those most susceptible began to die on the first day on which the temperature got up to 100 degrees (July 8) and were so quickly eliminated that on July 11, the last of the four hottest days, when the maximum temperature was 99 degrees, mortality in this group was only 0.38 percent. On the other hand fewer Leghorns died on the first two of the four hottest days than on the last two, which were not the worst. This suggests that even among those that succumbed during the heat wave resistance was somewhat greater in Leghorns than in the other two breeds.

Autopsy records reveal that the secondary peak of mortality in Leghorns on July 16 (Figure 1) was apparently independent of current temperature since in 9 of the 15 fowls dying on that day death was attributed to coccidiosis. Most fowls over one year of age have developed a high degree of immunity to the coccidia with which they are usually infested, and it seems possible, therefore, that the excessive mortality from coccidiosis following the heat wave may have resulted from a general lowering of resistance to these parasites in some fowls not killed outright by the heat.

To determine the significance, or otherwise, of the observed differences, mortality during the six-day period of extreme heat, July 7-12, has been compared in each group with that during a 12-day "control" period of moderate heat comprised by the six days immediately before and after the "test" period (Table 1). Mortality in the control period is based on the number of birds alive on July 1, less those that died from any cause during the heat wave. Mortality during the test period is based on those alive on the evening of July 6.

During these 12 days of moderate heat, none of the birds which died exhibited symptoms of heat-prostration and in post-mortem examination their deaths were found to have resulted from other causes. On the other hand, of the 72 birds which died during the six hot days, 34 exhibited before death the characteristic symptoms of extreme heat prostration previously described, and these undoubtedly perished as a direct result of the heat. Among those apparently dying from other causes in this period, some deaths were probably hastened by the extreme temperatures, but, since these birds did not show symptoms of heat-prostration, and, since post-mortem examination revealed in most cases some other cause of death, these birds have been considered separately.

Deaths during the test period from heat alone, excluding from the population those that died of other causes, were as follows:

	Percent and standard error
White Leghorns	1.79 ± 0.40
Rhode Island Reds	5.26 ± 2.09
Barred Plymouth Rocks	5.16 ± 1.78
Crossbreds	2.13 ± 1.49

Significance of the differences observed has been determined by application of the χ^2 test to the four-fold contingency tables by which any two breeds may be compared (Table 1). The values for P of .015 and <.01 show highly significant differences between the mortality from heat in White Leghorns and that from the same cause in greater heat-radiating surface, even though much of that surface is insulated with feathers. If this were true, or, if for any other reason the larger birds were more susceptible, the difference between breeds shown above could not be considered as demonstrating a true association of physiological characters with the morphological

TABLE 1.—*Mortality during 6 days of extreme heat and 12 days of moderate heat*

	Extreme heat					Moderate heat	
	Survived	Died from heat		Died from other causes		Survived	Died from other causes
Breed							
White Leghorns	1096	20		23		1067	46
Rhode Island Reds	108	6		4		104	5
Barred Plymouth Rocks	147	8		6		147	3
Crossbreds	92	2		3		91	2
		χ^2 tests for significance					
		χ^2	P	χ^2	P		χ^2 P
Differences between:							
White Leghorns and Rhode Island Reds		6.023	.015	1.091	.30		.051 .83
White Leghorns and Barred Plymouth Rocks		7.171	<.01	2.014	.165		1.613 .21
Rhode Island Reds and Barred Ply-Plymouth Rocks		0.0014	.973	0.022	.89		1.412 .24

either the Rhode Island Reds or Barred Rocks. The two heavy breeds did not differ from each other in this respect. In mortality from other causes, the Leghorns did not differ significantly from the Reds or Rocks during the heat wave and the control period.

Since in each of the three breeds there were representatives of two or more unrelated strains, the differences observed may safely be considered as differences between breeds and not merely between strains.

SUSCEPTIBILITY AND BODY SIZE

Since small animals have larger surface areas per unit of body weight than do large ones, it is quite conceivable that the advantage of the Leghorns might result from their smaller size giving them a somewhat ones constituting the breed. However, if the greater resistance of White Leghorns depended merely upon their smaller size, and the susceptibility of Reds and Rocks upon their greater size, then, within each breed, those dying from the heat should have been somewhat heavier than those which survived.

Weights of most of these birds had been taken when the first egg was laid, again in December, 1935, in some cases in February, 1936, and for most of the Leghorns in May, 1936. Where possible the weight of each hen dying during the heat wave (July 7-12) was paired with a control figure which was the average weight of two survivors of the same age (within two months) as the dead bird, in the same laying pen and having weights taken at the same time as the latest available weight for the dead

bird. Apart from these points the controls were drawn entirely at random. Adequate weights and controls were not available for every Leghorn that died, hence the number of these utilized is less than that given in Table 1. For 16 such comparisons in White Leghorns and 14 in Barred Rocks and Rhode Island Reds the mean weights, in grams, were as follows:

	Leghorns	Reds and Rocks
Died from heat	1736.7 ± 78.6	2785.0 ± 80.6
Controls	1848.4 ± 28.9	2543.9 ± 56.8

Significance of the differences was tested by Fisher's (1934, p. 119) modification of Student's pairing method, which yielded values for P of .22 for the Leghorns and .04 for the Reds.

While the birds dying from the heat in the two heavy breeds were 9 percent heavier than their controls, this situation was reversed in the Leghorns, among which the susceptible birds were 6 percent lighter than their controls. Neither difference was significant in the numbers available for study. Since these differences are in opposite directions it seems probable that resistance to extreme heat is not a function of body weight, but depends upon other conditions which are in some way associated with characteristics of the White Leghorn breed.

EGG PRODUCTION AND SUSCEPTIBILITY TO HEAT

(a) *Current Condition.* It is a common belief among poultrymen that birds in laying condition are more susceptible to heat than those not laying. This is probably true when the layers may be confined to trap-nests for some time or when an insufficient number of nests is available and two or more birds crowd into one nest. During the heat wave reported in this paper, special care was taken to examine the trap-nests about every half hour between 10 a.m. and 3 p.m. and to release any birds which had laid, or any which were evidently suffering from the heat. Under these conditions layers were no more susceptible than non-layers to the high temperatures. Considering that birds which laid on July 6, or later in the heat period, were in laying condition, the proportions of such birds, all breeds included, were as follows:

	Number of birds	Layers percent
Among those on hand, July 6	1,482	71.5
Among those dying from heat, July 7-12	36	72.2
Among those dying from other causes, July 7-12	30	20.0
Among survivors on hand, July 13	1,416	72.6

While the birds which died of other causes consisted mostly of non-layers, the population which died from the effects of heat did not differ significantly in proportion of layers from that which survived. This indicates that the laying condition does not necessarily lower the natural resistance to extreme heat. For emphasis this fact may be shown in another way. The mortality from July 7-12 from the effects of heat was:

Among 1,060 laying birds: 2.45 ± 0.47 percent. (Standard error)
Among 422 non-layers: 2.37 ± 0.74 percent.

The conclusion that layers, under conditions in this test, were no more susceptible than non-layers might still be erroneous if the comparatively resistant White Leghorns constituted a higher proportion of the laying population than of the non-layers. Actually the proportion of Leghorns was lower in the layers than in the non-layers. The more susceptible Reds and Rocks together made up 19.6 percent of the layers, but only 15.1 percent of the non-layers.

Breed differences in susceptibility would, therefore, tend to cause a somewhat higher death rate among the layers than among non-layers. Since, in spite of this, no significant difference occurred, the indications that the laying condition does not reduce

a fowl's capacity to tolerate extreme heat are all the more convincing.

(b) *Recent and antecedent production.* Even though layers are no more susceptible than non-layers to the effects of extreme heat, the better producers might be more susceptible, or even more resistant, than the poorer layers. To determine whether their current rate of egg laying or in antecedent production over a longer period. If, in comparing current production in this group, only birds laying during the week are considered, the comparison is narrowed to 27 pairs (one layer could not be paired with controls) in which mean production of those about to die was 4.18 eggs for the

TABLE 2.—*Relation of current and antecedent egg production to susceptibility to heat*

	Current production		Antecedent egg production	
	Died from:		Died from:	
	Heat	Other causes	Heat	Other causes
Number of birds	36	30	36	30
Mean eggs per bird	3.194± .376	1.267± .353	129.14±7.77	112.77±10.02
Mean eggs for controls	3.513± .216	3.316± .310	132.61±6.65	133.72± 6.90
Difference	.319	2.05	3.47	20.95
t	.705	4.296	.325	1.693
P	.487	<.01	.745	.10

or not any such relationship exists, the egg production of the 36 birds which died from heat was compared by Fisher's adaptation of Student's pairing method with that of 36 controls. The figure used as a control was the average egg production of two hens of the same breed, within two months of the same age and from the same pen as the hen which died (and with which the control was paired), but otherwise selected entirely at random. The periods of production utilized in the comparisons were:

(a) Current production, July 1-7.

(b) Antecedent production. For birds hatched in 1935, this was from first egg to July 1; for older birds, from October 1, 1935, to July 1, 1936.

The results of these analyses (Table 2) show that fowls dying from causes other than heat laid significantly fewer eggs than controls in the week before death and were somewhat lower in antecedent production than controls. On the other hand the values for P of .48 and .74 indicate that those dying from the heat did not differ significantly from those that survived either in week, against 4.52 for controls. The difference is not significant (P = .37).

AGE AND SUSCEPTIBILITY

The distribution among birds of different ages of deaths attributed solely to effects of the heat is shown in Table 3.

TABLE 3.—*Deaths from heat in birds of different ages*

Age	Number	Deaths percent
4 months or less	approximately 4000	0
1 year	1266	2.29
2 years	98	2.04
3 years	83	3.61
4 years	44	4.54
Over 4 years	35	0

Except for the extremely old birds, these figures show a tendency for the three- and four-year-old birds to be somewhat more susceptible than younger ones. It is possible that hot weather in 1933, when the temperature went above 95 degrees on eight days, may have eliminated some of the susceptible birds from the two older age-groups but there was little likelihood of

any such elimination in the others, since in 1934 and 1935 there were only six days on which the temperature reached 95 degrees.

DISCUSSION

The data presented above prove that White Leghorns are more resistant to the ill effects of extreme heat than are Barred Rocks and Rhode Island Reds. This attribute is independent of body size, laying condition, current rate of egg production, and genetic capacity for production. It is only slightly affected, if at all, by age.

While no satisfactory explanation for this difference is apparent, several possibilities must be considered. In spite of the familiar statement that the domestic fowl is descended from the jungle fowl, *Gallus gallus,* evidence for monophyletic descent is by no means conclusive. Proponents of the theory of polyphyletic origin have in the past relied chiefly upon differences in form for evidence that the so-called heavy breeds might have descended from some unknown extinct ancestor of birds like the Aseel of Malay, and the Mediterranean breeds from the jungle fowl. To such differences must now be added a number of physiological characters which are probably of equal importance. In comparison with heavy breeds, White Leghorns are less subject to broodiness, more resistant to a deficiency of vitamin B_1 (Nichita *et al.,* 1934, 1934a; Lamoreux and Hutt, 1937), more resistant to "slipped tendon" (Serfontein and Payne, 1934) which is a condition since shown to indicate an abnormally high requirement of manganese, more susceptible to the nematode *Ascaridia lineata* (Ackert, 1935) and more resistant to *Salmonella pullorum.*

With the possible exception of broodiness, none of these differences, nor the one demonstrated in this paper, could possibly have resulted from conscious artificial selection since domestication of the fowl. They may indicate lines of descent from different ancestors, or they may equally well have resulted from natural selection under exposures to entirely different environments of two geographic races descended from a common ancestral species. It seems equally probable that all these differences in physiological characters may be associated in some way with that indefinable attribute of Leghorns by virtue of which they are considered to have a more "nervous" temperament than heavy breeds. These possibilities remain to be explored.

Since the Leghorns used in this study were all white ones, while the heavy breeds were colored, the possibility exists that the capacities of white surfaces for reflecting heat rays and of colored surfaces for absorbing them may have influenced the difference. A comparison of black, brown or other colored Leghorns with colored heavy breeds or with White Leghorns would answer this question, as would a comparison of White Rocks with barred ones, provided that these combinations were available in adequate numbers at the right time and place for such a test. It is also possible that the comparatively loose feathering of the Rhode Island Reds and Rocks might provide enough more insulation than the tight feathering of the Leghorns to be an important factor in regulating body heat under extremes of temperature. Some evidence of this is found in the fact that cold weather affects the egg production of Leghorns more quickly than that of heavy breeds.

There is also the possibility that some breed characteristic of the White Leghorn may be responsible for the observed differences. It is difficult to believe that the ability to resist extreme heat could result from a single major gene which might be linked with some of those utilized in establishing breeds and varieties. If many genes

were responsible, then genetic linkage of the character with breed characteristics would seem unlikely. On the other hand, there is the possibility that some gene affecting most obviously some breed character of color or structure might through pleiotropic action profoundly influence some apparently unrelated physiological character. For example, the chemical processes responsible for dominant white, which prevent formation of melanin, or destroy it as fast as it is formed, and thus turn a genetically colored Leghorn into a white one, might have physiological effects as yet entirely unknown.

The physiological basis for fatal susceptibility to extreme heat in the fowl is not yet known. The very high normal temperature of the species (105-109°F.) suggests conditions of thermo-regulation different from those in mammals.

Rhoad (1936) has shown that during extremely high temperatures in Brazil, the Indian (Zebu) cattle are much less affected than European breeds. In the latter, respiratory rate, interpreted as indicating metabolic rate, rises rapidly with temperatures above 23°C. A significant difference between White Leghorns and Rhode Island Reds in the rate at which the chick temperature rises after hatching was found by Hutt (1935). After being incubated at around 100°F., newly-hatched chicks gradually raise the body temperature to that of adults. The Leghorns do this more quickly than the Reds. Whether or not their ability to do so is related to their greater capacity for tolerating extreme heat is as yet unknown, but it is clear that something about the White Leghorn gives that breed more control over its thermo-regulatory processes than is available in the two heavier breeds studied.

Fowls introduced from Europe and America to tropical countries are notoriously more susceptible than the native stock to disease and unfavorable climatic conditions, particularly to extreme heat. It is evident that under such conditions the White Leghorns would have a decided advantage over the two heavy breeds considered in this paper.

SUMMARY

During six days of heat more intense than had been experienced at Ithaca during at least 11 years, when maximum temperatures reached 101, 103, and 102°F. on three successive days, the mortality from heat prostration was recorded in three breeds not previously exposed to natural selection for resistance to such conditions.

Deaths from heat were 1.79 percent in White Leghorns, 5.26 percent in Rhode Island Reds, and 5.16 percent in Barred Rocks. The differences between Leghorns and each of the heavy breeds are statistically significant. There were no significant differences between breeds with respect to mortality from causes other than heat before, during, and after the heat wave.

The greater resistance of the Leghorns is apparently independent of their smaller body size.

It is shown that laying birds are no more susceptible than non-layers. Susceptibility to heat is independent of the current rate of egg production and of genetic capacity for laying as measured by the antecedent egg production for nine months or more.

Susceptibility to heat tends to increase with age, stock less than four months old being completely resistant to the conditions of this test.

The association of this physiological character and others with the morphological and color characteristics constituting the White Leghorn breed is briefly discussed. The genetic constitution of the White Leghorn appears to give that breed more control over its thermo-regulatory processes

than is available to the others compared with it.

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BREED DIFFERENCES IN RESISTANCE TO A DEFICIENCY OF VITAMIN B_1 IN THE FOWL[1]

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INTRODUCTION

The breeds and varieties of domestic fowls differ in size and shape of body, in structure and color of plumage and skin, in variation of the skeleton, and in the modification of structures, such as the comb and spurs, arising from the skin. Apart from colors, the differentiation of the breeds at the time of their establishment was apparently based upon morphological characters. A few differences between breeds in physiological characters, such as broodiness and color of egg, are now known, but it is not impossible that the present comparative freedom of Leghorns from broodiness and the ability of the same breed to lay white-shelled eggs may have resulted largely from artificial selection for those physiological characters as for other distinguishing breed characteristics. This paper reports an association between breed characteristics and an interesting physiological character—resistance to a deficiency of vitamin B_1.

The first indication that White Leghorns require comparatively little vitamin B_1, or through differential storage of it, or by other means, are markedly resistant to polyneuritis, was put forward by Nichita and Iftimesco (*9*).[2] On a diet deficient in vitamin B_1, or lacking it entirely, one of three White Leghorns, 10 months old at the start of the experiment, showed symptoms of polyneuritis after 41 days and died in 49 days. Another was entirely unaffected and was removed from the experiment at 78 days. A third showed no symptoms till the one hundred and seventh day and for 3 weeks thereafter maintained a subacute chronic state of polyneuritis, but quickly recovered when placed on a normal diet. On the other hand, Nichita, Tuschak, and Calcef (*10*) found that on diets deficient in vitamin B_1 six Rhode Island Reds about 10 months old showed symptoms of polyneuritis at from 7 to 19 days. Three of them were given yeast and quickly recovered, but the other three died of acute polyneuritis in 9, 18, and 21 days. In both experiments, controls on the experimental diet plus yeast were entirely normal.

The experiments of Nichita and his coworkers were not set up primarily to test the two breeds as to their requirements of vitamin B_1, and unfortunately the diets used differed considerably. The diet of the Rhode Island Reds included fresh beef, glucose, and starch, whereas that the White Leghorns did not. The latter received their carbohydrates from decorticated rice. Hence, while the experiments within each breed were well controlled, the fact that the diets were not identical makes any comparison of the two breeds with respect to their

[1] Received for publication May 2, 1938. This is No. 8 in the series by the junior author entitled "Genetics of the Fowl."

[2] Italic numbers in parentheses refer to Literature Cited, p. 316.

requirement of vitamin B_1 less accurate than might be desired. Accordingly, experiments were planned by the present writers to determine in chicks of these two breeds their resistance to a deficiency of vitamin B_1. The results are reported in this paper.

MATERIALS AND METHODS

In four separate trials Single Comb White Leghorn chicks were compared with Rhode Island Red chicks of the same age upon a diet deficient in vitamin B_1. Barred Plymouth Rocks were also included in the fourth trial. Environmental conditions were made identical for all chicks in any one experiment by brooding both lots together in the same brooder. To obtain samples of each breed affected as little as possible by differences between individuals and strains, the chicks used in each test were picked at random. Their dams were selected entirely at random. The population from which these chicks came included three strains of Rhode Island Reds and three of White Leghorns. The fowls used to produce chicks for any one test were not the same as those used for the other experiments. Since all the adult breeding stock received the same diet, which was made as complete as possible according to present knowledge of requirements for reproduction in the fowl, any possibility of differential viability in the chicks resulting from differences in the "carry-over" of essential nutrients in the egg was reduced to a minimum.

A diet lacking vitamin B_1 was obtained by the method of Keenan, Kline, Elvehjem, and Hart (*6*) who showed that by autoclaving a mixture of ground grains the vitamin B_1 could be completely destroyed without much loss of vitamin B_2 (riboflavin), or of B_4. The diet used was as follows:

	Pounds per 100
Ground yellow corn	40
Standard wheat middlings	20
Ground buckwheat	15
Wheat-germ meal	2
Soybean oil meal	5
Fish meal	7
Meat scrap	2
Liver meal	3
Alfalfa-leaf meal	3
Ground limestone	2
Salt (NaCl)	.5
High potency cod-liver oil	.5

This diet was used with uniformly good results for all chicks in the regular hatches of the department in 1936. It was autoclaved at 120° C. and 15 pounds pressure for 6 hours. An additional 0.25 percent of high potency cod-liver oil was added after autoclaving. This oil contained 250 units of vitamin D and 3,000 units of vitamin A per gram. The records for the controls proved that the diet not thus treated was entirely satisfactory.

That the data reported are an accurate measure of the requirements for vitamin B_1 and are not complicated by error due to the destruction of other nutrients was shown in a separate experiment with Rhode Island Red chicks. Twenty-five chicks receiving the autoclaved ration died at the mean age of 11 days, and all were dead 14 days after hatching. Of 23 chicks receiving the same ration plus 120 micrograms

of synthetic vitamin B_1 (Betabion, Merck) per 100 g of feed, none had died. Of 25 chicks receiving 240 micrograms of synthetic vitamin B_1 per 100 g of feed 2 died at 4 and 5 days of age. The remainder of the chicks appeared quite healthy at the end of the experiment.

The four experiments were so planned that in each one the dietary deficiency was initiated at a different age and (except in experiment 1) in chicks which, before introduction of the deficient diet or afterwards (experiment 2), had been for different periods upon the normal (unautoclaved) diet, with consequent different storages of vitamin B_1 as temporary defence against the deficiency. Only strong, vigorous chicks were used at the start of each trial. The four tests of the two breeds were as follows:

Experiment 1, initiated February 8, 1936. Deficient diet from hatching.
Experiment 2, initiated January 25, 1936. Deficient diet first 2 days; normal feed on the third day; deficient diet thereafter.
Experiment 3, initiated February 1, 1936. Normal feed to 14 days of age; deficient diet thereafter.
Experiment 4, initiated June 10, 1936. Normal feed to 21 days of age; deficient diet thereafter.

In experiments 3 and 4 all the chicks that died and those alive at the end of experiments were sexed. Since no evidence was found that either sex is more susceptible than the other, data for the two sexes were combined for all analyses.

RESULTS

The findings in the first three experiments (table 1) show conclusively that the White Leghorns are more resistant to a deficiency of vitamin B_1 than are the Rhode Island Reds.

TABLE 1.—*Comparative survival of Single Comb White Leghorn and Rhode Island Red chicks on diets lacking vitamin B_1*

Diet and breed	Chicks	Average body weight at—		Age at death	
		1 day	1 week	Range	Mean
	Number	*Grams*	*Grams*	*Days*	*Days*
Experiment 1. B_1-free diet from hatching:					
White Leghorn	21	35.0	41.1	5–26	12.86
Rhode Island Red	20	36.1	39.0	9–16	10.85
Experiment 2. B_1-free diet from hatching, normal diet on third day only:					
White Leghorn	15	32.0	46.5	15–62	22.13
Rhode Island Red	15	32.3	40.3	13–21	16.93
Experiment 3. Normal diet to 2 weeks, B_1-free diet thereafter:					
White Leghorn	12	36.1	44.1	29–49	34.17
Rhode Island Red	13	35.1	40.8	26–34	29.0

As was to be expected, the mean age at death is lowest for the chicks which never received any normal feed, highest for those on normal feed to 2 weeks of age, and intermediate for those which got normal feed on 1 day only. In each experiment, however, the mean age at death was higher for the White Leghorns than for the Rhode Island Reds. It is noteworthy also that the variation in susceptibility among individuals, as measured by the range in age at death, was much greater in the former than in the latter. In experiments 1, 2, and 3, chicks of the latter breed all died within 8, 9, and 9 days, respectively, whereas

deaths in the White Leghorns in the same trials were spread over 22, 48, and 21 days. The most resistant White Leghorn outlived the last surviving Rhode Island Red by 10, 41, and 15 days in experiments 1, 2, and 3, respectively. An exceptional White Leghorn chick in experiment 2 survived to 62 days of age.

It is commonly believed that animals growing most rapidly are more susceptible to nutritional deficiencies than are those in which growth proceeds more slowly. Weights of these chicks at 1 day (table 1) show the samples of the two breeds to be almost identical in size at the

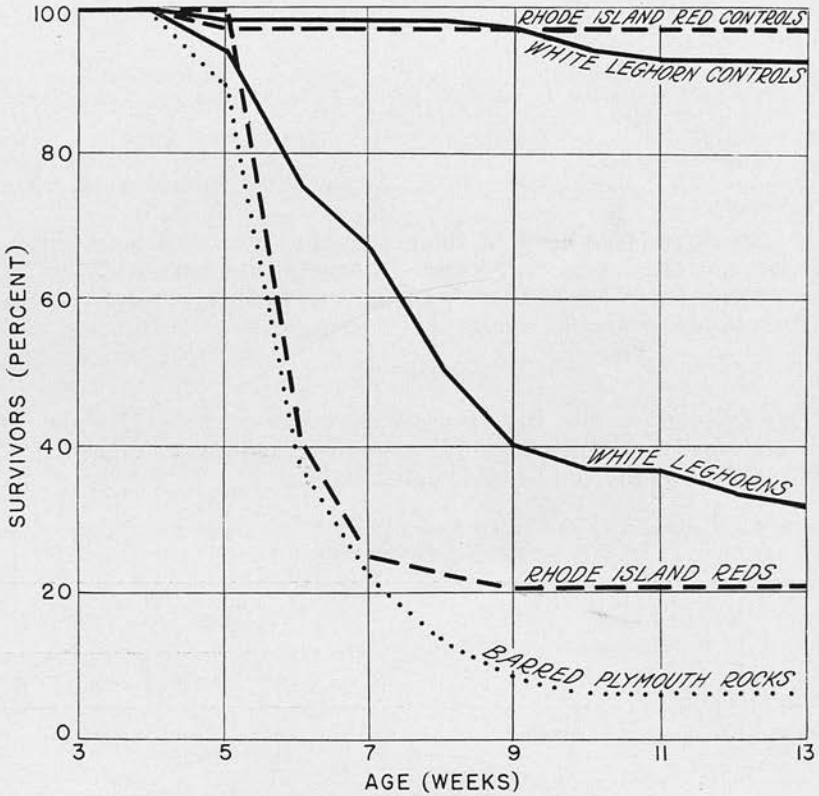

FIGURE 1.—Survival data for 59 White Leghorns, 39 Rhode Island Reds, and 38 Barred Plymouth Rocks placed on vitamin B_1-deficient feed at 3 weeks of age. The controls include 128 White Leghorns and 67 Rhode Island Reds.

beginning of the three tests. The increases in weights of these chicks from hatching to 1 week of age indicate that in all three experiments the White Leghorns grew somewhat more rapidly than the Rhode Island Reds. Ordinarily White Leghorns do not grow more rapidly than Rhode Island Reds in the first week. The differences observed may have resulted from chance or from adverse effects of the vitamin deficiency on the Rhode Island Reds. In any case, the greater resistance of the White Leghorns to a deficiency of vitamin B_1 cannot be ascribed to relatively slow growth in that breed.

Similar results were obtained in experiment 4. Since all the chicks did not die, the results in this test are not strictly comparable with

those in the first three and are therefore presented separately (figs. 1 and 2). Birds in this experiment were kept in battery brooders till 6 weeks old and confined thereafter in a 10-by-12-foot colony house with access to a wire-floored sun porch. Several cases of cannibalism occurred at from 8 to 12 weeks of age, and it is probable that some of the birds still alive after 10 weeks on the vitamin B_1-free diet (at 13 weeks of age, when the experiment was discontinued) were able to survive because of having received an extra supply of the vitamin from the birds eaten. Since these birds went out of the battery brooders and into the colony house at 6 weeks of age, it is possible that after that time some of the birds which survived the longest acquired a supply of the vitamin by coprophagy. It has not yet been proven,

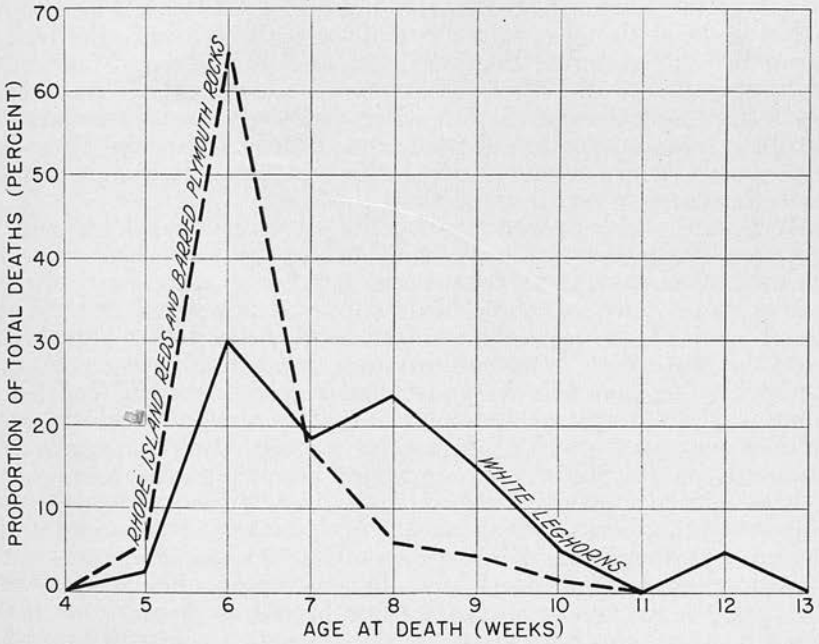

FIGURE 2.—Distribution of ages at death in 39 White Leghorns and 65 Rhode Island Reds and Barred Plymouth Rocks that died from deficiency of vitamin B_1.

however, that in the fowl, as in the rat (*3*) vitamin B^1 is synthesized in the digestive tract and voided with the feces. However, the critical period in this experiment was when the birds were from 5 to 7 weeks of age, at which time the greatest mortality was experienced by all three breeds. Since cannibalism was not serious till the ninth week, it is improbable that error from this source could have affected in any way the breed difference so conclusively demonstrated at from 5 to 8 weeks (fig. 1).

It is quite evident that whatever storage of vitamin B_1 had been accumulated by these chicks during 3 weeks on normal feed it was quickly exhausted (fig. 1). Some mortality occurred in the fourth week, but in the sixth week (third week on the deficient diet) mortality was extremely high in both the Barred Plymouth Rocks and the Rhode Island Reds. Only 39.5 and 41 percent, respectively, of these sur-

vived at the end of the sixth week of the 37 Rhode Island Reds and 34 Barred Plymouth Rocks placed on the deficient diet at 3 weeks of age. In contrast to these, 76.3 percent of the 59 White Leghorns put on the B_1-free diet at 3 weeks were still alive at the end of the sixth week. The percentages of survivors in the Rhode Island Reds, Barred Plymouth Rocks, and White Leghorns at 8 weeks of age were 23.1, 15.8, and 50.8, and at 13 weeks 20.5, 7.9, and 32.2, respectively, of the original numbers in each breed.

The greater resistance of the White Leghorns to a deficiency of this vitamin is demonstrated not only by the lower mortality in that breed but also by the fact that even those that did succumb were able to survive for a longer period than the Rhode Island Reds which died. Analyses of the ages at death up to 13 weeks show that, whereas 64.6 percent of the Plymouth Rocks and Rhode Island Reds that died did so during the sixth week, only 30.8 percent of the White Leghorns that succumbed did so during the sixth week and the rest lived for periods varying up to 7 weeks after the first peak of mortality. As a result the distributions of the ages at death in the two breeds differ markedly (fig. 2). Both curves are skewed; but the curve for the Plymouth Rocks and Rhode Island Reds is leptokurtic, while that for the White Leghorns is platykurtic.

Most of the chicks that died exhibited symptons of vitamin B_1 deficiency including unsteady gait, other signs of neuromuscular incoordination, and, in extreme cases, retraction of the head in characteristic opisthotonos. The birds appeared not to relish the autoclaved feed. This may have resulted from the fact that autoclaving made the mash dark in appearance and (presumably) less palatable, particularly to those chicks which had received normal feed before going on the autoclaved diet. On the other hand, the condition of anorexia may have resulted, in part or entirely, directly from lack of vitamin B_1 as Cowgill (*2*) has shown may happen in other species.

Other evidence of a deficiency of vitamin B_1 was provided by the retardation in growth of the experimental chicks. At 5 weeks of age, just prior to the onset of heavy mortality, the mean weights of the White Leghorns and Rhode Island Reds were respectively 87 and 97 g less than the normal weights for these breeds at 5 weeks, as determined by Card and Kirkpatrick (*1*). Since at 3 weeks of age, when these chicks were placed on the deficient diet, they had exceeded Card and Kirkpatrick's normal weights by 16.5 g in the White Leghorns and 13.7 g in the Rhode Island Reds, the experimental chicks obviously showed at 5 weeks a marked retardation of growth such as is commonly associated with a deficiency of vitamin B_1.

It seems reasonable to conclude, therefore, that the deaths in these chicks resulted primarily from polyneuritis attributable to the deficiency of vitamin B_1.

BREED DIFFERENCES ON A NORMAL DIET

It seemed possible that the differences between breeds with respect to mortality rate might be present even in birds raised under optimum conditions. If this were so, the differences found in experiments 1 to 4 might reflect a general debility of the Rhode Island Reds in comparison with White Leghorns, rather than a special susceptibility to a deficiency of vitamin B_1. To answer this question a comparison was made

of control populations of each breed drawn at random from the several thousand chicks hatched for other purposes from the same strains as those providing the chicks for these experiments.

The mortality curves for these populations show that the strains of White Leghorns and Rhode Island Reds used did not differ in this respect when on normal feed (fig. 1). Up to 3 weeks of age none of either breed died, and at 13 weeks the deaths were 1.5 percent in the Rhode Island Reds and 4.8 percent in the White Leghorns. The diet used for these controls was the same as that of the experimental birds except that it was not autoclaved. It was obviously satisfactory.

It seems safe to conclude that chicks of the two breeds used did not differ in mortality from other causes and that the differences observed in them on the experimental diets can be attributed solely to a difference between the breeds in susceptibility to a deficiency of vitamin B_1.

INDEPENDENCE OF SUSCEPTIBILITY AND BODY SIZE

The demonstration by Nichita et al. (*9, 10*) that adult Rhode Island Reds are more susceptible than adult White Leghorns to a lack of vitamin B_1 indicates a difference between breeds but raises the question whether or not that difference depends upon anything other than the difference in size of those two breeds. Cowgill (*2*) has presented some data indicating that in the larger species the amount of vitamin B_1 required per animal per day is somewhat less in proportion to body weight than in small ones. If this same principle were applicable to intraspecific races differing markedly in size, one would expect a somewhat lower requirement per unit of weight for Rhode Island Reds than for White Leghorns since the former breed exceeds the latter in weight at maturity by an average amount (in females) of around 900 g. This would be the reverse of what has actually been found.

However, the validity of Cowgill's formulas, which are based upon rather hypothetical "maximum normal weights" of the species considered, is open to question. Direct proof that the lower requirement of vitamin B_1 by White Leghorns is independent of their smaller body size at maturity is provided when the experimental animals are of the same size in the breeds compared. It was with this fact in mind that the experiments reported in this paper were carried out with young chicks. Comparisons of growth in White Leghorns and Rhode Island Reds made by Card and Kirkpatrick (*1*) and by Kempster and Parker (*7*) have shown that chicks of the two breeds do not differ in size till after 4 weeks. The present writers' data agree with their findings.

It follows, therefore, that in experiments 1 and 2, where all chicks but one died earlier than 4 weeks of age, and in experiment 3, where the mean age at death in the resistant white Leghorns was not quite 5 weeks, the markedly superior ability of the White Leghorns to withstand a deficiency of vitamin B_1 is a distinct racial characteristic entirely independent of body size. As it happened, the White Leghorns were slightly heavier than the Rhode Island Reds at 1 week of age in these three tests (table 1). In experiment 4, where the deficient diet was not introduced till the chicks were 3 weeks of age, the chicks of the two breeds on hand at 5 weeks of age weighed practically the same, 142.9 g for the Leghorns and 136.4 g for the Rhode Island

Reds. It was in the succeeding 2 weeks that both breeds experienced their heaviest losses (figs. 1 and 2). Obviously the superiority of the White Leghorns in this test was in no way related to body size.

CROSS OF RESISTANT × SUSCEPTIBLE BREEDS

While the primary object of this investigation was to determine whether or not there is a difference between White Leghorns and Rhode Island Reds with respect to their requirement of vitamin B_1, a test was also made to get some idea of the genetic basis for the lower requirement of the White Leghorns. The chicks used were from a cross of Rhode Island Red ♀♀ × White Leghorn ♂. These were tested concurrently with the purebreds in experiment 4 and in exactly the same manner. Mortality in the 53 cross-bred chicks was 73 percent up to 13 weeks of age, when the test was discontinued. The mortality in the same period was 68 percent for purebred White Leghorns and 79.5 percent for purebred Rhode Island Reds. It would appear, therefore, that the crossbred chicks were intermediate between the parent breeds in requirement of vitamin B_1. While further data are necessary to prove the point, this is some indication that the comparatively low requirement of this vitamin by the White Leghorns depend upon multiple factors.

DISCUSSION

These experiments with chicks yielded the same results with respect to breed differences in requirement of vitamin B_1 as did those of Nichita and Iftimesco (*9*) and Nichita et al (*10*) with adult birds. Since the strains of White Leghorns and of Rhode Island Reds used by the Rumanian workers were quite different from those used by the present authors, and since the birds of each breed used by the present writers came from more than one strain, there can be no doubt that the difference observed is really one between breeds and not merely between strains. The low requirement of vitamin B_1 is as truly a genetic breed character as are the dominant white of White Leghorns and the restricted black of the Rhode Island Reds. Whether it is peculiar to White Leghorns or occurs in all Leghorn varieties regardless of color remains to be determined.

The physiological basis for this special attribute of the White Leghorns is as yet quite unknown, and little or no explanation of its persistence as a breed characteristic can be offered. It is obviously different from practically all other distinguishing breed characteristics in that the latter are maintained by artificial selection, whereas it is impossible to see how the poultrymen, either modern or primitive, who helped to establish the White Leghorn breed, could have consciously selected for a lower requirement of vitamin B_1 as they did for morphological characteristics.

The character under consideration is not the only physiological one in which White Leghorns and Rhode Island Reds differ. Hutt (*4*) has shown that these two breeds differ in the rates at which the body temperature rises during the first 9 days after hatching. He (*5*) has also shown that adult White Leghorns are much more resistant to extreme heat than are Rhode Island Reds and Barred Plymouth Rocks. In a discussion of these and other physiological differences

between breeds, the junior author (*5*) has suggested ways in which such differences might have become established, but none of these can yet be considered as anything more than conjecture. The differences are probably between all Leghorns as one class and the so-called heavy breeds as another, rather than merely between the White Leghorns and the Rhode Island Reds.

So far as the writers can ascertain, apart from the work of Nichita and his coworkers, confirmed in this paper, it has not previously been demonstrated that breeds of the domestic fowl vary in their requirement of certain vitamins. Since most recent investigations of the nutritional requirements of fowls are based upon experiments with White Leghorns, there is much doubt about the extent to which findings reported for that breed are applicable to Rhode Island Reds and to other heavy breeds. There has been a tendency in recent years to establish standard requirements of various vitamins for poultry, i. e., so many units of vitamin A, of vitamin D, or of riboflavin per gram of feed. It is obvious that, with respect to vitamin B_1, any such requirement determined from experiments with White Leghorns only would not be accurate for Rhode Island Reds and vice versa.

Assay of vitamin B_1 with pigeons, according to the method of Kinnersley, Peters, and Reader (*8*) is now a standard procedure. These investigators tested pigeons of several different colors, but, so far as the present writers are aware, no experiments have yet been made to determine whether all of the many different breeds of pigeons have the same requirement of this vitamin, or whether some would yield entirely different results in such an assay. It is not impossible that some of the differences in such assays reported from different laboratories have arisen because the pigeon is not yet so well standardized as the commonly used rats of the Wistar Institute strain.

SUMMARY

In each of four experiments the ability of White Leghorn chicks to survive on a diet deficient, or lacking, in vitamin B_1 was greater than that of Rhode Island Reds. When chicks received the deficient diet from hatching, the mean ages at death were 12.9 days for the White Leghorns and 10.8 days for the Rhode Island Reds. For chicks similarly treated but given nondeficient feed on the third day only, the corresponding mean ages were 22.1 and 16.9 days. When deficient feed was supplied after the chicks had been for 2 weeks on the normal diet, the mean ages at death were 34.1 days in the White Leghorns and 29 days in the Rhode Island Reds. Typical symptoms of polyneuritis were observed.

Among chicks on normal feed to 3 weeks of age and the deficient diet thereafter, the percentages surviving to 13 weeks of age were 32 for the White Leghorns, 20.5 for the Rhode Island Reds, and 8 for Barred Plymouth Rocks.

In the White Leghorns deaths were delayed and spread out over a longer period, while in the heavy breeds they occurred earlier and were clustered somewhat narrowly around the mean age at death.

The superior resistance of the White Leghorns to this dietary deficiency is shown to be independent of body size and is not caused by differential mortality from causes other than polyneuritis.

F_1 progeny from a cross of Rhode Island Red ♀♀ × White Leghorn ♂ were intermediate between the parent breeds with respect to resistance to a deficiency of this vitamin.

These findings, along with those of Nichita and his coworkers in Rumania, prove that White Leghorns differ from Rhode Island Reds and Barred Plymouth Rocks in possessing as a hereditary breed characteristic a marked resistance to a deficiency of vitamin B_1.

The significance of this fact in poultry feeding, in experimental work in nutrition, and in the use of birds for quantitative assays of vitamin B_1 is discussed.

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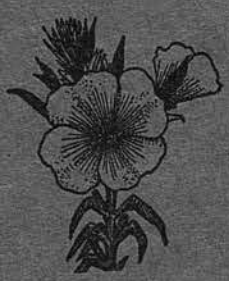

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Genetics of the Fowl

F. B. HUTT
AND
P. D. STURKIE

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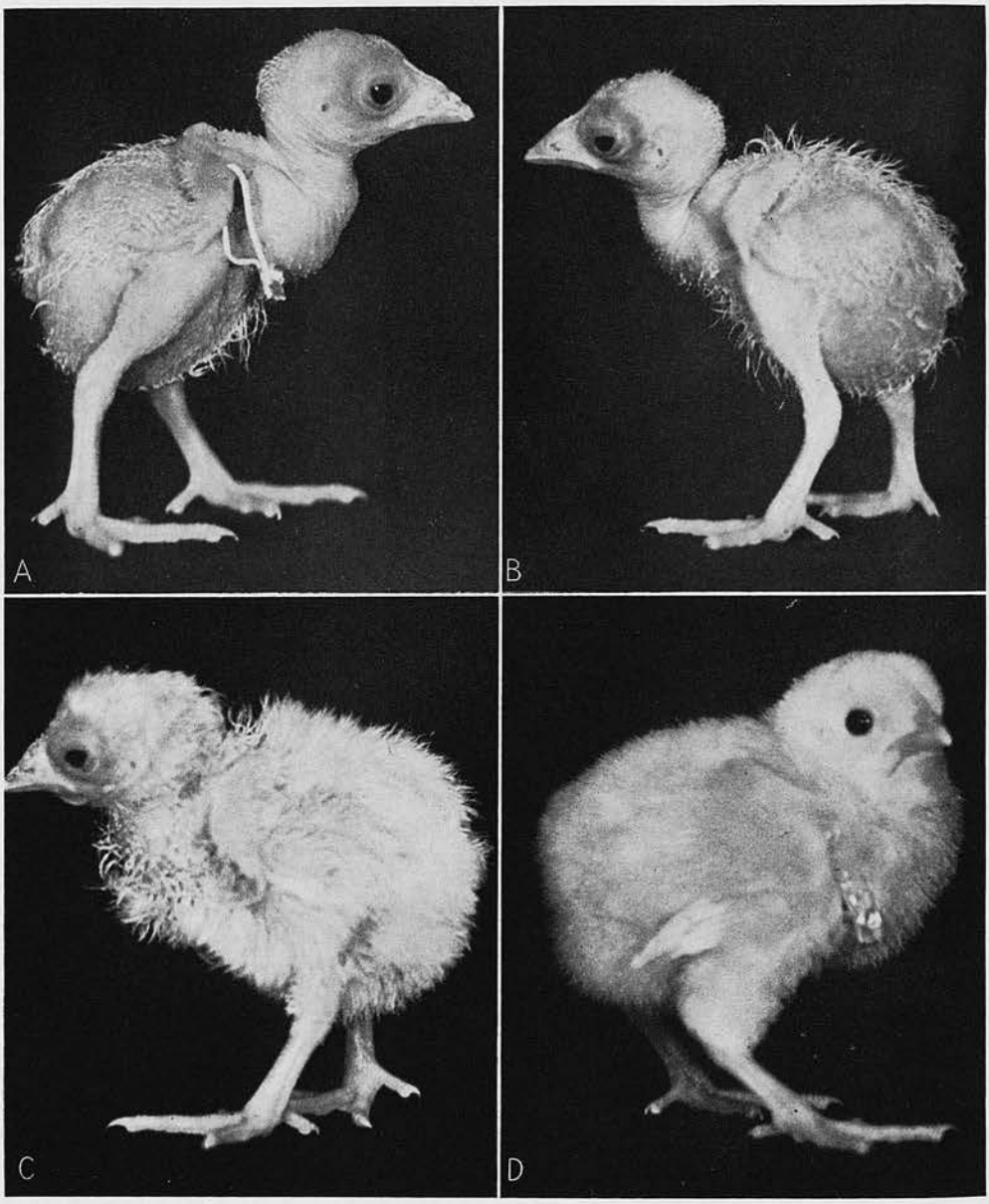

NAKED AND NORMAL CHICKS

Frontispiece

Newly hatched chicks showing three degrees of nakedness are compared with a normal chick (*D*). All of the three "naked" chicks (*A-C*) carry the *n* gene, and they show the range of variation observed. Chick *C* approaches normality more closely than any other of 150 naked chicks thus far hatched. The majority are like *A* and *B*.

GENETICS OF THE FOWL.

IX. Naked, A New Sex-Linked Mutation

F. B. Hutt and P. D. Sturkie

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A WHITE Leghorn chick closely resembling that shown in the Frontispiece (*A*) was brought in to this laboratory in 1935. The owner, who sought an explanation of the abnormality, stated that during the previous three years several others had occurred in the flock from whence it came. Fortunately, the chick lived to maturity. Genetic studies of its descendents have shown that the loss of down is caused by a recessive sex-linked mutation which is lethal to about half of the affected chicks during the last two or three days of incubation.

Description

A. Chicks

At hatching affected chicks show degrees of feathering varying from an almost complete lack of down to the condition shown in Frontispiece-*C* which shows the most completely covered chick yet obtained among over 150 naked chicks hatched from this stock. The majority are like the naked specimen shown in Frontispiece-*A* or the somewhat intermediate chick (*B*).

In the sparsely covered chicks there is complete absence of down in the apteria and the few strands of down on the pterylae seem wiry, short, thickened and sometimes curled or twisted. This appearance is caused by failure of the barbs to separate as in the normal feather (Figure 1). So firmly are they stuck together that even after soaking in water or alcohol it is difficult to separate them. In the modified naked chick (Frontispiece *B* and *C*) the down feathers show varying degrees of approach to the normal condition and some of them on the posterior spinal pteryla and in the abdominal region are indistinguishable from normal down feathers.

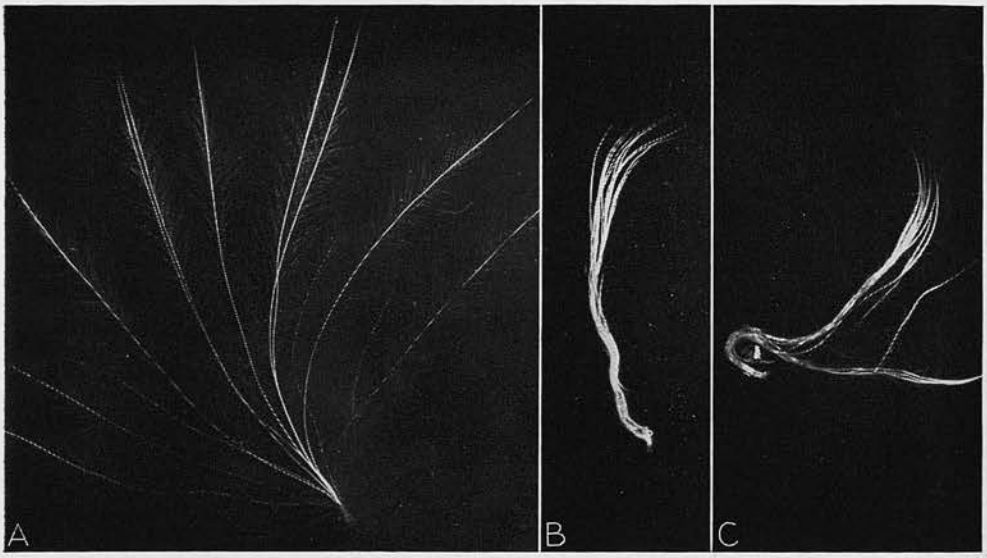
In general the anterior end of the chicks is more naked than the posterior one. The head, anterior spinal and alar feather tracts, as well as the anterior part of the ventral tract, may be quite bare, while the others are comparatively well covered, particularly the posterior spinal tract. This tendency toward an antero-posterior gradient of decreasing effect of the mutation is partially evident in the Frontispiece.

The arrangement and extent of the feather tracts are normal, and the follicles are quite distinct, somewhat as in a chick from which the down has been plucked.

Naked and normal chicks do not differ significantly in size at hatching, but the latter appear much larger because of their fluffy down (Frontispiece-*D*).

B. Juveniles

From about two to four weeks of age most of the naked chicks appear more naked than at any other period of their lives. The sparse, wiry down is worn off and feathers replacing it are very slow to develop. Even chicks partially covered at hatching may be quite naked in appearance at three weeks. In comparison with normal birds, this effect is heightened by the almost complete failure of the juvenile remiges to develop (Figure 2). The head tract is slowest in feathering and the humeral and femoral tracts usually show the longest feathers. The juvenile primary feathers do not appear in regular sequence from the inner to the outer one as in normal chicks. Some chicks may have at four weeks only the inner primary and one near the tip of the wing, perhaps the seventh or eighth, broken through the skin. Feather follicles not erupted fre-



BARBS OF "NAKED" FEATHERS DEFECTIVE

Figure 1

Single chick down feathers from normal (*A*) and naked (*B, C*) chicks. In the latter the barbs are folded and twisted together to form a single strand which, when softened, can be separated into its constituent barbs (*C*). This reveals that barbules are present in the abnormal feather as in the normal one.

quently appear swollen as if some constriction were preventing their contents from being pushed out. The rectrices are more successful than the remiges in breaking through their sheaths. In both a central rachis is present but the barbs are frequently spread out to form a rather downy feather like that of the Silky. Many of these feathers appear to be broken off right at the follicle, others at one to three centimeters from the skin.

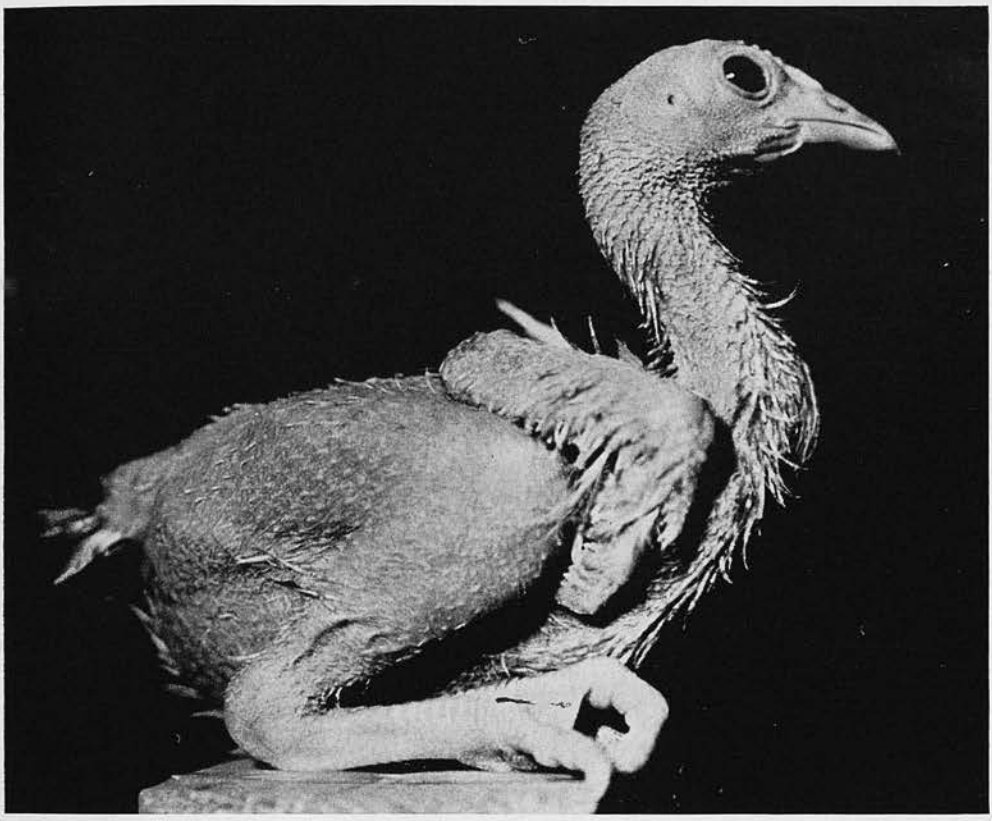
C. Adults

Mature birds have a much more complete covering of feathers than young ones but there is a great deal of variability in the extent of feathering. Pterylosis is normal. There are feathers on all of the pterylae, and down feathers in the apteria. The fluff plumage of the abdominal and ventral regions may be almost normal in density and length of feather (Figure 3*A*). Elsewhere the tips of the feathers are usually broken off and some have unusually long barbs at the distal end (Figure 4), so that the birds have a rough plumage instead of a smooth one.

The remiges are usually all missing, but stubs may remain of one or two that have broken off. The rectrices are better represented and up to four of them have been counted. Some of these attain full size and appear normal.

Areas lacking plumage usually show stubs of feathers which have broken off at distances of about one centimeter or less from the skin (Figure 3*B*). Some seem broken right inside the follicle. Some have blood clots at the broken end, indicating that the break has come shortly after eruption of the feather from the follicle sheath. In others the follicle is filled with dried blood. Subcutaneous hemorrhages are found in some follicles from which the feathers have not erupted. The scales of the feet are normal.

Under the microscope feathers from mature naked fowls are seen to be normal except that in most of them the apical barbs and the tip of the rachis are broken off (Figure 4). Some of the barbs are unusually long and lack



MAXIMUM NAKEDNESS

Figure 2

A naked chicken at five weeks of age, at about the end of the period when the feathers are most defective. The distribution of feather follicles is normal but the feathers are few and short.

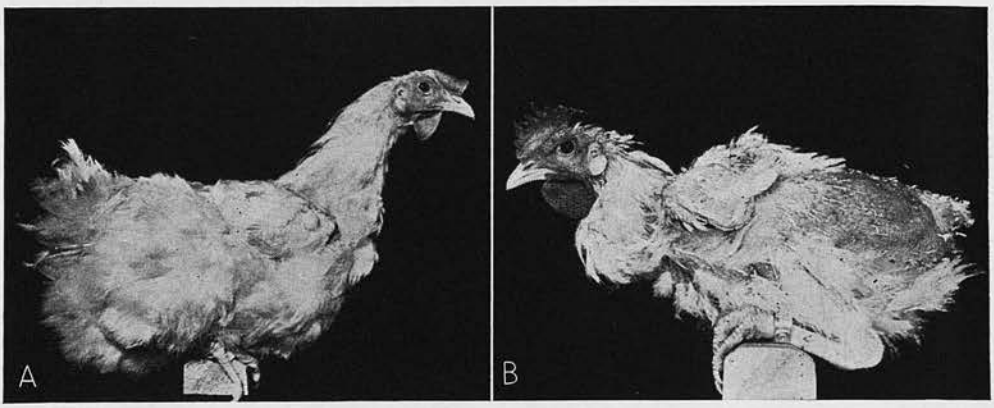
barbules on a greater portion of their distal extremities than do those on normal feathers. This gives the feathers of females somewhat the appearance of typical male neck and wing feathers, except that the feather is more rounded and the tip is broken off. Elsewhere, where barbules are present, they carry hamuli and barbicels like those in normal feathers and the result is a normal web.

Genetics

The original naked White Leghorn chick, secured in 1935, turned out to be a female. In the spring of 1936 she was mated (by artificial insemination) with an unrelated normal White Leghorn male and produced 13 chicks, all with normal down. Unfortunately, the original naked hen did not survive for another year and a backcross to her in 1937 was impossible. However, in that year one of her sons was mated to four of his full sisters and to some unrelated hens. Another son was mated with 12 Rhode Island Red hens.

It was soon apparent that both of these F_1 males produced featherless chicks just as readily from unrelated hens as from full sisters, that all of the naked ones (in these crosses) were females, and that considerably more than half of the affected embryos died during the last three days of incubation.

Four more such matings — of heterozygous males to females not carrying



MATURE NAKED FEMALES

Figure 3

The one on the left has almost normal plumage in the ventral regions and on the abdomen, but lacks the large wing feathers entirely. This is the extreme variation toward normality. The one on the right has only a scanty covering, and lacks both wing and tail feathers. The original naked chicken had fewer feathers than that in (*B*), but plumage in most adults is between the extremes shown.

the gene — were made in 1938. The results in all six of these matings are tabulated in Table I.

Assuming that there is no differential mortality of normal and naked chicks prior to the last three days of incubation, the expectation for a sex-linked recessive mutation is that among those hatched or dying during the last three days of incubation, one-quarter would be featherless. There is a close fit of observed to expected ratios in each of the six matings reported in Table I, and altogether there are 712 normal : 246 naked where the theoretical expectation is 719 :239.

Evidence of the six-linked nature of the mutation is shown in Table I and summarized (for the three matings in which sexes of all hatched chicks are known) by the following sex-ratios among 477 chicks hatched, or dead at 19-21 days of incubation:

	Normal		Naked	
	♂♂	♀♀	♂♂	♀♀
Observed	239	104	0	134
Expected	239	119	0	119

The observed numbers fit quite well to the expectation that among chicks not naked there should be a ratio of 2♂♂ : 1♀, and no males whatever among the naked chicks.

It is proposed to call this mutation "naked" and to designate it by the symbol *n*, which, so far as the writers can ascertain, has not yet been preempted for any other mutation in the fowl.

No effects of the gene are noticeable in heterozygous males. During the past six months matings were made to determine the effect of the gene on homozygotes. One might reasonably have expected that a mutation having such a marked effect in hemizygotes, and lethal to more than half of them, would have still greater effects upon the homozygous males. Such, however, is not the case.

Table I. Classifications in genetic analysis of the naked stock.

Male	Dead at 19-21 days of incubation				Hatched				Ratios Normal:Naked	
	Normal		Naked		Normal		Naked		Observed	Expected†
	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	N n	N n
Matings of Nn ♂♂ x N- ♀♀										
C 5919	17	7	0	39	75	37	0	21	136: 60	147: 49
C 5913	4	2	0	24	65	19	0	5	90: 29	89: 30
D 5976 (a)	6	2	0	21	72	37	0	24	117: 45	121: 40
D 5976 (b)	7	4	0	9	78*		21*		89: 30	89: 30
D 5983	5	3	0	19	180*		37*		188: 56	183: 61
D 5903	0	1	0	3	91*		23*		92: 26	89: 29
								Total	712:246	719:239
Matings of Nn ♂♂ x n- ♀♀										
D 5977	3	0	2	6	14	13	7	8	30: 25	27.5: 27.5
D 5960	1	2	5	4	3	7	2	5	13: 16	14.5: 14.5
Total	4	2	7	12	17	20	9	13	43: 41	42.0: 42.0

* These birds could not be classified for sex at the time of writing, but of the 81 naked chicks, 47 which have died were all females.

† To the nearest whole number.

Two males proven heterozygous were mated with sibling naked females. To avoid injuries during treading to the partially naked backs of these females, one mating was made by artificial insemination (♂ D 5977) and for the other, in which the hens were somewhat better covered, the male was placed in the pen only on every third day. The results, shown in the lower part of Table I, indicate that homozygous males suffer no higher mortality than hemizygous females during the last three days of incubation. It would also appear, since the ratio of 43 normal : 41 naked among those that hatched or died after 18 days of incubation is the same as the 1:1 zygotic ratio, that none of the naked chicks in these matings died earlier than 19 days. However, among the 41 naked there were 16♂♂ : 25♀♀. This is probable just a chance deviation from the 20.5 expected for each sex, but, if further data in matings of this type continue to show an excess of females, they would indicate that the mutation is lethal to some of the homozygotes at an earlier age than to the hemizygotes.

Lethal Action of the Gene

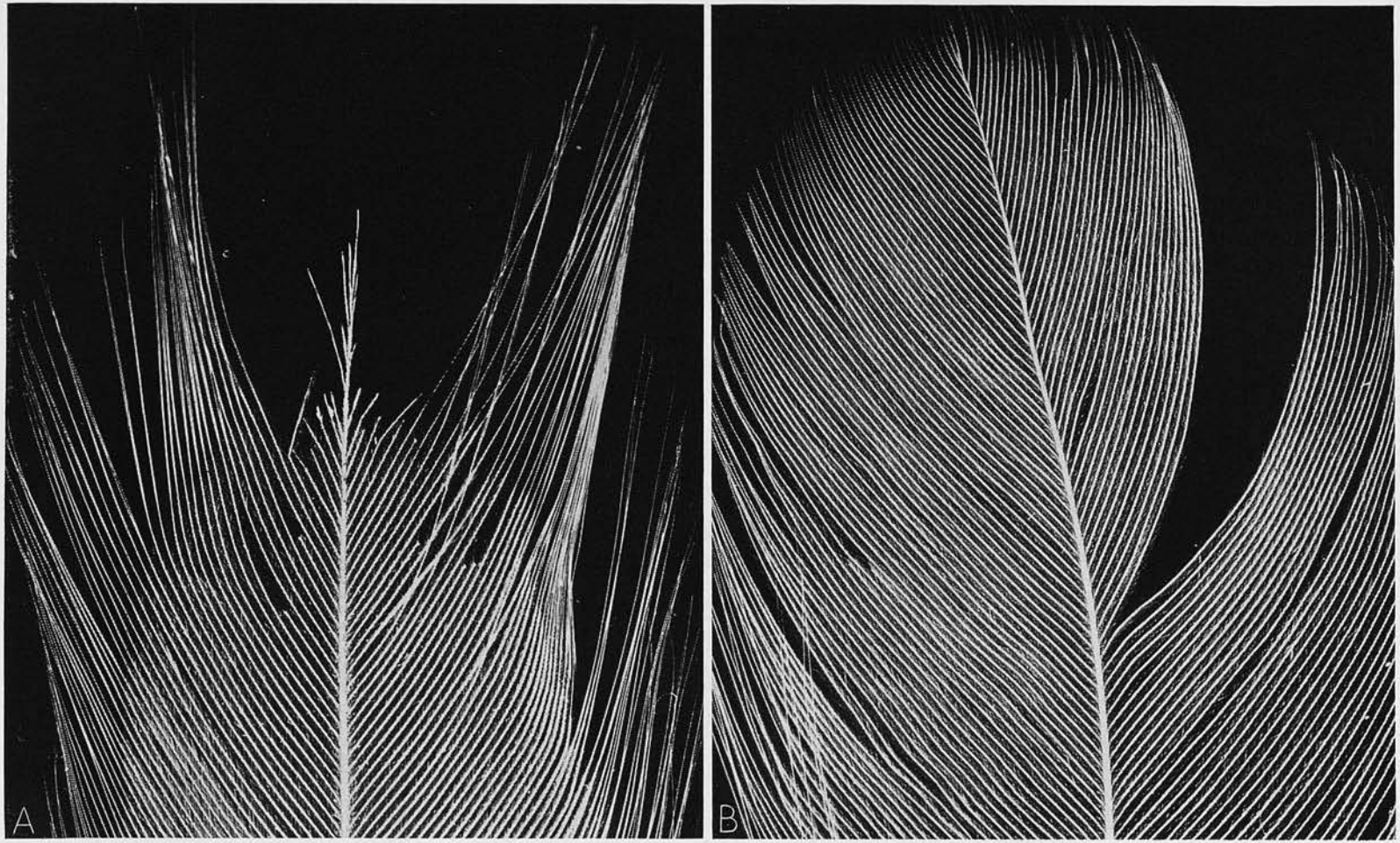
A. During Incubation

In the six matings of heterozygous males to normal females (Table I) the total number of naked chicks (246) among chicks hatched or dying after 18 days of incubation was slightly higher than that expected from the theoretical zygotic ratio (239). This proves that up to 18 days of incubation there is no greater mortality among naked embryos than in normal ones. During the last three days of incubation there is a marked difference, as is shown by the evidence from eight matings tabulated below. In order to restrict these data to the 19-21 day period, all unhatched eggs were broken for examination, and both normal and naked embryos which had died before 19 days were omitted from the class under consideration. The number of chicks hatched, plus those dead at 19-21 days, gives the total number alive on the eighteenth day. The following summary shows the mortality during the last three days in each mating, expressed in percentages, based separately upon the numbers of normal and naked chicks apparently alive on the eighteenth day:

	Died at 19-21 days	
	Normal %	Naked %
C 5919	17.6	65.0
C 5913	6.7	82.8
D 5976 (a)	6.9	46.7
D 5976 (b)	12.4	30.0
D 5983	4.3	33.9
D 5903	1.1	11.5
D 5977	10.0	40.0
D 5980	23.1	56.2
Weighted average:	8.5	46.7

The numbers in each mating are omitted but can be calculated, if desired, from Table I. The weighted averages are based on 755 normal and 287 naked chicks. In a brief earlier report of this condition by the senior author[6], it was stated that about three-quarters of the affected chicks die during the last three days of incubation. This was the case in the first two matings, but in later ones mortality among the naked chicks has been somewhat less, with an average of 46.7 per cent for all matings to date. While there is some evidence, as in the mating with ♂ D 5903, that when mortality in normal embryos is low deaths among the naked ones are correspondingly few, such a relationship is not always consistent. The highest mortality among naked embryos, 82.8 per cent, occurred in a mating with only 6.7 per cent mortality among normal ones. It seems probable, therefore, that while environmental agencies and genes affecting hatchability in general undoubtedly affect the hatchability of the naked chicks, there are also specific modifiers in some matings which particularly affect the naked chicks.

Those that die are full grown and in almost all cases have the yolk sac fully enclosed in the body. They do not appear to be different from those which hatch. It is probable, therefore, that most of the mortality occurs about the twentieth day of incubation.



TIPS OF NECK FEATHERS FROM MATURE FEMALES

Figure 4

The feather tips from a naked fowl (*A*) show the characteristic broken apical barbs and the elongation of barbules on a greater portion of their lengths than in normal feathers (*B*). The defects of the naked feathers are not due to a dietary deficiency, because feeding special diets had no effect, and transplants of normal skin to naked hosts grew a normal crop of feathers.

B. After Hatching

Mortality among naked chicks is higher during the first six weeks than in their normal siblings. Since many of the normal chicks were not raised in some of the matings, figures on mortality for all of those reported in Table I are not available. However, among sibling chicks which were raised under comparable environmental conditions mortality up to six weeks of age was as follows:

In 571 normal chicks: 13.1 per cent
In 150 naked chicks: 55.3 per cent

Mortality for both classes is somewhat high because these figures include chicks alive in pipped eggs which were helped out. Among them the death rate was unusually high. It is evident, however, that about half of the naked chicks which succeed in hatching succumb within six weeks. Since about 50 per cent die before hatching, this means that only about a quarter of the original zygotes survive to the age of six weeks after hatching.

Localization of the Defect

The question arises whether the abnormality induced by the *n* gene is localized in the feather follicles or is some general defect of metabolism. Martin and Gardner[7] reported that cysteine induced growth of hair in rats genetically hypotrichotic. This would indicate some metabolic defect, which these authors concluded was the lack of an enzyme necessary for breaking down "glutathione or other peptide linkages involving sulfur-containing groups" into amino acids. However, the fact that Roberts[8] was unable to confirm their results with cysteine and did show that skin from a normal rat would grow and maintain normal hair when grafted on a hypotrichotic one, indicates that the defect is in the hair follicles rather than in metabolism. A similar conclusion was reached by Crew[3] concerning the hairless mouse.

To measure the possible effects of cysteine on growth of feathers in the naked chicks, 7 of them, 5 days of age, were individually fed cysteine hydrochloride in aqueous solution as a supplement to an adequate stock ration. The amount given, 1 mg. per 3 grams of body weight, was greatly in excess of the dosages used by Martin and Gardner and by Roberts. It was kept reasonably constant by weighing the birds frequently and adjusting the amount of cysteine accordingly. After 21 days of this treatment the chicks fed cysteine did not differ from untreated controls in growth of feathers.

In a similar experiment using cystine with the same dosage as that for cysteine, no differences between eight experimental birds and their controls could be observed up to 26 days when the test was discontinued.

Mr. C. Dixon of this laboratory, who has successfully transplanted skin from naked donors to normal hosts and *vice versa,* finds that in persistent grafts the growth of feathers is the same as in the donor, regardless of the host. This shows that the mutation affects primarily the feather follicles.

Other Effects

Because of the high mortality before and after hatching it has thus far been difficult to obtain enough naked chicks of one age to permit reliable comparisons of them with their normal siblings in respect to growth, egg production, and other measures of physiological efficiency. Now that naked males have been secured that difficulty can be overcome. At hatching 18 naked chicks had an average weight of 39.8 grams compared with 40.55 grams for 18 normal siblings. At six weeks naked chicks and normal siblings had the following average weights in grams:

		♂♂	♀♀
Normal:	10 ♂♂, 8 ♀♀	466	427
Naked:	3 ♂♂, 7 ♀♀	515	392

Since the weaker naked chicks died and are not included, these figures merely indicate that growth in the best of that group is as good as that of normal chicks. Ten naked females for which records

are complete began to lay at a mean age of 178 days, with first eggs weighing on the average 45 grams. While these figures indicate that the naked birds are not radically different from other fowls, more extensive data on these points are desirable.

Discussion

Although different degrees of genetic hairlessness have been reported in the rat, house mice, deer mice, rabbits, cattle and swine, so far as the writers are aware this is the first case of generalized lack of plumage proven hereditary in birds. Professor L. J. Cole of the University of Wisconsin has informed the writers that he has some entirely featherless pigeons which are now being submitted to genetical analysis.

Aldrovandus[1] illustrated a nearly naked fowl which he labelled without further description as *"Gallina ferè petrificata,"* but his specimen differs from those reported in this paper in having the quills of the wing and tail feathers well developed, as are the shafts of feathers in other regions, but without normal barbs. It resembles more the "porcupine" mutation in pigeons reported by Cole and Hawkins[2] and is reproduced in the paper by those authors.

The naked condition differs from that in bare-neck or naked-neck fowls in having normal pterylosis but abnormal development in the feather follicles. Greenwood[4] has shown that the bare-neck condition, which is caused by an autosomal dominant gene, arises from restriction of the feather tracts in the anterior region of the body, and that where follicles are present normal feathers develop. The bare-necks lack down feathers and semiplumes in the apteria in all parts of the body, but the naked fowls may have almost normal feathers in the apteria particularly in the ventral and abdominal regions.

Embryos lacking down, or most of it, are occasionally found during the examination of eggs which have failed to hatch. The occurrence of three such embryos in related stock was reported by Serebrovsky and Petroff[9], who ascribed these abnormalities to genetic factors. However, Warren's[10] report of non-genetic complete downlessness shows that such an abnormality may arise entirely from extrinsic causes. Reduction of the down is not uncommon in embryos from fowls on diets deficient in riboflavin and in chondrodystrophic embryos, being most marked in both cases at about 13 or 14 days of incubation.

Naked chicks of about five weeks resemble somewhat the five-months-old birds illustrated by Warren[11], which were apparently homozygous for the autosomal dominant character, flightless. While the extent of the feathering is about the same in both, the flightless stock have defective beaks and toe nails, and the naked chicks do not. The former apparently have normal down at hatching. The flightless birds did not attain sexual maturity as the naked ones do. Moreover, at five months most of the naked chicks are much better covered than the two flightless specimens illustrated by Warren.

During the past year there appeared in the press a picture of an entirely nude chicken photographed by Mr. William LaVarre at Pisac, about twenty-five miles from Cuzco, in the mountains of Peru. In a personal communication to the senior author Mr. LaVarre has kindly provided additional information. Over fifty similar chickens were seen by him in the same district and he was sure that none had been plucked. The altitude is 12,000 feet, and the temperature between 20 and 60° F. The natives did not consider the naked birds unusual and stated that many years ago the wind had blown the feathers off many of their chickens, which subsequently produced featherless progeny. While this idea is open to question, it seems probable that there is a hereditary condition in Pisac which may be the same as that reported in this paper. The bird in Mr. LaVarre's photograph is considerably more naked than the adult stock thus far raised here. Its feather follicles seem normal in extent and distribution.

The genetically naked chickens discussed in this paper should not be con-

fused with those individuals extremely slow in feathering which are not uncommonly found in flocks of Rhode Island Reds, Plymouth Rocks and other heavy breeds, particularly in strains slow to mature. These have normal down at hatching and when they finally feather out are indistinguishable from normal chickens.

This new character should prove useful for clearing up some of the present uncertainties about the map of the sex chromosome in the fowl. For this the senior author[5] has recently suggested an arrangement different from those previously proposed. However, since of the seven genes which have thus far been assigned to that chromosome, five have effects measurable only in the plumage, the shortcomings of the new character, even apart from its lethal action, are obvious.

Freaks and mutations in poultry are reported continuously in the daily press. Many of the latter have been adopted as breed characteristics and others have been studied by geneticists. Since hereditary absence of feathers has not previously been established during the past 35 years of active genetic investigations with the fowl, and especially since sex-linked characters are particularly conspicuous, it seems probable that the mutation reported in this paper is of recent origin.

Summary

A hereditary character, naked, reducing the down of chicks and the plumage of adults is described. Pterylosis is normal, but the feathers do not erupt properly from their follicles. Remiges and rectrices are few or lacking. Naked fowls are easily distinguishable from normal ones at all ages.

This condition is caused by a sex-linked recessive gene, *n*, which on the average is lethal to about half of the affected chicks during the last two or three days of incubation. Among naked chicks hatched, mortality was 55 per cent during the first six weeks of life. Homozygous males are no more affected than hemizygous females.

Cysteine and cystine had no effect on the growth of feathers when fed in comparatively large doses. Skin transplants indicate that the defect is localized in the feather follicles.

Other conditions reducing the extent of the plumage in the fowl are briefly discussed and it is suggested that the naked mutation is of recent origin.

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A Note on Lambert's Mosaic in the Fowl

IN a recent number of this JOURNAL (20: 167-169) Dr. W. V. Lambert has described a most interesting case of an animal mosaic in which a fowl was white-skinned on the left half of the body and yellow-skinned on the right. He considered the most plausible explanation of the abnormality to be that it arose from non-disjunction, at an early cleavage, of the pair of chromosomes carrying the genes for yellow or white skin colour. This assumption presupposes that the bird was heterozygous for skin colour. The evidence from two similar cases suggests that his alternative explanation,—that of a gene mutation—is somewhat more likely to be the correct one.

If it were a case of non-disjunction, one would expect that in addition to the factor for white skin, the chromosome lost from the cell giving rise to the yellow side would also carry other genes. The loss of some of these, particularly those affecting the size of the whole organism or any part thereof, would also be expected to be shown as well as the absence of the dominant gene for white skin. It would be expected, therefore, that the yellow half would be smaller than the white side or would be in some respects disproportionate.

Exactly such a case has recently been described by Crew (*Jour. Genet.* 20: 179-186). A male fowl from a mating of light Sussex female × Rhode Island Red male (and therefore heterozygous for white and yellow skin) was found to be not only yellow in one leg and white in the other, but also to have bones distinctly smaller on the yellow side than the corresponding bones on the white side. The difference in size between the two halves of the body was almost equal to that normally existing between males and females, although some bones were more affected than others. All these facts are in accord with the explanation suggested by Crew that the condition arose from non-disjunction of the chromosomes carrying not only the gene* for skin colour but also enough size factors to produce the marked lateral asymmetry of the skeleton.

Somewhat similar is the case described by Mackin (*J. Exper. Zool.* 38:355-368) in which a gynandromorph fowl had not only testicular tissue on the right side and ovarian tissue on the left, but also differences in size between bones of the right and left limbs which the present writer has shown (*Poultry Sci.* 8:202-218) represented very closely the normal sex dimorphism in the appendicular skeleton of the fowl. From this case, apparently a true gynandromorph, it was evident that the loss of the sex chromosome from one side also meant the loss of those size factors which normally bring about the differences in size between male and female skeletons.

These two cases indicating a relationship in the fowl between chromosome aberrations and size disturbances are in accord with our knowledge of modifications in size occurring in polyploid plants and animals and in cases of gynandromorphism in insects. Crew's case is quite consistent with the view that early non-disjunction of the chromosomes carrying the gene for yellow or white skin colour is associated with marked size disturbances. Lambert makes no mention of size differences between right and left sides of his mosaic, nor are any evident from the photograph.

In view of these facts, it seems more likely that this abnormality arose from a mutation in the skin colour gene at some time in early cleavage. From Lambert's description and from the fact that the earlobes appear more completely white than would be expected in a hybrid between a Leghorn and any white-skinned breed (practically all of which have red ear-lobes) it appears most probable that the fowl was a Leghorn and therefore homozygous for yellow skin. If such were

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the case the mosaic represents a dominant somatic mutation (from *ww* to *Ww*). However, as Lambert points out, if the fowl were heterozygous for skin colour and a recessive mutation had occurred in the *W* gene, the same effect could have been produced.

The similarity and the difference between Lambert's and Crew's cases—both having lateral asymmetry with respect to skin colour, but only one case exhibiting irregularities in size—suggest that the presence or absence of such irregularities should be a valuable aid in distinguishing between animal chimaeras resulting from non-disjunction of chromosomes and those arising from factor mutations in somatic cells, where either phenomenon has occurred at an early stage of ontogeny.

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SEX DIMORPHISM AND VARIABILITY IN THE APPENDICULAR SKELETON OF THE LEGHORN FOWL

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SEX DIMORPHISM AND VARIABILITY IN THE APPENDICULAR SKELETON OF THE LEGHORN FOWL

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During the past five years there has been sent to this department a number of male fowls suspected by their owners of having been originally females and of having turned into functional males in a manner similar to that of the cases described in the fowl by Crew[5] and in the pigeon by Riddle.[17] In nearly every instance it has been impossible to verify the accuracy of the case history by breeding tests or by post mortem examination.

Sex dimorphism, in size as well as in plumage and colour, is more conspicuous in the Gallinae than in the majority of other birds. It seemed possible that this dimorphism might be so great that skeletal measurements would give a clue to the original sex of such individuals as those mentioned above. It was recognized that the range in length of any single bone in one sex would probably overlap the range of the same bone in the other sex, as Schneider and Dunn[18] have already demonstrated for several bones of the fowl. However, casual observations suggested that the difference in size between male and female fowls is more marked in the tarso-metatarsus than in other bones of the appendicular skeleton. This bone was not measured by the workers quoted. There was also some evidence from a pseudo-gynandromorph studied at this department that the sex dimorphism is greater in the proximal bones of the extremities than in the distal ones.* Accordingly, at the suggestion of Professor Crew, the writer undertook to determine the extent of sex dimorphism in the appendicular skeleton of the fowl and to see if a significant dimorphism prevailed either in absolute length, or in proportional length of one or more bones, which might be of value in

* In this bird, a report of which is being given elsewhere by Prof. Crew, the measurements of the appendicular skeleton were those of a male on one side and approximately those of a female on the other. Differences in shank colour showed that the disturbance had resulted from the loss of an autosome, not of a sex chromosome as would be at first suspected.

determining the original sex of fowls which had undergone partial or complete sex reversal.

MATERIAL

The material studied included practically all the mature fowls which died or were killed at this department from September 1927 to September 1928, as well as a number of carcasses kindly supplied by the Royal (Dick) Veterinary College, Edinburgh, to which they had been sent for autopsy. One hundred and fifty-three skeletons were measured but the data used in the present report are confined to those of Leghorn fowls only. Of these there were 53 females, 36 males and 16 capons. Theoretically the measurements for all varieties of this breed should be comparable but it is probable that some, notably the whites, are slightly larger on the average than others. The majority of the birds used in the present investigation were Brown Leghorns which were in some cases slightly below standard weights for the breed.

The fact that a number of the birds were diseased should in no way prejudice the reliability of the bone measurements since most diseases are probably unable to affect the size of any bone once growth is completed. Malformed and broken bones were not measured and one skeleton affected with erythromyelosis was discarded.

METHODS

After autopsy both legs and both wings of each bird were skinned and boiled until the flesh could be easily removed. When two or more skeletons were boiled together, cotton bags were used to keep them separate. The individual bones were scraped clean and allowed to dry for at least 18 hours at room temperature after which any remaining pieces of cartilage were removed from the epiphyses and measurements were taken. This was done by marking on a sheet of paper the extremities of each bone (using a sharp pencil) and measuring the distance between the two points. The procedure was simplified by using a vertical line as the starting point for all measurements. Care was taken to ensure that distances were always measured between the same points on each bone. These were as follows:

Humerus—from the proximal point of the head to the distal extremity of the ulnar tubercle.

Radius—from the margin of the concavity in the proximal end to the extremity of the tubercle on the distal convex surface.

Ulna—from the proximal point of the olecranon to the distal extremity of the inferior flange of the trochlear surface.

Carpo-metacarpus—from the extremity of the flange dorsal to the articular surface on the proximal end to the outermost point of the tuberculum articulare on the distal end.

*Two phalanges of third digit**—the maximum distance between the proximal and distal extremities.

Femur—from the proximal extremity of the trochanter to the end of the external condyle.

Tibio-tarsus—from the projecting point of the cnemial process to the farthest point on the external condyle.

Tarso-metatarsus—from the end of the ridge between the two surfaces for articulation of the tibio-tarsal condyles to the extremity of the central trochlea.

Four phalanges of third digit—from the tubercle dividing the articular grooves on the proximal surface to the extremity of the condyles on the distal end. The claw was removed from the ungual phalanx before that bone was measured.

Measurements of all bones were made to the nearest half millimetre. In every case (except where odd ones were lost) all the bones listed above were measured in duplicate, *i. e.* in both right and left limbs, the average of each pair being taken as the correct length for that bone. It is evident therefore that the average measurement might be expressed to 0.25 mm. In the majority of cases the measurements were coincident and only in rare cases was there a greater difference than 0.5 mm.

In statistical analysis of the absolute lengths, the humerus, radius, ulna, femur, tibio-tarsus and tarso-metatarsus were grouped in 1.0 mm. classes and the remaining bones into 0.5 mm. classes.

I ABSOLUTE BONE LENGTHS

The biometric constants for absolute lengths of the bones of

* This terminology is based on the embryological evidence as presented by Lillie[12] which indicates that the first and fifth digits are lost in development while the second, third, and fourth persist. Kaupp[8] and Bradley[2] refer to these two phalanges as constituting the second digit.

TABLE 1. BIOMETRIC CONSTANTS OF ABSOLUTE BONE LENGTHS

Bone	No.	Mean. (mm)	Standard Deviation (mm)	Coefficient of Variation (%)
		(a) Females		
Humerus	53	72.377 ± .350	3.778 ± .247	5.219 ± .342
Radius	52	64.154 ± .287	3.065 ± .203	4.777 ± .316
Ulna	52	70.943 ± .334	3.575 ± .236	5.039 ± .333
Carpo-metacarpus	51	39.206 ± .094	1.000 ± .067	2.551 ± .170
Phal. 1 of Dig. III	52	15.019 ± .038	.404 ± .027	2.623 ± .173
Phal. 2 of Dig. III	52	14.221 ± .047	.506 ± .033	3.558 ± .235
Femur	53	82.075 ± .401	4.330 ± .283	5.275 ± .346
Tibio-tarsus	53	116.566 ± .627	6.699 ± .439	5.748 ± .376
Tarso-metatarsus	52	78.615 ± .476	5.088 ± .366	6.472 ± .428
Phal. 1 of Dig. III	51	19.235 ± .063	.667 ± .044	3.415 ± .228
Phal. 2 of Dig. III	51	15.412 ± .048	.507 ± .034	3.289 ± .219
Phal. 3 of Dig. III	52	14.375 ± .046	.492 ± .032	3.422 ± .226
Phal. 4 of Dig. III	50	12.970 ± .046	.488 ± .032	3.762 ± .253
		(b) Males		
Humerus	36	80.639 ± .419	3.720 ± .296	4.613 ± .367
Radius	36	72.505 ± .385	3.427 ± .272	4.723 ± .375
Ulna	36	80.472 ± .421	3.745 ± .286	4.654 ± .369
Carpo-metacarpus	36	44.319 ± .103	.921 ± .073	2.078 ± .165
Phal. 1 of Dig. III	35	16.686 ± .056	.429 ± .030	2.571 ± .207
Phal. 2 of Dig. III	35	16.386 ± .056	.489 ± .035	2.984 ± .240
Femur	36	92.288 ± .496	4.413 ± .350	4.782 ± .380
Tibio-tarsus	36	133.528 ± .709	6.316 ± .502	4.731 ± .376
Tarso-metatarsus	35	91.629 ± .609	5.340 ± .430	5.828 ± .469
Phal. 1 of Dig. III	35	20.900 ± .069	.589 ± .047	2.818 ± .227
Phal. 2 of Dig. III	35	16.843 ± .049	.438 ± .035	2.600 ± .209
Phal. 3 of Dig. III	35	15.829 ± .054	.478 ± .038	3.019 ± .243
Phal. 4 of Dig. III	35	13.857 ± .058	.509 ± .041	3.673 ± .296
		(c) Capons		
Humerus	16	82.700 ± .633	3.753 ± .447	4.538 ± .541
Radius	16	74.012 ± .589	3.495 ± .417	4.587 ± .547
Ulna	16	82.419 ± .638	3.757 ± .448	4.557 ± .543
Carpo-metacarpus	16	45.312 ± .368	2.186 ± .260	4.824 ± .575
Phal. 1 of Dig. III	15	16.913 ± .131	.754 ± .093	4.458 ± .549
Phal. 2 of Dig. III	15	16.127 ± .116	.942 ± .116	5.841 ± .719
Femur	16	95.094 ± .698	4.138 ± .493	4.351 ± .519
Tibio-tarsus	16	138.109 ± 1.143	6.779 ± .808	4.908 ± .585
Tarso-metatarsus	16	95.218 ± 1.106	6.029 ± .719	6.332 ± .755
Phal. 1 of Dig. III	15	21.846 ± .236	1.355 ± .166	6.202 ± .764
Phal. 2 of Dig. III	16	17.425 ± .172	1.021 ± .121	5.859 ± .699
Phal. 3 of Dig. III	16	16.328 ± .196	1.165 ± .139	7.135 ± .851
Phal. 4 of Dig. III	16	14.969 ± .185	1.099 ± .131	7.342 ± .875

the males, females and capons studied are given in Table 1. Variation in the numbers measured in any class merely indicates that some bones were lost, broken or malformed.

(a) Variability

In such material it is to be expected that the absolute variation, as measured by the standard deviation, would be in general proportional to the magnitude of the object measured. For comparison of organs of different size the coefficient of variation must be employed and when this is done the present data afford some interesting exceptions. In males, females and capons the tarso-metatarsus proved to be relatively more variable, as indicated by the coefficient of variation, than any other bone in the appendicular skeleton, though it was exceeded in mean length by the tibia in all three classes and by the femur in males and females.

There is in all three classes distinct evidence that the variability of the phalanges (in both wing and leg) increases progressively toward the distal extremity while the mean lengths of these bones decrease progressively in the same direction. If this increased variability were apparent only in the standard deviation, it might be construed as having arisen from the progressive decrease in the means, but since it is also evident in the coefficients of variation (which are corrected for the magnitudes of the means), a definitely greater variability in the lengths of the distal phalanges is indicated. The same phenomenon is apparent in the measurements and variation constants recently given for the phalanges of all four digits of the fowl by Alpatov and Boschko-Stepanenko.[1] Assuming that the length of any bone is a function both of the rate of growth and of the duration of the growth period, the problem arises of why either or both of these processes should be more variable in the distal than in the proximal phalanges. No solution can be attempted in this paper.

The coefficients of variation for the humerus, ulna, femur and tibia in this material are slightly higher than those given by Schneider and Dunn (loc. cit.) for the same bones from 348 female White Leghorns and 46 males of the same variety. Since the difference is equally as apparent in the males, where the numbers were approximately equal, as in the females, where their

number of individuals was over six times that of the writer, it may be concluded that the greater variability of the writer's material results from the greater heterogeneity of the stock rather than from the comparatively small numbers available.

(b) Sex Dimorphism

In the mean lengths of each bone measured there is a statistically significant sex dimorphism as shown in Table 2. The males are consistently larger than the females and the difference is in no case less than twelve times its probable error.

TABLE 2. COMPARISON OF MALES WITH FEMALES

Bone	Difference in Mean Length in mm. ♂—♀	Range in mm. ♂	Range in mm. ♀	$\frac{(♂—♀)}{♀} \times 100$
Humerus	8.262 ± .553	14.75	15.50	11.41
Radius	8.351 ± .480	13.25	13.75	13.02
Ulna	9.529 ± .537	15.50	14.00	13.43
Carpo-metacarpus	5.113 ± .139	7.50	8.50	13.04
Phal. 1 of Dig. III	1.667 ± .067	3.75	3.50	11.09
Phal. 2 of Dig. III	2.165 ± .072	3.50	4.50	15.22
Femur	10.213 ± .637	18.00	17.25	12.44
Tibio-tarsus	17.062 ± .946	23.25	27.25	14.55
Tarso-metatarsus	13.014 ± .772	21.75	21.00	16.55
Phal. 1 of Dig. III	1.665 ± .093	4.00	6.25	8.66
Phal. 2 of Dig. III	1.431 ± .068	4.00	4.50	9.29
Phal. 3 of Dig. III	1.454 ± .070	4.50	4.50	10.11
Phal. 4 of Dig. III	.887 ± .073	4.50	4.75	6.84

In Table 2 the extent of the range between minimum and maximum bone length in each sex is also given. Comparison of these with the means (Table 1) shows that for every bone the range of absolute bone length in one sex overlaps the limit of that bone in the other sex. The absolute length of any one bone is therefore of no value in diagnosing the sex of doubtful individuals despite the significant differences in mean length. Since the range of any of these bones in one sex may overlap the limit of variation of the same bone in the other sex, it follows that the range of the total length of all these bones in males may overlap the limits of the same figure in females and therefore that total absolute length of any number of bones in the appendicular skeleton will not give a figure confined solely to either sex.

The range for any one bone is approximately the same in each sex. This confirms the suggestion of Schneider and Dunn *(loc. cit.)* that the distinctly smaller ranges found in the bones of their males were due to the smaller numbers of that sex studied.

Considering only the humerus, ulna, femur and tibio-tarsus, the male bones are 13.4 per cent greater on the average than those of the female. For these same bones Schneider and Dunn give a difference of 16 per cent. In the data of Latimer[10] for these four bones from approximately 20 mature individuals of each sex, a difference (computed by the present writer from Latimer's averages) of 15.6 per cent is found. The greater sex difference in the material of these other workers may possibly have resulted from the somewhat greater average size of their birds.

The difference between males and females is most extreme in the tarso-metatarsus. This is of special significance when considered along with the fact that in both sexes this bone displays a greater relative variability than any other of those measured. The observations of Latimer *(loc. cit.)* on the ages at which ossification of various bones is completed explain both of these phenomena. Whereas in the humerus, radius, ulna, femur and tibio-tarsus ossification was found to be completed from 10 to 47 days later in males than in females, ossification in the tarso-metatarsus was not completed in males till 56 days later than in females. Latimer's data suggest that in comparison with other bones of similar size, ossification of the tarso-metatarsus is completed relatively early in females and relatively late in males. Why this phenomenon should occur is an unsolved problem, but it is attested both by Latimer's observations and by the greater variability and sex dimorphism observed in this bone by the present writer.

The greater percentage growth of the male skeleton is less in the toe bones than in the longer bones of the leg and wing.

II. PROPORTIONAL BONE LENGTHS

In order to determine if a significant sex dimorphism existed in the proportional lengths of bones in the appendicular skeleton, for every bird measured there was calculated the ratio of each bone to a more proximal bone in the same limb. The hum-

erus and femur were chosen purely arbitrarily as bases for the indices. In every skeleton the length of each of these bones was taken as 100 and the proportional length of every other bone in the same limb expressed on this basis. The biometric constants for these data are shown in Table 3.

(a) Variability

As is to be expected, the variability in the proportional length (Table 3) of every bone is much less than that in the absolute lengths (Table 1). In general those bones which showed the greatest variability in absolute length also exhibit the greatest variability in proportional length. As the extremity of each limb is approached the ratio of each phalanx to the humerus or femur becomes more variable. In the ratio of the tarso-metatarsus to the femur, a greater variability is evident than in any other index.

The ratios found and the degrees of variability are not in entire accord with the report of Latimer *(loc. cit.)* who states that the ratio of radius to humerus remains constant at 92.5 per cent after 1300 grams of body weight. In the present writer's material this index ranged from 85 to 91 per cent in females and from 87 to 93 per cent in males with a mean of 88.5 for 53 females and of 89.9 for 36 males. A few of the females were under 1300 grams but for these Latimer gives an even higher index. Similarly his statement that "the adult tibio-femoral ratio in the chicken is 147" can hardly apply to all individuals since in the material described in this paper the tibio-femoral index ranged from 137 to 148 with a mean figure of 141.7 for females and of 144 for males. However, Latimer was dealing not only with smaller numbers but also with larger birds of a less heterogeneous stock and on that account would probably find less variability. His measurements were taken to include the maximum length of each bone and should therefore be comparable with those made by the present writer.*

Among the skeletons of other breeds not included in this

* Latimer's statement (page 37) that in females the ulna is on the average 0.05 cm. greater than the humerus is obviously a misprint since his averages are 7.52 cm. for the humerus and 7.47 cm. for the ulna.

TABLE 3. BIOMETRIC CONSTANTS OF PROPORTIONAL BONE LENGTHS

Bone	No.	Mean. (mm)	Standard Deviation (mm)	Coefficient of Variation (%)
(a) Females				
Humerus	53	100.	0	0
Radius	52	88.529 ± .073	.779 ± .051	.879 ± .058
Ulna	52	98.144 ± .056	.599 ± .039	.613 ± .039
Carpo-metacarpus	51	54.171 ± .024	.257 ± .017	.474 ± .031
Phal. 1 of Dig. III	52	20.793 ± .018	.190 ± .012	.914 ± .060
Phal. 2 of Dig. III	51	19.705 ± .024	.259 ± .017	1.314 ± .087
Femur	53	100.	0	0
Tibio-tarsus	53	141.735 ± .109	1.172 ± .076	.827 ± .054
Tarso-metatarsus	52	95.548 ± .128	1.367 ± .090	1.431 ± .094
Phal. 1 of Dig. III	51	23.333 ± .023	.244 ± .016	1.045 ± .069
Phal. 2 of Dig. III	51	18.731 ± .016	.174 ± .011	.929 ± .062
Phal. 3 of Dig. III	52	17.562 ± .017	.187 ± .012	1.065 ± .070
Phal. 4 of Dig. III	50	15.775 ± .019	.201 ± .013	1.274 ± .085
(b) Males				
Humerus	36	100.	0	0
Radius	36	89.972 ± .082	.731 ± .058	.813 ± .065
Ulna	36	100.055 ± .045	.565 ± .045	.564 ± .045
Carpo-metacarpus	36	54.833 ± .026	.232 ± .018	.423 ± .034
Phal. 1 of Dig. III	35	20.757 ± .017	.152 ± .012	.732 ± .059
Phal. 2 of Dig. III	35	20.200 ± .026	.232 ± .019	1.152 ± .093
Femur	36	100.	0	0
Tibio-tarsus	36	144.279 ± .116	1.033 ± .082	.716 ± .057
Tarso-metatarsus	35	99.201 ± .161	1.431 ± .114	1.443 ± .115
Phal. 1 of Dig. III	35	22.707 ± .023	.198 ± .016	.872 ± .073
Phal. 2 of Dig. III	35	18.257 ± .014	.127 ± .010	.696 ± .056
Phal. 3 of Dig. III	35	17.135 ± .024	.214 ± .017	1.248 ± .101
Phal. 4 of Dig. III	35	15.328 ± .025	.218 ± .017	1.422 ± .114
(c) Capons				
Humerus	16	100.	0	0
Radius	16	89.623 ± .158	.936 ± .112	1.044 ± .124
Ulna	16	99.690 ± .102	.606 ± .072	.608 ± .072
Carpo-metacarpus	16	54.314 ± .158	.939 ± .112	1.729 ± .206
Phal. 1 of Dig. III	15	20.524 ± .079	.451 ± .056	2.197 ± .270
Phal. 2 of Dig. III	15	19.566 ± .234	1.342 ± .165	6.859 ± .845
Femur	16	100.	0	0
Tibio-tarsus	16	145.233 ± .338	2.004 ± .241	1.310 ± .156
Tarso-metatarsus	16	100.085 ± .479	2.843 ± .339	2.845 ± .339
Rhal. 1 of Dig. III	15	23.002 ± .120	.690 ± .085	2.999 ± .369
Phal. 2 of Dig. III	16	18.366 ± .112	.667 ± .079	3.632 ± .433
Phal. 3 of Dig. III	16	16.908 ± .115	.686 ± .082	4.057 ± .484
Phal. 4 of Dig. III	16	15.726 ± .133	.788 ± .094	5.011 ± .597

study were those of three dwarf fowls in which, owing to an unfavourable environment, growth had been stunted. In these, the tarso-metatarsus exhibited a greater deviation from its normal ratio to the femur than did any other bone (Table 4).

TABLE 4. RATIO TO FEMUR OF LEG BONES OF THREE DWARF FOWLS

	♀ 35	♂ 68	♂ 69
Tibio-tarsus	139.13	143.57	136.54
Tarso-metatarsus	84.78	88.86	84.62
Phalanx 1 of third digit	22.82	22.43	21.77

In fowls of other breeds the proportional lengths of the bones were found to be very similar to those in Leghorns. Comparison of the data for these three dwarfs with the ratios in Table 3 shows that while the phalanx and the tibio-tarsus exhibit little deviation from the normal ratio, the tarso-metatarsus is much shorter in every case. The inference is that this bone has not only greater potentialities for growth than the others in the appendicular skeleton, but is also distinctly more susceptible to conditions unfavourable for growth.

(b) Sex Dimorphism

A statistically significant sex dimorphism exists in the mean ratio of each wing bone (except Phalanx 1) to the humerus and of each leg bone to the femur. The differences, though not given here, have been calculated and (except in Phalanx 1 of the wing) are in each case more than 13 times their probable errors. In the phalanges of the toe, the proportional length is greater in females than in males. For the other leg bones and those of the wing the ratio is greater in males than in females. The first phalanx in the third digit in the wings presents an exception for which no explanation can be offered.

As in absolute length, the range of proportional length for any bone in the male was found to overlap the limit of that index in the same bone in the female. Such an index can not therefore be used with accuracy in determining the sex of an individual fowl. The same finding applies to several other ratios tried.

Latimer (loc. cit.) observed a sex difference in the weights, plotted against gross body weight, of the humerus, tibio-tarsus and tarso-metatarsus but not in other long bones studied. He also

noted sex-dimorphism in the lengths of the three proximal bones in the wing and leg when plotted against body weight and age. The present writer attempted to establish indices of bone length to body weight but found that these overlapped in the two sexes even more than did the other ratios reported above. Schneider and Dunn[18] and Kopec[9] present data to show that body weight is three to six times as variable as are bone measurements. Latimer also found a sex difference in the weight of bone per unit of length in the humerus and tarso-metatarsus (but not in others) when plotted against body weight.

III. CAPON SKELETONS

The range from minimum to maximum in every bone is less in the capons than in the normal males as is to be expected from the smaller numbers measured. The greater variability of the capons in absolute bone lengths (Table 1) suggests that a disturbance in growth results from castration but larger numbers are desirable before this point can be considered proven.

With the exception of the distal phalanx of the wing the mean absolute lengths for all bones in the capons are greater than the means for the same bones in normal males (Table 5). The 6 larger bones are on the average 2.9% greater in capons than in the males.

Similar results have been observed following castration in rabbits by Poncet[16], in guinea-pigs by Pirsche[15], in rats by Hatai[7] and in steers and ovariotomised cows by Tandler and Keller[22].

In these capons the increased size of the bones compared with those of males is statistically significant in the humerus, tibio-tarsus and three phalanges of the toe. If greater numbers had been available the probable errors of the means would have been considerably less and the differences would probably be significant in most cases, since the majority of them are even now either greater than three times their probable error or almost that magnitude. It is also significant that the increase in size is consistent for all the bones except one phalanx of the wing.

One important principle is evident, viz. that the increased growth of capons is greater in the posterior than in the anterior limb. The same phenomenon has been observed in eunuchs and

TABLE 5. COMPARISON OF CAPONS WITH MALES

Bone	Mean Absolute Length		
	Capon minus ♂ mm.	Difference / P. E.	(Capon—♂) x100 / ♂
Humerus	2.061 ± .759	2.7	2.56
Radius	1.507 ± .703	2.1	2.08
Ulna	1.947 ± .764	2.7	2.42
Carpo-metacarpus	.993 ± .382	2.6	2.24
Phal. 1 of Dig. III	.227 ± .142	1.6	1.36
Phal. 2 of Dig. III	—.259 ± .128	2.0	—1.58
Femur	2.806 ± .856	3.3	3.04
Tibio-tarsus	4.581 ± 1.345	3.4	3.44
Tarso-metatarsus	3.589 ± 1.262	2.8	3.92
Phal. 1 of Dig. III	.946 ± .245	3.9	4.52
Phal. 2 of Dig. III	.582 ± .178	3.3	3.46
Phal. 3 of Dig. III	.499 ± .202	2.4	3.15
Phal. 4 of Dig. III	1.112 ± .193	5.7	8.03

eunuchoid individuals by Tandler and Grosz[21], Launois and Roy[11] and in rabbits by Poncet *(loc. cit.)*. Launois and Roy cite the investigations of several other workers also reporting the same phenomenon in eunuchs and cases of gigantism associated with infantilism. The writer has been unable to find any other measurements of capon skeletons but the principle is amply demonstrated by the indices in Table 6, taken from the material described in this paper.

It is evident that in capons not only is the femur greater in proportion to the humerus but the tarso-metatarsus is greater in proportion to the femur and the whole posterior limb is greater in proportion to the anterior limb than in males. Comparison of the data on capons and males in Table 3 reveals that, whereas in the anterior limb the proportions of all bones to the humerus are less in capons than in males, in the posterior limb the ratios of all bones to the femur are greater than in males except in one phalanx. It is evident, however, that in all three of the indices used there is a marked difference between females and normal males. As stated previously this difference results largely from growth being protracted in males after it has ceased in females. In the opinion of the writer the still greater differences in these indices for the capons result from a protraction of growth in that class after it has ceased in the males. The increase in these

TABLE 6. INDICES SHOWING THE ELONGATION OF THE POSTERIOR LIMB IN CAPONS

	No.	Femur x 100 / Humerus	7 leg bones x 100 / 6 wing bones	Tarso-metatarsus x 100 / Femur
Female	52	113.4	122.9	95.5
Males	35	114.4	123.7	99.2
Capons	16	114.9	125.7	100.0

indices and the elongation of the posterior limb result, therefore, from a continuation of a differentiating process which is already operative, rather than from any special heterogony to be included among the sequelae of gonadectomy.

The slight bilateral asymmetry observed in eunuchs by Tandler and Grosz (loc. cit.) was not apparent in the capons.

Mitchell, Card and Hamilton[14] found that before 18 weeks and after 32 weeks there was little difference between the weights of cockerels and capons in White Plymouth Rocks but their records show the capons to have exceeded the cockerels in weight from the 18th to the 32nd week. Waite[24], also using White Rocks, found that up to 24 weeks of age there was little difference but that from 24 to 42 weeks the capons exceeded the cockerels. Titus and Jull[23] computed from the equation of a curve of diminishing increment that in Rhode Island Reds, cockerels and pullets mature at 21 to 23 months and capons at 17.4 months.

While there is a great difference between the rates of growth of Leghorns and the heavier breeds, it seems logical to assume that if in the latter the capons do not grow faster than normal cockerels up to 5 or 6 months of age, neither will they do so in Leghorns up to a corresponding period of 4½ to 6 months. Nevertheless the capon skeletons are larger than those of the males and one is therefore led to the conclusion that skeletal growth is protracted in capons after it has ceased in normal males. This is in accord with the findings of Mitchell, Card and Hamilton and of Waite, although it is impossible to say how much of the greater gains made by their capons after six months represent growth of skeleton and how much is accumulation of fat. Such a conclusion is not necessarily a contradiction to the computation of Titus and Jull that capons mature sooner than normal males

since the question of what constitutes maturity is open to different interpretations and theirs can obviously not be based on skeletal growth. It is particularly difficult to decide what constitutes maturity in a capon, or if a capon may be said to mature at all in the same sense as a normal male, since its condition corresponds to one of indefinitely protracted sexual infantilism. The data of Schneider and Dunn[18] show that in Leghorns maximum growth of the skeleton is attained before eight months.

DISCUSSION

The data in Table 2 shed some light on the case of the gynandromorph fowl described by Macklin[13] and her bird in turn gives a clue to the principles underlying sex dimorphism in general. Macklin's fowl (judging from photographs of the head) was a Barred Plymouth Rock. Nevertheless it is evident (Table 7) that the difference in skeletal measurements between left and

TABLE 7. COMPARISON OF NORMAL SEX DIMORPHISM WITH THE BILATERAL ASYMMETRY OF MACKLIN'S GYNANDROMORPH

	Hutt	Macklin
	Ratio $\frac{♀}{♂}$	Ratio $\frac{\text{Left}}{\text{Right}}$
Humerus	.89	.86
Radius	.88	.87
Ulna	.88	.85
Femur	.88	.86
Tibio-tarsus	.87	.82

right sides of this bird was almost identical with that between females and males in the present writer's material.

In Macklin's gynandromorph both testis and ovotestis were found at autopsy. Whether or not these had both been present during the growth period is not known but as Macklin says "the secretions of any endocrine glands concerned with growth were equally available to the two sides, so that the explanation must rest upon the different genetic constitutions of the two halves." Presumably sex dimorphism in normal fowls is also due to the genetic constitution of the soma rather than to the endocrine secretion of the gonad. This does not mean that endocrine secretions play no role whatever in growth. The data on gonadecto-

mized animals show a distinctly abnormal growth in that class resulting apparently from a disturbed inter-endocrine balance affecting the pituitary. It is evident, however, that the ultimate growth of any individual depends upon the ability of the soma to respond to all the factors stimulating growth, and that presumably this ability is determined by its genetic constitution.

Contrary to the opinion of Castle[3] [4], but in accord with those of Davenport[6], Wright[25], Sumner[19] [20] and Kopec[9] the writer is led to the conclusion that while there are genetic size factors affecting all parts of the body, there must also be special factors affecting only certain regions and certain individual bones. This is attested by the extreme variability and sex dimorphism in the tarso-metatarsus, by the fact that the greater size of bones in males is not uniform for all bones and by the elongation of the posterior limb to a greater extent than the wing when growth is protracted in males and capons.

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SUMMARY

The appendicular skeletons of 53 females, 36 males and 16 capons were measured and subjected to biometrical study.

A statistically significant sex-dimorphism was found in the mean absolute length of all bones measured. With the exception of one phalanx in the wing, a statistically significant sex dimorphism was also found in the mean ratio of every wing bone to the humerus and of every leg bone to the femur. In the phalanges of the toe the length proportional to the femur is greater in females than in males. In all other bones the ratio to the humerus or femur is greater in males than in females.

The longer bones in males fowls were 13.4 per cent greater than in females. The increase in the males was not constant for all bones but varied from 6.8 to 16.5 per cent.

The tarso-metatarsus exhibited a greater variability both in absolute length and in proportional length than any other bone. It also represented the extreme sex dimorphism being 16.5 per

cent greater in males than in females. In dwarfed fowls the growth of the tarso-metatarsus was found to be retarded more than that of other bones. It is concluded that this bone differs from others in its potentialities for growth and its susceptibility to environmental conditions.

Variability in the absolute and proportional lengths of the phalanges was found to increase progressively toward the distal extremity of either limb.

Capons were found to be slightly larger in all bones (except one) than males. In the six longer bones the average difference was 2.9 per cent. It was shown that in capons the increase in size is greatest in the posterior limb but reasons are given for the opinion that the elongation of that limb results from the continuation of a process already operative in normal males and not from a heterogonic growth induced by gonadectomy.

It is concluded that sex dimorphism results from a differential response of the soma, caused by its genetic constitution, to the different factors promoting growth.

No index was found which would permit diagnosis of sex in doubtful individuals from a study of the appendicular skeleton.

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EIGHT NEW MUTATIONS IN THE DOMESTIC FOWL

F. B. Hutt
University of Minnesota, St. Paul, Minnesota

Reprinted from the
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EIGHT NEW MUTATIONS IN THE DOMESTIC FOWL[1]

F. B. Hutt, University of Minnesota, St. Paul, Minnesota

Eight hitherto unreported genetic characters in the fowl are being investigated. These are: (1). Congenital palsy. Affected chicks exhibit various degrees of palsy at hatching. In some cases this is so extreme that the bird is unable to stand, while, in others, the chick appears normal except for a tremor which is perceptible as long as the bird stands. Affected chicks are free from palsy when squatting and adopt that position more than do normal chicks. Extremely palsied birds die within a week of hatching. Others have been kept alive for nearly three months, but none have attained sexual maturity. At two months, palsied chicks are only one-third the size of normal sibs of the same age. The character is apparently a simple autosomal recessive, but there is a deficiency of palsied chicks, apparently due to prenatal mortality. It was found in White Leghorns. (2). Supraorbital featherless areas. Small irregular areas on the top of the head are entirely devoid of down at hatching and of feathers at all subsequent stages. The defect may be bilateral, with affected areas separated by a strip of normal down, or may be unilateral, in which case the featherless spot occurs more commonly on the right side. Occasionally it occurs in the center of the top of the head. The character is evident in embryos at eleven days and is therefore a useful one for genetic investigations. Affected birds appear to be otherwise normal. This character has been found in three unrelated strains of White Leghorns, and is apparently a simple recessive. (3). Double oil gland. There are two uropygial glands instead of one. In most cases the two parts are quite distinct and may extend from each other at right angles, but in some cases only the tip of the gland is bifurcated. Breeding results to date indicate that a single dominant gene is responsible, but the fact that heterozygotes mated to unrelated fowls with single glands yield an excess of singles suggests that there may be modifying factors involved. The line has yielded some birds entirely devoid of the gland at maturity. There is evidence that these are homozygous for the character and that at hatching such homozygotes have two vestigial and atrophic oil glands. Since the character is dominant and recognizable in the 14-day embryo it is valuable for linkage studies. (4). Frizzling modifier. A single recessive gene when homozygous almost completely suppresses the frizzling character (dominant) in fowls heterozygous for frizzling. Fowls homozygous for frizzling and for the modifier exhibit a less extreme type of frizzling than is common to unmodi-

[1] Paper No. 1107 of the Journal Series of the MINNESOTA AGRICULTURE EXPERIMENT STATION.

fied homozygotes. Contrary to the usual condition with modifying factors, there is a sharp distinction between the four types: *Ff* unmodified, *Ff* modified, *FF* unmodified, *FF* modified. (5). Abnormal osteogenesis. Two of nine fowls from one dam exhibited extreme thickening of the tarso-metatarsi. Both died before breeding age. Other bones of the skeleton were affected, particularly the carpometacarpus. The character is apparently genetic and recessive. (6). Twisted beak. Eight fowls with twisted upper beaks appeared in the progeny of three hens mated to a common sire. The character is sublethal, since affected birds can only survive when hopper-fed and when the beak is regularly trimmed. From ratios to date it appears to be a simple recessive. (7). Club foot. The genetic basis for varying degrees of club foot in fowls is being investigated. As in man, the character is extremely variable both in its expression and in its genetic behaviour. (8). Shrivelled wattles. In a strain of White Leghorns there was found a hereditary reduction of one or both wattles. The character appears to be sex-limited (not sex-linked) and has so far been found only in males. It is of interest because the related species *G. varius* has only a single wattle, and apparently of value because affected wattles are not frozen when those of normal fowls are.

B. MAMMALIAN GENETICS

Bovine Quadruplets

F. B. HUTT

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CORRIGENDA

p. 342, column 2, paragraph 3 should read:

A similar secondary break in the pleural patch is evident on the left side of B and on the right side of A . . . . . pigment which has slipped down into the secondary break of B (left side) whereas this break is unspotted in A (right side).

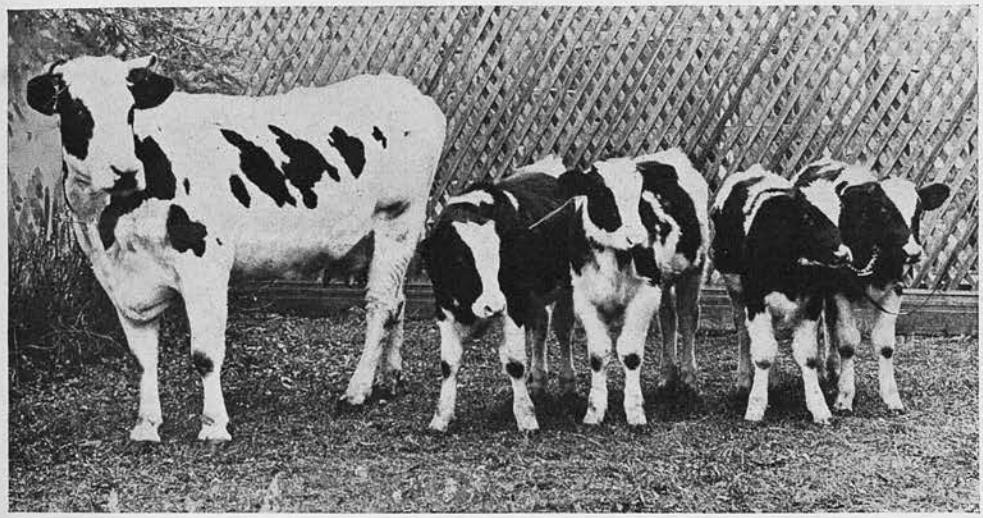
p. 343, Fig. 3. The author is not responsible for the black switch and other retouching done by the engraver in the lower photograph.

BOVINE QUADRUPLETS

Including Twins Apparently Monozygotic

F. B. HUTT

Division of Poultry Husbandry, University of Minnesota



AAGIE SEGIS CALAMITY AND HER FOUR CALVES

Figure 1

From left to right—dam, bull, free-martin, heifer, heifer, the latter two being the pair considered monozygotic. If the twins are actually identical two very unusual phenomena are thus represented, for quadruplet calves occur only with extreme rarity, (probably not more often than two sets in a million births), and identical twins in cattle are so uncommon that some biologists doubt their existence.

SOME time ago there came to the attention of the writer a case of multiple births in cattle which presents some features of particular interest to biologists. It has not been possible to secure as much information as could be desired about this case, but further study has been rendered impossible by the slaughtering of the animals concerned. The data available are therefore presented herewith.

On July 2, 1925, Aaggie Segis Calamity, a Holstein-Friesian cow belonging to Mr. W. E. Lawson, of Hayfield, Manitoba, Canada, and registered in the herd books of The Holstein-Friesian Association of Canada as No. 83496, gave birth to four living calves (Figure 1). These were sired by the bull, Hector Colanthus Fayne, No. 56351.

The dam was born on Sept. 24th, 1918, and was thus almost seven years old when her quadruplets were dropped. Prior to her greatest achievement this cow had had two bull calves at single births and had produced 19,215 lbs. of milk, with an average fat content of 3.47 per cent, in her second lactation period. At her fourth calving (*i. e.* the one following

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the birth of quadruplets) Aaggie Segis Calamity dropped twins, a bull and a heifer, the latter of which died soon after.

Quadruplets

Special interest is centred, not in the dam, but in the quadruplets. They have naturally received a considerable amount of newspaper publicity and have also been illustrated in several journals.[2, 3, 21]. They made an interesting side-show exhibit for an amusement company operating at the Manitoba Provincial Fair in July, 1926, and three of them were also entered for competition in the regular classes where two of them (heifers) were placed third and fourth and another (the bull) won fifth prize in their respective sections.

Births of three or more at a time are unusual in uniparous species, but, when they do occur, it is still more unusual for all of such offspring to be alive and healthy as were these four. De Bruin[5] reviews several cases of multiple bovine pregnancies reported in veterinary literature, including two cases of quadruplets, but does not state how many of these calves lived to maturity. In one instance, quintuplets lived to eight days. In another sextuplets were prematurely born following an injury. This author and Pearl[19] both quote from McGillivary's *Manual of Veterinary Science and Practice* (1857) the case of a cow which produced 25 calves in eight years, including two sets of quadruplets (not those referred to above) and one set of sextuplets. All of the latter died prematurely. Concerning one set of quadruplets no record of death or survival was given but in the other case three of the four calves lived. Other instances of bovine quadruplets are cited by Bissonnette.[4]

The number of possible ways in which quadruplets might arise is rather large to permit of any accurate calculation of the theoretical frequency with which such an event might be expected to occur. This is especially so in the case of the domestic ox, for in this species there has been some doubt whether monozygotic twinning ever occurs or not, so that the extent to which it might influence the frequency of quadruplets can not be computed.

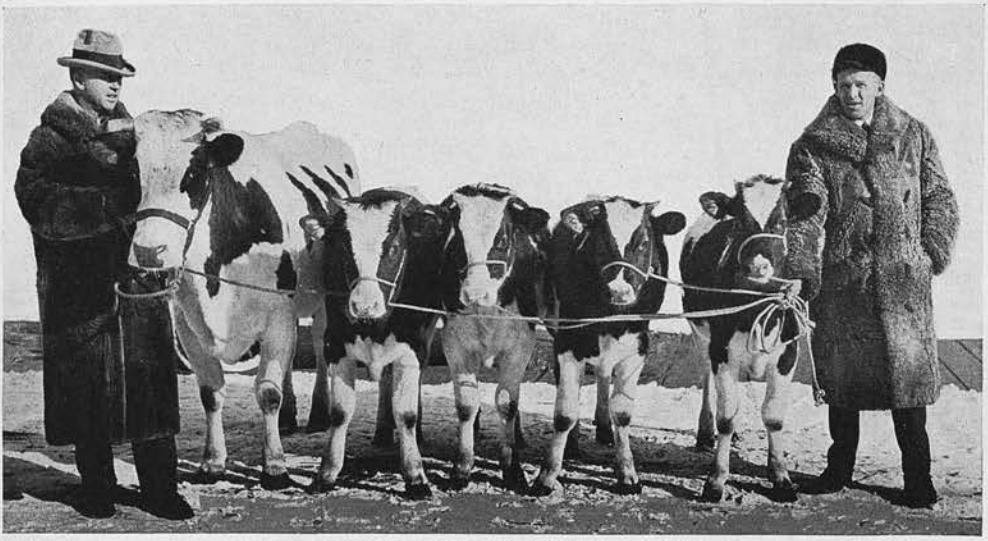
Jones and Rouse[10] found in a study of Hereford and Aberdeen Angus herd books only one case of quadruplets among 747,100 births. This number of births is not enough to establish the frequency of bovine quadruplets, especially since these records do not include still-born calves, but the ratio is not out of line with those given for man, also an uniparous species, by various investigators, as recently summarized by Jenkins[9] and Greulich:[7]

	Number of births	*Quadruplets birth ratio*
Veit. Prussia, 1826-1849	13 million	1 : 371,694
Neefe, 1877	50 million	1 : 551,368
Prinzing, Germany, 1871-80	63 million	1 : 778,688
Arey		1 : 750,000
Guzzoni		1 : 757,000
Greulich	120 million	1 : 670,734

Monozygotic Twins

The writer is of the opinion that the case reported in this paper persents a phenomenon even rarer than the birth of quadruplets, inasmuch as there are good reasons for believing that this particular set resulted from coincident dizygotic and monozygotic twinning.

The quadruplets included one bull and three heifers. One of the latter was found upon examination by Dr. Harry Ross, of Brandon, the attendant veterinarian, to be a free-martin, and although attempts were subsequently made to get her bred, these were unsuccessful. When the four calves were seen by the writer in July, 1926, it was at once evident that the two other heifers, both apparently normal, presented every indication of being identical twins.



THE QUADRUPLETS AT 7½ MONTHS

Figure 2

From left to right—dam, bull, free-martin, heifer, heifer. Quadruplet calves rarely survive the first few days of life, and to have all of them reach maturity is most unusual.

Reversed Asymmetry

This was attested in part by the striking uniformity of size and conformation which they exhibited, but particularly by the distinct reversed asymmetry, or "mirror-imaging," displayed in their colour patterns.

This phenomenon is most apparent in conjoined monsters where it is frequently found as *situs inversus viscerum,* a condition wherein one individual (*i. e.* one half of the monster) has such unpaired organs as the heart, aortic arch and stomach in the normal position on one side of the body, while in the other half of the monster those same organs are found on the opposite side of the body. This condition is apparently no commoner in separate human twins than in persons born at single births, but Wilder[23] has shown that reversed asymmetry is sometimes found in the palm or finger patterns of monozygotic twins. In such cases the pattern of the left hand, or of the left index-finger of one twin, resembles the right hand or right index-finger pattern of the other and vice versa. Other examples of mirror-imaging in human twins, including non-genetic left handedness and counter-clockwise hair-whorl, are discussed by Verschuer[22] and Newman.[17, 18] The latter writer found reversed asymmetry of double bands and other characters so frequent in the armadillo, *Dasypus novemcinctus,* that he concluded that "the occurrence of symmetry reversal or mirror-imaging in twins or double monsters may safely be taken as a criterion of their monozygotic origin."

Presumably this condition arises from the early separation of primordia which were originally destined to give rise to the right and left sides of the body. Most vertebrates are in general bilaterally symmetrical but exhibit asymmetry in certain unpaired organs and in such small details as the variations in colour pattern, and (in man) right or left-handedness, or the direction of hair-whorl. When one zygote gives rise to two individuals, this asymmetry is not always retained in each animal and in some instances the phenomenon of reversed asymmetry is brought about. A con-

siderable body of evidence brought together by Newman[18] indicates that whether non-conjoined twins appear identical or exhibit asymmetry reversal depends, respectively, upon whether twinning takes place before the symmetry and asymmetry of the embryo are established or during that process.

The writer is not aware of any published account of mirror-imaging of any character in separate bovine twins. Such a condition is more likely to be noticed in those breeds in which white spotting is a characteristic than in self-coloured animals.

Kröning[11] in his study of the colour patterns of double monsters in cattle, swine, cats and goats, illustrates two cases of conjoined calves which exhibit marked reversed asymmetry. In one of these (Case 10.) the white blaze on one forehead lies much to one side and is of an unusual pattern. This is replicated almost identically on the other head but as a mirror image. Similarly his Case 23, in which the two calves are joined only at the thighs, illustrates the same phenomenon but with a considerable degree of variation in small details.

Williams[24] illustrates three specimens of conjoined monsters in the museum of the New York State Veterinary College concerning which he says: "As far as the fission extends, the markings are inversely identical. The proximal sides of the divided parts are marked inversely alike, and the distal sides follow the same rule. Had the fission been completed and the plan of marking persisted, the color markings, when the calves were standing side by side or head to tail would have been unlike, but when standing head to head or tail to tail, would have been inversely identical. That is, a photographic negative of either side of one calf, turned end for end, would be the negative of the opposite side of the other calf."

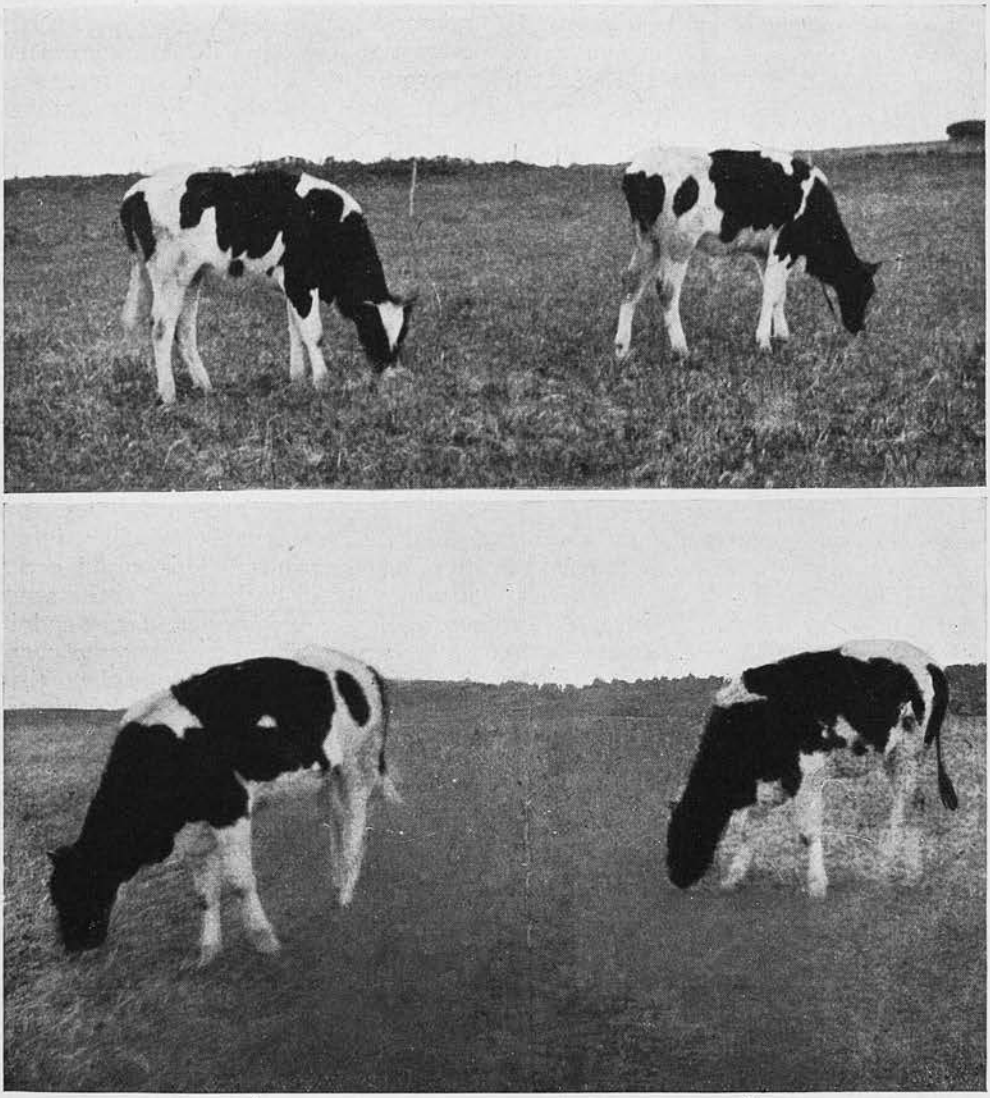
Mirror-imaging is certainly not found in all conjoined monsters nor is it evident in more than a small percentage of monozygotic twins. Where present, however, it is, as Newman points out, a reliable indication of their common origin. This is the condition found in the two heifers of the quadruplets.

Photographs taken by the writer when the calves were a few days more than a year old illustrate a fairly clear case of mirror-imaging of their black and white markings. (Figures 2 and 3).

One is first struck with the similarity between the large black spot in the right lumbar region of *B* and that in the corresponding location on the left side of *A*. Both are in the same region, of approximately the same size and shape and both point in the same direction. This would not be significant if this particular region were one of the six centres from which, as Allen[1] has pointed out, pigment formation tends to spread in mammals and birds. This, however, is not the case. The large spot on the flank comes as a result of a "secondary break" in the large pleural (side) patch of black pigment. Allen's studies indicate that in cattle two secondary breaks tend to cut the pleural pigment patch into three regions, one just behind the shoulder, a second over the last ribs and the third in the lumbar region. In this case the lumbar area has been entirely cut off and appears as a separate patch.

A similar secondary break in the pleural patch is evident on the left side of *A* and on the right side of *B* but in neither case has it progressed as far as on the opposite sides of these animals. It is of almost the same extent in both cases but this is partially obscured by a small spot of black pigment which has slipped down into the secondary break of *B* (right side) whereas this break is unspotted in *A* (left side).

Marked similarity is also evident between the lower margins of the side



A B

MIRROR IMAGE PATTERNS

Figure 3

The two heifers, *A* and *B*, considered monozygotic, from the right side, (above), and from the left side, (below). Note that the right side of *A* resembles the left side of *B*, and vice versa. This mirror image relation of the patterns is strong evidence that the heifers are identical twins.

patch on the right of *A* and that on the left of *B*. Both tend to run to a point and both have a small spot of black isolated from the main pigmented area at about the same point. The resemblance of these two sides arises from the "primary break" between side and shoulder patches being of approximately the same extent in both cases although it is less so on the dorsal margin than on the ventral one. On these same sides the shoulder patch extends down on the leg to the same extent in both cases although more anteriorly in *B* than in *A*.

The pleural patch on the right of *B* terminates ventrally in a spot well down on the flank. This is copied on the left flank of *A*.

The pigmented areas of the shoulder and neck centres overlap to some extent in these two animals so that a sharp division between them is not evident. However, from one or both of these patches there is produced an extension on the right fore-leg of *B* which closely resembles in shape and location the corresponding extension on the left fore-leg of *A*—more so than either of these patches resembles that on its own opposite side. At the dorsal margin a narrow primary break is evident between the neck and shoulder patches on the right side of *B*. The same break is quite evident at the ventral margin in the left of *A*, but, though present, it does not stand out at the dorsal surface as in *B* because the shoulder patch,—already small in *B*—has disappeared there in *A*.

The case for mirror-imaging may be summed up by saying that the right side of *A* resembles the left side of *B* more closely than it does *A's* own left side and, conversely, the left of *A* is more like the right of *B* than like *A's* own right.

There are small differences, it is true. However, white spotting is one of the most variable of characters and apparently one that is much influenced by irregularities in development. Kröning's investigation (*loc. cit.*) shows conclusively that there may be great differences between the colour patterns of individual members of conjoined monsters which, *ipso facto*, must be genotypically identical. This same fact has also been demonstrated in another way by Wright[25] who, working with guinea pigs, found that even after 20 generations of close inbreeding, when a family had been reduced almost to a pure line, the amounts of white spotting exhibited by litter mates might range anywhere from 20 to 90 per cent. The close mirror-imaging of the right and left sides of the two heifers described above is therefore all the more remarkable because of the natural variability of the white spotting character.

Wright calculated that in members of such highly inbred families the variations in pattern were determined about 3 per cent by heredity, 5 per cent by tangible environmental factors and 92 per cent by irregularities in development. One cannot argue that the same conditions apply to ungulates as to rodents, but even if they do so only in part, the close resemblance of the sides compared above indicates that both animals were exposed to remarkably similar intrauterine conditions. This could occur most easily if both were in the same horn of the bicornuate uterus and if both were enclosed in the same chorion. Evidence is given below that these two conditions, which, of course, would be expected in monozygotic twins, were present.

It should be pointed out also that the extent of the black colour in each of the primary pigment areas,—aural, nuchal, scapular, pleural and sacral (Allen[1])—is essentially the same in both calves. The bull and the freemartin differed greatly in this respect and similar differences are apparent in the several pairs of Holstein twins illustrated in previous numbers of this journal.[8, 16]

It is significant also that the triangular white blaze and the amount of white on the muzzle are practically identical in the two heifers. (Figure 2). In the heads of ten bovine monsters illustrated by Kröning[11] there is a wide range of markings such as is seen in any Holstein-Friesian herd, but between the two heads on any one monster there is in most cases a remarkably close resemblance such as is shown by the two animals herein compared. The bull and freemartin of these quadruplets appear at first

glance to be alike in this respect but the latter has much more white on the sides of the face.

Breeding Behaviour

Unfortunately the writer was unable at the time these calves were first seen to take measurements or to make a more detailed examination of their resemblance. On his return to Brandon after being away from Manitoba over a year it was found that one of the heifers had turned out to be a "shy breeder" and had therefore been slaughtered. Further comparisons were thus made impossible but through the courtesy of Mr. W. E. Lawson, Sheriff M. McGregor and Dr. Harry Ross, considerable additional data were obtained through correspondence and on the occasion of a visit to Brandon in the summer of 1929.

The two heifers came in heat at 10 months and 12 days but neither of them ever became pregnant. The butcher who dressed the carcass of one of the heifers informed the writer that its reproductive organs were not normal and were in some respects like those of the free-martin of the quadruplets, which he had also dressed. It is unfortunate that a detailed and accurate description of the anatomy of the reproductive system could not have been obtained. The other heifer changed owners twice but Dr. Ross has reported that it had never been in calf. One cannot say definitely that these two heifers were free-martins and that they had been made so through the usual medium of the testicular hormone from the bull calf but this is the most probable explanation.

The bull has sired a few calves. This indicates that the mechanism of hormone action whereby free-martins are produced is not a reversible reaction which can be upset by altering the *amounts* of the male and female hormones. In other words one bull calf produced enough testicular hormone to affect seriously the reproductive systems of the three females, but although there were apparently vascular connections between the bull and all three heifers, the combined endocrine secretions of the latter were unable to affect the reproductive system of the bull. This is in accord with the conclusions of Lillie[13] and of Bissonnette[4] that the modification of the female by the hormones of the male is an all-or-none reaction, dependent for the intensity of its action upon the time of onset of the testicular hormone rather than upon any quantitative differences in the two conflicting forces.

Position of the Calves *in Utero*

Dr. Ross, who removed the placentae, writes that "the foetal envelopes were three." This was to be expected if two of the four calves were monozygotic, but it cannot be taken as proof positive because of the tendency, observed by Lillie,[12] for chorions to fuse in cases of dizygotic twinning. However, the fact that there were only three sets of foetal envelopes is especially significant when considered along with the birth order, which was—bull, heifer, heifer, free-martin.

It is obvious that the bull, born first, must have been at the base of one of the two horns of the uterus, and that the free-martin, born last, must have been nearer the tip of one of the horns. It was to be expected that the two heifers would be born consecutively if they were monozygotic, since both would be enclosed in the same chorion (even possibly in the same amnion) and its rupture would facilitate the birth of the second twin right after that of the first. On the other hand it must be pointed out that had there been four chorions instead of three, even if the two were dizygotic twins, their consecutive birth could be expected in twelve cases out of the twenty-four possible

ways in which the births of the four calves might then have been arranged. However, there were only three chorions and it seems most probable that whatever two of the four animals were in one common chorion would be born consecutively. On this basis, and considering the birth order, the most logical interpretation is that the bull and the free-martin were in separate chorions in one horn of the uterus while the two heifers were in the other horn.

If this were not the case, there must have been one of the heifers in the same horn as the bull which would mean that the other heifer must have been between the bull and the free-martin since the latter could only have been near the tip of one horn. In such a condition it is impossible to see how the free-martin, being furthest away, could be so much more affected than the heifer between it and the bull, since the opportunity would obviously be greater for vascular anastomosis between the chorions of the bull and the adjacent heifer than between those of the bull and the more remote free-martin. This alternative is therefore untenable and the original premise that the two heifers were in one horn and presumably in a common chorion seems the more logical interpretation. As pointed out previously, such a condition would be expected if the two were monozygotic and would almost be necessary in addition to genotypic identity to bring about the close resemblance in colour pattern of the sides to which, by the separation of the original primordia of right and left sides of one animal, there had been conveyed the genetic potentialities for identical patterns.

The fact that the free-martin was obviously more affected by the testicular hormone than were the two heifers also suggests that it and the bull were in one horn since such an arrangement would facilitate an earlier anastomosis of chorionic blood vessels, which, in turn, would permit the maximum effect of the hormone from the bull. Conversely, one would expect less effect (as was evident) upon the two calves in the opposite horn because of the opportunity for vascular anastomosis between their chorion and that of the bull being delayed longer than if both chorions were in the same horn.

The greater degree of abnormality exhibited by the free-martin compared with that of the two heifers, and the fact that both of the latter were apparently affected to the same extent (since both came in heat at the same time, appeared normal and were not considered free-martins till found to be shy breeders) are quite easily explained by the evidence from Lillie's work that the modification of the free-martin is an "all or none" reaction, varying in degree according to the time of onset of the testicular hormone, and by the two heifers being in a common chorion in the same horn of the uterus. There is still the possibility that this common chorion may have arisen from the fusion of two separate membranes, but in view of the other evidence for the two animals being monozygotic, this seems less likely.

A necessary corollary to the arrangement of the four calves *in utero* suggested above is that, while there must have been a fusion of the chorions of the bull and the free-martin sufficient to permit vascular anastomosis, such a fusion should not have been extensive enough to prevent them being recognized upon removal as two distinct chorions, comprising two of the three sets of foetal envelopes mentioned by Dr. Ross. A survey of the literature reveals nothing to indicate that a bull and a free-martin must necessarily be enclosed in a single chorion.

Weights

The only weights available were taken when the calves were 13 days of age. They were recorded as follows:

Bull	72½ lbs.
Heifer	63 lbs.
Heifer	60½ lbs.
Heifer	59½ lbs.

Dr. Ross writes that the free-martin was the heaviest of the three heifers. This would mean that the two considered monozygotic weighed within one pound of each other at 13 days. Such a close agreement at that age is even more significant than if it had been observed in the weights at birth.

Discussion

The rarity of monozygotic twins in cattle has been discussed by Lillie,[12, 13] Gowen[6] and other writers, some of whom have expressed doubt whether such twins ever occur. While it is true that a case of monozygotic bovine twins has not yet been demonstrated to the satisfaction of all biologists; nevertheless it has not yet been shown that such an event may not occur.

The best embryological evidence for monozygotic twinning is the finding of only one corpus luteum in both ovaries of a cow carrying twin embryos. Out of 126 cases of twin bovine embryos, where both ovaries were examined by Keller and Tandler (cited from Lillie), or by Lillie,[13] one instance was found by the latter in which only one corpus luteum was present. This must have been a case of monozygotic twinning unless the Graafian follicle had liberated two ova.

Pearl[19] has suggested that two of the triplet calves described by him might be monozygotic. Lush[14] has described and pictured twin bull zebus (*Bos indicus*) which were so alike in conformation and markings that they might be considered identical and twin Jerseys[15] which from the standpoints of colour, conformation, breeding behaviour and milk yield seemed almost certainly identical. It seems possible that although monozygotic bovine twins are quite rare, other cases could be brought to light and definitely established if veterinarians, animal husbandmen and others who have frequent opportunity to observe twin calves would attempt to get evidence from every angle bearing on the case. Such studies would be facilitated if a means could be found to distinguish between a chorion originally single and one arising from the fusion of two separate chorions. Reichle[20] has recently pointed out that in man such a distinction is possible by examination of the septum to see if chorionic tissue be present between the two amniotic layers. Whether or not the same distinction can be made in bovine birth membranes has not yet been proved.

It is probable that cases of monozygotic twins will be more easily recognized in breeds exhibiting white spotting such as the Holstein-Friesian, Guernsey and Ayrshire, than in self-coloured breeds. It should be pointed out, however, that from analogy with twins in man, reversed asymmetry, or mirror imaging, such as has been described in this paper, is to be expected less frequently than cases where the right side of one twin resembles the right side of the other, with similar correspondence being shown in the two left sides.

Apart from the biological interest attached to bovine monozygotic twins, such animals would be particularly valuable for investigations in nutrition, physiology and husbandry because of their being genotypically identical. A feeding experiment repeated upon each animal and using first one twin and then the other as a control would probably yield more conclusive results than if a dozen animals of greater genetic heterogeneity and of different ages were used for the same test.

Summary

A case of living bovine quadruplets is reported in which there were one bull, one free-martin and two heifers which, being sterile, were probably also free-martins affected to a lesser degree than their "litter" sister. All four animals lived to breeding age. The bull produced a few calves.

The two heifers least affected exhibited reversed asymmetry or mirror-imaging in their black and white colour patterns. They were practically identical in facial markings. Their weights at thirteen days varied only half a pound from the average of the two. From the birth order and foetal membranes of the quadruplets it is most probable that the two heifers were enclosed in a common chorion in one horn of the uterus.

In view of all these facts, it is considered that the two heifers constitute an authentic case of so-called "identical" or monozygotic twinning in cattle.

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Congenital Taillessness in the Rat

F. B. Hutt
and
O. Mydland

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CONGENITAL TAILLESSNESS IN THE RAT*

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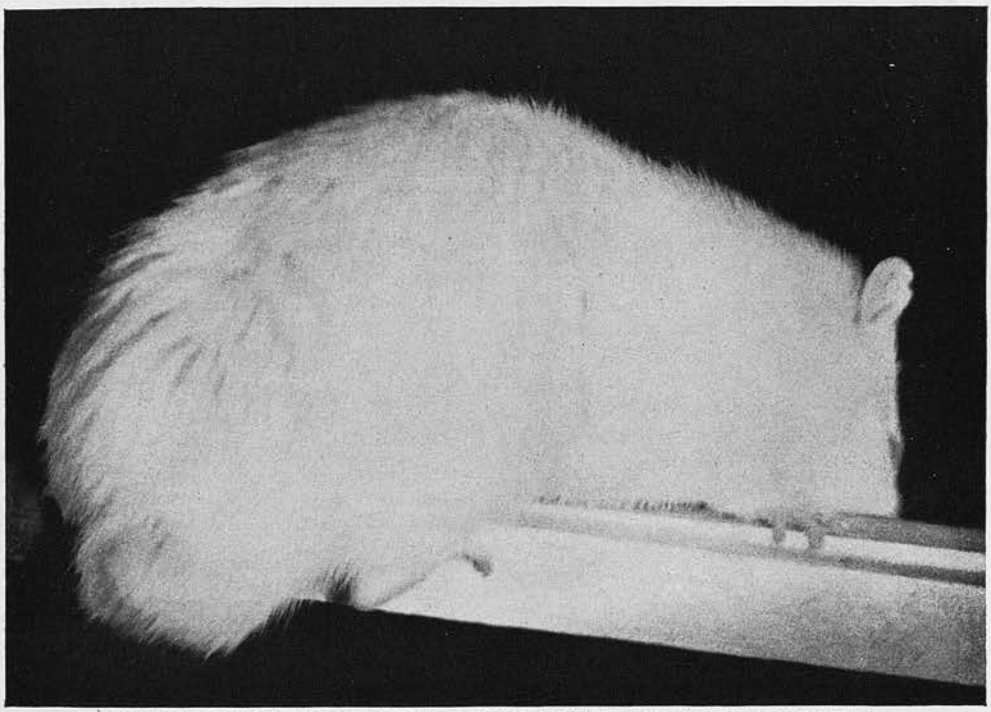

TAILLESS IN APPEARANCE BUT GENOTYPICALLY TAILED

Figure 13

The 229 descendants of this rat all had conventional tails. This fairly conclusively proves that the loss of this rat's caudal appendage was due to accidents of development rather than to any change in the animal's genetic constitution.

IN January, 1931, through the kindness of Mr. Ivan G. Grettum, the writers of this note secured a tailless male albino rat. (*Rattus norvegicus* Erxleben.) This animal had appeared in the rat colony of the Champion Animal Food Company of Minneapolis.

Neither at birth nor when full grown (Figure 13) did the rat have the slightest external evidence of any tail. By the absence of this appendage the animal's scrotum was made much more conspicuous than in tailed rats.

Tailless albino rats were described by Conrow,[1,2] but up to the time when this specimen was secured no breeding trials had been reported, so that it was impossible to say whether this condition arose from genetic causes or from some accident during development. The latter possibility seemed the more likely in view of the rarity of the tailless condition at the Wistar Institute as reported by Miss Conrow. On the other hand, Lang[12] has reported the investigations of Nägeli which made it evident that in the closely related house mouse, *Mus musculus*, the tailless or short-tailed condition is hereditary. In the house mouse this abnormality is apparently due to a

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author, and there are occasional references to the same condition in horses, sheep and swine.

If animals which should have tails can be deprived of them through accidents during development, there is compensatory justice in the occasional persistence of embryonic tails at birth among animals in which tails are decidedly superfluous. The list of such occurrences in man numbers over eighty-five. Their distribution reveals that the event may even occur in Tennessee and shortly after the Scopes trial!

Finally, hereditary modifications of tail-length are common. The genetic nature of the tailless or "bob-tailed" condition in the Manx cat has been established by several investigators. Less well known is the evidence of Godron[6] and of Klodnitzky and Spett[9] for a dominant gene or genes shortening the tail in the dog. Experiments of Nägeli (reported by Lang[12]), Dubosq[4] and Dobrovolksaia-Zavadskaia[3] make it clear that there is a hereditary type of taillessness in the house mouse and that it is dominant and lethal in the homozygous state, but the exact genetic basis for the condition has not yet been definitely determined. Landauer and Dunn[10] and Landauer[11] have found that in fowls there are two types of hereditary rumplessness as well as the non-genetic congenital rumplessness referred to above.

In addition to these specific cases, most geneticists would agree that genes are responsible for the differences in tail length which are characteristics of certain species. Within the Rodentia one finds that the number of caudal vertebræ may vary from 30 in the rat down to the rudimentary tail of the guinea pig. Similar differences are found in the Primates. Even in closely related species, and within a species, there may be great hereditary differences in length of tail. For example, in sheep the number of caudal vertebræ varies in so-called tailless, short-tailed and long-tailed varieties from three to twenty-four. Genetic factors affecting the caudal vertebræ are obviously among those which have played a conspicuous rôle in the evolution of species.

Since several of the mammals have achieved apparent taillessness by the acquisition of genes which inhibit development of the tail beyond the embryonic stages, it is not impossible that hereditary taillessness may yet be found in the rat. During the course of this investigation several lady members of a class in nutrition expressed the hope that a race of tailless rats might be produced. Their interest arose from the feeling that if the rat could modify his long snaky tail, replacing it with either a short one like that of the rabbit or none at all, like the guinea pig, their aversion to handling the animals would be overcome, and, as a result, they would enjoy more fully certain phases of a laboratory course in nutrition.

However desirable from this standpoint a gene for taillessness might be, there is reason to believe that such a mutation could not be utilized to add to the prestige of the laboratory rat. Investigations with short-tailed mice show that homozygosity for short tail is lethal and that there is apparently a reduction of viability in the heterozygote. Evidence of reduced viability in short-tailed cats is presented by Hind[7] and by Schwangart and Grau[13], while the report of Klodnitzky and Spett[9] suggests that some short-tailed dogs are similarly afflicted. If a reduction in vigor is inevitably associated with a reduction in the tail, that character would be undesirable in most laboratories, even if the rat could attain it.

Because hereditary taillessness effects vitality, it does not follow that accidental congenital taillessness must have the same effect. However, Miss King[8] reports that the tailless rats studied at the Wistar Institute were somewhat below the average in size, vigor and fertility. These defects were not noted in our specimen. The average

size of 10 litters from him was 7.2, which is slightly above the average for the albino rat. It is possible that females may be more adversely affected than males. Several years ago there appeared in the rat colony of the Division of Agricultural Biochemistry here, the only tailless rat observed in that department among a number estimated to be over ten thousand. The pelvic girdle of this animal, a female, was so deformed that she could not give birth to her litter. That such a condition is most unusual is shown by the fact that Miss King got several litters from four tailless females.

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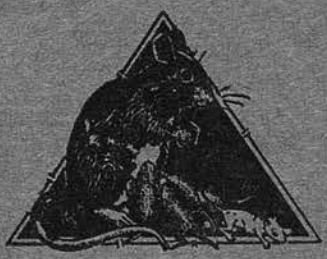

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A HEREDITARY LETHAL MUSCLE CONTRACTURE IN CATTLE

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SOME time ago Dr. J. E. Thompson, city chemist and veterinarian of Ely, Minnesota, reported to the writer a peculiar set of circumstances encountered in his veterinary practice during 1931. Upon subsequent investigation the condition responsible for them has proved to be one of considerable interest to geneticists, veterinarians and breeders of live stock.

In three herds of Holstein-Friesian cattle which had been under his observation for many years, and which during that time had been practically free from even the simplest forms of dystocia, there occurred in one year five cases in which the calves were alive at full term, but were afflicted with an abnormality so extreme that only two could be delivered entire and then only with great difficulty. To deliver the other three it was necessary to dismember them in the uterus.

All of these five calves were sired by the same bull. They were so much alike that, after encountering two of them in the *A* herd, Dr. Thompson was able to diagnose the condition at once upon manual examination of two cases of difficult parturition in two other herds and to predict before being told that the afflicted calves had been sired by Mr. *A*'s bull.

None of these calves were seen by the writer of this article but the description given by Dr. Thompson is adequate to permit ready identification of the condition. The affected calves apparently caused distress to their dams before the end of the gestation period but were alive at full term. The head was drawn up toward the back, apparently by contraction of the cervical muscles, and the neck was extremely rigid. The fore and hind limbs were folded and almost wrapped around the body. Like the neck, they were characterized by extreme rigidity, there being very little movement, if any, at the joints. All five calves were the same. The sexes were not noted.

Allegedly an Acquired Character

A peculiar coincidence was that in the year before these calves were encountered their sire had been injured by getting a chain embedded in his neck. After the wound healed the neck was stiff for a long time. The owner stated that when the bull was taken out for service the painful condition and stiffness in the neck seemed even more pronounced than at other times. When this situation was followed by the appearance among his offspring of five calves exhibiting among other complications extreme rigidity of the neck, it was not unnatural that the owner should ascribe the abnormality of the calves to the injury of the sire. This interpretation seemed all the more likely because, although the bull was nearly six years old, his progeny had all been normal prior to the injury to his neck, which had occurred in his fifth year.

Disregarding the Lamarckian aspect of the case, it seemed fairly certain that the abnormality was a hereditary muscle contracture similar to that found by Roberts[5] in lambs. The possibility that it might have been occasioned by nutritional disorders seemed unlikely because of its occurrence in three separate herds. Furthermore, while one might expect occasional teratological conditions in any herd, it was extremely unlikely that five cases so much alike should

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appear in the progeny of one sire unless the condition were hereditary. Moreover, the abnormality does not present any of the usual teratological conditions. Dr. Thompson stated that he had never encountered anything like it in many years of practice until these five cases occurred, all in one year.

Accordingly the writer visited the owners of the three herds in company with Dr. Thompson, and, thanks entirely to the latter's proficiency with the Finnish language, managed to trace the relationships of the cattle concerned.

Genetic Evidence

The sire of these calves had been born at the *A* farm. Though both his sire and his dam had been purebreds and registered, this bull had never been registered. For convenience he will be designated as Bull *X*.

The five cows producing the abnormal calves were as follows:

Herd A

No. 1. Daughter of Bull *X*.

No. 2. Daughter of Bull *X*.

No. 3. Half-sister to Bull *X*, by the same sire but from a different dam.

Herd B

No. 4. Daughter of Bull *X*.

Herd C

No. 5. The descent of this cow could not be definitely traced but it was found that a number of the *C* cows had been sired by bulls in the *A* herd. It was therefore quite probable that No. 5 had been sired either by Bull *X*, or by *X*'s sire. It will be shown later that she might have gotten the gene or genes for the muscle contractures from either of these bulls.

It is obvious from these facts that the condition is hereditary. Its appearance late in the breeding life of *X* was in no way related to the injury to that bull's neck, but rather wholly the result of his then being mated to his own daughters, some of whom carried the same gene or genes for muscle contractures as he did. By chance three of them produced calves homozygous for the abnormality.

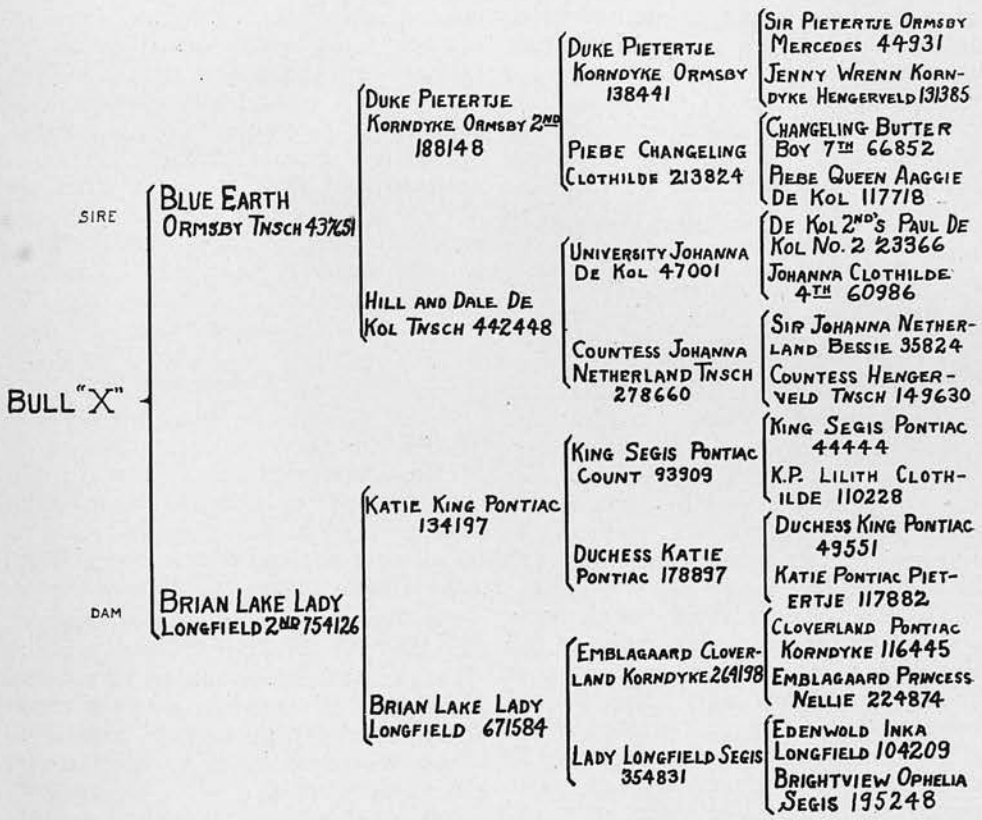
Owing to incompleteness of the available records, no attempt was made to determine the ratio of affected calves to normal ones, or the proportion of carriers of the gene to non-carriers among the progeny and half sisters of Bull *X*. The bull was slaughtered after these calves appeared, so that further breeding trials were impossible. There can be little doubt, however, that the muscle contracture is a simple recessive character. If it were dominant it would immediately be eliminated from the germ plasm. If it were dependent upon duplicate recessive genes, or were inherited in any other way, it is extremely unlikely that three daughters out of a very limited number should have produced it in one season.

Cow No. 3 had given birth to four normal calves sired by Bull *X*, prior to the one with the muscle contractures. Such a ratio is strongly suggestive of a simple recessive character although the data are limited. Moreover, the available evidence that the abnormality is a unifactorial recessive character is supported by the fact that a condition very similar to that described in this paper, if not identical with it, has been shown to behave as a simple recessive charac-

MUSCLE CONTRACTURES FATAL

Figure 13

Calf born at full term with contractures of the neck and limbs. This condition is not only fatal to the calf, but in some instances to the dam as well. The photograph shown above is of a calf born in Norway, and is reproduced through the courtesy of Dr. Otto Mohr. Similar lethal abnormalities have been reported in sheep and swine.



PEDIGREE OF BULL *X*

Figure 14

Pedigree of the bull producing the lethal muscle contracture. The evidence indicates that he inherited the gene for the lethal character from his sire, Blue Earth Ormsby Tnsch.

ter in two other species of the same sub-order of mammals.

Effects of the Abnormality

The abnormality is not a character that lends itself to the usual genetic analyses based on the ratio of affected calves to normal ones in the progenies of various types of matings. The homozygous recessive condition is not only lethal to the calf but, in some cases, also to the dam. Of the five females producing calves with contractures, Numbers 1 and 2 were heifers carrying their first calves. Both of the latter had to be dismembered to overcome dystocia. In spite of every care, one heifer was fatally injured and the other suffered such irreparable damage that she could not subsequently be gotten in calf. Cow No. 3 was apparently unaffected but the later history of the other two is not known. It seems probable that the birth of a calf with contractures is fraught with grave risk for all the dams and that that risk may be accentuated for primiparae.

The facts that birth of a homozygote may eliminate a female heterozygote from further breeding tests, and that the chance of such elimination occurring may be less if the heterozygote has already produced one or more normal calves, would not be conducive to the securing of 3:1 and 7:1 ratios unless more females were tested than is ordinarily possible with

cattle. The alternative method is to determine the proportion of animals carrying the gene in two different kinds of populations. These, and the proportion of carriers expected in each if the character be a simple autosomal recessive, are as follows:

Type of Mating	Heterozygotes expected among living progeny
(1) Heterozygote x normal	one-half
(2) Heterozygote x Heterozygote	two-thirds

Pedigree of Bull *X*

In view of the possibility that the congenital lethal muscle contracture may be encountered in cattle elsewhere, and to point out lines of descent in which it may be expected to occur, the pedigree of Bull *X* is shown for four generations. (Figure 14.)

There is some evidence that the gene for contractures has descended in the sire's side of the pedigree, rather than in the dam's side. Bull *X* had been mated to two half-sisters in the *A* herd, one from the same sire as *X*, the other from the same dam. The owner stated that the latter cow had produced five normal calves by Bull *X*. The other was Cow No. 3, who had produced by *X* four normal calves and one with the abnormality. It would seem therefore that both Bull *X* and Cow No. 3 had inherited a gene for the lethal muscle contractures from their common sire, Blue Earth Ormsby Tnsch.

There is no way of knowing at present whether the mutation had arisen in this animal or whether he had inherited it from his parents. It is hoped that the publication of this note may lead to recognition of the condition if it occurs elsewhere, and that further studies of the pedigrees concerned will reveal the common ancestor responsible for the dissemination of the gene.

Geographical Distribution of the Gene

After most of this investigation had been completed the writer learned that a condition very similar to the muscle contracture described in this paper, and probably identical with it, had been reported and illustrated by Mohr.[4] Although his communication gives few details of the condition, a comparison of notes with Professor Mohr at the Sixth Genetics Congress made it fairly certain that the character found in northern Minnesota was identical with that discovered by him near Bergen, Norway. By courtesy of Professor Mohr, one of the two such specimens reported there up to August, 1932, is shown in Figure 13.

Dr. Thompson states that Professor Mohr's specimen resembles his except that in the case at Ely the head was not twisted to one side but was drawn up toward the back.

In his report of a similar condition in the pig, Hallquist[2] briefly states that he and Dr. Bonnier have found in a race of Swedish cattle a condition which is apparently similar to that described in this paper if not identical with it.

If the abnormality had occurred in man instead of in cattle some connection might be suspected between its occurrence in Minnesota and in the Scandinavian countries. It seems more probable that the mutation has arisen independently in the American Holstein-Friesians than that it has been distributed to Norway, Sweden and America from some single source, but the latter possibility cannot be ruled out.

A hereditary condition somewhat resembling that discussed in this paper has been found in Red Danish cattle by Loje.[3] The affected foetuses have the same rigidity of the limbs, but differ in having short necks and in being dead and partially mummified at birth. Death apparently occurs about the eighth month of gestation. The differences between this abnormality and that found in Minnesota may mean that two different genes are producing characters some-

what alike, or may possibly result from different modifying factors in the two breeds interacting with a single gene for contractures.

It is entirely probable that the abnormality has occurred elsewhere in this country, but has not been recognized as being hereditary. To his description of 16 teratological specimens Williams[6] adds accounts (Case 17) of two calves characterized by extreme rigidity of the body and legs. One of these was alive at full term and had to be dismembered before delivery. It had the same upward bend of the neck as had Dr. Thompson's specimens. It is not improbable that these calves were also afflicted with hereditary contractures, but that, in the absence of inbreeding, not enough abnormal ones were produced to permit recognition of the hereditary nature of the condition.

Comparative Genetics

It is of special interest that hereditary lethal contraction of the limbs has also been found in two other species of the Artiodactyla. Roberts[5] has conclusively proven it to be a simple recessive condition in sheep, and Hallquist[2] has made extensive studies of it in swine with the same results. In swine the symptoms would appear to be somewhat less extreme than those in cattle and sheep. The hind limbs are not commonly affected, and Hallquist does not mention rigidity of the neck. In three of Roberts's affected lambs examined in detail by Mr. Wm. C. Miller, joints of both fore and hind limbs were affected. Two of the three had wryneck. The contractures in sheep would appear to resemble those in cattle more closely than does Hallquist's "krummsteifbeinige" condition in swine. This is to be expected.

From the standpoint of comparative genetics it would be of interest to learn if this abnormality ever occurs in other species of the Artiodactyla or in species outside of that sub-order. It is not impossible that it may yet be recognized in some of the army of laboratory rodents or in man.

Discussion

The lethal condition described in this paper is one of a considerable number of fatal abnormalities of domestic animals which have been shown to be hereditary. Thirty of these are listed in a recent review.[7]

At least eleven such characters are now known in cattle. It is remarkable, however, that apart from the occurrence of bull-dog calves in the few imported Dexter cattle, only one other hereditary lethal character has been found in domesticated mammals in North America. That one is the epithelial defect studied in calves by Hadley and Cole.[1]

One reason why these lethal characters have been found more frequently elsewhere is probably that in some of the smaller countries of Europe there has been somewhat more inbreeding of stock than has been practised in this country. This is particularly likely to occur in some of the geographically isolated districts in the Scandinavian countries where free interchange of stock is less common and where several lethals have been found. Such a condition was responsible for the discovery of the condition at Ely, Minnesota. The *A* herd had one of the few purebred Holstein bulls in a comparatively isolated district and accordingly the bull was utilized for several years by the three herds in which the abnormality occurred. The resultant inbreeding brought the hereditary contractures to light.

It is even more probable, however, that the discovery of so many lethal factors in Europe is the direct result of the researches of such investigators as Professor Otto Mohr and the late Christian Wriedt, and of the general interest stimulated by their findings. There can be little doubt that many lethal factors are interfering with the reproductive efficiency of domestic animals the world over. It is to be hoped that breeders of live stock and veterinarians will become

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C. HUMAN GENETICS

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SEX DIFFERENCES IN THE EXPRESSION OF AUTOSOMAL GENES AFFECTING HUMAN DENTITION

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During the past three years the writer has studied the genetic basis for the occurrence of a gap between the upper central incisor teeth of man. This condition is known to arise from an abnormal enlargement and attachment of the *frenum labium*, a fold of mucous membrane which normally runs from the centre of the upper lip to the gum, where it is attached about five millimetres above the gingiva. In persons exhibiting a gap between the upper central incisors, it is usually found that this fold is enlarged to form a sort of fibrous ligament which, instead of stopping above the teeth, runs right between the central incisors and is attached behind them.

In this study there were encountered a number of persons in which such a space had arisen from some other cause and in whose pedigrees there was no history of a similar condition. The majority of cases, however, present a definite family history; and in the six pedigrees studied by the writer (and to be published later) the peculiarity has behaved as a unifactorial autosomal dominant character.

However, in two of these pedigrees, and in two others not yet completed, there occurred apparent exceptions to the rule in that parents reported to have no space between the incisors had children in which such a space is quite marked. In all four cases the apparently unaffected transmitter was a mother with a family history of spaced central incisors. Two of these could not be examined, one had incisors close together and the fourth was found on examination to have a gap of about 1 mm. in width. In two other cases girls reported not to show the peculiarity were later found to have small gaps between the upper central teeth. Furthermore, in these six pedigrees slightly fewer females than males had the gap; the figures for sibships completely traced are given in table 1.

This difference is not in itself significant, but all the findings together suggested that the character was less common in women than in men. In order to get data on the incidence of the condition in the general population an arrangement was made whereby determination of its presence or absence was made by competent dentists during their routine examination of 3,368

first-year students who entered the University of Minnesota in the fall of 1931. Among these the frequency of spaced upper central incisors was found to be:

	per cent
In 1842 men	11.5 ± 0.72
In 1526 women	8.7 ± 0.72
Difference	2.8 ± 1.01

This difference is 2.78 times its (standard) error and therefore indicates a significantly greater frequency of the condition in males than in females. The figures given must include a number of cases in which the gap was not

TABLE 1

Sex distribution of spaced incisors in six pedigrees

SEX	NUMBER	AFFECTED	
		Number	Per cent
Male	25	15	60.0
Female	31	17	54.8

TABLE 2

Distribution of spaced incisors in relatives of affected students

CLASS	NUMBER OF REPLIES
Father affected	27
Mother affected	13
Both parents affected	5
Space in sibs or near relatives but not in either parent	25
No known family history	28

hereditary, and from other data it would seem that if these could have been eliminated the difference might have been still greater.

To those in whom the spaced incisors were noted a questionnaire was sent asking for information about its occurrence in relatives. Out of 112 replies, 12 were discarded because of inadequate data and two because the condition arose from missing lateral incisors. The remaining 98 were distributed as shown in table 2.

There was thus a family history of the condition in 71.4 per cent of the affected persons. Downs (1927) has reported that of eleven cases of "spaced uppers" in which the family history could be traced, only one was negative.

The general findings from the students' replies, as presented in table 3,

confirm the other evidence that the gap is less common in women than in men.

If there were no sex difference in the expression of this character, the numbers and percentages in these four classes should be approximately equal in the two sexes. That they are not so in the 95 students who replied to the questionnaire can hardly be considered more indicative of a sex difference than of a tendency for college boys to answer their professors' questionnaires more faithfully than do college girls. However, the fact that among 50

TABLE 3

Sex distribution of spaced incisors

	MALES	FEMALES
(A) Sex distribution in affected persons:		
1. In 50 affected parents	32	18
2. In 95 affected students of known sex	63	32
(B) Percentages of each sex affected:		
3. In 70 sibships with family history:*		
(a) in 86 males	46.5%	
(b) in 83 females		38.5%
4. In 45 sibships from affected parents*	61.9%	40.0%

* Excluding the propositus in every sibship.

TABLE 4

Width of gap between spaced incisors

SEX	NUMBER MEASURED	RANGE	AVERAGE
		mm.	*mm.*
Male	6	1.8–4.4	3.00
Female	6	1.0–4.5	2.25

affected parents nearly twice as many fathers had the condition as did mothers corroborates the other evidence that the character is sometimes suppressed in females.

Since the sex ratio of replying students is so abnormal (63:32) these students cannot be used in any determination of the incidence of spaced incisors in their sibships. Accordingly in class B_3 of table 3 there have been included *only the brothers and sisters* of those students who reported the condition in either parent or in some near relative. This is an adaptation of the "brother and sister" method introduced by Weinberg (cited from Baur, Fischer and

Lenz, 1931). The elimination of the propositus does not entail any great error since the chance of the character appearing in any brother or sister was as great as that of its appearing in the discarded propositus. The method does reduce the frequency of the condition because in many families the numbers were too small to obtain a representative ratio after the elimination of the propositus. This is to some extent compensated for when a considerable number of sibships are considered.

In this case the sibships yielded, as was expected, a normal sex ratio of 86♂♂ to 83♀♀, but only 38.5 per cent of the females were affected in contrast to the figure of 46.5 per cent for males. Since this group may possibly have included some cases of non-genetic spaced teeth, there are considered in class B_4 the sibships of these students having definite evidence of a hereditary gap in the form of an affected parent. In this group the sex difference is much more pronounced.

Actual measurements of the gap have as yet been made on only a small number of persons but in these the space is somewhat smaller in males than in females. (See table 4.)

DISCUSSION

In consideration of the evidence from these several angles it is consistent with the data to conclude that the space between the upper central incisors caused by hereditary abnormality of the *frenum labium* is sometimes suppressed in whole or in part in females.

On this basis one can account for the pedigrees in which the character appears to have skipped a generation, *i.e.*, to be a recessive. On the other hand one must recognize that such suppression or modification is not found in every case. This is attested by the fact that the widest gap thus far encountered, 4.5 mm., was in a girl. Presumably there are modifiers for this gene as for others. The degree of expression of the character must depend upon the interaction of the causative gene with its modifiers and its environment. The sex difference in its expression may be considered the result of differential responses of the causative gene and its modifiers to the different endocrine environments found in the two sexes.

In this connection the findings of Downs (1927) are of special interest. In studying the occurrence of various abnormalities of dentition in combination with sixteen different pathological conditions indicative of endocrine dyscrasias, no association was found between any specific dental anomaly and any specific endocrine disorder but abnormalities in dentition were approximately three times as frequent in those with endocrine dyscrasia as in normal persons. From these data the present writer has com-

puted the following frequencies of the condition which Downs designates as "spaced uppers" and which is probably wholly or partly the same as that considered in this paper:

	per cent
In 375 persons with endocrine disorders	12.27 ± 1.68
In 271 normal persons	2.58 ± 1.04
Difference	9.69 ± 1.97

Since this difference is nearly five times its standard error, it indicates that the greater frequency of spaced uppers in persons with endocrine disorders is significant. Unfortunately Downs did not give the distribution of these cases in the two sexes.

It is probably a coincidence that an endocrine basis for sex-limitation of an autosomal gene is found in two other characters affecting the dentition of man. One of these is found in the pedigree of female-sex-limited premature decay and loss of teeth reported by Sedgwick (cited by Gates, 1929) in which 10 of 11 daughters in two generations were affected while none of four sons showed the character. A similar tendency is evident in a pedigree of female-sex-limited missing lateral incisors given by Schultz (1932). This last is of particular interest because the character has been found in other pedigrees to be very irregular in its expression. Adequate data on its sex distribution are not available but the influence of sex on its expression is definitely shown in Schultz's pedigree and it may well be a factor in causing the other irregularities.

It is desirable to point out that while a male-sex-limited character, such as is found in Schofield's (1921) pedigree of webbed toes, can be accounted for apart from endocrines by postulating a gene in the Y-chromosome, a female-sex-limited character such as the two just cited can not be adequately accounted for by sex-linked genes, since in the offspring of an affected female the character would appear in both males and females were it not for the modifying influence of the endocrine secretions.

The hereditary basis for spaced upper incisors is apparently intermediate between that of a simple dominant character appearing equally in both sexes and that for such a character as pattern baldness which, from the studies of Osborn (1916), would appear to be manifested in males when they are heterozygous for it, but in females only when they are homozygous. In the latter case a single gene can be fully expressed in males but not in females, where the duplex condition is necessary to overcome the inhibiting forces of the endocrine environment.

These data on spaced incisors are presented to show that sex differences in the expression of an autosomal character may obscure its genetic basis un-

less such differences are recognized. It is suggested that a more widespread recognition of the fact that such differences exist may help to account for some of the frequent cases of apparently irregular genetic behaviour of inherited characters in man.

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AN EARLIER RECORD OF THE TOOTHLESS MEN OF SIND

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IN Thadani's recent addition[4] to his former note[3] on sex-linked anadontia associated with anhidrosis in the "Bhudas" of Sind, he states that "no records, either written or from memory are available about the first two generations." Since the following sentences from Darwin[1] have apparently not been noticed by Thadani or by Gates, Popenoe, Castle, Roberts and others who have cited his first report in their writings on heredity in man, their repetition here seems justified.

> . . . . I may give an analogous case, communicated to me by Mr. W. Wedderburn, of a Hindoo family in Scinde, in which ten men, in the course of four generations, were furnished, in both jaws taken together, with only four small and weak incisor teeth and with eight posterior molars. The men thus affected have very little hair on the body, and become bald early in life. They also suffer much during hot weather from excessive dryness of the skin. It is remarkable that no instance has occurred of a daughter being thus affected; and this fact reminds us how much more liable men are in England to become bald than women. Though the daughters in the above family are never affected, they transmit the tendency to their sons; and no case has occurred of a son transmitting it to his sons. The affection thus appears only in alternate generations, or after long intervals . . . .

The syndrome reported by Darwin's correspondent is obviously identical with that described by Thadani. Since both accounts come from the province of Sind in north-west India, it is reasonably certain that the Darwinian quotation refers to the earlier generations of the family reported by Thadani (The Sind of today and the Scinde of Darwin's day are identical.) Darwin's account is given in the second edition of "Animals and Plants," which appeared in 1875, but not in the first edition of the same work, published in 1868. This suggests that his correspondent wrote between these two dates. Assuming that in this country there would be a span of approximately twenty years between generations, it would appear that Mr. Wedderburn's four generations cover the period from about 1780 to 1860 (since he refers to "men" and does not mention any children), while Thadani's four generations probably extend from about 1850 to 1930.

The data, incomplete though they be, apparently include from six to eight generations. One wonders if Darwin's correspondent gave any more details than are found in the quotation above. Possibly a search through Darwiniana at Down House, Burlington House, the British Museum and elsewhere might bring Mr. Wedderburn's letter to light. Should it permit the formation of a pedigree chart covering these earlier generations, it should not be difficult to fit the earlier history to the later one, and, with some amplification of Thadani's data, it might be possible to construct a pedigree chart covering seven or more generations of this interesting sex-linked character.

The fact that four affected females occurred in Thadani's later generations, while none were known to Darwin's correspondent, suggests that in this pedigree, as in those of some other sex-linked characters, there have arisen aberrations in the mechanism of inheritance, or in the behavior of the gene. However, among five generations of an American family exhibiting exactly the same syndrome, Roberts[2] found five partially affected females, some of whom showed no more evidence of it than occasional patches of skin lacking sweat glands. Such a partial manifestation might have been entirely missed by Darwin's informant. It seems more likely, therefore, that in the Indian family the

exceptions to the conventional behavior for recessive sex-linked characters occur because the gene is not completely recessive in heterozygotes (which are always females). Such a condition is not to be unexpected for a gene having such profound effects in hemizygous (XY) persons.

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D. EMBRYONIC MORTALITY IN THE FOWL

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X.–XII.—Studies in Embryonic Mortality in the Fowl.

I. The Frequencies of Various Malpositions of the Chick Embryo and their Significance.

By F. B. Hutt, B.S.A.

II. Chondrodystrophy in the Chick.

III. Chick Monsters in Relation to Embryonic Mortality.

By F. B. Hutt, B.S.A., and A. W. Greenwood, Ph.D.

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X.—Studies in Embryonic Mortality in the Fowl. I. The Frequencies of Various Malpositions of the Chick Embryo and their Significance.

By F. B. Hutt, B.S.A., Animal Breeding Research Department, University of Edinburgh. *Communicated by* Dr F. A. E. Crew. (With One Plate.)

(MS. received October 5, 1928. Read January 7, 1929.)

Introduction.

The loss to the poultryman from mortality among embryos during incubation is probably second only to that from disease. In the attempt to reduce this loss many investigations have been made of the influence on incubation of such environmental factors as temperature, humidity, oxygen supply, methods of turning and cooling, etc., as well as of the influence of the ration fed to the breeding stock. However, even when conditions of nutrition and incubation are ideal so far as present knowledge goes, there is still a mortality in the incubator that may range as high as 50 per cent. of the fertile eggs and is seldom less than 25 per cent.

The investigations reported in this and the two following papers of the series were made to determine the extent to which various types of abnormalities occasionally observed in the chick embryo were responsible for the high mortality rate.

Material.

This investigation is based upon examination of over 12,000 eggs which had failed to hatch, including dead germs at first test (eight or nine days) and eggs unhatched at twenty-one days, from the following four sources, all in Scotland:—

Animal Breeding Research Department.
Romanno Bridge Poultry Farm, West Linton, Peeblesshire.
W. Johnston & Co., Cowden, Comrie, Perthshire.
Sussex Hatcheries, Glasgow.

To the three firms mentioned we express our thanks for the eggs and data so willingly supplied and without which this investigation could not have been made. These sources will hereafter be designated A, B,

C, and D, but these letters will not represent the firms in the same order as they are listed above.

The number of eggs received from each source, together with the breeds kept and incubators used, are given in Table I.

TABLE I.—MATERIAL USED IN THE INVESTIGATION.

Source.	Dead Germs. 1–10 days.	D.I.S. 10–21 days.	Breeds.*	Incubators.	Times turned daily.
A	1775	8059	W.L., Bl.L., W.W.	Blue Hen Miller	3 1
B	271	744	W.L., R.I.R., W.W., B.R., L.Suss. × R.I.R.	Glevum (mammoth)	5
C	...	654	W.L., Anc., R.I.R., R.I.R. × L.S.		
D	104	190	Friz., Br.Leg.	Hearson	2
	2150 †	9647 †			

* Abbreviations as follows :—

W.L.—White Leghorn
Bl.L.—Black Leghorn
Br.L.—Brown Leghorn
L.S.—Light Sussex
Friz.—Frizzle
W.W.—White Wyandotte
R.I.R.—Rhode Island Red
B.R.—Barred Plymouth Rock
Anc.—Ancona.

† Exclusive of eggs from "chondrodystrophy matings" at this Department.

Practically all of the eggs examined came from settings between 12th February and 1st May, with the exception of some later and earlier settings at this Department. The total of 11,797 unhatched eggs examined represents settings of 39,760 eggs from nine different breeds, and in several types of incubators under different management, and may therefore be considered as a fairly representative sample of incubated eggs in general. In some cases eggs removed at first test were not sent to us, and from some lots (*e.g.* all from C) only those dead at hatching were received. Actually, therefore, the total number of dead embryos from the 39,760 eggs set was somewhat greater than the 11,797 examined by us. However, since data was obtained from each hatch, even where

the eggs were not examined, we are able to determine for each source the average infertility, dead at first test, dead at hatching, and chicks hatched during the season's operations. These data are given in Table II.

TABLE II.—SEASON'S AVERAGE INCUBATION RESULTS FOR EACH SOURCE OF MATERIAL.

Source.	Of Eggs set.		Of Eggs fertile.		
	Infertile. Per cent.	Hatched. Per cent.	Dead Germs. Per cent.	Dead-in-shell. Per cent.	Hatched. Per cent.
A	6·75	57·07	9·75	29·04	61·21
B	8·19	72·95	6·80	17·24	75·96
C	8·48	65·11	8·81	20·06	71·13
D	5·04	64·08	11·50	21·02	67·48

"Dead germs" in this paper refers to those eggs in which the embryo was distinctly dead at eight or nine days when the first test was made. It includes embryos ranging from one to eight days old.

"Dead-in-shell" refers to those embryos found in eggs unhatched after twenty-two days of incubation. Since doubtful cases are often left in at first test, this class will include some embryos of less than eight days, but the majority will range from eight to twenty-one days. The proportion of embryos eighteen days and over is given in Table III.

METHOD.

Eggs were received from the contributors a few days after the termination of each hatch. Each egg was broken, the embryo examined and recorded as "too young to detect abnormality," "18-day normal," "Position II," "teratological abnormality," etc., in its proper category by checking it off in a counting-board consisting of six rows of differently coloured wooden beads. All teratological abnormalities were separated and re-sorted into their various classes.

The abnormalities observed in this survey were of three distinct classes, and will therefore be discussed separately under the following heads:—

1. The Frequency of Various Malpositions of the Chick Embryo and their Significance.
2. Chondrodystrophy in the Chick.
3. Chick Monsters in Relation to Embryonic Mortality.

I. The Frequency of Various Malpositions of the Chick Embryo and their Significance.

During the examination of the first two lots of eggs from one of these farms, it was found that many of the fully formed dead embryos were apparently abnormal only in their positions. Accordingly data were kept on the frequency of the most common malpositions found among the eggs subsequently examined. Such abnormal positions were recorded only among the embryos approximately eighteen days old or over, since prior to this stage of development, persistent malpositions could not be determined with any degree of accuracy. Among 8295 unhatched embryos (excluding all that had been discarded at first test on the eighth day of incubation), 1935 had died before the tenth day and 929 from the tenth to seventeenth days. The remaining 5050 which were approximately fully formed provided the data given below.

The normal position for the chick just before emerging is as follows: The head is towards the larger end of the egg (or toward the air-cell in oval eggs). The neck is so bent as to bring the head to the right side of the body and backwards with the beak under the right wing and just external to the femorotibial joint (fig. 1). As a consequence of this position the tip of the beak rests near the shell at a point just where the inner shell membrane separates from the outer to form the air-cell. The feet are folded on the ventral side of the body almost exactly the same as in a trussed fowl, except that the toes reach to the head.

The deviations from this position which we have most frequently observed, the first three of which have also been noted by Sanctuary (1925), are as follows:—

Position I.—The head is not bent to the right but extends down the median ventral line and is buried between the thighs (figs. 2 and 3). In some cases of this class the head is turned slightly to the left, but the beak is still caught between the thighs. In the less extreme cases of this type of abnormality in position the beak is just held enough by the tibia to prevent it from reaching the shell. A number of embryos which had died at twelve to fourteen days were found in Position I.

Position II.—The chick is turned completely upside down so that the head is in the small end of the egg (figs. 4 and 5).

Position III.—The head of the chick is in the large end of the egg but is turned to the left instead of to the right. In most cases this brings

the beak near the shell at a point directly opposite the air-cell (figs. 6 and 7).

Position IV.—The chick lies with the head toward the large end and the beak under the right wing as in the normal position, but is rotated within the shell, so that the beak is buried opposite to the air-cell (fig. 8). This abnormality was not noticed as being of frequent occurrence till toward the end of the incubation season, hence data concerning it are available for only 1085 dead embryos of eighteen days or over.

Several chicks were found in combinations of Positions I and II or of Positions II and III. Other abnormalities found in much smaller numbers included chicks normal in every respect except that the head was not under the wing, and some in which the beak pointed toward the large end of the egg instead of toward the side.

The frequency of embryos in Positions I, II, and III is shown in Table III.

TABLE III.—FREQUENCY OF POSITIONS I, II, AND III.

Source.	Dead-in-shell at Hatching.	18-day Embryos.	Normal (incl. Pos. IV.)	I.	II.	III.	I and II.	II and III.	Total Abnormal.
A	6707	4114	2693	363	758	272	16	12	1449
B	744	458	270	41	85	56	2	4	194
C	654	380	229	48	62	37	2	2	155
D	190	98	53	15	20	9	...	1	46
Totals.	8295	5050	3245	467	925	374	20	19	1844

It should be particularly noted that in the above table the embryos classified as normal include those in Position IV, since records of this abnormality were not kept till towards the end of the season. The data on Position IV are given in Table IV.

It is reasonable to assume that these smaller lots may be taken as a representative sample of the dead-in-shell from each source, and that the percentage of Position IV embryos found in these samples would be approximately correct for the entire lot of dead-in-shell from each source. On this basis we have deducted from the embryos classed in Table III as normal the calculated number in Position IV, and have thus been able to determine the frequency in percentage of all four abnormal positions in proportion to the true normal positions among all the 18-day

TABLE IV.—FREQUENCY OF POSITION IV.

Source.	Dead-in-shell at Hatching.	18-day Embryos.	Position IV.	
			No.	Per cent. of 18-day Embryos.
Poultry Farm A	1222	850	176	20·70
„ B	129	117	21	17·95
„ C	230	188	35	18·08
„ D	79	41	6	14·63

(or over) embryos from each source. These data are presented in Table V.

TABLE V.—RELATIVE FREQUENCY OF POSITIONS I, II, III, AND IV.

Source.	18-day Embryos.	Normal, per cent.	Pos. I, per cent.	Pos. II, per cent.	Pos. III, per cent.	Pos. IV, per cent.	I and II, per cent.	II and III, per cent.	Total Abnormal Positions, per cent.
A	4114	44·77	8·82	18·42	6·61	20·70	·38	·29	55·22
B	458	41·00	8·95	18·56	12·23	17·95	·44	·87	59·00
C	380	42·20	12·63	16·29	9·74	18·08	·53	·53	57·80
D	98	39·65	15·31	20·20	9·18	14·63	...	1·02	60·34
Average (weighted)	5050	44·14	9·25	18·31	7·41	20·12	·39	·37	55·85

SIGNIFICANCE OF ABNORMAL POSITIONS.

It cannot yet be definitely stated to what extent all these abnormal positions prevent chicks otherwise healthy and normal from emerging.

It is reasonably certain that none of the chicks in Position I (head buried between the thighs) would be able to break the shell. Even the normal chick is unable to raise the neck till some time after it has left the shell, and in most cases chicks in Position I had the head so tightly wedged between the thighs that to raise it would be much more difficult than in the normal chick. In a very few of these chicks the beak was close enough to the shell to break it if the chick had been able to do so. Since none of the 467 embryos found by us in Position I nor the 196 reported by Sanctuary, had pipped the shell it is reasonable to conclude that chicks in Position I are unable to hatch. From our data this would account for 9·25 per cent. of the fully formed dead-in-shell at hatching

time. Sanctuary found this abnormality in 196 cases out of 1490 "full-time" embryos, or 13·15 per cent., it being more common in his material than either Positions II or III.

In order to realise the extent to which Positions II, III, and IV may prevent or hinder hatching, some understanding of the normal process is essential. The most detailed available account of the actual process of hatching is still that of Réaumur (1751), first published in 1749. He describes the position of the chick in the shell, elaborates on the difficulty of its task to pierce the tough membrane and the hard shell wall, and goes on to describe how the first crack is extended till the shell is broken almost all the way around the egg, thus permitting the chick to burst the two portions asunder and tumble out. Since the head of the chick remains under the right wing up to the time when the two parts of the shell are separated, this extension of the first opening can be made only if the chick rotates in the shell. Réaumur was perplexed to know whether this rotation was done by means of the beak, or the only other probable agency, the feet. He finally satisfied himself by a simple experiment that the latter were responsible for the rotation. He first determined that the fracture was lengthened from left to right, then by removing shell and membrane in advance of the chick's beak, removed the only object against which the beak could exert any force. Since the chick was able to rotate and hatch as in normal cases, Réaumur concluded that the feet were responsible for the rotation.

Our own observations are essentially in accord with those just quoted, except that we are inclined to think that one of the most important steps in hatching occurs before any of the process described by Réaumur—*i.e.* the penetration of the beak through the inner shell membrane into the air-cell, so that the allantoic respiration is supplemented by normal breathing through the lungs. That this does happen is attested by the peeping of the chicks, which is usually heard before the eggs are pipped. Possibly Réaumur took this part of the process for granted. The number of chicks which we have found dead-in-shell with the head far removed from the air-cell suggests that the initiation of pulmonary respiration is of prime importance if the chick is to emerge successfully.

The physiological changes in the embryo prior to hatching as described by von Baer also emphasise this point. (We quote from Lillie's (1919) summary.)

"Important changes preparatory to hatching take place on the seventeenth to the nineteenth days. The fluid decreases in the amnion. The neck acquires a double bend so that the head is turned forwards, and in

consequence the beak is towards that part of the membranes next to the air-chamber. The intestine is retracted completely into the body-cavity, and on the nineteenth day the yolk-sac begins to enter the body-cavity. On the twentieth day the yolk-sac is completely included, and practically all the amniotic fluid has disappeared. The chick now occupies practically all the space within the egg, outside of the air-chamber. The umbilicus is closing over. The ductus arteriosi begin to contract, so that more blood flows through the lungs. The external wall of the allantois fused with the chorion still remains very vascular.

"Now, if the chick raises* its head, the beak readily pierces the membranes and enters the air-chamber. It then begins to breathe slowly the contained air; the chick may be heard, in some cases, to peep within the shell two days before hatching, a sure sign that breathing has begun. But the circulation in the allantois is still maintained, and it still preserves its respiratory function. When the chick makes the first small opening in the shell, which usually takes place on the twentieth day, it begins to breathe normally, and then the allantois begins to dry up and the circulation in it rapidly ceases. It then becomes separated from the umbilicus, and the remainder of the act of hatching is completed, usually on the twenty-first day."

Since the allantoic circulation is maintained till the twentieth day it is quite possible that strong chicks may be able to pierce the shell without ever having breathed through the air-cell. This is evidenced by the chicks occasionally found hatching (or pipping the shell) at the small end of the egg. Although in certain of these cases the air-cell may be at the small end, it is more likely that such chicks were strong enough to break the shell and begin normal breathing before allantoic circulation stopped, and without the intermediate use of the air-cell. It is probable, however, that the extra energy consumed by the physical exertion attendant upon the attempts to pierce the shell puts a burden on the respiratory apparatus greater than can be accommodated by the allantois alone. The normal chick has both pulmonary and allantoic respiration to meet this burden. The chick in Position II, III, or IV is in most cases unable to reach the air-cell, and is therefore dependent on the allantois alone. It is reasonable to assume that only the strongest chicks are able to overcome this handicap and hatch successfully.

* The chick does not "raise" its head in the usual sense of the expression, *i.e.* extension (in contradistinction to flexion) of the head upon the neck. Actually it thrusts the beak forward really toward its back, and so pierces the inner shell membrane to enter the air-cell (see fig. 1).

Some idea of the importance of pulmonary respiration at this stage is conveyed by the observations of Dr F. R. Horner (quoted by Tegetmeier, 1867), that before breaking the shell the chick breathes at the rate of about eighty times a minute, but that this rate decreases after a hole has been made in the shell large enough to admit air freely.

Histological examination of lung tissue from chicks found fully formed, but dead in Positions II and III, showed plainly that the lungs, though well supplied with blood, had never been used.

In addition to the handicap imposed by the inability to breathe through the lungs, many of the chicks in Positions II and III are hampered mechanically in their efforts to break the shell. It is manifest that a chick with the head in the small end of the egg is very cramped. Many have been observed in positions where the head had apparently no room whatsoever to move. Others were placed so that even if they did strike the shell the blows would be in such a slanting direction that they would have very little effect.

It should be noted that the breaking of the shell membranes, and shell is facilitated—if not accomplished entirely—by the egg tooth. The position of this structure on top of the upper beak suggests that the taps on the shell are made by upward movements of the beak rather than by direct forward hits. The observations of Breed (1912) confirm this suggestion. Some of the chicks found by us in Positions II and III were so situated that it was difficult to see how the egg tooth could be brought into efficient action against the shell, even had the cramped quarters of the chick permitted any upward thrusts of the beak.

In some cases ruptured blood-vessels in the chorio-allantoic membrane surrounding chicks dead in abnormal positions, in unpipped eggs, suggested that their efforts to break the shell had not only failed but had probably wrought their own destruction by injuring the only available means of respiration.

It is difficult to say whether or not the failure of the head to be under the wing affects the ability to hatch of the chicks occasionally found in this position. Réaumur was of the opinion that the wing served to guide the thrusts of the beak.

Causes.

From the fact that the proportions of the various abnormalities do not vary a great deal among the eggs from different sources, it is doubtful if they are due to any one method of incubation, turning, or handling. It would be of interest in this connection to determine the

frequency of these abnormal positions in eggs incubated under hens, which are turned much more than those in incubators. It seems possible that Position IV in which the embryo is normal except in being turned from the air-cell, may be caused by its being turned to that position just before it becomes too large to move freely within the shell. If so, the critical point after which it would be safer not to turn the eggs, comes before the period at which turning is usually stopped, *i.e.* the end of the 18th or 19th day. In this connection the recent experiments at the South Dakota Experiment Station (1927) are of special interest. It was found that eggs turned only to the 15th day gave better hatches than those turned to the 19th day. If turning beyond the critical point be the cause of Position IV, the fact that only a part of the embryos examined by us were affected can be explained by their being not all at exactly the same stage of development.

It is probable that some of the abnormalities (particularly Positions II or III of the full grown embryos) result directly from an incorrect orientation established by the first few cleavage divisions. Normally the embryo first lies at right angles to the long axis of the egg, and in such a way that when the small end of the egg is pointed away from the observer the head of the embryo is directed to the left. This orientation is preserved until about fourteen days of incubation, at which time the embryo becomes adjusted to the shape of the egg.

Duval (quoted from Lillie) found that out of 166 fertile eggs observed at an early stage, 124 embryos were oriented exactly as described, in 39 the axis was slightly oblique, 2 had the head toward the large end and 1 was completely reversed from the normal position. Similarly, rearrangement of Féré's (1899) data shows that of 296 embryos examined by him, 42 deviated from the correct axis—3 by 15°, 27 by 45°, 7 by 90°, and 5 by 180°. Féré classed these with other abnormalities induced by exposure to fumes of alcohol, but since they occurred just as frequently in his controls as in treated eggs, it is evident that the incorrect orientation occurred naturally and was not due to experimental procedure.

These observations afford quite a likely explanation of at least some of the abnormalities in position, but it remains to be shown what factors cause the embryonic axis to vary from its normal position at right angles to the long axis of the egg as delineated by the chalazæ. The extent to which different methods of storing, handling, turning, and incubating eggs can cause these abnormal positions must also be determined experimentally.

The incidence of embryos in two malpositions combined suggests that no single cause is responsible for more than one abnormal position. The average frequency of Position I is 9·25/100 (Table V). The average frequency of Position II is 18·31/100. If each of these malpositions was caused by entirely independent agencies one would expect them to occur together purely by chance in (9·25/100) (18·31/100) = 1·69/100 cases. If both of these positions could be induced by the same cause, the frequency of embryos combining Positions I and II would be greater than 1·69 per cent. expected by purely chance association. Actually these two malpositions occurred together in only 0·39 per cent. of the total number, *i.e.* considerably less than would be expected on the basis of two independent causal agencies. It is difficult to believe that any embryos in both Positions I and II could possibly have hatched. The data may therefore be taken to indicate not only that two separate factors are responsible for Positions I and II, but also (since their coincidence is less than would be expected by chance association) that the two causes are probably somewhat antagonistic.

Similarly the frequencies of Positions II and III are 18·31 and 7·41 per cent. respectively of all the embryos examined. On the basis of a purely chance association, one would expect them to occur together in 1·35 per cent. of the population. Only 0·37 per cent. were recorded in II and III together. Here, again, the evidence shows that two distinct agencies are at work, one causing Position II, the other Position III, and that they tend to be antagonistic rather than associated. Such an antagonism may be nothing more than the greater mechanical restriction imposed upon an embryo with the head in the small end, than on one with the head in the large end, so that in the former case it is more difficult for the head to become turned to the left away from the normal position.

The analysis just given indicates that in the attempt to eliminate malpositions a distinct cause for each one must be sought.

Since in the case of the majority of the eggs examined it was impossible to trace those from individual hens, we are unable to say if the tendency to produce embryos in these abnormal positions is hereditary or not. There is some evidence from our own flock that this may be so.

No significant difference was observed between the frequencies or proportions of these abnormal positions in eggs examined early in the season (February) and those from April and May settings.

Summary.

1. An examination has been made of 11,797 eggs which had failed to hatch, among which were 5050 embryos which had died after the eighteenth day.

2. Four major malpositions of the chick embryo are described and the frequency of each given.

3. It is concluded that one of these, in which the head is buried between the legs, definitely prevents hatching. In the material examined, this abnormality was responsible for 9·25 per cent. of the mortality among embryos of eighteen days or over.

4. It is suggested that the other three malpositions usually result fatally, by reason of their preventing pulmonary respiration in the embryo as well as by mechanical hindrance.

5. Of the embryos over eighteen days, nearly 56 per cent. were in one or another of the four major malpositions.

6. Possible causes are discussed, and the suggestion made that some of the abnormal positions result from an incorrect orientation of the embryo established early in cleavage.

Acknowledgments.

The writer is indebted to the Romanno Bridge Poultry Farm, West Linton, W. Johnston & Co., Comrie, and the Sussex Hatcheries, Glasgow, for material supplied for this investigation. He is especially indebted to Dr A. W. Greenwood for valuable assistance in examining the eggs. Thanks are also due to Professor F. A. E. Crew for constructive criticism and advice, and to Mr J. M. A. Chisholm, of this Department, who did all the photography.

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EXPLANATION OF PLATE.

Fig. 1. Normal position prior to hatching.

Figs. 2 and 3. Position I. Head buried between the thighs.

Fig. 4. Position II. Head in the small end of the egg.

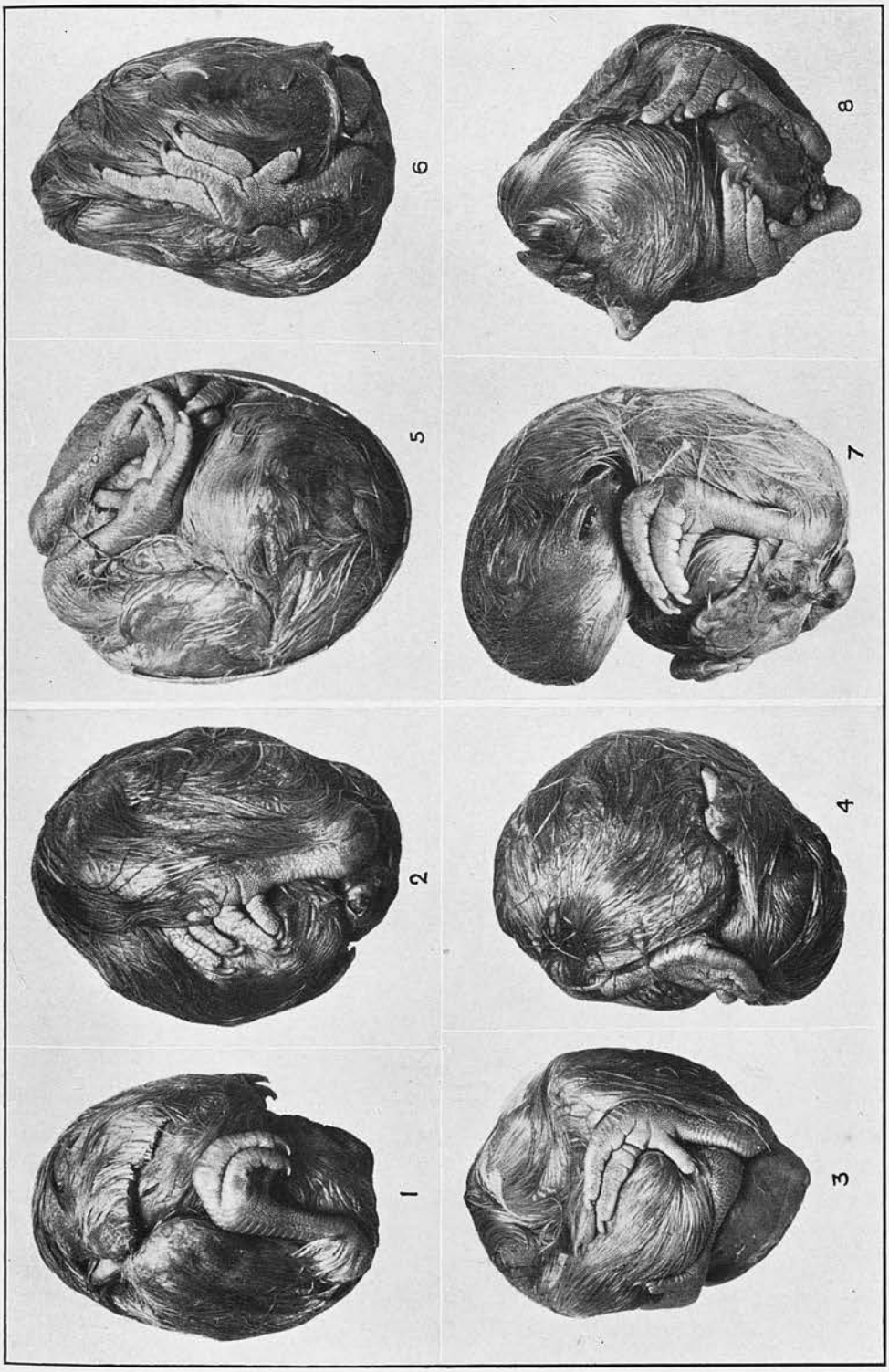
Fig. 5. Positions II and III combined. Head in the small end of the egg, and turned to the left instead of to the right.

Fig. 6. Position III. Head turned to the left and in this case extending to the small end of the egg.

Fig. 7. Position III. Head to the left.

Fig. 8. Position IV. The embryo is turned so that the beak is buried away from the air-cell.

(*Issued separately April* 1, 1929.)



F. B. Hutt. I.—Plate I.

XI.—Studies in Embryonic Mortality in the Fowl. II. Chondrodystrophy in the Chick.

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Chondrodystrophy.

Among the eggs incubated in January 1928 at this Department a number of embryos were found with the parrot beak and short legs peculiar to that abnormality first observed in chick embryos by Dunn (1923), and tentatively termed by him the "parrot" embryo. More recently Landauer's (1927) extensive study of the morphology and histology of these embryos has shown that the condition corresponds closely to the *chondrodystrophia fœtalis* or *achondroplasia* of mammals, of which the "bull-dog" calf of the Dexter and the achondroplastic dwarf in the human are well-known examples.

All embryos dying during incubation at this Department were thereafter systematically examined till by March it was found that 21 hens out of 76 from which eggs had been incubated had produced one or more chondrodystrophic individuals. Of these the 18 which gave most were put in one pen on 7th March, and all their eggs, till 1st April, were examined at 11 to 13 days of incubation. Thereafter all their eggs were allowed to incubate 21 days. When it was found that very few chondrodystrophics were obtained from the April eggs, breeding was discontinued except for random samples from these 18 hens set during May and June.

Description of Chondrodystrophy.

The more extreme cases of chondrodystrophy are characteristically and markedly abnormal. The lower beak is much shortened, and the upper beak bends down over it, giving the characteristic "parrot beak" appearance (Pl. I, figs. 1, 2, and 4). The frontal and parietal bones of the skull are vaulted anteriorly to make a much rounder head than is found in the normal embryos. The legs are exceptionally short. This is caused partly by a general shortening and thickening of the bones and partly by a bending which may be in the femur, tarso-metatarsus, or tibia (Pl. I, fig. 6). In our material, bending of the tibia was most

evident, although in some cases the tarso-metatarsus or femur was also badly bent. The effect of this bending is to turn the plantar surface of the foot toward the body. The epiphyses of the long bones are often markedly enlarged.

In less extreme cases there may be no trace of prognathia in the upper beak, but the bend in the tibia is still present in varying degrees, some of which can be best detected by microscopic examination of sectioned bones.

In our examination of eleven- and thirteen-day living embryos it was noticed that feather growth was so retarded in extreme chondrodystrophics that in some cases less down was present at 13 days than is found on the normal embryo at 10 days (Pl. I, figs. 1, 2, and 3). In chondrodystrophic embryos found dead in other material at 16 to 18 days it was noted that the down was somewhat thinner and seemed somewhat curled, so that in some cases one could identify a chondrodystrophic individual before the beak or tibia had been examined (Pl. I, fig. 5).

In the young living embryos (11–13 days) it was also noticed that the embryos had a swollen appearance, caused by a gelatinous substance just beneath the skin. Traces of this were apparent in older embryos.

Extreme cases which have survived to the 18th day present the characteristic appearance shown in Pl. I, fig. 5. The yolk-sac has been retracted, and is so much larger in proportion to the dwarfed "chondro" embryo than to the normal chick, that the general appearance of the former is a small head protruding from a spherical mass, mostly yolk. Less extreme cases, 18 days or over (Pl. I, fig. 6), may approach the normal so closely as to require sectioning of the tibia and microscopic examination.

For a complete account of the pathology and histology of the chondrodystrophic skeleton the reader is referred to Landauer's (1927) detailed study.

Dead chondrodystrophic embryos were found by us at all stages from 8 to 21 days of incubation. The majority of the eggs from our own chondrodystrophy matings were examined at 13 days, at which time the abnormality is easily recognised. In some cases the embryos had died before the tenth day. In the eggs from outside sources the majority of chondrodystrophic embryos had died during the second week of incubation. We have not known one to hatch, but found one chondrodystrophic embryo alive in the shell at the twenty-second day.

Chondrodystrophy has been found combined with microphthalmia and exencephaly, but only in a very small and insignificant number of cases.

Frequency of Chondrodystrophic Embryos.

It was very difficult to determine the exact number of chondrodystrophics in the material from the three outside sources—Commercial Poultry Farms A, B, and C. (Full details of this material are given in the first paper of this series.) Decomposition had become well advanced in many cases, so that in most of the embryos under 10 days definite diagnosis was impossible. Only 6 chondrodystrophics were positively identified in the 2046 "first test" eggs (*i.e.* dead before 9 days) examined, and these were all from one lot of 280 such eggs removed at Poultry Farm A from eggs set 26th February. Of the eggs classed as dead-in-shell, 24 per cent. were too young or too decomposed to be of value. The incidence of chondrodystrophy in the remaining "valid" embryos is shown in Table I. Only those in which identification was positive have been included.

TABLE I.—INCIDENCE OF CHONDROS FROM CONTRIBUTING FLOCKS.

Source.	D.I.S. examined.	"Valid" Embryos.	Chondrodystrophics.	Per cent. of valid Embyros.
Poultry Farm A	8059	5990	48	·80
" " B	744	653	52	7·96
" " C	654	492	12	2·44
Our own stock D	...	1900 (eggs set)	124	6·53

At this Department 124 chondrodystrophic embryos were found. They first appeared in the eggs from some of 65 hens being used to test the fertility of partially castrated males. These eggs were incubated only till the sex of the embryo could be easily determined, *i.e.* to about the eleventh day. As soon as chondrodystrophic embryos were observed careful examination was made for them in all eggs set in this and other experiments. By 8th March, 21 hens had been found to produce one or more specimens of this abnormality, and 18 of these were then penned separately. One other hen subsequently produced 2 chondrodystrophic embryos. All eggs from these 18 were opened at the eleventh or thirteenth day, until early in April, when it became apparent that the chondros were decreasing. Thereafter the chicks were allowed to hatch, and only the unhatched eggs were examined for abnormalities.

Out of a total of approximately 1900 eggs set at this Department from 126 hens, 124 chondrodystrophic embryos were obtained, all in the progeny of 22 hens.

Seasonal Incidence of Chondrodystrophy.

The data just given and those in Table I give little indication of the extent to which chondrodystrophy may be responsible for embryo mortality. This is because the condition was found much more frequently early in the season than in the later hatches. Thus, of the 48 chondrodystrophics out of 5990 "valid" embryos from A, 37 appeared among 2453 embryos from February incubations. The remaining 3537 dead embryos from March and April settings contained only 11 specimens of this abnormality. Similarly, in B's eggs the frequency of chondrodystrophics was 8·5 per cent. of the valid embryos in February, 6·9 per cent. in March, and 3·3 per cent. in April.

In our own "chondrodystrophy matings" (*i.e.* the 21 chondrodystrophy-producing hens) the frequency of this abnormality was as high as 34·78 per cent. *of the eggs set* in January, but declined steadily to a complete absence in June (Table II).

TABLE II.—SEASONAL DISTRIBUTION OF CHONDRODYSTROPHIC EMBRYOS FROM 21 CHONDRODYSTROPHY-PRODUCING MOTHERS AT THE A.B.R.D.

Month of Laying.	Hens Laying.	Fertile Eggs Set.	Chondrodystrophic Embryos.	
			No.	Per cent.
January	6	23	9	34·78
February	21	150	48	32·00
March	20	204	59	28·92
April	13	47	5	10·64
May	8	18	1	5·55
June	9	38	0	0
Total		480	122	

Dunn (1927) found a distinct tendency toward a decrease in chondrodystrophy in his "C" series as the season advanced, but was dealing with smaller numbers over a shorter season, and therefore made no definite conclusion. The data in Table II, supplemented by that from the A, B, and C eggs, establish fairly conclusively that the occurrence of chondrodystrophy is influenced by season, and drops to a comparatively low figure in the summer months. Two chondrodystrophics have been obtained here from eggs laid in July, and therefore it cannot be said that it does disappear entirely. It was noted, particularly in the eggs from

Poultry Farm A, that chondrodystrophic embryos found later in the season were "low grades," *i.e.* much less abnormal than those observed from February settings.

One hen, Brown Leghorn 115, had a chondrodystrophic embryo in her egg of 4th February, but seventeen eggs recorded between that date and 7th May contained only normal embryos. The egg laid on the latter day yielded an extreme chondrodystrophic embryo, with parrot beak and an angle of almost 90° in the tibiæ. Another hen produced two chondrodystrophics only—1 in an egg laid in February and 1 from the egg of 9th May. Our data suggest no reason for such a discontinuous distribution.

♀ No. 92 produced 28 chondros out of 40 fertile eggs laid between 19th January and 22nd March. Three of the remaining 12 eggs contained normal embryos and 9 died at an early stage of development. This laying period was one continuous cycle, with the good record of 40 eggs in 73 days. The hen went broody on 23rd March, and did not resume laying till 6th April. The eggs laid on 19th, 20th, and 22nd March all gave rise to chondrodystrophic embryos, but in not a single embryo out of 23 eggs incubated after the broody period was there any trace of this abnormal condition. This suggests that the two weeks' abstinence from laying permitted the righting of whatever condition had been abnormal during the long winter cycle.

Evidence of a Genetic Basis for Chondrodystrophy.

From the facts that the incidence of chondrodystrophy from different hens ranged from 1 up to 28, and that there was no consistent semblance of genetic ratios, evidence for the abnormality being due to the genetic constitution *of the embryo* is negative. However, the greater frequency of the abnormality in the progeny of certain birds of our flock than from others suggests strongly that the tendency to produce it is inherited. In addition to No. 92's 28 chondrodystrophics, another hen had 14, and still two others 10 each. In contrast to these, many hens under identical conditions in the same pen had no chondrodystrophics whatever in progenies of over 25 embryos examined. The records of the 21 hens in the chondrodystrophy-matings are given in Table III.

Since not all the eggs from each hen were incubated during April and May, we have not attempted to compare the frequency of chondrodystrophics from different mothers on a percentage basis. If the chondrodystrophic progenies of the same size were grouped, the distribution of these groups would not even approximate a Poissons series

TABLE III.—PRODUCE OF FEMALES IN CHONDRO MATINGS.

Hen.	Fertile Eggs Incubated.	Chondrodystrophic Embryos. No.
92	61	28
142	37	14
35	24	10
68	25	10
105	19	8
156	26	8
8	17	6
143	38	4
114	30	4
20	10	5
61	31	3
370	13	3
139	41	3
95	9	3
106	14	3
117	20	2
30	20	2
115	20	2
38	15	2
58	12	1
24	9	1

such as would be expected if the higher incidence of the abnormality in the progeny of certain females were due to chance. The chance of getting progenies of 28 and 14 in such a series would be infinitely small, and the progenies of only one chondrodystrophic would be much more frequent than were actually found.

Since the high incidence of chondrodystrophic embryos in the progenies of certain hens cannot be due to chance, and since only certain hens showed the tendency while others under identical environmental conditions produced only normal embryos, we are led to believe that the causal agency is an hereditary physiological abnormality in the dam which, under certain environmental conditions, results in the production of chondrodystrophy in the embryo.

Breeds.

The majority of the birds used for breeding at this Department are Brown Leghorns. Eighteen of the 22 hens which produced chondrodystrophic progenies were of this breed, the other four being White Wyandotte, Rhode Island Red, Frizzle, and Cross-bred. Our own data and that from the three outside sources give little indication that any one breed is more likely to produce chondrodystrophic embryos than another,

except that they were more frequent in the progeny from Light Sussex ♀ × R.I.R. ♂ at Poultry Farm B than in other breeds at the same place. Their occurrence, however, was not as frequent there as in the Brown Leghorns at this Department; and, since records of individual hens at B were not available to us, we are unable to say if their greater frequency in this cross was due to a general tendency of many hens toward chondrodystrophy or to one or two individuals with a marked tendency such as was exhibited by our ♀ No. 92. The latter is a White Wyandotte. Chondrodystrophic embryos were found in eggs from all the nine breeds and varieties represented by the material examined from the four sources, a list of which is given in the first paper of this series.

Age of Dam.

Of the 22 chondrodystrophy-producing mothers found at this Department, 1 was 3 years old, 3 were 2 years old, and the rest were pullets almost a year old at breeding. Since this represents fairly well the proportions of hens of these ages bred this season, it is doubtful if age of dam has much to do with the incidence of chondrodystrophy.

Sex of Chondrodystrophic Embryos.

Males predominated among the first chondrodystrophic embryos to appear at this Department. This was not in accord with Dunn's finding that of 51 chondrodystrophics sexed by him 26 were males and 25 were females, but it seemed explicable on the basis of the proportion of males being normally higher early in the season, as the work of Jull (1924) would indicate. We have determined the sex of 83 chondrodystrophics obtained from chondrodystrophy-producing matings at this Department before 1st April, the ratio being 50 males to 33 females. Of 91 normal embryos from the same hens and of the same period, 41 were males and 50 females. The sex-ratio for the whole 174 embryos was 109·64.* The numbers expected on this basis in the four classes are given in Table IV.

At first glance it would seem that the number of males is significantly high in the chondrodystrophics and proportionately low in the normals. However, on application of the X^2 test for goodness of fit to this distribution, it is found that the value of P is 0·107. This has been calculated by the method and table of Fisher (1928), taking n as 2. This means that one would expect as poor fit (or worse) of observed

* Males per 100 females.

TABLE IV.—SEX OF CHONDRODYSTROPHIC AND NORMAL EMBRYOS AND EXPECTED PROPORTIONS IN EACH CLASS ON THE BASIS OF A SEX-RATIO OF 109·64 FOR THE ENTIRE POPULATION.

Class.	Observed.	Expected.
Chondrodystrophic ♂	50	43
Chondrodystrophic ♀	33	40
Normal ♂	41	48
Normal ♀	50	43
Total . .	174	174

to expected ratios in about 11 per cent. of cases, and that the higher proportion of males observed in chondrodystrophic embryos is not significant.

Relation of Chondrodystrophy to Mortality.

From Dunn's observations and those made at this Department it may safely be assumed that chondrodystrophic embryos do not hatch but usually die before 19 days. Table I shows that this abnormality caused 0·80, 7·96, and 2·44 per cent. of the mortality among such dead-in-shell as could be classified at Poultry Farms A, B, and C respectively, covering the whole season. Allowing for doubtful cases discarded and for the many embryos of less than 8 days in which classification was not attempted, it is probable that these percentages would not be far wrong if applied to all the mortality at each place. However, based on positively identified chondrodystrophics only, the condition was responsible for 0·55 per cent. *of the total mortality* at all ages at A and 5·12 per cent. of the same at B. These figures cover the whole season. As stated previously, chondrodystrophy has a much greater effect on the earlier hatches.

The records of our own flock show that the abnormality may be more serious than at A, B, or C. Out of approximately 1900 *eggs set* from January to June, 122, or 6·4 per cent., produced chondros. Table II shows that the frequency of this abnormality from the group of chondro mothers ranged from 34·78 per cent. to 0·0 per cent. of the fertile eggs set according to season. The record of individual hens may be much worse, *e.g.* that of ♀ No. 92, who had only 3 normal embryos out of 40 fertile eggs set up to 22nd March.

There is some evidence from the birds mated here that chondrodystrophy may be associated with an unusually high number of deaths

before 7 or 8 days, *i.e.* apart from the recognised chondrodystrophics. In addition to No. 92's record, hen No. 156, from whom 31 fertile eggs were set up to 2nd April, produced 5 normal embryos, 8 chondrodystrophics, and the remaining 18 embryos died at various stages, practically all before 7 days. Other females producing this abnormality showed a similar tendency. Unfortunately, there are not adequate controls to establish this point.

It should be noted that the percentage frequency of chondrodystrophy varied considerably among eggs from the four sources from which they were obtained. To some extent this would be expected, even if the actual frequency of chondrodystrophics were the same in all stocks. Since the percentage hatch was lower in some cases than others (*vide* Table II in the preceding paper of this series), one would expect a greater number of normal embryos among the dead-in-shell from such hatches, and consequently a lower percentage of abnormals. Possibly this accounts to a slight extent for the low percentage of abnormals from Poultry Farm A. However, at Farms B, C, and at this Department, where the percentage hatches were much the same, the frequency of chondrodystrophics varied much more than did that of teratological abnormalities and malpositions. It must not be inferred, therefore, that chondrodystrophy is responsible for mortality to the same extent in all flocks.

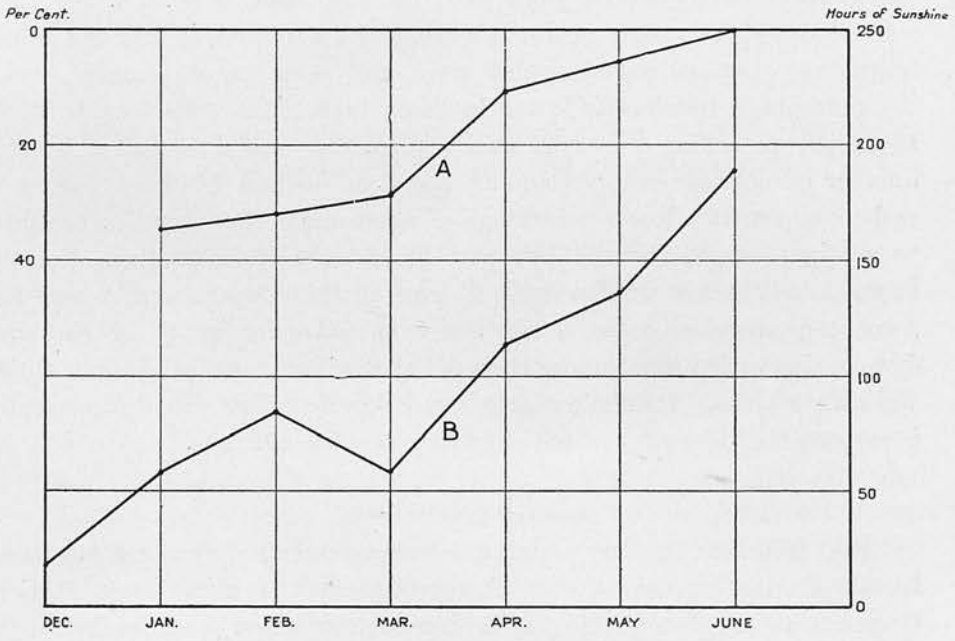
Discussion.

The fact that, so far as we are aware, chondrodystrophy has been found in America only at the Storrs Agricultural Experiment Station, Connecticut, suggests that it may be less common there than here, where it was obtained in all four sources from which eggs were received. Additional evidence for this is found in Dunn's report that the abnormality constituted about 1·5 per cent. of 4644 embryos dead after the 7th day examined at Storrs, whereas, in three out of four flocks here, the frequency was much higher.

In attempting to determine factors concerned in the etiology of chondrodystrophy this difference must be considered. The marked decline in its frequency from January to June in our own chondro matings, together with a similar decline in eggs from Poultry Farms A, B, and C over a shorter period, is still more important. Both together suggest that some difference in weather is a factor. To find that factor, one must look for something which is aggravated in this locality in January, February, and March, but becomes ameliorated from March on, and which is more acute in this climate than in the United States. Lack of

sunshine is at once suggested. Edinburgh is at 55° 36′ North Latitude, and is therefore subject to very short days in winter. This condition is often aggravated in winter by mist and cloudy skies, so that the lack of sunshine is quite evident to any visitor.

Examination of the meteorological data at the Royal Observatory, Edinburgh, led to the conclusion that sunlight was of more importance than temperature, humidity, barometric pressure, or any other determiner of climate. Text-figure 1 shows in graphic form the total hours of sun-



Text-fig. 1.—Graphs showing (A) the monthly frequency, in percentage, of chondrodystrophic embryos from the 21 hens in the chondrodystrophy-producing matings at the Animal Breeding Research Department from January to June 1928. (B) The total hours of sunshine per month at Edinburgh, from December 1927 to June 1928.

shine as recorded at the Observatory, half a mile from the poultry-yards of this Department, from December 1927 to June 1928. The decrease in the frequency of chondrodystrophic embryos at this Department, also recorded, parallels the increase in sunshine closely.

In support of this hypothesis, the average hours of sunshine per month from December to June, over a period of 28 years at Edinburgh, is compared in Table V with the same data for a period of 45 years at New York. The latter data are taken from the records of the New York Meteorological Observatory, kindly furnished by the Royal Observatory, Edinburgh.

TABLE V.—AVERAGE HOURS OF SUNSHINE PER MONTH AT EDINBURGH AND NEW YORK.

Month.	Edinburgh, 28 years.	New York, 45 years.
December . .	41·0	141·0
January . . .	44·8	140·7
February. . .	70·7	159·2
March . . .	106·1	205·7
April . . .	142·2	229·3
May	162·1	270·5
June . . .	192·4	292·2

Admittedly the hours of sunshine at New York do not hold for all other parts of the United States, but they should apply reasonably closely for nearby Connecticut. The difference between Edinburgh and New York sunshine is so marked as to suggest at once why chondrodystrophy has been of such rare occurrence at the Storrs Connecticut Station and in the United States generally, compared with the A.B.R.D. flock here and other flocks nearby.

Chondrodystrophy must not be confused with leg weakness or avian rickets, which is caused by a lack of Vitamin D or of adequate minerals or both, and cured by direct sunshine, ultra-violet light, or cod-liver oil plus proper minerals. At this Department the flock received a grain ration, consisting of maize, wheat, and oats, and a mash mixture of wheat middlings, bran, ground oats, soy-bean meal, alfalfa meal, fish meal, bone meal, and salt. Oyster-shell was supplied in hoppers, and crude cod-liver oil was fed in the mash till well on in April. Cabbage was given occasionally. All birds were out of doors daily. There was no evidence of leg weakness in the laying stock, and no unusual number of soft- or thin-shelled eggs. No. 92's eggs were all of particularly firm, sound shell. The rations of Poultry Farms A and B had no deficiency of any of the vitamins or minerals recognised as essential. Both were feeding cod-liver oil. Farm C's rations are not known.

Hart, Steenbock, *et al.* (1925) have shown that the hatchability of eggs from hens deprived of sunshine for several weeks declines to a low figure, but can be quickly restored by irradiation with ultra-violet light and much more slowly restored by feeding cod-liver oil. The deficiency of ultra-violet light in this locality during the fall and winter months is even more acute than is indicated by the small number of hours of sunshine. The relative humidity here averaged 85 per cent. for December, January, February, and March. Under such conditions the amount of

ultra-violet light actually reaching the fowls would be considerably less than in a drier atmosphere. It seems possible that under such acute shortage of ultra-violet light, cod-liver oil may supply enough Vitamin D to permit normal calcium assimilation, but cannot make up for *other beneficial effects* of the radiant energy. The same reason may explain its slower curative action in the experiments just cited. That the value of sunshine is not confined to its catalytic rôle in calcium and phosphorus metabolism is evidenced by its bactericidal powers, its value in the treatment of tuberculosis, and its ability to increase the resistance of the body to toxins and to invading micro-organisms.

Evidence of a genetic basis for chondrodystrophy has already been given, and the belief expressed that the tendency to produce the abnormality is inherited. Such a statement is incompatible with Dunn's (1927) finding that there was no greater incidence of this abnormality among his inbred chondrodystrophy series than among controls. However, it is evident that, whether or not the hereditary tendency be present in the same degree in all affected individuals, it is not manifested in the same degree in their offspring, and in any case is manifested only under unfavourable conditions. Many of our fowls produced only 1 or 2 chondrodystrophic embryos; none of Dunn's produced more than 6. If the abnormality is most frequently expressed under sunshine starvation, it is quite conceivable that most of the individuals in the flock at Storrs were safely past the border-line of such starvation by the middle of February, when incubation was begun, and that the tendency to produce chondrodystrophic embryos was not manifested to the same extent as it might have been under less favourable conditions. Table V indicates that the average sunshine is almost as great at New York in February as it is here in May, at which latter season chondrodystrophy had decreased in our chondrodystrophy-matings to one-sixth of its earlier intensity.

What rôle sunlight may play in the etiology of chondrodystrophy the writers cannot state. Since two embryos exhibiting the abnormality were obtained in eggs from Poultry Farm B, set in July, it is doubtful if lack of sunshine is the sole cause. The provisional hypothesis is suggested that extreme lack of sunshine superimposed upon an inherited susceptibility results in the production of eggs deficient or incorrectly balanced with respect to some essential constituent, and that such eggs give rise to chondrodystrophic embryos.

The writers are indebted to the Romanno Bridge Poultry Farm, West Linton; W. Johnston & Co., Comrie; and the Sussex Hatcheries,

Glasgow, for eggs supplied for this investigation. Thanks are also due to Professor Crew for his interest and constructive criticism; to the Royal Observatory, Edinburgh, for meteorological data; and to Mr J. M. A. Chisholm for the photographs.

SUMMARY.

1. Among 7135 chick embryos of nine days or over, obtained from unhatched eggs from three poultry farms, 112 cases of chondrodystrophy were observed. From 1900 eggs set at this Department, 124 chondrodystrophic embryos were obtained.

2. The mortality caused by this condition varied in different flocks from 0·80 to 7·96 per cent. of the embryos dying after the eighth day; at this Department it was responsible for loss of 6·5 per cent. of the eggs set.

3. The incidence of chondrodystrophic embryos was found to be greatest in January and February, and to decline steadily thereafter to an almost complete absence in June.

4. The occurrence of chondrodystrophy appears to be independent of breed of fowl, sex of embryo, and age of dam.

5. The number of these abnormal embryos produced by individual hens ranged from 1 to 28.

6. Evidence is advanced in support of the theory that the causal agency is an hereditary physiological abnormality in the dam which, under certain unfavourable conditions, results in the production of chondrodystrophy in the embryo.

7. It is shown that the incidence of chondrodystrophy appears to be inversely proportional to the amount of sunshine, and the suggestion is made that lack of direct sunlight is a factor in the etiology of the abnormality.

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EXPLANATION OF PLATE.

(All photographs are natural size.)

Fig. 1. Three eleven-day embryos from eggs set on the same day and in the same incubator. The one on the right is normal; the other two are from ♀ No. 92, and are typically chondrodystrophic. In addition to the vaulted skull, parrot beak, and shortened bent legs of the two abnormal embryos, a difference in the rate of growth of the down is already apparent.

Fig. 2. A thirteen-day chondrodystrophic embryo from ♀ No. 92. The retarded growth of down is characteristic of acute chondrodystrophy.

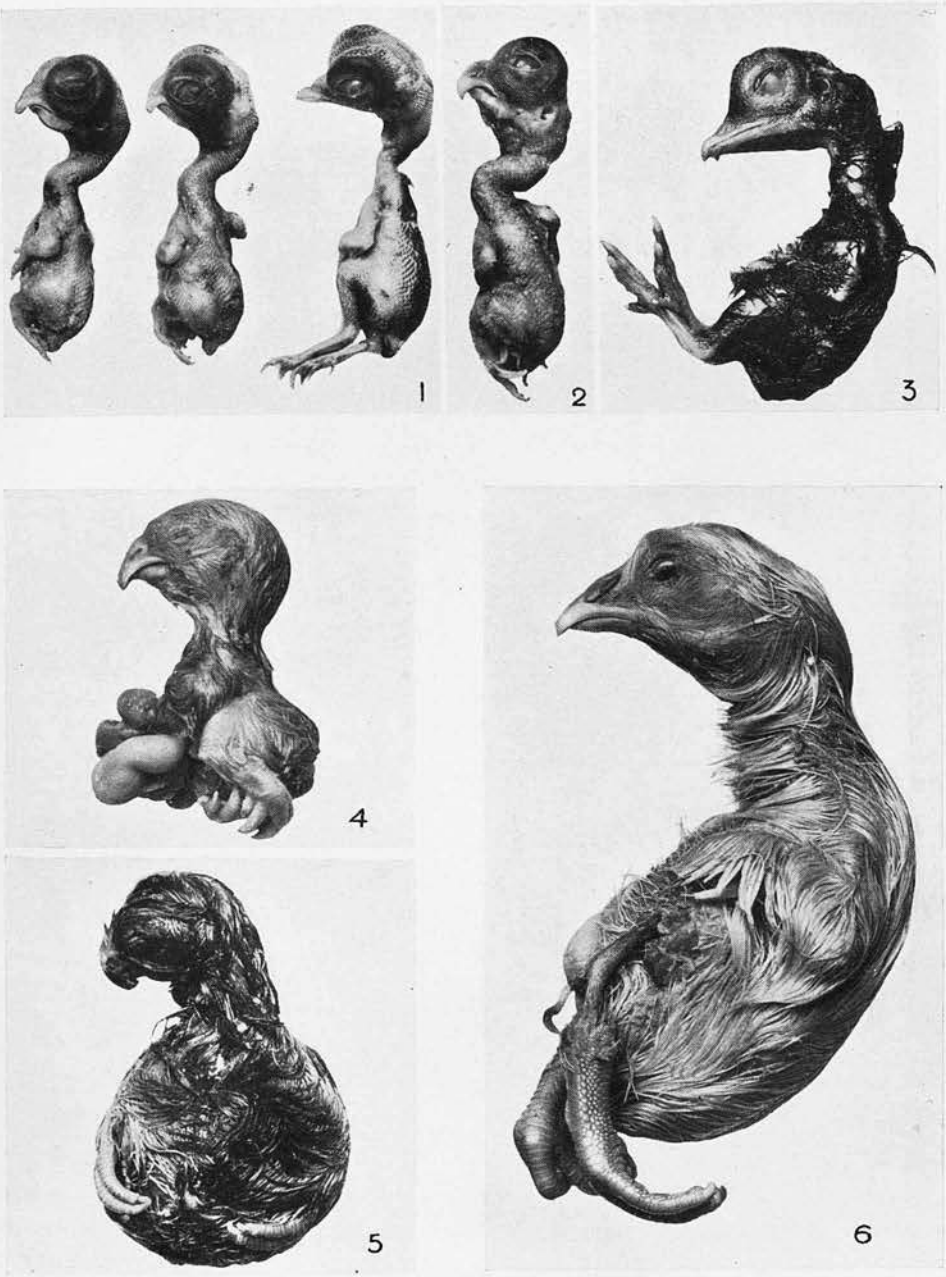
Fig. 3. A thirteen-day normal embryo from the same setting as fig. 2. The difference in length of the legs and in the direction of the plantar surfaces of the feet in fig. 2 and fig. 3 is well marked.

Fig. 4. A specimen, estimated about 18 days, exhibiting extreme chondrodystrophy complicated with ectopia. The sharp angle of the bend in the tibia is evident; a similar bend is also present in the much reduced tarso-metatarsus.

Fig. 5. Extreme chondrodystrophy in an embryo, estimated at 20 days. The yolk-sac has been drawn into the body cavity, and gives the embryo the appearance of a round ball, from which the feet project. The curly appearance of the down is characteristic of extreme chondrodystrophy.

Fig. 6. A less extreme case of chondrodystrophy in a twenty-one-day chick. The slight protrusion of the upper beak might have passed unnoticed, but the backward turning of the feet suggested the typical bending of the tibia revealed when the muscles covering that bone were removed.

(*Issued separately April* 1, 1929.)



F. B. Hutt and A. W. Greenwood. II.—Plate I.

XII.—Studies in Embryonic Mortality in the Fowl. III. Chick Monsters in Relation to Embryonic Mortality.

By F. B. Hutt, B.S.A., and A. W. Greenwood, Ph.D., Animal Breeding Research Department, University of Edinburgh. (With Three Half-tone Plates.)

(MS. received October 5, 1928. Read January 7, 1929.)

During the course of an examination of some 12,000 eggs which had failed to hatch, abnormal embryos of three distinct classes were encountered. Abnormalities of position and chondrodystrophy have been discussed in previous communications of this series (Hutt 1928, Hutt and Greenwood 1928).

The present paper deals with various types of monsters already well described in the literature of teratology. Their description in this paper will therefore be no greater than is necessary to establish their identity.

Full details of the material examined, which came from three large commercial poultry farms and from this Department, have been given in the first paper of the series.

Types of Monsters Encountered.

1. *Hyperencephaly.*—The characteristic features of this monstrosity include a complete absence of the roof of the cranium, marked reduction or absence of the upper beak, and almost invariably absence of both eyes. There is usually extreme shortening of the neck, and ectopia is conspicuous in over 75 per cent. of the specimens (figs. 1, 2, and 3).

2. *Exencephaly.*—Included in this class are various types of meningocele in which a portion of the brain extrudes through the cranium but is still confined by the meninges. It is usually due either to an incomplete ossification of the brain case or to portions of the brain being pushed up so that the bones cannot meet in the normal suture lines. Such monsters are easily recognised by the protrusion from the head of a bare mass of tissue free from down (figs. 4, 6, and 7). In some cases this is in the occipital region (notencephaly), where it consists of an extrusion of the optic lobes; but in our material it occurred more commonly as an extrusion of part of the cerebral hemispheres in the anterior part of the skull (proencephaly). The terminology used is that of Dareste (1891), who classified hyperencephaly as a subdivision of the exencephaly group.

3. *Microphthalmia.*—The characteristic feature of this group is the failure of one or both eyes to develop normally (fig. 5). Dissection of several of these specimens showed that a small pigmented optic cup is present in many cases which appear to have no eye whatever. It is evident that anophthalmia without other complication is merely an advanced degree of microphthalmia, and therefore these two conditions are classed together in this report. The defect may be bilateral, but more commonly only one side is affected. Unilateral microphthalmia is accompanied by shortening of the face on the affected side, with the result that the upper beak is twisted to the short side (fig. 5).

Embryos were found with both microphthalmia and exencephaly (figs. 13, 14, and 15). In some of these there was also a reduction of the fronto-nasal process, suggesting that the condition was allied to a low grade of hyperencephaly. Ninety-three per cent. of all monsters observed in this survey were of one or another of the three types described above. Other miscellaneous abnormalities occurring in small numbers included the following:—

Ectopia—Eversion of the viscera.

Prognathia—Protrusion of the upper or lower beak beyond its fellow.

Cyclopia—A condition in which there is a single median eye. The cerebral hemispheres are absent, the mandible and tongue reduced, and the upper beak represented by a small proboscis-like structure above the eye (figs. 8 and 9).

Duplicity—Duplication of parts ranging in degree in this material from two upper beaks to two separate heads or duplication of the whole body except the head (figs. 10 to 12 and 16 to 20).

Otocephaly—Characterised by various degrees of approach of the ears on the ventral surface of the head, and different grades of reduction of the brain, extending down to entire absence of the head.

Malformed Limbs—Including thickened and flattened tarso-metatarsus, unilateral absence of leg muscles, and absence of one or more toes. Minor cases of twisted feet or toes were not included.

Absence of Premaxillæ—One embryo was abnormal only in the absence of these bones; another lacked also the nasals.

Frequency.

The number of these various monsters observed among 11,797 unhatched eggs examined are shown in Table I.

TABLE I.—FREQUENCY OF TERATOLOGICAL ABNORMALITIES.

Source.	Eggs.	Hyperencephaly.	Exencephaly.	Microphthalmia.	Both Micro. and Exenceph.	Miscellaneous.	Total.
A	9834	161	80	72	22	22	357
		1·74%	·81%	·73%	·22%	·22%	3·63%
B	1015	16	13	11	4	3	47
		1·57%	1·29%	1·08%	·39%	·29%	4·63%
C	654	1	5	7	3	2	18
		·15%	·77%	1·07%	·46%	·30%	2·75%
D	294	1	2	6	0	2	11
		·34%	·68%	2·04%	0%	·68%	3·74%
Total	11797	179	100	96	29	29	433
Per cent.		1·52	·84	·81	·25	·25	3·67

These defects can be recognised in chick embryos of 6 days or even less without microscopic examination, and were quite common in the dead germs removed at 8 or 9 days. For this reason the percentages given in Table I are calculated for the total numbers of eggs examined, including both dead-in-shell at hatching and those dying before the first test (usually made at 8 days). Many of the latter were too small or too decomposed to permit recognition of teratological abnormalities; but, since in our material these were found dead at all stages from 20 days right down to the limit of recognition, it is probable that the same types occurred beyond that limit. Dareste, who studied chiefly the earlier stages of chick embryos, found other types of abnormalities (*e.g.* omphalocephaly and arrested development of blood islands) which invariably caused death in the first week of incubation. It is practically certain, therefore, that the total loss from teratological monsters is much greater than is indicated by the percentage given above. Indeed, Stockard (1921), in his exhaustive studies of abnormalities induced experimentally in *Fundulus* (minnow), found that "there may be such minor defects as would escape observation until the hatched embryos were found to be unable to right themselves and swim." Possibly a similar condition is responsible in part for some of the chick embryos found fully formed dead in the shell, apparently normal but unable to hatch, and generally classed as "too weak to hatch."

Out of 96 specimens of microphthalmia encountered in this study, the defect was bilateral in 13 and unilateral in 83 Data for 55 specimens

of unilateral microphthalmia show that the left eye was affected in 31 cases and the right one in 24.

Ectopia, or extrusion of the viscera, was not recognised as being a distinct type of abnormality till well on in the investigation. Its frequency, therefore, is probably greater than the six specimens recorded would indicate.

Dareste states that otocephaly is a very rare occurrence in the chick. Only one specimen in our material, a chick with practically no head, fitted into his definition of this class, although, if Wright's (1923) grades of otocephaly were followed, the two cases of cyclopia observed would also be included.

The remainder of the 29 miscellaneous monsters included 8 cases of prognathia, 8 specimens exhibiting duplicity, 3 with malformed limbs, and 2 with prenexillæ or nasal bones absent.

Seasonal Fluctuations.

The monthly distribution of teratological monsters in the eggs from Poultry Farms A, B, and C is given in Table II. The total number of monsters from each source is given in brackets, and their monthly frequency is expressed as a percentage of the number of unhatched eggs examined. The month refers to the dates of setting, not of hatching.

Table II.—Seasonal Distribution of Monsters.

Month.	Farm A (357).	Farm B (47).	Farm C (18).
	Monsters.	Monsters.	Monsters.
February	4·77	5·16	...
March	3·51	5·21	2·80
April	1·65	2·40	2·61

A tendency toward a decrease in teratological abnormalities as the season advanced is evident in all stocks. The material from Farms B and C is rather small, and permits of greater variation and error; but the numbers from Farm A are large enough to indicate a steady decline from February to April, which may be considered reasonably significant.

Sex.

Wright found that otocephaly was much more common in females than males in his inbred guinea-pigs, the ratio being 55 ♀ : 26 ♂, or 32·1 per cent. males. We have records of sex of 147 monstrosities, representing the types most common in our material, but are unable to find any significant deviation from the normal sex-ratio in any class. The data are given in Table III.

Table III.—Sex of Monsters.

Class.	♂	♀	Sex-Ratio.*
Hyperencephaly . . .	30	34	88·23
Exencephaly	22	17	129·41
Microphthalmia . . .	21	17	123·53
Micro. + Exenceph. . . .	3	3	100·00
All classes	76	71	107·04

* Expressed as the number of males per 100 females.

Mortality.

Chicks exhibiting a low grade of microphthalmia recognisable by their twisted beaks occasionally hatch, but perish sooner or later. The comparatively large numbers found dead in this investigation lead to the belief that the majority affected with this abnormality die in the shell. Similarly, exencephalic chicks may very rarely hatch, but their death soon follows. We know of no case in which a hyperencephalic chick has hatched. Table IV shows the proportions of the different monstrosities found dead in the first, second, and third weeks of incubation.

Table IV.—Period of Mortality of Teratological Abnormalities.

	First Week, per cent.	Second Week, per cent.	Third Week, per cent.
Hyperencephaly . . .	28·57	32·97	38·46
Microphthalmia . . .	7·59	13·92	78·48
Exencephaly	...	12·50	87·50
Micro. + Exenceph. . . .	...	9·52	90·48

The majority of the last three classes in this table were embryos of 18 to 20 days. In many cases it was possible to estimate the age of

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The majority of the last three classes in this table were embryos of 18 to 20 days. In many cases it was possible to estimate the age of

hyperencephalic embryos only by the size of the posterior limbs. Very few of those that survived to the third week were fully formed. Apart from the occasional exceptions listed above, there is every reason to concur in Dareste's statement that "les monstres simples autosites produit chez les oiseaux périssent fatalement dans la coquille, avant l'éclosion."

Discussion.

The older teratologists expressed the belief that monsters were caused by an arrest in development which in some cases was believed to be brought about by pressure of the amnion or injury to that organ. In recent years it has been shown that the same abnormalities can be produced experimentally in fish embryos (Stockard 1921 and others) in which no amnion is present, as in the Amniota, and hence that the amnion is not responsible to the extent hitherto believed. The same abnormalities can be produced by different means, chief of which are chilling, lack of oxygen, exposure to fumes of alcohol, ether or other gases, and the use of such chemical substances as magnesium and butyric acid. An excellent review of such experimental teratology in the chick has recently been given by Hyman (1927). The common feature of all these methods is that they cause an arrest in the development of the embryo. Fairly conclusive proof of this is given by Stockard's demonstration that by chilling the embryo of Fundulus at a critical period he could produce every known type of monster, such types being dependent upon the period of interference with growth.

Whether or not all monsters occurring in the absence of experimentation are due to this same cause is another question; but the discontinuous mode of development of the chick embryo suggests that an arrest of growth may well be the cause of abnormalities in that species. Stockard found that, prior to the formation of the gastrula, the embryo was particularly susceptible to an arrest of development. The fowl's egg is ready to be laid at 21 to 27 hours after ovulation. The variation in the time required to traverse the oviduct results in corresponding differences in development at the time of reaching the vagina. This variation is made still greater by the fact that some eggs may be ready for laying at the end of the day but be retained by the hen till the following morning. Others may reach the end of the oviduct in the morning and be laid at 21 or 22 hours after ovulation. It has been shown by Patterson (1909) and others that gastrulation takes place very close to the time of laying, and it is therefore quite conceivable that an egg laid only 21 or 22 hours after ovulation may be just at the commence-

ment of gastrulation. Such an egg would be very susceptible to the chilling to which it is subjected after laying. The majority have completed gastrulation at laying, and are therefore better able to withstand the cessation or retardation of growth.

Another possibility must be considered. Alsop (1919) has shown that high or low incubation temperatures during the first three days will produce various types of defects in the eye and brain. However, since the abnormalities produced experimentally were dissimilar to those found in her controls it is questionable whether faulty temperature is responsible for the monsters found in our material. If there were a great number in any one hatch this would be suspected; but, since the actual frequency is very low, sometimes only one in the eggs remaining from an 85 per cent. hatch, it seems more probable that incubator conditions are not responsible in the majority of cases. There may be a differential susceptibility to slightly high or low temperature, but this again would depend largely on the degree of development when the egg went into the incubator.

The actual *modus operandi* of the arrest is best explained on the assumption that there is a critical period for the development of each organ of the body. At this time the anlage of that organ develops at a greater rate than other parts of the embryo. An arrest in development at such a stage slows or stops growth, so that all parts of the embryo are equal. When cell division is resumed, the particular region previously accelerated is not again able to assume its differential rate, and is therefore suppressed or poorly developed. The rest of the body may develop normally, but the affected anlage can never recover from its reverse and fulfil its original destiny.

In most experimental teratology with the chick, embryos are not incubated and observed beyond the first week, and it is therefore doubtful to what extent the abnormalities induced artificially correspond to those commonly found in our material which was incubated under supposedly optimum conditions. Our data afford one interesting case of agreement under natural conditions with results obtained experimentally. Stockard (1921) found that the period at which an arrest of development could affect the eyes of Fundulus was close to the critical period for the primary brain ventricles, and that as a result combinations of defects in these regions were common in groups of experimental embryos subjected to the same treatment. In our material the frequency of eye defects was 0·81 per cent. and of exencephaly 0·84 per cent. If these were induced by different causes one would expect them to be associated

by chance in only 0·0068 per cent. of cases, or less than once in the 11,797 eggs examined. Actually 29 embryos were found with both microphthalmia and exencephaly, a frequency of 0·25 per cent., which is over 33 times the expectation. This indicates that in the chick embryo, defects of eye and brain arising under optimum conditions tend to be associated, and that presumably the critical periods of development for each are close together. The chief difference between Stockard's findings in the minnow and these in the fowl is that in the former case a continuous development was arrested by experimental procedure, while in the latter a naturally discontinuous development was subjected under natural conditions to an arrest equally effective because operative at a critical period.

Injuries have been induced simultaneously in brain and eye regions of the chick by Hyman (1927) and Hinrichs (1927), but, since these were done with chemicals and ultra-violet light at various stages of incubation, they are not comparable with those abnormalities occurring naturally in the chick or induced by a chilling in the minnow. The work of these two investigators, however, shows that there is an antero-posterior gradient of susceptibility to injury in the chick, and that the greater frequency of head abnormalities than all other types is probably due to a higher level of metabolism in the anterior end of the embryo. Such an interpretation is in accord with Child's axial gradient hypothesis.

Monsters produced in the departmental stock were few in number, and no evidence was found of an hereditary tendency underlying their production. Wright (1923) found that otocephaly (similar in some respects to monsters described in this paper) increased to a marked extent in certain inbred families of guinea-pigs. In one branch the frequency of this abnormality was 20 per cent. in the nineteenth generation, compared with 1·5 per cent. for the family and 0·2 per cent. for the entire population. While no genetic ratios were demonstrable, the increase of abnormalities thus brought about by inbreeding could only be interpreted as the intensification of genetic factors rendering the individual carrying them susceptible to any unfavourable environmental condition predisposing toward the abnormality.

It is probable that a non-lethal grade of exencephaly has become incorporated as a breed characteristic in such breeds as the Polish, Crèvecœur, and Houdan, in which cerebral hernia is typical. Dunn (1927) believes that this character is entirely distinct from exencephaly. Dareste (1891), on the other hand, after making a study of young chicks of these breeds, concluded that the condition was quite comparable to

proencephaly, differing only in that the cerebral hemispheres were upthrust entirely instead of only in part. This resulted in spreading apart the frontal bones (which were normally developed), and the overlying membrane gradually became ossified to form the large brain case typical of such fowls. Tegetmeier's (1867) study of Polish skulls supports Dareste's findings. Dareste concluded that these races were produced by the spontaneous appearance and hereditary transmission of an exencephaly of a grade or kind that was not fatal to its exhibitor. If this be so, then the tendency to produce similar abnormalities, lethal in effect, may also be hereditary.

Summary.

In an examination of 11,797 eggs which had failed to hatch, 433 specimens exhibiting teratological abnormalities were obtained.

The various types of monsters observed are briefly described and the frequency of each given.

The most common types were characterised by various degrees of abnormality either in the brain, cranium, or eyes, or in two of these structures, or in all three. Hyperencephaly, exencephaly, and microphthalmia constituted 93 per cent. of all the monsters observed.

Other teratological abnormalities encountered included prognathia, duplicity, ectopia, cyclopia, otocephaly, malformed limbs, and absence of premaxillæ.

Monsters accounted for at least 3·6 per cent. of all mortality in the material examined. Since this material included many embryos too decomposed for detection of teratological abnormality, the actual loss is probably considerably higher.

No significant deviations from the normal sex-ratio were evident in any class of monsters.

A decline in the frequency of such abnormalities from February to April was evident.

Evidence is adduced from Stockard's experimental teratology in fish eggs, to show that a very probable cause of the production of monsters in the chick is an arrest of development of the embryo at a critical stage. Presumably such a condition results from the chilling of those eggs laid in the early stages of gastrulation.

Some evidence to indicate the possibility of an hereditary tendency to produce monsters is briefly discussed.

ACKNOWLEDGMENTS.

The writers are greatly indebted to the Romanno Bridge Poultry Farm, West Linton; W. Johnston & Co., Comrie; and the Sussex Hatcheries, Glasgow, for supplying the majority of the eggs examined. Thanks are also due to Professor F. A. E. Crew for suggestions and advice, and to Mr J. M. A. Chisholm, of this Department, for all the photographs.

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EXPLANATION OF PLATES.

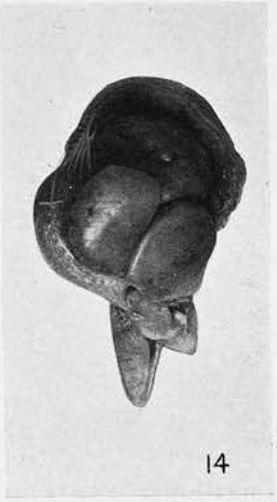
PLATE I.

Fig. 1. Hyperencephaly in an embryo of about 8 days. × 3.

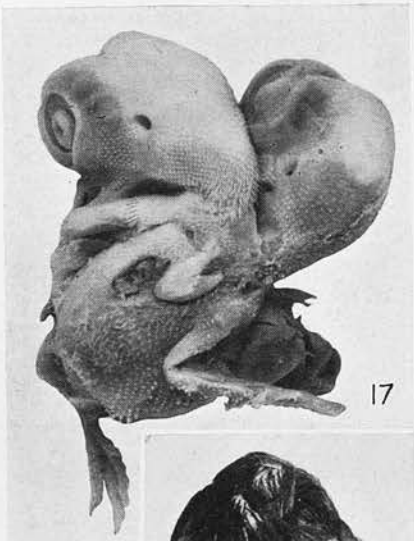
Fig. 2. A hyperencephalic embryo of about 16 days enclosed in the amnion. Natural size.

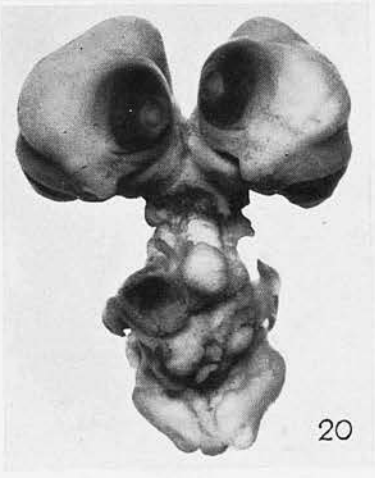
Fig. 3. A hyperencephalic embryo of about 18 days, with the membranes and yolk-sac removed, showing the characteristic absence of upper beak, eyes, and brain covering. Ectopia is conspicuous. Natural size.

F. B. Hutt and A. W. Greenwood. III.—Plate I.



F. B. Hutt and A. W. Greenwood. III.—Plate II.





F. B. Hutt and A. W. Greenwood. III.—Plate III.

Figs. 4 and 6. Exencephaly. Nineteen-day embryos exhibiting anterior encephalomeningoceles (proencephaly) of different sizes. × 1·4.

Fig. 5. Unilateral microphthalmia in a twenty-day chick. The down has been plucked from around the ear and eye. The black dot is made by the reduced eyelids. The twisted upper beak is characteristic. × 1·4.

Fig. 7. Exencephaly extended to include both the occipital region and that of the cerebral hemispheres. × 1·4.

Fig. 8. Anterior aspect of the head of a cyclopean embryo. × 1·4.

Fig. 9. Anterior aspect of the head of an embryo in which cyclopia is not complete. The two lenses have come together in the median line, but are not completely fused. The reduced lower beak, the tongue, and the characteristic proboscis above the eye are conspicuous. The absence of cerebral hemispheres is less so. × 1·4.

Plate II.

Fig. 10. Partial duplicity and exencephaly in the head of a sixteen-day chick. The two upper beaks cross the lower ones, which are seen in part from the side. Two combs are evident, but duplication of the rest of the head, though present, is less obvious. × 1·4.

Fig. 11. One lower beak, but two upper ones and two combs. The dark spot between the two combs is a small opening from the median eye, resulting from fusion of the inner eyes of the parts which almost became two heads. × 1·4.

Fig. 12. Duplicity more advanced than in figs. 10 and 11. The opening of the median eye is distinct. × 1·4.

Figs. 13, 14, and 15. Various degrees of exencephaly combined with unilateral microphthalmia.

Plate III.

Fig. 16. A fully formed embryo to which were attached by a strand of tissue the hind limbs only of a second embryo. Natural size.

Fig. 17. Cranial dichotomy in a ten-day embryo. × 2.

Fig. 18. A chick of about 16 days exhibiting duplicity extending anteriorly as far as the neck. Down has been plucked to show the four wings. Natural size.

Fig. 19. Caudal dichotomy in a microphthalmic chick of about 9 days. × 2.

Fig. 20. Cranial dichotomy in a chick of about 7 days. × 3.

(*Issued separately April* 1, 1929.)

STUDIES IN EMBRYONIC MORTALITY IN THE FOWL

IV: Comparative Mortality Rates in Eggs Laid At Different Periods of the Day and Their Bearing On Theories of the Origin of Monsters

F. B. HUTT and A. M. PILKEY

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STUDIES IN EMBRYONIC MORTALITY IN THE FOWL

IV: Comparative Mortality Rates in Eggs Laid At Different Periods of the Day and Their Bearing On Theories of the Origin of Monsters

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Various studies upon the development of eggs of fowls and of pigeons have shown that the processes of formation of the egg and of development of the embryo during the passage of the egg down the oviduct are continuous throughout the twenty-four hours of the day. They are involuntary reactions. On the other hand the actual laying of the egg is a reflex for which the necessary sensory stimulus usually does not occur while the hen is on the roost at night.

This means that if an egg be fully formed and ready to lay at 2 A. M., it is retained in the uterus till the following morning. The embryo of such an egg (when fertile) would presumably have undergone some six hours further development than if the egg had been fully formed at 2 P. M. and had been laid at 2:30 P. M. Such a view is consistent with the observations of Patterson[1] who found considerable variation in the degrees to which embryos had developed by the time of laying.

The problem arises of whether or not such variation in the development of the embryo at the time of egg laying, (when the egg is chilled and growth is arrested) affects its chances of surviving after it is put in the incubator. The work of Stockard[2] on eggs of *Fundulus* indicates that in eggs chilled at an early stage of gastrulation, or prior to initiation of that process, abnormalities in development are more likely to occur, while eggs chilled after gastrulation is completed are better able to withstand the resultant arrest of growth and to continue development normally when the necessary temperature is restored. Since Patterson[3] and others have shown that in eggs of the fowl and of the pigeon gastrulation takes place very close to the time of laying, it is to be expected that in eggs laid as

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soon as fully formed, (and therefore closer to the gastrulation period), one would find a high proportion of abnormalities and a higher mortality rate. Conversely, in eggs retained in the body for some time after their formation has been completed, gastrulation is probably completed, abnormalities in development should be fewer and consequently the mortality rate should be less.

In a previous paper of this series, Hutt and Greenwood[4] reported that the frequency of teratological monsters detected in the macroscopic examination of 11797 dead fowl embryos was 3.67 per cent. This figure did not include those that may have occurred in the embryos dead before six days, but the frequency with which such cases of abnormal development are encountered in embryos of one to five days in embryological laboratories suggests that mortality from this source is somewhat greater than is indicated by the percentage given above. Moreover, since Stockard pointed out that chilling at a critical stage also caused such minor defects as could be detected only when the hatched embryos were found to be unable to right themselves and to swim, it is probable that chilling of chick embryos at critical stages may also cause abnormalities not readily detected but sufficient to prevent the chick from hatching.

It is to be expected that among eggs laid early in the morning there will be a greater proportion which have been held in the body beyond the normal period, while in the eggs laid in the afternoon there should be a higher proportion of those which are laid as soon as they are fully formed. Such a view is supported by the observation of Atwood[5] that the mean weight of eggs is greater in those laid early in the morning and declines as the day advances. Accordingly there is to be expected a slightly higher mortality rate among the afternoon eggs than in those laid early in the morning. The present paper reports an attempt to measure the difference.

MATERIAL AND METHODS

All eggs incubated at the poultry departments at University Farm and at the Crookston Branch Station in 1929 were marked with the time at which they were taken from the trapnests. Collections were made six times daily. No eggs were

held longer than 7 days before incubation and at both stations they were kept in a room in which the temperature, though variable, was never high enough to induce development.

All eggs were candled at 9 days. Infertile eggs and those with dead embryos were removed, broken individually and examined to make sure that diagnosis of infertility or mortality was correct. A similar inspection was made of all eggs which failed to hatch. All eggs from both stations were examined at University Farm.

The records cover eggs laid from March 13th to May 6th, 1929, at University Farm, and those from March 5th to April 19th, 1929, at Crookston.

RESULTS

The data obtained are shown in Table 1.

Mortality is based on the numbers of fertile eggs. For many individuals the numbers of eggs laid in one or more periods were too small to give an accurate measure of the mortality rate in the eggs of these periods from any one hen. Accordingly the data have been treated as a whole. The probable errors have been calculated by the formula:

$$\text{P. E. (in per cent)} = .6745\sqrt{\frac{N.\ p.\ q}{N}} \times 100$$

where N = number of fertile eggs.

p = probable (*i. e.* observed) per cent. mortality.

q = probable (*i. e.* observed) per cent. hatch,
$= 1 - p$.

It is evident that while the fertility was much higher at Crookston than at University Farm, there were no consistent differences between the fertility rates at different periods of the day. On the other hand, there is a higher mortality rate, consistent in both sets of data, in the afternoon eggs than in those laid prior to 9 A. M., in which period the mortality rate is lowest at both stations. The differences between periods are so slight in the data from University Farm that they cannot be found statistically significant for that material alone by the probable error method (Table 2). However, the numbers of eggs and

TABLE 1.—RATES OF INFERTILITY AND OF EMBRYONIC MORTALITY IN EGGS LAID AT DIFFERENT PERIODS OF THE DAY.

PERIOD		UNIVERSITY FARM			CROOKSTON			COMBINED DATA	
Hours of Laying	Designation	Infertile %	Fertile eggs	Embryonic Mortality %	Infertile %	Fertile eggs	Embryonic Mortality %	Fertile eggs	Embryonic Mortality %
To 9 A. M.	A	13.39	1196	32.61±.91	2.60	1907	31.62± .72	3103	32.00±.56
9:05—12 M.	B	15.37	2345	33.65±.66	3.75	2697	33.78± .61	5042	33.71±.45
12:05—2 P. M.	C	16.20	1148	34.75±.94	3.25	1073	38.77±1.00	2221	36.69±.69
After 2 P. M.	D	15.47	1229	34.90±.91	2.33	1133	35.39± .95	2362	35.14±.66

TABLE 2.—DIFFERENCES SHOWING HIGHER RATES OF EMBRYONIC MORTALITY IN THE AFTERNOON EGGS.

PERIODS	UNIVERSITY FARM		CROOKSTON		COMBINED DATA	
	Difference	Diff. / P. E.	Difference	Diff. / P. E.	Difference	Diff. / P. E.
B—A	1.04±1.11	.93	2.16± .94	2.29	1.71±.71	2.41
C—A	2.14±1.30	1.64	7.16±1.23	5.82	4.69±.88	5.33
D—A	2.29±1.28	1.78	3.77±1.18	3.19	3.14±.86	3.65
C—B	1.10±1.14	.96	3.99±1.17	3.41	2.98±.82	3.63
D—C	.15±1.30	.12	—3.38±1.38	2.45	—1.55±.95	1.63
D—B	1.25±1.11	1.12	1.61±1.12	1.44	1.43±.79	1.81
(C+D)—(A+B)	1.54± .83	1.85	4.15± .82	5.06	2.95±.59	5.00
(C+D)—A	2.22±1.12	1.98	5.42± .99	5.47	3.89±.74	5.26

the mortality rates at the two stations are so much alike that the data from both sources may legitimately be combined. When this is done, the probable errors are reduced and it is found that there are statistically significant differences (Table 2) indicating a lower mortality rate in the eggs laid before 9 A. M. than in those laid during the afternoon.

The difference between mortality rates in periods A and B is only 2.41 times its probable error and therefore barely significant.

The mortality rate for all eggs laid after 12 o'clock is also significantly higher than for all eggs laid in the morning, although not so great as when it is compared with the eggs laid before 9 A. M. exclusively, where a difference of $3.89 \pm .74\%$ is found. The slightly higher mortality among the eggs laid from 12 to 2 than in those laid after 2 is only 1.63 times its probable error and is therefore not significant. It results from a peak of mortality in the former period at Crookston which was not duplicated at University Farm.

Further evidence in support of these observations is afforded by comparisons of the mortality rates in eggs of birds laying only at limited periods of the day. (Table 3). In computing these data, only those fowls have been included which laid five fertile eggs or more during the season.

TABLE 3—MORTALITY RATES IN FERTILE EGGS OF BIRDS LAYING ONLY IN LIMITED PERIODS OF THE DAY.

Periods of laying*	No. of Birds	Mean Rate of Embryonic Mortality (%)
A, B and C	33	45.30±3.17
A, B and D	25	47.00±3.44
B, C and D	41	58.90±2.97

*For designations of periods see Table 1.

The difference between the mean embryonic mortality in eggs of hens laying *only after 9 A. M.* (B, C and D) and those *not laying after 2 P. M.* (A, B and C) is 13.60 ± 4.34. Since this is 3.13 times its probable error, a significantly lower rate in the latter group is indicated.

It is of interest to note that the mortality rates in eggs of these three groups of hens laying at limited periods are greater than those for the whole population. Some hens laid at only two of

the four periods, but their numbers and their eggs were too few for statistical treatment. Ten birds laid only in the morning but none laid only in the afternoon.

THE INFLUENCE OF SEASON ON MORTALITY RATES

If the entire hatching season be divided into semi-monthly periods and the difference be determined between the mortality rates among eggs laid in the afternoon and those laid prior to 9 A. M., it is found that there is a gradual reduction of this difference as the season advances. (Table 4) This reduction is very slight but it is found in the data from both University Farm and Crookston, so may therefore be considered fairly significant. The data indicate that as the season advances the

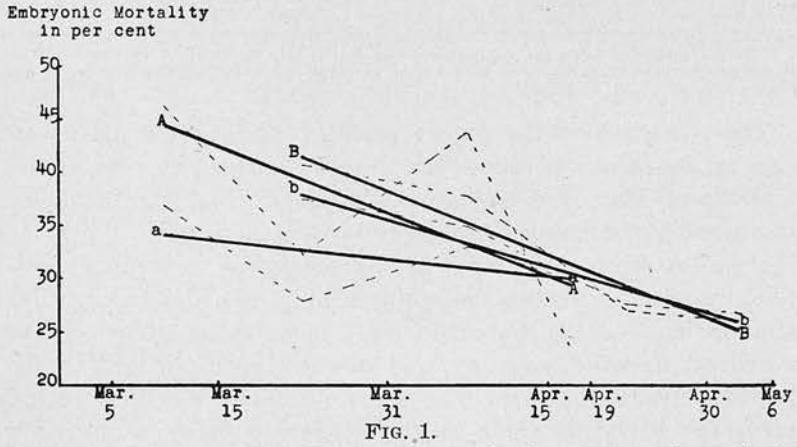

FIG. 1.

Graphs showing the decline, as the incubation season advanced, in embryonic mortality at University Farm and at Crookston. Straight lines fitted to the distributions show that at each station the reduction in the mortality rate is greater for the afternoon eggs (capital letters) than for those laid prior to 9 A. M. (small letters).

Equations:

A — A (Crookston, after 12.), $Y = -.397x + 26.54$
a — a (Crookston, before 9.), $Y = -.118x + 14.99$
B — B (University Farm, after 12.), $Y = -.404x + 21.68$
b — b (University Farm, before 9.), $Y = -.291x + 17.99$

mortality rate declines in both early morning and afternoon eggs, but that the reduction is more marked in the latter class. (Fig. 1). The straight lines and their equations show that the rate of decline of the mortality in afternoon eggs was practically identical at both stations.

TABLE 4—SEASONAL CHANGES IN MORTALITY RATES.

Season of Laying	Fertile eggs laid before 9 A. M. (A)		Fertile eggs laid after 12 M. (C+D)		Difference (C+D)—A
	No.*	Mortality %	No.*	Mortality %	
		CROOKSTON			
Mar. 5-15	496	37.09±1.46	570	46.49±1.53	9.40±1.56
Mar. 16-31	631	28.05±1.11	694	32.42±1.19	4.37±1.62
Apr. 1-15	686	33.09±1.21	895	43.91±1.21	10.82±1.71
Apr. 15-19	236	30.08±2.01	197	23.86±2.05	—6.12±2.87
		UNIVERSITY FARM			
Mar. 15-31	234	37.61±2.14	577	40.73±1.37	3.12±2.53
Apr. 1-15	394	34.77±1.62	860	37.91±1.11	3.14±1.96
Apr. 16-30	410	27.56±1.48	730	26.99±1.10	— .57±1.84
Apr. 30	131	26.72±2.61	216	25.93±2.01	— .79±3.29
May 6					

* The numbers here do not agree with the totals in Table 1 because of the inclusion here of floor eggs at Crookston and the omission of the few eggs set prior to Mar. 15 at U. Farm.

The reduction of the higher mortality among the afternoon eggs as the season advances is of special interest in view of the findings of Hutt and Greenwood *(loc. cit.)* that the incidence of observed teratological monsters in their material declined as the season advanced. Such an agreement is not proof positive, but it does lend some support to Stockard's theory that abnormalities of development result from arrest of growth at a critical stage of ontogeny, and to the theory outlined in the introduction to this paper that eggs laid at such a critical period are more likely to occur in the afternoon than in the early morning. Whether these changes as the season advances are due to a general rise in temperature, so that chilling of the egg following laying is less rapid, or to a lengthening of the working day or to some other factor can not yet be definitely stated.

OBSERVED ABNORMALITIES

The incidence of teratological monsters in the material reported in this paper, including cases of microphthalmia, anophthalmia, hyperencephaly, exencephaly, duplicity, otocephaly, ectopia and prognathia (but exclusive of chondrodystrophy) is shown in Table 5.

In both sets of data the frequency of observed abnormalities is lowest in the eggs laid prior to 9 A. M., increases gradually during the day and in the eggs laid after 2 P. M. is approximately twice the figure for the early morning eggs. It is logi-

cal in view of all the data presented in this paper to assume that these abnormalities of development and other lesser ones that could not be detected macroscopically are responsible for the slightly higher mortality rate observed in the afternoon eggs.

TABLE 5—FREQUENCY OF OBSERVED TERATOLOGICAL ABNORMALITIES.

Period of Laying	CROOKSTON		UNIVERSITY FARM	
	Fertile Eggs	Frequency of Monsters %	Fertile Eggs	Frequency of Monsters %
To 9 A. M.	1907	.26	1196	.75
9:05 — 12 M.	2697	.37	2345	.94
12:05 — 2 P. M.	1073	.37	1148	1.74
After 2 P. M.	1133	.53	1229	1.46

The frequency of teratological monsters observed in all the material from both stations is 0.70 per cent. of the fertile eggs, somewhat less than that for the Edinburgh data. This can be accounted for in part by the higher frequency of such specimens in the February and early March eggs at Edinburgh, whereas no eggs for this season were included in the present data. It is probable, in view of both years' data, that eggs set here in January and February would show not only a higher incidence of observed abnormalities in development, but also a greater difference between mortality rates in early morning and in afternoon eggs than were observed in the short season covered by the data in this paper. It is also possible that the difference in latitude with the attendant differences in the length of day and night at Edinburgh and at this station may be in part responsible for the greater incidence of abnormalities at the former place. It is quite conceivable that when the working day of the fowl is shortened, as occurs most noticeably in northern latitudes prior to the vernal equinox, there may be a tendency on the part of the birds to retain the eggs for a shorter time in the body than when, later in the season or in southern latitudes, a longer day provides ample time for feeding and for egg laying at leisure. University Farm and Crookston are at 45°, and 47° 45′ North Latitude respectively, whereas Edinburgh is at 55° 36′ North Latitude. All observations reported in this paper were taken by Central Standard time.

PRACTICAL IMPORTANCE

The difference between mortality rates in late afternoon and in early morning eggs is too small to be of much practical importance at this latitude after March 15th. If a still higher mortality rate among afternoon eggs were found in eggs incubated in January or February, or in more northern latitudes, it might be worth while to attempt to reduce it. Presumably the best way to do so would be to take all afternoon eggs from the nests before they were chilled and to incubate them for a period of four or five hours. By so doing those with embryos in which gastrulation had not yet been completed would be developed beyond the critical period at which chilling and arrest of growth could result in abnormal development.

SUMMARY

The mortality rate of embryos in eggs laid after 12 M. was found to be slightly higher than in those laid before 9 A. M.

This difference was greatest in March and was not observed after April 15th. Up to this date the difference was from 4 to 10 per cent. in the different periods at one station and a little over 3 per cent. at the other.

The frequency of observed teratological abnormalities was least in the eggs laid prior to 9 A. M. and increased gradually during the day, being approximately twice as high in eggs laid after 2 P. M. as in those laid before 9 A. M.

With respect to these three observations, the data at both stations were consistent.

All of these findings are compatible with the following theories:

(1) Abnormalities in development result from the arrest of growth occurring when embryos are chilled at a critical period of ontogeny, *i. e.* prior to gastrulation or early in that process.

(2) The proportion of embryos developed beyond the critical stages is greatest in the eggs laid prior to 9 A. M. and least in the eggs in the afternoon.

(3) The frequency of abnormalities in development in embryos of the fowl decreases as the spring laying season advances.

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STUDIES IN EMBRYONIC MORTALITY IN THE FOWL, V. RELATIONSHIPS BETWEEN POSITIONS OF THE EGG AND FREQUENCIES OF MALPOSITIONS

BY

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Studies in Embryonic Mortality in the Fowl, V. Relationships Between Positions of the Egg and Frequencies of Malpositions*

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(Received for Publication June 14, 1933)

THE incidence of malpositions found in chick embryos which had died after the eighteenth day of incubation has been reported as follows:

	No. of such embryos	Malpositions	
		Percent	Types included
Sanctuary (1925)	1490	51.7	I to III
Hutt (1929)	5050	55.8	I to IV
Hutt and Cavers (1931)	4253	52.1	I to VI*

* Excluding V.

From these data, and the similar reports of other workers based on smaller numbers or on "dead in shell," it is obvious that malpositions are largely responsible for the peak of mortality occurring during the last three days of incubation.

In the representative material reported by Hutt and Cavers (1931) based on the examination of all dead embryos from 24,660 fertile eggs incubated at two experiment stations in three years, malpositions in embryos of 18 days or older were apparently responsible for 24.4 percent of the total mortality.

It is obvious that to reduce the present high embryonic mortality universally encountered in artificial incubation the origin of these malpositions must be discovered, as well as some means of eliminating them or of reducing their frequency. An investigation in that direction is reported in this paper.

MALPOSITIONS CONSIDERED

The six major malpositions thus far reported may be briefly described as follows:

* Paper No. 1191 of the Journal Series of the Minnesota Agricultural Experiment Station.

† The junior author is not responsible for any imperfections of analysis, of deductions or of English in this paper.

Position I	(Sanctuary, 1925)	Head between the thighs.
Position II	(Reaumur, 1751)	Head in small end.
Position III	(Sanctuary, 1925)	Head to left instead of under right wing.
Position IV	(Hutt, 1929)	Head normal, but embryo rotated so that the beak is buried away from the air cell.
Position V	(Smith, 1930)	Feet over head.

Position VI This malposition has been studied in this laboratory since 1929. It was briefly described by Hutt and Cavers (1931) but merits a more complete description here. The embryo is normal in all respects save that the beak lies above the right wing instead of being under it as in the normal position. In some cases the beak lies under the humero-radial joint but the distal extremity of the wing is buried under the head. The beak thus lies in a fork of which the upper branch is formed by the radius and ulna and the lower part by the distal portion of the wing. In this condition the chick is unable to strike the shell with force, often because the femoro-tibial joint of the right leg keeps the beak wedged in this fork. When the whole wing is under the beak, the blows of the latter are less impeded but are ill-directed. Chicks in Position VI are commonly found in "pipped" eggs, particularly in those having large round holes pecked nearer to the large end of the egg than is usual.

Over a five-year period Position VI has been more common in eggs incubated at this laboratory than has any other single malposition, but the fact that so many of the affected embryos can pip the shell suggests that it may not be as great a barrier to hatching as are some of the others. The handicap imposed by Position VI is undoubtedly much less severe than those from the other five malpositions because it is the only one that does not prevent the beak from entering the air cell and the rapid pulmonary respiration following that step.

The six malpositions listed above are all subject to minor variations. Embryos in Position II may also be in Positions I or III and those in Position III may also be in Position VI. In both of these cases the position first named has been considered the more significant deviation from normal, and the embryo has therefore been recorded as being in that position.

Illustrations of Positions I, II, III and IV are shown by Hutt (1929).

OBJECT OF THE EXPERIMENT

Some evidence was secured in our early studies that Positions II and IV were more frequent in eggs incubated in a horizontal position than in those incubated (in Buckeye and Petersime incubators) with the large end up and the long axis of the egg at an angle of about 45 degrees from the horizontal. This suggested that the position of the egg during incubation might be a factor in the production of malpositions, and a three-year experiment was planned to find the frequencies of Positions I, II, III, IV, and VI in eggs incubated either in a horizontal position or tilted with the long axis 45 degrees from the horizontal.

PROCEDURE

Controlled experiments were carried out with eggs set at University Farm, St. Paul, in 1931 and 1932 and at the Northwest School and Experiment Station, Crookston, Minnesota, in 1930, 1931 and 1932. The general procedure was to incubate some eggs in: (1) tilted trays with the large end of the egg at an angle of 45 degrees above the horizontal, others (2) on identical trays kept horizontal. At each station all eggs were incubated in the same large forced-draught Buckeye incubator. Tilted eggs were turned in the usual way by rotating the whole tray through an angle of 90 degrees. The others were turned by hand. Both lots were turned twice daily, and at the same time, with the exception that in 1932 both lots at University Farm were turned thrice daily. Following the usual practice, on the eighteenth day of incubation all eggs

were placed in a horizontal position in pedigree baskets (at University Farm) or in hatching trays (at Crookston).

All eggs used were from White Leghorns and were allocated at random to the horizontal and tilted trays. At each station all eggs used each season came from fowls in uniform environmental conditions receiving the same ration. Moreover, the samples incubated each year in the two different positions were sufficiently large at both stations to overcome any possible error from variation between individual fowls with respect to the frequency of malpositions in their progeny. Since the air in both incubators was kept in constant rapid circulation by electric fans, the environmental conditions which might affect embryonic viability (temperature, relative humidity and carbon-dioxide content of the air) were identical for both tilted and horizontal eggs.

In order to get adequate data, eggs were incubated in the manner outlined above in nearly every setting at the two stations in the years mentioned. These settings were made between March 1 and May 15 and are therefore representative of the normal incubation period in this latitude. The controlled data presented below are actually composed of contributions from 32 separate and distinct settings, in each of which comparable eggs were incubated concurrently in the two positions in the same incubator, that incubator being of the same type at both stations.

All unhatched eggs from both sources were broken and examined in the laboratory of the senior author, either by him or his assistant, J. R. Cavers. From the number of eggs set there were deducted all the infertiles and those dying up to 18 days of incubation. Malpositions may cause mortality before the eighteenth day, but, since the evidence indicates that in most cases the lethal effect is exerted after that age, and since the definitive position cannot in all cases be ascertained with certainty before the eighteenth day, the consideration of malpositions in this paper is restricted to those encountered in embryos 18 days of age or older. The number of these in any given setting is obtained by adding to the number of hatched chicks the number of embryos older than 18 days, as indicated by the degree of inclusion of the yolk sac and by size.

In the data shown, the number of malpositions is expressed as a percentage of the number of embryos alive at 18 days. Some slight error is entailed because a few of the chicks in Positions II and VI may hatch, but whatever error there may be would be apportioned equally to the tilted and horizontal eggs regardless of their numbers.

Additional data bearing on the problem under consideration are available from other eggs incubated at University Farm in 1930 and 1933. In the latter year malpositions were recorded in tilted eggs from the Buckeye machine and from others incubated horizontally in an Oakes forced-draught incubator. These were all White Leghorn eggs, but about one-quarter of those in the Buckeye were from older hens, while all the remainder (in both incubators) were from birds one year old.

In 1930 data from tilted eggs in a Petersime incubator were available for comparisons with corresponding data from eggs incubated horizontally in Prairie State and Cyphers incubators. These are less comparable because the eggs used came from different breeds and because all incubators did not have the same attendant. Nevertheless, the number of eggs involved is sufficient to reduce somewhat the error from these sources, and it will be seen that these data conform well to those from the controlled experiment.

RESULTS

The gross data concerning these various settings are shown in Table 1.

Although there was some variation in the mortality of the embryos from season to season, and also some differences between hatches at the two stations, when either station is considered for any given year, there was very little difference between the percentage hatched of 18-day embryos in eggs incubated horizontally and that of those set in tilted trays. In fact when the whole 5,030 embryos alive at that age in tilted eggs are compared with the 3,040 in eggs incubated horizontally, the percentages hatched differ by only 0.5 percent. This shows: (1) that these two positions of the incubating egg have apparently no effect upon the embryonic mortality after 18 days; and (2) that for purposes of this experiment the samples of tilted and horizontal eggs were sufficiently large to eliminate genetic and environmental differences which might otherwise obscure the correct interpretation of the data.

In 1932, at the suggestion of Dr. T. C. Byerly, some eggs were incubated with the small end up (in tilted trays) for a period of 48 hours at the beginning of incubation and thereafter in the usual way with the large end up (Tables 1 and 2, U.F. '32a). It is noteworthy that the frequency of each malposition was lower in these eggs than in tilted controls of the same year and station, but, since the figures are within the range for each malposition encountered in other lots, the data have been included in the computation of totals and averages. Only 715 18-day embryos were involved so that the apparent reduction of all malpositions cannot yet be considered significant.

The frequency of malpositions among the 18-21-day dead embryos varied from 42 to 85 percent, with an average for the tilted eggs of 60.4 and for the others of 65.9 percent. We are unable to account satisfactorily for the remarkably high incidence of malpositions among the eggs incubated at Crookston in 1932. It is certainly not due to personal bias on the part of the examiners for in the eggs from University Farm

TABLE 1.—*Malpositions in White Leghorn eggs (Buckeye incubators)*

Source	Embryos alive at 18 days			Malpositions among 18–21 day dead expressed as percent of:	
	Number	Hatched percent	Died 18–21 days number	18-day living embryos	18–21 day dead
Tilted (large end raised 45°)					
Univ. Farm 1931	1157	90.6	108	7.9	85.2
Univ. Farm 1932	1355	81.7	247	10.6	57.9
Univ. Farm 1932a*	715	88.5	82	8.4	73.2
Crookston 1930	893	84.1	142	7.6	42.2
Crookston 1931	527	75.5	129	12.5	51.2
Crookston 1932	383	65.5	132	22.4	65.1
Totals and average	5030	83.3	840	10.1	60.4
Horizontal					
Univ. Farm 1931	372	88.9	41	8.5	85.3
Univ. Farm 1932	809	85.3	119	8.8	59.7
Crookston 1930	884	86.6	118	8.1	61.0
Crookston 1931	548	85.4	80	10.6	72.5
Crookston 1932	427	68.4	135	20.8	65.9
Totals and average	3040	83.8	493	10.7	65.9

* Incubated with the small end up for the first 48 hours, thereafter with the large end up.

settings, examined concurrently by them, a rather low frequency of malpositions was found.

The frequencies of Malpositions I, II, III, IV and VI in the various lots are shown in Table 2.

In all controlled lots Position I was more frequent among the tilted eggs than among those incubated horizontally. Conversely, in every case Position II was more frequent among the horizontally incubated eggs than more embryos in Position I among the eggs from small incubators than among the tilted ones cannot be stated, but since these machines were operated by inexperienced students, this deviation from the uniform results found in the controlled experiment is not a serious one.

The differences between the tilted and horizontal eggs with respect to the frequencies of Positions I, II, IV and VI are so consistent in every year and at both sta-

TABLE 2.—*Frequencies of malpositions among eggs incubated in tilted or horizontal positions (expressed as percentages of the numbers of 18-day living embryos)*

Source	Position I Head between thighs		Position II Head small end		Position III Head left		Position IV Rotated		Position VI Beak over wing	
	Tilted	Horizontal	Tilted	Horizontal	Tilted	Horizontal	Tilted	Horizontal	Tilted	Horizontal
Controlled										
U.F. '31	2.59	0.54	0.86	1.88	1.29	2.68	0.17	2.15	3.02	2.15
U.F. '31[a]	—	2.57	—	3.14	—	0.85	—	0.57	—	5.14
U.F. '32	1.69	0.61	1.03	2.10	3.24	1.98	0.80	1.61	3.76	2.47
U.F '32[a]	1.39	—	0.70	—	2.79	—	0.42	—	3.01	—
Cr. '30	0.56	0.34	0.89	2.37	1.46	1.58	0.56	1.47	3.24	2.37
Cr. '31	2.65	1.28	1.14	1.64	2.85	1.82	1.32	1.82	4.55	4.01
Cr. '32	2.35	2.81	2.35	3.51	5.48	3.51	1.30	4.92	10.96	6.09
Others										
U.F. '30[1]	1.23	1.46	3.03	3.18	3.19	1.12	0.90	1.37	4.01	2.75
U.F. '33[2]	2.31	1.88	1.00	3.76	1.10	2.03	1.20	0.92	16.76	2.31

'31[a] Based on 350 18-day embryos not turned after the 14th day; not included in any totals or averages.
'32[a] Small end up for first 48 hours; large end up thereafter.
[1] Based on 1222 18-day living embryos in a Petersime (tilted) and 1165 in small incubators (horizontal).
[2] Based on 996 18-day living embryos in a Buckeye (tilted) and 691 in an Oakes (horizontal).

in the tilted ones. Position III tends to be more common in the tilted eggs but in two cases this situation is reversed. Position IV is very much more frequent among the eggs incubated horizontally than in those tilted, the difference being evident in every lot. On the other hand the frequency of Position VI in the tilted eggs exceeds that in the horizontal eggs in every lot.

The supplementary data provided from other eggs set at University Farm in 1930 and 1933 are, with one slight exception, in complete accordance with the conditions found in the more carefully controlled incubations. Just why there should be a few tions that they are undoubtedly significant. This interpretation is confirmed by statistical analyses of the summarized data. (Table 3.)

In this table the error used is not the probable error but the standard error, and a difference exceeding twice its standard error may therefore be considered significant. Accordingly all the differences are real except that for Position III. Although the difference between the frequencies of Position VI in tilted and in horizontal eggs is only 1.9 times its standard error, there can be little doubt, in view of the uniformity of the difference in all lots (Table 2), that

Position VI is more frequent among the eggs incubated with the large end raised 45 degrees from the horizontal.

Probabilities of Assuming Malpositions

The results are particularly interesting because they show that, while the position of the egg has *apparently* no effect upon the mean hatchability of large samples of 18-day embryos there is actually a decided risk for the embryo in an egg lying horizontally and an almost equally great risk, though of a different kind, if the egg be tilted. It is because the two risks are almost equally great that eggs incubated horizontally have practically the same mortality rate as do those incubated in a tilted position.

For example, if an embryo in our material survived to the eighteenth day in a horizontal tray, it would have 0.95 chances per 100 trials of subsequently dying in Position I, compared with exactly twice that chance of the same event happening if it were in a tilted tray. However, its comparative security in this respect would be counteracted by the fact that it would have 2.14 chances per 100 of ending up in Position IV (apparently always lethal), whereas, had it been in a tilted tray, the likelihood of that event would have been only 0.65 per 100.

The maximum and minimum probabilities of embryos in this material getting caught in one or other of the five malpositions in eggs incubated in tilted or horizontal positions are shown in Table 4. These probabilities are based, as before, on the assumption that of all the embryos alive at 18 days, all in malpositions were subsequently unable to hatch. This assumption is probably completely correct for Positions I, III, and IV which are apparently always lethal, but it is not completely correct with respect to Position II, some of which actually hatch, or to Position VI, which is probably less of a handicap than any of the others. However, there was no way of ascertaining the number of these which did hatch, and, since the proportion of embryos in Positions II and VI which did hatch should have been the same for both tilted and horizontal eggs (all being horizontal for the last three days), the method used is the only feasible one. It should accurately reveal the relative frequencies of each malposition in the tilted and horizontal eggs.

It is evident that the minimum frequency of these malpositions (7.96 percent) would be attained if some method of incubation could be devised to utilize the advantages of both the tilted and horizontal positions. The former caused an increase of Positions I, III and VI to the extent of 2.10 percent above the minimum, whereas the horizontal position resulted in an increase of Positions II and IV to a figure 2.73 per cent higher

TABLE 3.—*Summarized comparisons of the frequencies of malpositions in tilted and horizontal eggs**

	Tilted		Horizontal		Difference Percent	Difference
	Number	Percent	Number	Percent		$E_{diff.}$
18-day embryos	5030		3040			
Position I	91	1.81±.14	29	0.95±.18	0.86±.23	3.7
Position II	52	1.03±.13	69	2.27±.27	1.24±.29	4.3
Position III	128	2.54±.21	65	2.14±.28	0.40±.35	1.1
Position IV	33	0.65±.12	65	2.14±.28	1.49±.30	4.9
Position VI	203	4.03±.27	97	3.19±.35	0.84±.44	1.9
All five malpositions	507	10.06±.42	325	10.69±.59	0.63±.72	0.8

* Including only experimental and control White Leghorn eggs incubated concurrently in Buckeye incubators, and excluding the "others" of Table 2.

than the minimum. If the good features of both systems of incubation could have been utilized, the number of embryos dying per hundred after the eighteenth day would have been reduced from 16.5 (the observed rate) to around 14.4 or 13.7, which would be equivalent to a reduction of the mortality after 18 days by from 12.8 to 16.5 percent. Since nearly 50 percent of all embryonic mortality in the fowl occurs after the 18th day, the net result would be to *decrease the total mortality by approximately 6 to 8 percent.*

would be eliminated or very greatly reduced.

It is worth noting, however, that in one hitherto unmentioned setting of eggs on horizontal trays in small incubators the frequency of Position I was 16.9 percent in 136 embryos alive at 18 days. This is nine times its frequency in the tilted eggs and eighteen times its average frequency in the horizontal eggs of the controlled experiment. The machine was operated by a student and failure to turn the eggs may have been a factor. In one composite series of 350 eighteen-day embryos which had been in-

TABLE 4.—*Probabilities of 18-day living embryos having acquired different malpositions in incubating eggs lying horizontally (H) or tilted (T)*

Malposition	Minimum probability and source	Maximum probability and source	Excess above minimum probability	
			in tilted	in horizontal
Position I	0.95 H	1.81 T	0.86	
Position II	1.03 T	2.27 H		1.24
Position III	2.14 H	2.54 T	0.40	
Position IV	0.65 T	2.14 H		1.49
Position VI	3.19 H	4.03 T	0.84	
Total	7.96	12.79	2.10	2.73

ORIGINS OF MALPOSITIONS

The object of this investigation was to ascertain some of the conditions affecting the frequency of malpositions. These may be briefly considered in turn.

Position I (head between thighs).—Kuo (1932) observed that some embryos were unable to turn the head at about 16 to 19 days of incubation so that the beak pointed under or toward the right wing, while the left face lay on the breast. In embryos unable to attain this position, normally preceding hatching, the beak was sometimes buried between the thighs. Our data indicate that this process of turning the head is more difficult in an egg with the large end up than in one lying on its side. Probably this is because in the former case not only would the retraction and turning of the head be required but also the lifting of it against the force of gravity. In the horizontal egg, the adverse affect of gravity

cubated on their sides, but had not been turned over after the fourteenth day, the frequency of Position I was 2.57 percent (Table 2 U.F. '31a). This is higher than that in six out of the seven other lots incubated in a horizontal position (Table 2). It suggests that turning frequently at the critical stages from the fourteenth to the eighteenth day may assist in retraction of the head and thus reduce the frequency of this malposition.

Position II (head in small end).—It is obvious that at some stage the normally-developing embryo must be forced into the larger end of the egg. The observations of living embryos by Kuo (1932) indicated that such a stage is ordinarily reached between the fifth and ninth days and led him to conclude that it must be reached by the ninth if the embryo is subsequently to assume the normal position. This is confirmed by the finding of Byerly and Olsen

(1933) that Position II is apparently determined during the second week of incubation and by their earlier evidence (1931) that its frequency can be increased by incubating the eggs with the small end up instead of the large one. The smaller percentage of embryos in Position II among eggs tilted with the large end up is obviously the result of the greater opportunity for the yolk and embryo to float to the large end than is afforded in eggs lying horizontally. Among the latter some eggs are sure to be tilted occasionally with the small end slightly higher than the large end. Others may contain embryos oriented (abnormally) with the head toward the small end right from the primitive-streak stage. Taylor's (1932) report that Position II is more commonly assumed by embryos originally deviating from the normal orientation is substantiated by unpublished data in this laboratory. Together they suggest that certain embryos require special treatment to be gotten safely into the large end. Obviously this treatment is to incubate the egg with the large end up during the critical period, which Kuo reports to be from the fifth to the tenth day of incubation. It is probable that frequent turning in more than one plane would also facilitate the passage of the yolk and embryo to the large end.

Position III (head left).—With respect to this malposition the data are equivocal. In four out of seven trials, it is more frequent in the tilted eggs. Byerly and Olsen (1931) also found it more frequent in upright than in horizontal eggs. However, since in three cases out of seven it was more frequent in the eggs incubated in a horizontal position, the available data give little or no clue to its origin.

The frequency of Position III was lower among the horizontal eggs not turned after the fourteenth day (Table 2. U.F. '31a) than in any other of the sixteen settings. Since only 350 embryos are involved the difference, though striking, should not be considered significant unless it be confirmed in larger numbers.

Position IV (Rotated away from air cell).—It was suggested (Hutt, 1929) that this position is assumed at some turning late in incubation when the embryo has been caught with its head or beak past the air cell and has been unable to return to its normal position with the beak underlying the chamber. This probably occurs just at the time when the dimensions of the chick are too great to permit its turning except by the great exertions at hatching time which are possible only with the aid of pulmonary respiration. If eggs in a horizontal tray are not turned on or after the eighteenth day, one finds that practically all which subsequently hatch are pipped *on the upper surface.* If the eggs are turned on the nineteenth and twentieth days, they are not pipped on the upper surface any more than on any other because an embryo of that age is no longer able to change its position or that of the air cell to lie with the beak and air cell uppermost. If, before turning is stopped on the eighteenth day, an embryo is just at the point when rotation within the egg is becoming impossible, the last turning may roll it with the beak past the air cell. If the embryo is then unable to move it becomes fixed in Position IV.

Embryos in eggs tilted with the large ends up are turned with the long axis of the egg. There is thus much less likelihood of rotating the embryo past the air cell than with the rolling motion, in a plane at right angles to the long axis, which is the usual method of turning eggs lying horizontally in a flat tray.

It is not surprising, therefore, that Position IV should be more than thrice as common in the horizontal eggs as in the tilted ones (Table 3) and consistently so in each series (Table 2). The mode of its origin suggested above is substantiated by the few

settings at University Farm in 1931 in which the eggs were not turned after the fourteenth day. In these 350 embryos alive at 18 days the frequency of Position IV was only 0.57 percent, which is much less than for the seven other settings of horizontal eggs. However, the frequency of other malpositions (I, II and VI) was slightly higher in these same eggs, so that if there be an age when cessation of turning will prevent the assumption of Position IV, or lessen its frequency, that age would have to be somewhat later than the fourteenth day if a decrease in Position IV is not to be offset by an increase in others.*

Byerly and Olsen (1931) considered Position IV as a stage intermediate between the normal condition and Position II. The evidence for this is not clear, although both II and IV are increased in eggs incubated in a horizontal position and in the few eggs incubated by them with the small ends up. If the mode of origin of Position IV suggested above be the correct one, the two malpositions are quite distinct.

Position V (feet over head).—In our earlier work this condition was rare and therefore not considered as a distinct malposition, but Smith (1930) is undoubtedly correct in so designating it. Its frequency in our material was as follows: In 5,359 tilted 18-day embryos—0.31 percent. In 3,688 horizontal 18-day embryos—0.46 percent.

The difference between these figures and the apparently higher incidence of this malposition in Smith's (1930) material may possibly be due to differences in classification.

Position VI (beak over wing).—This malposition is consistently more frequent in the tilted eggs. The first explanation would appear to be that in them the beak must be raised and wedged under the wing against the force of gravity. An objection to this interpretation is that in many cases of Position VI the beak has been raised even higher above the body, and turned further toward the large end than is necessary to assume the normal position. Moreover, from examination of 19-day living embryos it would appear that the beak does not go under the wing till that age or later, at any rate at a stage when all eggs are lying horizontally, ready for hatching. The disadvantage associated with the large-end-up position must be incurred before that time, but the data do not permit anything more definite than speculation as to where that disadvantage originates.

DISCUSSION

The results of this investigation suggest that there are stages of incubation when it is advantageous to have the eggs with the large ends up and others when the horizontal position is preferable. Incubating with large end up until the twelfth or fourteenth day should reduce the frequency of Position II. Thereafter the advantage would appear to lie with the horizontal eggs if Positions I, VI (and possible III) are to be kept at a minimum. This in turn increases

*It is to be hoped that Byerly's (1930) statement that Positions III and IV are of doubtful importance as barriers to hatching will not deter investigators from seeking to find ways of preventing their occurrence. Our earlier suggestion (Hutt, 1929) that these malpositions usually result fatally by reason of their preventing pulmonary respiration as well as by mechanical hindrance has been substantiated by additional observations over a six-year period which indicate that these two are almost invariably fatal if not so always. A similar viewpoint has been expressed by Kuo (1932). In the Edinburgh material (Hutt, 1929) the combined frequencies of Positions III and IV in 5,050 embryos dying after the eighteenth day totalled 27.5 percent. In 1,333 such embryos reported in this paper (Table 1) the frequencies of Positions III and IV together made 21.8 percent. Since they are evidently responsible for about a quarter of all mortality after the eighteenth day, or about one-eighth of the total mortality during incubation, they present a problem of primary importance to the investigator of embryonic mortality.

the chance of forcing embryos into Position IV, unless that risk can be offset by ceasing to turn before the critical stage for Position IV is reached. Such a system would mean that the eggs would be turned in one plane for the first two weeks of incubation and in another during the last four days of turning. It is quite possible that if some means could be found to turn them alternately in both planes the advantages of both the horizontal and upright positions could be combined and the disadvantages of both eliminated. It is also probable that the lower embryonic mortality observed by several investigators when eggs were turned four to six times daily, compared with that resulting when eggs were turned only twice, may be to some extent associated with a lowered frequency of malpositions. For this reason it is to be hoped that future investigators of the effects on hatchability of such mechanical manipulations during incubation as different amounts of turning and different positions of the egg, will consider not only total mortality but also the frequency of the various malpositions contributing to that mortality.

This discussion has not considered the conclusion of Byerly and Olson (1931) that "gravity and air hunger are the principal factors determining the position of the chick embryo in the egg at hatching time." Since gravity draws the heavier albumen to the bottom of the egg, permitting the yolk to rise to the top, and also causes the embryo to be at the highest point of the yolk in early stages there can be little doubt that gravity is an important factor, perhaps the most important factor in determining the position of the embryo. However, it is extremely doubtful that gravity acts more strongly on any one egg than on another, and, therefore, if in eggs incubated small-end-up there is found a high proportion of embryos in Position II, that result must be ascribed, not to gravity, but to the position of the egg. Fortunately the latter is more amenable to artificial manipulation than the former.

On the other hand it can hardly be said that there is as yet any evidence that "air-hunger" is a factor in the causation of either the normal position or of malpositions under the usual conditions of incubation. Byerly and Olsen found that when one end of the egg was coated with paraffine there was a tendency for the embryo to be found in the opposite end. Accordingly in eggs incubated large-end-up-and-paraffined there was a high incidence of Position II, which these authors ascribed to efforts of the embryo to leave the paraffined end and to seek that providing a better air supply. It is obvious, however, that any response to an unfavorable air supply would be made, not by the embryo, but by its respiratory organ, the allantois. In this connection it is well to recall that as early as 1855 Dareste observed that in eggs having the large end varnished the allantois apparently moved to the small end. This he first attributed to efforts of the allantois to secure an adequate air supply. Later (Dareste, 1863) he concluded that the phenomenon was entirely mechanical and that it resulted from persistence under the varnish of early adhesions of the amnion and chorion. These adhesions prevented the spreading of the allantois between these two membranes under the varnished region, with the result that it grew only toward the small end. Whether the reason be this or the fact noted by Baudrimont and Martin-Saint-Ange (1847, p. 250) that the allantois would not develop under regions of the shell coated with a substance impermeable by air, it is obvious that the embryos found in the small ends of the varnished eggs were drawn there because the allantois was confined to that end. The reason for their assuming Position II was probably not "air-hunger" but the fact that they were prevented from getting into the

large end almost as effectively as if they had been incubated with the small end up.

The underlying cause of Position II is thus neither gravity nor "air hunger" but any conditions which prevent the embryo from getting into the large end of the egg at the period set by Kuo (1932) at from 5 to 10 days. Under normal conditions of incubation the most important factors would seem to be the position of the egg at this period and the orientation of the primitive streak.

SUMMARY

The frequencies of five malpositions of late chick embryos have been determined in eggs incubated horizontally and in others incubated concurrently in the same incubators but with the large ends raised about 45 degrees up to the eighteenth day.

In horizontal eggs the frequency of malpositions was 10.1 percent of 5,030 embryos alive at 18 days, or 60.4 percent in those dying after that age.

In eggs with the large end raised the frequency of malpositions was 10.7 percent in 3,040 embryos alive at 18 days, or 65.9 percent in those dying after that age.

Embryonic mortality after 18 days was practically equal in both lots.

The frequency of Position I (head between thighs) was twice as great in tilted as in horizontal eggs, and that of Position VI (beak over wing) was 25 percent higher in the former than in the horizontal eggs.

Conversely, Position II (head in small end) was over twice as common, and Position IV (rotated from air cell) over thrice as common in horizontal as in tilted eggs.

These differences were consistent in eggs from two experiment stations over a three-year period and are statistically significant.

Position III (head left) was slightly more frequent in the tilted eggs but not consistently so.

It is shown that if the good features of both the horizontal and tilted positions for incubation could be combined, the total mortality during incubation would be reduced by approximately 6 to 8 percent.

In the light of these and other data the possible modes of origin of these malpositions and means of lessening their frequencies are discussed.

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THE RELATION BETWEEN ABNORMAL ORIENTATION OF THE 4-DAY EMBRYO AND POSITION OF THE CHICK AT HATCHING

BY

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(Contribution from Minnesota Agricultural Experiment Station)

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THE RELATION BETWEEN ABNORMAL ORIENTATION OF THE 4-DAY EMBRYO AND POSITION OF THE CHICK AT HATCHING [1]

By J. R. CAVERS, *research assistant in poultry husbandry*, and F. B. HUTT, *poultry husbandman and animal geneticist, Minnesota Agricultural Experiment Station*

INTRODUCTION

Despite the extensive knowledge at hand concerning the early embryology of the chick and the steadily accumulating information regarding its behavior at the time of hatching, there is very little known of the relationships which exist between the two stages of development. Because of their importance as factors causing embryonic mortality, malpositions of the fully formed embryo have been the object of study at this station for several years. The data herein presented deal with abnormal orientations of the young embryo and their possible effects upon the subsequent position of the chick within the egg.

MALPOSITIONS

The completion of the incubation period usually finds the body of the embryo parallel to the long axis of the egg, with the head towards the large end of the shell. It has been generally considered that the embryo accommodates itself thus to the shape of the egg at the end of the second week, but Kuo (*9*),[2] who has described the manner in which the final position is attained, found that the time of its fixation is usually about the tenth day.

Several well-defined deviations from the normal position at hatching, referred to as malpositions, have been described.

The method of designating normal and abnormal positions of fully formed embryos at this laboratory is as follows:

N. The normal position. Body parallel to long axis of egg and head in large end; beak under right wing and toward air cell (fig. 3, *A*).
I. Head buried between thighs.
II. Embryo upside down with head in small end of egg (fig. 3, *B*).
III. Head turned to left and away from air cell.
IV. Body rotated so that head is away from air cell; otherwise normal.
VI. Head not beneath wing, but above or away from it.

More complete descriptions of these malpositions, data on their frequencies, and discussions concerning their relation to embryonic mortality, have been given by Sanctuary (*12*), Hutt (*6*), and others. A detailed description of position VI has been given by Hutt and Pilkey (*7*). The malpositions differ in the extent to which they prevent or hinder hatching. Position I is undoubtedly always lethal, and positions III and IV appear to be fatal in nearly all cases, if not in all. Some of the embryos in positions II are able to hatch. Position VI is probably a considerable hindrance to the chick, if not an actual barrier to hatching.

[1] Received for publication Nov. 8, 1933; issued June, 1934. Paper No. 1224 of the Journal Series of the Minnesota Agricultural Experiment Station. The sixth paper of the series of "Studies in embryonic mortality in the fowl."

[2] Reference is made by number (italic) to Literature Cited, p. 530.

Although some chicks in malpositions may hatch and their positions then be unknown, the influence of abnormal orientations can still be studied in the embryos which fail to hatch.

In the first paper of this series, Hutt (*6*) suggested that some of the abnormal positions of fully formed embryos may result from the aberrant orientations of the embryo, which several embryologists have shown to be not infrequent, and which are known to be established at early cleavage. Such an origin seemed especially likely in the case of position II (head in small end of egg) since very young embryos are sometimes found parallel to the long axis of the egg (instead of at right angles to it), with the head pointing toward the small end. Accordingly an investigation was begun in 1931 to determine whether or not any relation exists between the early orientation and the later position of the embryo. Since the investigation was begun Taylor (*13*) has reported that from embryos originally abnormally oriented there resulted 50 percent more embryos in position II than there were from embryos having normal orientation at 6 days.

ORIENTATION

The position of the avian embryo on the yolk during early stages of development has a fairly definite relation to the axes of the egg. When an egg remains in a horizontal position for a few moments, the yolk comes to rest with the blastoderm on top. The embryo may then readily be observed by removing a portion of the shell above it. Embryos of the domestic fowl at 2 or 3 days of age were described by Von Baer (*1*) as lying at right angles to the long axis of the egg, with the head to the left when the small end of the egg is directed away from the observer. Early embryologists considered the normal orientation given by Von Baer to be relatively constant in all eggs, and used it in studies of segmentation as a means of predicting the future caudal and cephalic regions of the undifferentiated blastoderm. Later investigations have shown that marked deviations may occur from the normal orientation. Féré (*4*) measured orientations of a large number of chick embryos and found that 25 percent of them deviated by more than 45° to the right or left of the normal axis, This is in agreement with the findings of Dalton (*3*), Rabaud (*10*). Kopsch (*8*), and Taylor (*13*).

PROCEDURE

Since it was necessary not only to determine the orientation but also to give the embryo an opportunity to hatch, it was essential that the position of the embryo at an early age be determined without breaking the shell as had been done in most previous studies. Moreover, it was desired to obtain the readings of orientation as early as possible in the incubation process in order to ascertain more nearly the original orientation established during cleavage. For these reasons special apparatus had to be designed to permit observation of young embryos through the shell.

APPARATUS

A candling apparatus was constructed which contained two 500-watt projection lamps and an electric fan to cool them. The box was designed to give the maximum illumination of an egg placed in a

horizontal position in an egg-shaped hole in the top. Very little light could emerge except through the egg. The eggs of White Leghorn fowls were used, since their chalk-white shells transmit the light readily.

The top of the box was marked with lines which radiated out from the center of the egg-shaped hole every 15° throughout an entire circle. The line which represented the normal orientation of the embryo, being perpendicular to the long axis of the egg and 90° counterclockwise from the small end, was marked 0°. Other lines were marked 15°, 30°, 45°, etc., to the right or left of this line (fig. 2).

DETERMINATION OF ORIENTATION

The apparatus just described made readings of orientations possible at 3 days of incubation. The vitelline blood vessels of the normal embryo showed fairly well through the shell at 60 hours, and soon thereafter the body itself was visible. At 72 hours the cephalic and caudal regions could be distinguished, and in many cases the beats of the heart as well. Since the cranial and cervical flexures are at that age well advanced, the embryo itself is not entirely suitable for accurate measurements. It was found that the vitelline veins and arteries could be utilized along with the body of the embryo in determining the angle of orientation.

After about 72 hours of incubation the vitelline blood vessels were distinctly visible through the shell. These pass bilaterally from the embryo at right angles to its long axis and divide into two main branches, which extend in anterior and posterior directions. The forward branches develop more readily to form a wide fork, the arms of which are more or less parallel to the body of the embryo. The fork formed by the posterior branches occupies a similar relation to the embryo, but it is not so clearly defined until about 80 hours. By 90 hours, however, these vessels may have attained an equal or even greater length than those extending forward (fig. 1).

Since the object of the experiment was to determine the relationship between the definitive position just prior to hatching and the original orientation established in the early cleavage stages, it was desirable to determine that orientation at the earliest possible age consistent with accurate readings. After numerous trials this was found to be between 84 and 90 hours. At that time the embryo and the blood vessels both serve to differentiate the two ends of the body. The angle of orientation is indicated more exactly by the blood vessels than by the embryo itself, for the reason that they extend farther over the yolk and they are more plainly visible through the shell. The extra-embryonic blood vessels are less subject to changes of position than is the embryo, especially when the latter is enclosed within the amnion from the fourth day on. Moreover, it was found by breaking eggs at 48 hours that the branches of the blood vessels described above are almost exactly parallel to the straight line which the embryo's body then forms.

In eggs incubated in a horizontal position, the 4-day embryo is found on top of the yolk and near the highest point of the shell. Readings were quickly made in such eggs directly upon removal from the incubator. A number of the eggs used were incubated with the large end up, and the long axis of the egg about 45° from the vertical. In these some difficulty was at first encountered because, as the

eggs cooled, the embryonic membranes adhered to the inner shell membrane in the region of the air cell. This obstacle was easily overcome by placing all eggs in a horizontal position immediately upon their removal from the incubator, a procedure which caused the yolk to float freely so that the orientation of the embryo could be determined without difficulty. Readings were made to the nearest 15° radius.

DISTRIBUTION OF ORIENTATIONS

The orientations of 4,721 chick embryos were observed by candling on the fourth day of incubation. Of these 2,906 were in eggs incubated in a horizontal position and 1,815 were in eggs incubated in the tilted position described above. Figure 2 shows the distribution of all these embryos at 24 different angles on the yolk.

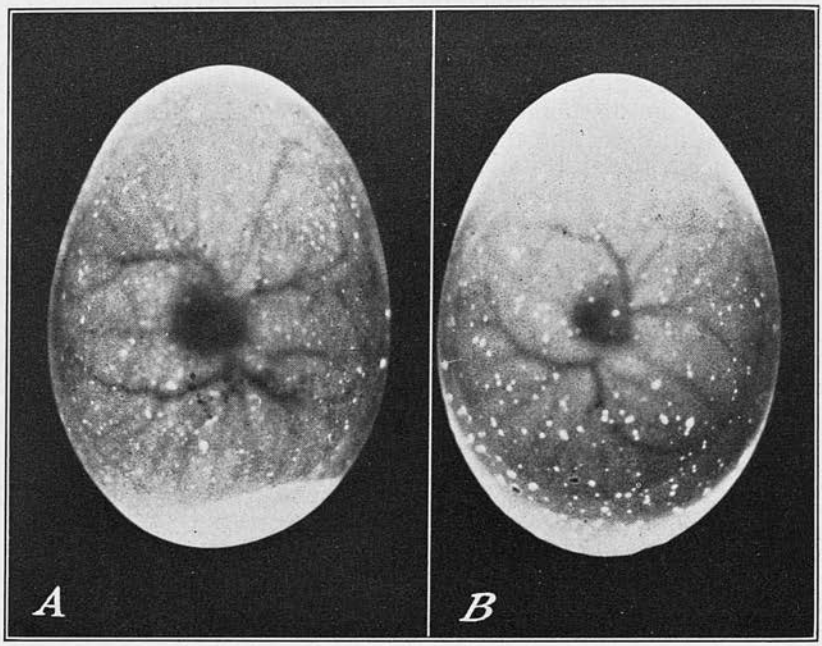

FIGURE 1.—Photographs of living chick embryos at 90 hours of incubation, taken through the shell of the egg. Owing to slight movements during the 10-minute exposures used in taking these pictures, the bodies of the embryos are less distinct here than when orientations were measured: *A*, Normal orientation; *B*, an embryo deviating to the right of the normal orientation. In both cases the anterior end of the embryo is to the observer's left.

The skewed distribution shown in figure 2 indicates that deviations occur more often to the right of the normal axis, i.e., toward the small end of the egg, than to the left. A similar condition is apparent also in the rather limited numbers reported by Dalton (*3*) and Kopsch (*8*). Unfortunately Féré's extensive data are not presented in sufficient detail to permit a comparison. Other data in this laboratory indicate that this skewed distribution of orientations arises from differences between individual hens with respect to the early orientations of their progeny.

The class with the center at 0° and a range of 7.5° on each side contains 1,491 embryos, or about one third of the total number.

Obviously the definition of normal orientation cannot be limited to such a narrow class. It has seemed preferable to consider as normal all orientations falling within the quadrant bounded by radii at 45° right and left of the so-called normal axis at 0° (fig. 2). The classes exactly at 45° right and 45° left contain 480 and 117 embryos, respectively. These cannot all be classed either as normal or abnormal, but by allotting them to the groups of normal and abnormal orientations in the proportion of 566 : 291 (right) and 243 : 76 (left)—the frequencies in the two adjacent classes of orientation—it was possible to determine the number belonging in the normal quadrant.

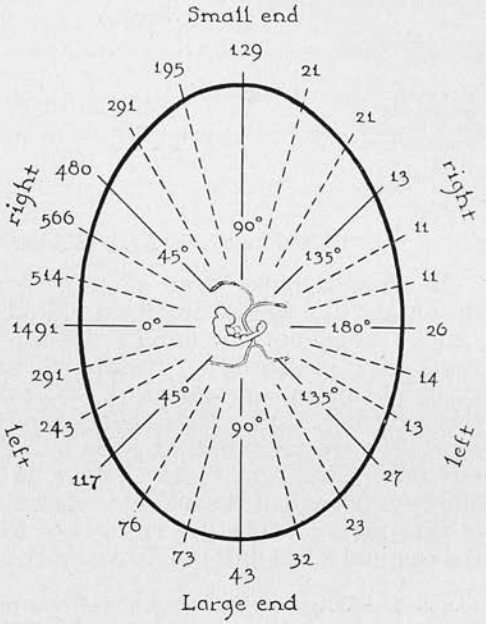

FIGURE 2.—Distribution of 4,721 chick embryos oriented at different angles with respect to the principal axes of the egg at 4 days of incubation.

This permitted a comparison of the writers' determinations with those of Féré (*4*), Rabaud (*10*), and Kopsch (*8*), which is shown in table 1. It is evident from these data that with large numbers of chick embryos 75 percent of their orientations are to be expected within the normal quadrant.

TABLE 1.—*The proportion of normal orientations in the present data compared with those in other investigations*

Investigators	Method of observation	Total orientations	Orientations within normal quadrant
		Number	*Percent*
Cavers and Hutt	Through shell	4,721	74.4
Féré (*4*)	Shell removed	2,926	74.9
Kopsch (*8*)	do	155	62.6
Rabaud (*10*)	do	100	76.0

The accuracy of the method was tested by making a second measurement on 204 embryos 12 hours after the first. The eggs were turned in the incubator between readings, a procedure which would change the orientation if that were not relatively constant. The results, shown in table 2, indicate that the method used is remarkably accurate. Moreover, it has been shown (table 1) that the type of distribution is similar to that obtained by other workers. It is concluded therefore that the method of observing positions through the shell and using largely the vitelline blood vessels to indicate the angles of orientation gives essentially the same result as when the body is observed directly after breaking the egg. It has the special advantage of not interfering with the viability of the embryo.

TABLE 2.—*Comparison of two readings of orientation taken 12 hours apart on the same embryos, the eggs having been turned between readings*

Orientation	Eggs in indicated deviation class	
	Number	*Percent*
Same orientation at both readings	109	53.4
Deviation of 15°	80	39.2
Deviation of 30°	13	6.4
Deviation of 45°	2	1.0
Total	204	100.0

ORIENTATION AND SUBSEQUENT MORTALITY

In order that the dead embryos might be examined in good condition and the age at death estimated, the eggs were candled several times throughout the hatch. Of the 4,721 embryos whose orientations were measured on the fourth day, 3,864 were utilized in the study of subsequent mortality. The distribution of mortality among these during three subsequent periods of incubation and the total mortality are presented in table 3. The percentages of mortality in any one period e.g., 11 to 17 days, are based on the number of live embryos in each of the different classes of orientation at the beginning of that period. The percentages of total mortality are calculated on the original 3,864 embryos alive on the fourth day.

TABLE 3.—*Embryonic mortality at different periods of incubation in embryos originally having different orientations*

Orientation on fourth day (degrees)	Mortality in each period based on the number of embryos alive at the beginning of that period				Total mortality, 4–21 days	
	Embryos	4–10 days	11–17 days	18–21 days		
	Number	*Percent*	*Percent*	*Percent*	*Number*	*Percent*
135±22.5, left	54	5.6	0	5.9	6	11.1
90±22.5, left	131	4.6	4.8	20.1	36	27.5
45±22.5, left	357	3.9	4.7	17.1	86	24.2
0±22.5	1,971	5.0	5.0	15.5	467	23.7
45±22.5, right	1,002	7.5	5.7	17.3	279	28.0
90±22.5, right	271	5.5	5.9	20.3	79	29.2
135±22.5, right	38	7.9	2.9	14.7	9	23.7
180±22.5	40	2.5	7.7	16.7	10	25.0
Total or average	3,864	5.6	5.1	16.4	972	25.2

Of 3,461 embryos which survived to the nineteenth day, 222 were then removed and examined without being allowed to hatch. The expected 18-to-21-day mortality for these has been calculated to the nearest whole number in each class of orientation at the rate prevailing for that class in the same period among the remaining 3,239 embryos. For example, 26 of those removed had originally been oriented at 45° ± 22.5° left. The mortality after 18 days among 301 embryos originally so oriented, but given an opportunity to hatch, was 52, or 17.3 percent. Accordingly, the expected mortality for the 26 removed was 4 and the total 18-to-21-day mortality in the class was taken as 56. The number among which mortality was actually observed was so large that the calculation for the comparatively small number removed is quite justifiable.

For convenience, and to avoid fluctuations arising from small numbers, the embryos have been grouped in 8 classes of orientation. Each of these 8 includes 3 of the original 24 classes used when readings

were made. For example, the class at 45° ± 22.5° right includes those embryos originally at 30°, 45°, or 60° to the right of the normal position, and, since these are mid-class values, the actual limits of the large class are at 22.5° right and left of the radius at 45° right.

The distribution of mortality is fairly uniform throughout the different classes of orientation except where the numbers are small to begin with. Embryos in the class at 135° ± 22.5° left are especially favored, the rate of total deaths being less than half that found in the rest of the population. This might indicate an advantage to the embryo in being oriented toward the air cell of that egg were it not for the fact that in the class at 90° ± 22.5° left, mortality is slightly in excess of that of the total population. High or low death rates in any of the classes with few embryos are accompanied by compensating fluctuations in adjacent classes and may therefore be attributed to chance. It is worthy of note, however, that the lowest total mortality (except in two classes containing small numbers) occurs in the modal class (0° ± 22.5°), and that this class is the one containing embryos deviating not more than 22.5° from the orientation considered the norm for this species. A comparison of the mortality rate in this group with that for all other embryos reveals the following figures:

	Mortality (percent)
Normally oriented (1,971)	23. 69 ± 0. 97
All others (1,893)	26. 67 ± 1. 02
Difference	2. 98 ± 1. 40
$\frac{\text{Difference}}{\text{Standard error}}$	2. 1

Since the difference between the mortality rates for these two groups is 2.1 times its standard error, it is statisticially significant, and since the rates for every class of abnormal orientation having adequate numbers are uniformly higher than that for the normal class, it may also safely be considered as biologically significant. This agrees with the finding of Taylor (*13*) that eggs oriented normally at 6 days hatched better than others by about 5 percent. The normal orientation is evidently slightly more conducive to the survival of the embryo than are deviations from it.

It is obvious that the excessive mortality among the abnormally oriented embryos occurs in the last 4 days of incubation and that it is to a large extent attributable to the comparatively high rate of 29.2 percent prevailing for embryos originally oriented at right angles to the normal orientation and with the head toward the small end of the egg (90° ± 22.5° right). The number in this class, however, is too small to indicate by statistical methods that its mortality rate is significantly higher than that for the normal class (23.7 percent) or for the entire population (25.2 percent). The excessive mortality in this class occurred chiefly in the last 4 days of incubation, and it will be shown later that it resulted from an excess of the head-in-small-end malposition.

The same class of orientation also exhibited high mortality during the period from 11 to 17 days. More extensive data would be necessary to prove that these embryos are under a handicap during this period, but such a possibility is indicated. Apart from this the mortality prior to the eighteenth day of incubation does not appear to be related to the original orientation of the embryo.

ORIENTATION AND MALPOSITIONS

All eggs failing to hatch were examined on the twenty-second day. The positions were recorded of embryos which appeared to have survived beyond the eighteenth day of incubation, as indicated by size and by the amount of yolk enclosed within the body. Of 3,239 embryos which survived to the eighteenth day or beyond and which were given the opportunity to hatch, 533 failed to emerge from the shell. Some were alive and even pipped, but since they had not hatched on the twenty-second day they were considered as dead. Almost 65 percent of the 533 embryos were in one or other of the five malpositions described on page 517.

The early orientation of each embryo which died at 18 to 21 days is presented in table 4. Included with the frequency of each position at that age (o) is the expected frequency (c). The latter numbers were derived by allocating the embryos dying in a given position at 18 to 21 days to the various classes of orientation according to the proportions of the 3,239 embryos in those same classes at 4 days. If abnormal orientation has no relation to the occurrence of malpositions, there should be no significant difference between the observed and calculated distributions. The results of tests for goodness of fit are given in the two lower lines of the table. In making these calculations the classes having small numbers were combined, as is recommended by Fisher (*5*), to avoid the use of an expected number lower than 5. The values of P obtained for the distributions of positions normal, I, III, IV, and VI are well above the 0.05 level of significance, and differences between the observed and expected distributions of these positions may therefore be ascribed to chance. On the contrary, the distribution of position II gives a P value much smaller than 0.01, and the difference between the observed and expected values in this case is therefore highly significant. This means that more embryos in position II are associated with certain orientations than would be expected from chance alone.

TABLE 4.—*The early orientations of 533 fully formed embryos found dead in normal and abnormal positions at age of 18 to 21 days*

Orientation on fourth day (degrees)	Observed (o) and expected (c) frequencies of positions											
	Normal position		Position I		Position II		Position III		Position IV		Position VI	
	o	c	o	c	o	c	o	c	o	c	o	c
135±22.5, left	----	2.7	----	0.9	1	1.0	2	0.8	----	0.5	----	1.7
90±22.5, left	5	6.8	7	2.4	2	2.4	1	1.9	----	1.2	8	4.3
45±22.5, left	15	17.7	7	6.1	5	6.3	5	5.0	5	3.2	15	11.2
0±22.5	101	98.0	29	34.0	19	35.1	29	27.8	18	17.5	62	62.4
45±22.5, right	50	47.6	15	16.5	26	17.0	11	13.5	10	8.5	28	30.3
90±22.5, right	15	13.3	8	4.6	13	4.8	4	3.8	1	2.4	5	8.5
135±22.5, right	2	1.9	----	.7	1	.7	2	.6	----	.3	----	1.2
180±22.5	2	2.0	----	.7	1	.7	----	.6	----	.4	3	1.3
Total	190	190.0	66	65.9	68	68.0	54	54.0	34	34.0	121	120.9
χ'^2	2.34		5.72		19.76		0.73		0.77		3.64	
P	.80		.22		<.01		.68		.68		.46	

The relation of abnormal orientation to the occurrence of position II is clearly demonstrated in table 4. The observed occurrences of this malposition are deficient in the 0° class and excessive in classes at 45°

and 90° right. It will be recalled that 90° right represents an orientation toward the small end of the egg and that similarly in malposition II the chick is upside down with its head in the small end of the egg (fig. 3, *B*). Abnormal orientation at an early stage is not the sole cause of position II, however, for 19 of the 68 embryos dying in that position at 18 to 21 days had been normally oriented on the fourth day, and 8 had actually been directed toward the large end of the egg.

The relation of abnormal orientations to subsequent positions of the embryo at hatching may also be measured in another way by considering what eventually happened to those originally having different orientations. The only limitation in such an analysis is that the positions of hatched chicks cannot be known with certainty and the analysis must be confined to those found dead at 18 to 21 days of incubation. Only the results for position II are presented

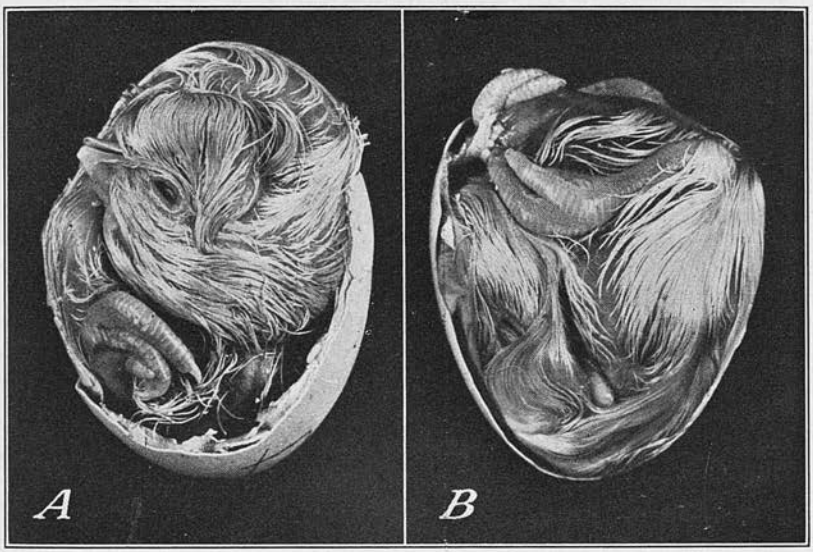

FIGURE 3.—Fully formed chicks which failed to hatch: *A*, Normal position, the head under the right wing and the beak just entering the air cell; *B*, position II, the embryo completely reversed with the head in the small end of the egg.

(table 5) because early orientation apparently had no relation whatever to the occurrence of the other malpositions. In table 5 the frequency of embryos dead in position II for each class of orientation is expressed as a percentage of the total number of embryos which were in that class on the fourth day and which survived to 18 days.

TABLE 5.—*Frequencies of position II in unhatched eggs, expressed as percentages of the 18-day live embryos in each class of original orientation*

Orientation on fourth day (degrees)	18-day embryos	Embryos dead in position II		Orientation on fourth day (degrees)	18-day embryos	Embryos dead in position II	
	Number	*Number*	*Percent*		*Number*	*Number*	*Percent*
135±22.5, left	46	1	2.2	90±22.5, right	227	13	5.7
90±22.5, left	116	2	1.7	135±22.5, right	33	1	3.0
45±22.5, left	301	5	1.7	180±22.5	35	1	2.9
0±22.5	1,670	19	1.1				
45±22.5, right	811	26	3.2	Total	3,239	68	2.1

The 68 cases of position II found among chicks failing to hatch represent 2.1 percent of all the embryos alive at 18 days. While the frequency of this malposition among the 1,670 embryos alive at 18 days and originally normally oriented ($0° \pm 22.5°$) was only 1.1 percent, its incidence among 1,569 similar embryos originally oriented otherwise was 3.1 percent, and among those originally oriented with the head toward the small end ($90° \pm 22.5°$ right) it attained the maximum frequency of 5.7 percent. This means that, apart from all other causes of mortality, the chance of dying in the head-in-small-end malposition during the last 4 days of incubation was, in the writers' material as a whole, over five times as great for the embryo directed toward the small end of the egg at 4 days of incubation as for the one not deviating more than 22.5° from the normal orientation at the same age. It will be shown later that this chance was lower among eggs incubated in one position than for those in another.

Taylor (*13*) has reported finding 50 percent more embryos in the head-in-small-end malposition at hatching time among those abnormally oriented at 6 days than among those normally oriented at the same age. From his abstract it would appear that all those not deviating more than 45° were considered as normal, and on this basis his results would seem comparable to those just given.

Further and somewhat more direct evidence of the influence of abnormal orientation upon the occurrence of malposition II is furnished by the data for 222 embryos which were all examined on the nineteenth day, and none of which were allowed to hatch (table 6). Position II occurred in 6.3 percent of these embryos. The numbers involved are small for the purpose of calculating percentages, but they do illustrate the marked tendency of embryos oriented toward the small end of the egg on the fourth day to be in a similar position at hatching.

TABLE 6.—*Occurrence of position II in embryos of known orientation, from eggs broken and examined on the nineteenth day*

Orientation on fourth day (degrees)	19-day embryos	Embryos in position II		Orientation on fourth day (degrees)	19-day embryos	Embryos in position II	
	Number	*Number*	*Percent*		*Number*	*Number*	*Percent*
135±22.5, left	5	1	20.0	90±22.5, right	14	5	35.7
90±22.5, left	3	0	0	135±22.5, right	1	0	0
45±22.5, left	26	0	0	180±22.5	1	0	0
0±22.5	109	4	3.7				
45±22.5, right	63	4	6.3	Total	222	14	6.3

The higher frequency of position II among these embryos than in those failing to hatch might be taken to indicate that a considerable proportion of chicks in this malposition are able to hatch were it not that the numbers involved are too small to permit a conclusion and that these eggs were all incubated in the horizontal position. As will be shown later, this position during incubation is particularly conducive to the assumption of the head-in-small-end malposition.

On the other hand, 9 of the 14 embryos originally oriented directly toward the small end of the egg (class 90° right, table 6), had succeeded in correcting their position before the nineteenth day. Of the

78 embryos oriented in the half of the egg away from the air cell (45° 90°, and 135° right) only 9, or 11.5 percent, retained that position. It is evident, therefore, that only a small proportion of these abnormal orientations have resulted in position II. Other malpositions in these same 222 eggs opened on the nineteenth day occurred independently of the early orientation.

REDUCTION OF THE RISK FOR ABNORMALLY ORIENTED EMBRYOS

In the fifth paper of this series Hutt and Pilkey (7) showed that position II is significantly more frequent in eggs incubated in a horizontal position than in those tilted at 45° with the large end up. The question naturally arises whether the tilted position makes it easier for all embryos to assume the correct position for hatching or whether it gains its advantage chiefly by facilitating the escape from the head-in-small-end malposition of the embryos originally oriented toward the small end of the egg. Analysis of the material upon which the present paper is based sheds some light upon this problem.

Of the 3,864 embryos of known orientation which were utilized in the study of subsequent mortality and malpositions, 2,049 were incubated in the horizontal position and the remainder in the tilted position, all being horizontal after the eighteenth day. In table 7 there is shown the effect of the tilted position upon the incidence of position II in embryos originally oriented (1) at $90° \pm 37.5°$ right and (2) any and all other ways including the normal orientaton at $0° \pm 25°$. The 222 embryos examined on the nineteenth day were deducted in determining the number of 18-day embryos. Accordingly, the figures record the incidence of chicks dead in position II among embryos alive at 18 days and given the opportunity to hatch. The inevitable slight error from the probability that some of those in that malposition had hatched is distributed equally to the two groups and therefore does not prejudice the data.

TABLE 7.—*Effect of the position of the incubating egg upon the incidence of position II in embryos originally oriented with the head toward the small end and in those oriented otherwise*

Position during incubation	Total embryos at 4 days	Embryos oriented in the given class		Embryos alive at 18 days	Embryos subsequently dead in position II		Difference between percentages dead in position II in horizontal and tilted positions
	Number	*Number*	*Percent*	*Number*	*Number*	*Percent*	
Embryos originally oriented 90°±37.5°, right:							
Horizontal	2,049	274	13.4	210	19	9.05	7.14±2.19
Tilted	1,815	239	13.2	209	4	1.91	
Embryos in all other orientations:							
Horizontal	2,049	1,775	86.6	1,388	23	1.66	0.12±0.47
Tilted	1,815	1,576	86.8	1,432	22	1.54	

The percentages of the total number of embryos which were oriented toward the small end do not differ significantly in the horizontal and tilted eggs. This is to be expected if the orientation is established in early stages of cleavage. The fact that the expectation was realized indicates that the method used has permitted the

determination of the original orientation at a stage before it might be concealed by movement of the embryo or modified by the position of the incubating egg.

Among all the embryos not originally oriented within the sector bounded by radii at 37.5° right and left of the small end, there is no significant difference between the frequencies of position II in the horizontal and tilted eggs. These embryos constitute 87 percent of those alive at the eighteenth day and are sufficient in number to reveal even a small difference between the horizontal and tilted positions if there were one. On the other hand, among those originally oriented within 37.5° of the small end, the frequency of position II is nearly five times as great in the eggs incubated in a horizontal position as in the tilted ones. The difference—7.14 percent—is 3.3 times its standard error and therefore statistically significant even in the relatively small numbers available for comparison.

On the basis of this analysis and of the data in table 5 it seems safe to conclude that for all embryos surviving to 18 days there is some risk of subsequently dying in position II and that for about 87 percent of them that risk is only slightly lessened, if at all, by incubation in the tilted position. Table 5 indicates that this risk is smallest for those not originally deviating more than 15° from the normal orientation.

The chances of death in the head-in-small-end malposition after the eighteenth day were approximately as follows for embryos in the different classes of orientation at 4 days or earlier in the writers' material:

For embryos at $0° \pm 22.5°$ (normal), 1 per 100.
For other embryos, not within 37.5° of small end, 1.6 per 100.
For those at $90° \pm 37.5°$ right of normal, if tilted, 2 per 100.
For those at $90° \pm 37.5°$ right of normal, if horizontal, 9 per 100.

Hutt and Pilkey (*7*) pointed out that one way to reduce the mortality attributable to this malposition would be to incubate the eggs in the tilted position with the large end up during the critical period when the embryo is becoming fixed in its position with relation to the long axis of the egg. From the studies of Byerly and Olsen (*2*) this period would appear to be during the second week of incubation, while Kuo (*9*) terminates it by the eleventh day. The analysis given above shows why such treatment would be effective.

DISCUSSION

The frequent failure of chick embryos to attain the normal position for hatching undoubtedly accounts for the peak of mortality which occurs during the last few days of incubation. The head-in-small-end malposition (fig. 3, B), which has been shown in this study to result in some cases from incorrect orientation, places the embryo at a double disadvantage. With its head in the small end of the egg there is usually much less room for the embryo to work while initiating and carrying out the process of hatching. Moreover the air cell is inaccessible as a source of air to supplement the allantoic respiration before the shell is pipped.

Evidence has been gathered at this laboratory which shows that position II is likely to be fatal in the majority of cases, but on the other hand, a few chicks in this malposition have been definitely observed by the writers to hatch. It would appear, therefore, that though the

head-in-small-end position is usually fatal, it is not so invariably. This is not in accord with the opinion of Réaumur (*11*), who noted the malposition as early as 1751 but did not consider it a handicap.

Although position II seems to be of relatively infrequent occurrence when the total number of fertile eggs is considered, it accounts for no small portion of the mortality in eggs incubated in a horizontal position. Over 18 percent of 5,050 embryos dying on or after the eighteenth day in eggs so incubated were found by Hutt (*6*) to be in position II. From the data in table 7, which indicate that at least 19 out of 42 cases of position II in horizontal eggs resulted from abnormal orientation, it would appear that about 45 percent of such deaths, or about 8 percent of the mortality in the last 4 days of incubation, is traceable in horizontal eggs to abnormal orientations. This amounts to a little less than 4 percent of the total mortality. From the data in table 7 it would appear that for tilted eggs the mortality from this cause is considerably less than this figure.

Successive readings of orientation in the present study have shown that the extra-embryonic blood vessels and the yolk have a relatively constant relationship to the long axis of the egg from 72 to 96 hours, and that this relationship is maintained in most cases until the end of the sixth day. During this period the vitelline blood vessels serve to anchor the embryo in a fixed position upon the yolk, and the chalazae, in turn, maintain the yolk in a fairly constant relationship to the long axis of the egg. Kuo (*9*) points out that the restraint exercised upon the movements of the embryo by these two factors persists in varying degrees up to the tenth day of incubation. This is undoubtedly why some of the embryos originally directed toward the small end are never able to escape from that initial handicap. On the other hand, after the fifth day the embryo moves with increasing freedom up to the tenth day or slightly later (Kuo). This, in turn, explains why, in the writers' material, 98 percent of 209 embryos originally oriented within 37.5° of the small end were subsequently able to assume the normal head-in-large-end position in eggs incubated in the tilted position. Evidently the freedom of movement which permits such a shifting is somewhat more restricted in the eggs incubated in the horizontal position.

Further tests will be necessary to determine whether or not the occurrence of position II independently of the original orientation can be overcome by such manipulations as multiple turnings or turning the eggs in more than one plane during the critical second week when the head-in-small-end position is apparently established.

It is particularly interesting that the orientation associated with the lowest total mortality rate should also be that in which the greatest number of embryos is found (table 3). The rates 11.1 and 23.7 percent in embryos oriented at $135° \pm 22.5°$ left and $135° \pm 22.5°$ right are based on small numbers, and can therefore hardly be compared with the others which are based on over 100 embryos in each class. Apart from these two classes, the embryos not deviating more than 22.5° to right or left of the normal position have the lowest rate of mortality, one which was earlier shown to be significantly lower than that for all other orientations combined. The numbers in the five largest classes seem amply large to rule out the possibility of this being a coincidence, but further data bearing on this point are desirable.

The special interest lies in the probability that the orientation at right angles to the long axis of the egg, and with the head to the left when the small end of the egg is directed away from the observer, has become the normal and the modal orientation because of its survival value. Such an explanation is tenable only if the angle of orientation of the embryo is in some measure an inherited character. Obviously it is not a character of the embryo, but rather one that is maternally determined. If of two hens one should produce eggs containing embryos oriented within a very narrow range from $0° \pm 22.5°$, and another yield embryos with a much wider scatter of orientations, or within a narrow range but one deviating from the normal, the progeny of the first would have a slight advantage over that of the second. If the tendency exhibited by the first hen were inherited it would be preserved by natural selection and would in time become established as the normal for the species. Unpublished data collected by the writers support this hypothesis.

SUMMARY

The orientation of 4,721 chick embryos was accurately determined by candling, without breaking the shell, at 84 to 90 hours of incubation. Seventy-five percent of these embryos lay within 45° right or left of a line at right angles to the long axis of the egg and with their heads to the left when the small end of the egg was directed away from the observer.

The mortality rate for embryos originally normally oriented was significantly lower than that for all the remaining embryos.

Abnormal early orientation had apparently no markedly adverse effect upon the viability of the embryo until the last 4 days of incubation.

One malposition of the fully formed embryo, that in which the body is upside down with the head in the small end of the egg, was five times as frequent among embryos which had been oriented toward the small end of the egg on the fourth day as among those then normally oriented.

When eggs containing embryos originally oriented within 37.5° of the small end of the egg were incubated in a tilted position with the large end up, the frequency of the head-in-small-end malposition was reduced to 1.9 percent, but among such eggs incubated in a horizontal position its frequency was 9 percent. Tilting the eggs did not materially reduce the frequency of this malposition among embryos having other initial orientations.

Four other malpositions appeared to be independent of the original orientation of the embryo.

It is suggested that the normal orientation of the chick embryo has been evolved and established by virtue of the greater survival value which it confers upon the embryo.

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EMBRYONIC MORTALITY IN THE FOWL. VII. ON THE RELATION OF MALPOSITIONS TO THE SIZE AND SHAPE OF EGGS

BY

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Embryonic Mortality in the Fowl

VII. ON THE RELATION OF MALPOSITIONS TO THE SIZE AND SHAPE OF EGGS

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MALPOSITION III (head under left wing) is probably the most important malposition because of its frequency, which may range up to 5 percent of the embryos still alive after 18 days of incubation, and the fact that it is almost always lethal. Its cause is quite unknown, except that it is more frequent in eggs incubated with the large end up than in those incubated horizontally (Byerly and Olsen, 1936). During examination of some 40,000 unhatched eggs it appeared to the writer that malposition III was more frequent in the larger eggs, and, since definite information on this point was lacking, an investigation was undertaken to determine the relationships, if any, between the frequencies of various malpositions of the embryo and the size and shape of eggs.

MATERIAL AND METHODS

During 1933 and 1934 all eggs incubated in the regular hatches at the Minnesota Agricultural Experiment Station were utilized. The fowls producing these eggs all received the same ration. The eggs were incubated in forced draught incubators, in the tilted position, to 16 or 18 days and then laid horizontally in hatching trays. The conditions of incubation were the same in both years.

In 1933 the following measurements were taken on all eggs set:

Weight, to nearest 0.5 gm., taken on the next morning after laying.

Length, to nearest 0.1 mm.

Maximum diameter, to nearest 0.1 mm.

Diameter, 13 mm. from large end, to nearest millimeter.

Diameter, 13 mm. from small end, to nearest millimeter.

Length and maximum diameter were obtained with vernier calipers.

The last two measurements in the above list were considered necessary for a more accurate indication of shape than is provided by the others. They were taken in a special apparatus consisting of a thin brass plate supported rigidly 13 mm. above a base plate and containing 20 round holes varying in diameter from 23 to 43 millimeters in which eggs were fitted. The smallest hole permitting one end of an egg to touch the bottom plate measured to the nearest millimeter the diameter of that egg at a point 13 mm. from the end in the hole.

All these measurements were recorded on the egg and on the record of incubation.

In 1934 weight was recorded as before but the other measurements were taken after the hatch and only on eggs containing embryos which had died after the second candling at 16 days. This procedure did not supply any data (except weight) for the eggs which hatched but did provide measurements for all eggs containing embryos in malpositions or dead in the normal position. Weights were not taken on the first four settings of eggs in genetic experiments, so those measured in 1934

[1] Paper No. 1582 of the Journal Series of the Minnesota Agricultural Experiment Station, where the data were gathered and most of the statistical analyses were made.

exceed in number those weighed (Tables 1 and 2).

In both years all eggs failing to hatch were examined and the position of the embryo recorded for those over 18 days old. It is difficult to recognize with accuracy the age of embryos from 18 to 21 days old. In this investigation, as in the author's previous work on malpositions, embryos with the major portion of the yolk sac still outside the body have been considered as too young to have attained the definitive 18- to-21-day group. By restricting the data to embryos with the yolk sac drawn into the body, or almost entirely so, few if any of those considered are likely to be only 18 days old. Most of them are more likely over 19 or 20 days of age. However, since even with normal incubation some embryos are found alive at 22 days with the yolk sac outside of the body, it is obvious that ages cannot be assigned with complete accuracy to those dying after 18 days. For that reason it seems preferable to refer to them as 18- to-21-day dead embryos, with the understanding that this class includes only fully-formed chicks, in all of which the yolk sac, or the major part of it, has been drawn into the body.

Since each egg carried the hen's number and date of laying, there was no difficulty in maintaining the identity of every egg and adding the data on its ultimate fate (for example, hatched, dead in position III, and so forth) to the earlier record of its weight and dimensions.

The eggs used in this study came from different breeds and from both pullets and older birds. For analyses of data they were separated into two groups consisting of those from:

(1) S. C. White Leghorns only.

(2) Heavy breeds, including Rhode Island Reds, Plymouth Rocks, and a few from White Wyandottes.

ANALYSIS OF DATA

Within each of these groups comparisons with respect to weight and the four measurements were made as follows:

(1) Between (a) eggs which hatched and (b) eggs containing embryos dead after 18 days and in the normal position.

(2) Five separate comparisons, one for each malposition, of (a) eggs containing embryos dead after 18 days in one malposition, and (b) a normal control population of eggs which hatched and those containing late embryos in normal position, that is, the two groups of the first comparison.

All eggs containing embryos of 18-21 days in malpositions were included in the analyses. The sample of hatched eggs (data of 1933) was drawn only from birds which yielded any one of the five malpositions considered as well as hatched chicks and from the records for such birds every third egg which hatched was taken for the sample. Since all eggs were listed on the records in the order of laying from the beginning of the incubation season this method of sampling reduced to the minimum any possible error from genetic, seasonal, or individual variation in size of egg or hatchability.

The sample of eggs containing chicks dead in the normal position (data of 1934) included all such eggs only from hens which also produced malpositions. The normal position was considered as that in which the head is in the large end of the egg, with the beak under the right wing and pointing toward the air cell.

The five malpositions for which the egg measurements were compared with normal controls have been described in detail by Hutt and Pilkey (1934) and are therefore here only designated briefly as follows:

Position I: head between the thighs.

Position II: head in small end of egg.

Position III: head in large end but under or over left wing.

TABLE 1.—*Comparison of measurements of eggs which hatched and eggs containing fully-formed chicks dead in the normal position*†

Group	Number of eggs	Weight§ grams	Length mm.	Maximum diameter mm.	Diameter 13 mm. from:	
					Large end mm.	Small end mm.
White Leghorns						
(a) Hatched.....	340	56.97±0.19	59.24±0.10	41.60±0.06	36.20±0.05	32.31±0.07
(b) Dead, normal position.....	110	57.83±0.48	59.23±0.22	42.01±0.15	36.12±0.12	32.54±0.15
Difference.....		0.86±0.52	0.01±0.24	0.41±0.16*	0.08±0.13	0.23±0.16
Heavy Breeds						
(a) Hatched.....	319	57.39±0.25	57.31±0.13	42.33±0.08	36.86±0.07	34.22±0.08
(b) Dead, normal position.....	73	57.00±0.79	57.49±0.28	42.12±0.17	36.41±0.16	33.63±0.18
Difference.....		0.39±0.83	0.18±0.31	0.21±0.19	0.45±0.17*	0.59±0.20*

* Statistically significant differences.

† Means, differences and standard errors were calculated to five places of decimals, though only two are shown.

§ For weight the numbers of dead in normal position were 72 for Leghorns and 38 for heavy breeds.

Position IV: embryo rotated and beak away from air cell.

Position VI: beak over right wing, instead of under it.

RESULTS

In the first of these comparisons the means with their standard errors (Table 1) revealed that in the five measures considered there were no consistent differences between (a) eggs which hatched, and, (b) eggs containing fully-formed chicks dead in the normal position.

It is true that the differences between these two groups in the maximum diameter of Leghorn eggs and in the "13 mm. large end" and "13 mm. small end" diameters for eggs of the heavy breeds were respectively 2.56, 2.60, and 2.93 times their standard errors and therefore statistically significant. However, these differences were far from consistent. In Leghorns the "deads" had a greater maximum diameter than those that hatched, in the other group the hatched eggs were the wider. Similarly, with respect to the diameter 13 mm. from the small end, the difference is one way in Leghorns and the opposite way in the heavy breeds. Accordingly, since the seven other differences were not significant, and especially since the diameters at 13 mm. from either end were later found to have little or no relation to malpositions, the samples of hatched eggs and of those containing embryos dead in the normal position were considered sufficiently uniform to justify their being combined in one large sample, hereinafter designated as the control group, for comparisons with eggs containing embryos in malpositions. Since the "normal" eggs for the two years could be treated as one group, the 1933 and 1934 measurements of eggs with any one malposition were also combined, but the Leghorn eggs were considered separately from those of heavy breeds. This merely means that (for example) in studying the length of Leghorn eggs containing embryos dead in position I, instead of comparing 45 of these with 340 controls which hatched in 1933, and again 32 of them with 110 controls dead in the normal position in 1934, one comparison was made of 77 such eggs with 450 controls.

Comparisons of Eggs Containing Malpositions with Controls

In Table 2 are given the means, with their standard errors, of each of the five measures

TABLE 2.—*Mean measurements of control eggs and eggs containing specific malpositions*†

Group	Numbers weighed	Weight grams	Numbers measured	Length mm.	Maximum diameter mm.	Diameter 13 mm. from:	
						Large end mm.	Small end mm.
White Leghorns							
(a) Controls	412	57.12±0.19	450	59.24±0.10	41.70±0.06	36.18±0.05	32.36±0.06
(b) Position I	68	57.90±0.63	77	59.01±0.25	42.15±0.17	36.39±0.11	32.92±0.15
Position II	48	56.62±0.63	65	58.82±0.29	41.80±0.24	35.97±0.15	32.31±0.19
Position III	95	59.60±0.44	119	60.01±0.22	42.32±0.14	36.21±0.11	32.55±0.15
Position IV	30	58.20±1.13	33	59.86±0.40	41.95±0.30	36.18±0.20	32.21±0.23
Position VI	300	57.76±0.22	312	59.44±0.12	41.84±0.06	36.27±0.06	32.41±0.07
Heavy Breeds							
(a) Controls	357	57.35±0.24	392	57.35±0.12	42.30±0.08	36.78±0.06	34.11±0.07
(b) Position I	86	58.13±0.48	92	57.47±0.22	42.58±0.14	37.02±0.11	34.36±0.13
Position II	45	58.62±0.66	53	57.48±0.39	42.52±0.15	36.77±0.15	34.49±0.21
Position III	74	59.20±0.53	100	58.25±0.24	42.55±0.13	36.87±0.11	34.32±0.15
Position IV§	18	58.39	23	57.81	42.50	36.74	34.22
Position VI	274	58.08±0.28	296	57.42±0.14	42.41±0.08	36.93±0.07	34.42±0.09

† Though only two places of decimals are shown, means were calculated to five places and standard errors to three.

§ Significance tested by Fisher's *t* test instead of by standard errors.

considered for eggs containing any one malposition, and for controls.

Of the 50 measurements (2 breeds × 5 malpositions × 5 measures) of "malposition eggs," 41 are greater than the corresponding measurements for their controls, whereas by chance about 25 would be expected to be slightly greater and 25 slightly less than controls. However, these differences are not in all cases statistically significant when considered in relation to their standard errors, nor is it certain that all the statistically significant differences have also biological importance. Statistically significant differences are shown in Table 3. To save space differences less than twice their standard errors are omitted.

Position III (head under left wing) clearly occurs in eggs significantly heavier and longer than those containing normal embryos, the differences in these respects being highly significant in both Leghorns and

TABLE 3.—*Statistically significant differences by which measurements of eggs containing specific malpositions exceed those of control eggs*

	White Leghorns		Heavy Breeds	
	Difference	Diff./S.E.	Difference	Diff./S.E.
Position I				
Max. diam.	0.442±0.181 mm.	2.4		
Diam. 13 mm. from small end	0.557±0.164 mm.	3.4		
Position III				
Weight	2.476±0.473 gms.	5.2	1.853±0.581 gms.	3.2
Length	0.779±0.237 mm.	3.3	0.906±0.267 mm.	3.4
Max. diam.	0.617±0.156 mm.	4.0		
Position VI				
Weight	0.639±0.290 gms.	2.2	0.734±0.365 gms.	2.0
Diam. 13 mm. from small end			0.310±0.114 mm.	2.7

heavy breeds. The maximum diameter was also significantly greater by 0.617 mm. in Leghorn eggs containing this malposition than in controls, but in heavy breeds the 0.22 mm. by which this dimension in eggs containing malposition III exceeded that in controls was not significant. In all of 10 measures for the two breeds, the weights and dimensions of eggs containing malposition III exceeded those of eggs which did not.

Position VI (beak over wing) occurred in eggs somewhat heavier than controls in both groups, but the differences were comparatively small and would be considered of no importance had there been a difference in only one group. Since both groups were consistent in this respect, it is possible that this malposition is more likely to occur in larger eggs, and that even though the samples number 274 and 300, still larger numbers might be necessary to establish more significant differences. Eggs containing malposition VI exceeded those which did not in all five measures for each of the two groups.

Position I (head between thighs) occurred in Leghorn eggs somewhat greater in maximum diameter than controls, but significantly greater in diameter at a point 13 mm. from the small end. Eggs of heavy breeds containing this malposition were also somewhat larger in these dimensions, but the differences between them and controls were small. The data in Table 2 agree with common knowledge in showing the Leghorn eggs, though differing little from the others in weight, to be considerably longer, narrower and more pointed than those of heavy breeds. The analysis suggests the possibility that in such Leghorn eggs the chick may be less able to extricate itself from Position I if the small end be somewhat wider than average. In the shorter, wider eggs of heavy breeds, the similar trend is far from significant. Just why there should be any difference between breeds in this respect is not evident and future work may show that the tendency apparent in Leghorn eggs in this investigation can be ascribed to chance.

Position II, in contrast to those previously mentioned, appears to be entirely independent of the size and dimensions of the egg. Eggs containing fully formed embryos dead in that malposition are smaller than controls in four of five measures for Leghorns, and greater than controls in four of five measures for heavy breeds. None of the differences are statistically significant and all are small. This is quite in accord with the earlier evidence of Cavers and Hutt (1934) that abnormal orientation of the embryo is primarily responsible for the head-in-small-end malposition.

Indices of Shape

Since single dimensions do not indicate shape so well as indices derived from two or more dimensions, similar comparisons to those already considered were made, using as indices of shape (1) length/maximum diameter, and (2) maximum diameter/diameter 13 mm. from the small end.

Since neither of these indices varied significantly and consistently one way or the other in any groups of eggs containing a specific malposition, and since both of them can be computed, if desired, from the data in Tables 1 and 2, the 16 determinations for each are not given in detail here.

Means for the first index of the two mentioned above varied in Leghorns from 1.410 to 1.427 in different malposition-groups, with 1.424 for those that hatched. In the heavy breeds the mean indices for eggs with specific malpositions varied from 1.350 to 1.369, with 1.354 for eggs that hatched.

Means of the other index varied in Leghorns from 1.280 to 1.302, with 1.287 for those that hatched, and in heavy breeds only from 1.232 to 1.242 with 1.237 for eggs that hatched.

OTHER EVIDENCE

The foregoing evidence that malposition III is more likely to occur in the larger eggs is supported by its distribution among 6,616 fertile eggs incubated at the experiment station during the spring hatching seasons of 1933 and 1934. All of these were weighed on the day after they were laid. Since in a number of genetic matings there had been no selection whatever for size of eggs, there were many eggs smaller than those usually incubated in commercial operations. After eliminating from consideration all eggs in which embryos died prior to 18 days of incubation, the frequencies of deaths in malposition III in the various size classes were determined. These are shown in Figure 1 as percentages of the numbers of embryos alive on the eighteenth day.

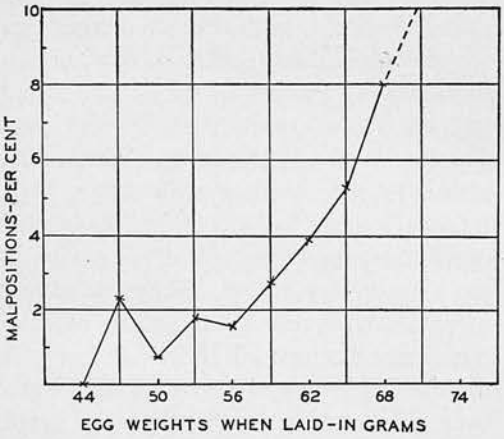

FIG. 1. Incidence of head-left malpositions in unhatched eggs, expressed as percentages of those alive at 18 days.

It is evident that the frequency of malposition III increases rather sharply in eggs heavier than 55 to 57 grams. However, since the frequency of this abnormality can be ascertained only in eggs which have failed to hatch, it follows that the association with size of egg shown in Figure 1 would be spurious if the general mortality rate also increased as sharply in eggs heavier than 55 to 57 grams. Since it is well known that mortality is somewhat higher in large eggs than in those of medium size, this possibility requires examination.

In Table 4, where the various classes of Figure 1 are assembled in three major size groups, it is shown that the relation between deaths in malposition III and egg size is genuine and is not a result of the slightly higher general mortality experienced in the largest eggs. While the mortality after 18 days in the largest eggs (21.56 percent) is, as was to be expected, somewhat higher than that in the eggs of medium size, it is slightly less than that in the smallest eggs (21.97 percent). At the same time the frequency of malposition III among dead embryos in the latter group is only 3.95 percent, whereas it is 20.14 percent in the dead embryos of the largest eggs.

Whether the frequency of deaths in malposition III be expressed as a percentage of the embryos alive at 18 days, or as a percentage of the total deaths after 18 days, the difference between that frequency in (a) either the medium or the largest egg-size group of Table 4 and (b) one or both

TABLE 4.—*The frequency of embryos dead in malposition III in eggs of different sizes*

Egg weight	Living 18-day embryos No.	Mortality 18–21 days		Dead in position III expressed as percent of embryos:		Excess above nearest smaller group		Diff. / S.E.	
		No.	Percent	(a) Alive at 18 days	(b) Dead at 18–21 days	(a)	(b)	(a)	(b)
40–51 grams	346	76	21.97	0.87±0.499	3.95±2.234				
52–60 grams	4019	780	19.41	2.01±0.221	10.38±1.092	1.14±0.546	6.43±2.487	2.1	2.6
61–78 grams	1359	293	21.56	4.34±0.552	20.14±2.345	2.33±0.594	9.76±2.586	3.9	3.8

groups of smaller eggs is statistically significant.

DISCUSSION

The data present no adequate or consistent evidence that the *shape* of an egg, as measured in this investigation, influences the probability of the contained embryo dying in any malposition. The fact that the maximum diameter and the diameter near the small end of the Leghorn eggs containing malposition I are greater than in controls appears biologically unimportant because this same trend is not pronounced in the eggs of heavy breeds.

However, the evidence that the *size* of the egg has an important relation to the probability of the contained embryo dying in the head-under-wing position is incontrovertible. There is also some evidence that positions I, IV and VI are more likely to occur in larger eggs (Table 2), since in all comparisons of eggs containing them with controls, the latter are smaller and narrower. Additional data and some new approach to the problem are needed to show whether or not this apparent trend is significant. It is clear, however, that the incidence of malposition II bears no relationship to size of egg.

Since the frequency of malposition III is over five times as great in eggs heavier than 60 grams as in eggs smaller than 52 grams, it is reasonable to conclude that this abnormality is one of the more important factors contributing to the well-known higher mortality rate prevailing in the larger eggs. Certain data in Table 4 suggest that it may even be the sole factor responsible for the greater mortality in such eggs. The mortality rate in the largest eggs (21.56 percent) exceeds that in the medium-sized ones by 2.15 percent. The incidence of deaths in malposition III in the largest eggs (4.34 percent) exceeds that in the medium-sized ones by 2.33 percent. Since all of these figures are percentages based upon the numbers alive in the respective classes at 18 days, it is obvious that the increase in deaths in malposition III is slightly more than enough to account for the entire rise in mortality in the larger eggs.

In the population of fowls from which these eggs were drawn a few lines had not been bred at all for large eggs, and in most of the stock breeding to increase egg size had not proceeded more than three generations. However, birds laying small eggs were eliminated from most of the pens. It is quite probable that in other strains, where selection and progeny testing for the production of large eggs has been conducted for several years, the rise in the frequency of malposition III might not begin at 56 grams (Fig. 1) but at some greater weight. This is particularly likely to be the case where such breeding has been combined with efforts to improve hatchalibity by breeding.

The peculiarities of development which render embryos in the large eggs more likely than those in small ones to be caught with the head on the left side and under (or over) the left wing have yet to be discovered. In an earlier brief presentation of the facts brought out in this paper (Hutt, 1934) it was suggested that this malposition is more likely to occur when the ratio $\frac{\text{size of egg}}{\text{size of embryo}}$ exceeds an optimum figure The fact that large eggs differ from small ones in the proportions of parts, in surface area, and in other respects suggests several theories but little is to be gained by an elaboration of these until more studies have been made. The present contribution suggests that in further investigations of the ætiology of this malposition, experimental treatments applied equally to small eggs and to very large ones might provide useful information.

In view of some recently advanced theories, as yet unsupported by evidence, which dispose of malpositions in general as indica-

tions of deaths resulting from malnutrition, debility, retardation of development and other vague causes, it seems desirable to emphasize the fact that malposition III is a distinct entity, the frequency of which is largely influenced by conditions (size of egg) not affecting seriously, if at all, the frequency of other malpositions. Similarly, it has been shown by Cavers and Hutt (1934) that the frequency of malposition II (head in small end) is largely affected by the early orientation of the embryo, but that other malpositions are entirely independent of the original orientation of the embryo. The incidence of II is greater in eggs incubated horizontally; III is more common in those with the large end up. Obviously, the causes of one of these malpositions have little in common with causes of the other.

SUMMARY

Weights and measures of length, breadth, and diameters at 13 mm. from each end were taken on eggs of White Leghorns and heavy breeds incubated during two hatching seasons. Comparisons were based upon 659 which hatched, 183 in which embryos died at 18-21 days in the normal position, and 1,170 in which embryos of 18-21 days were found dead in malpositions.

Eggs containing embryos dead in this period but in the normal position did not differ significantly and consistently in size, dimensions, or shape from those which hatched.

In each of the two breed-groups eggs in which embryos died in malposition III (head-under-left-wing) exceeded controls in all dimensions and by statistically significant differences in weight and length.

Position VI (beak over right wing) occurred in eggs consistently larger in every comparison in both breed groups, but not significantly so. Other malpositions were not significantly related to egg size.

Among 5,724 embryos alive at 18 days, the frequency of subsequent deaths in malposition III increased sharply with increasing size of egg. In those heavier than 60 grams, this abnormality occurred in 20.14 per cent of late dead embryos, a frequency over five times as great as its incidence of only 3.95 percent in eggs smaller than 52 grams.

It is suggested that this malposition is to a large extent responsible for the well-known fact that embryonic mortality rates are higher in large eggs than in those of medium size.

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On the Origin, Common Types and Economic Significance of Teratological Monsters in Embryos of the Domestic Fowl.

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It is recognized that the viability of chick embryos during incubation is influenced by such different environmental factors as temperature, supply of oxygen and of carbon dioxide, humidity, methods of turning and also by the ration fed to the flock from which the eggs came. Nevertheless, even when these conditions are at the optimum, so far as present knowledge permits, there is still a mortality during the 21 days of incubation that may range from 25 to 50 per cent. The question arises, " If conditions of nutrition and incubation are good enough to let 65 per cent. of the chicks hatch, what is wrong with the other chicks that die under exactly the same circumstances ? "

In the attempt to answer this question and particularly to determine the extent to which hereditary lethal factors are involved, the writer has examined, during 1928 and 1929, 17,771 fertile eggs which had been incubated but had failed to hatch. It was found that the most frequent deviations from normal development were abnormalities in the position of the embryo within the shell. The significance of these has been discussed elsewhere (Hutt, 1929). Apart from these the most commonly observed abnormalities were various types of teratological monsters, of which a total of 559 have been encountered.

Common Types.—The most common types of teratisms observed in this work are described below. The majority of them have been illustrated in a previous communication by Hutt and Greenwood (1929).

HYPERENCEPHALY.—In this condition there is no upper beak, usually no eyes and always a complete absence of the roof of the cranium. Ectopia is usually associated with extreme cases of hyperencephaly.

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MICROPHTHALMIA.—The distinctive characteristic of this type is the lack of development in one or both eyes. The eye may be any degree less than full size, ranging right down to cases where it is represented only by a small spot of black pigment. Cases of the latter type, in which there is no perceptible development whatever, are the most common. Unilateral microphthalmia, which is much more frequent than the bilateral condition, is accompanied by reduction of the face on the affected side so that the upper beak is twisted across the lower one and toward the side with the small eye. Anophthalmia, or bilateral absence of eyes, is here included with microphthalmia since it appears to be only an extreme case of the latter condition. Neither right nor left eye is more likely to be affected than the other.

EXENCEPHALY.—This class includes various types of cerebral hernia and of extrusion of the optic lobes. It appears to arise from incomplete ossification of the cranium or from portions of the brain becoming so displaced that the frontal or parietal bones do not meet in the normal suture lines. This condition is frequently associated with varying degrees of reduction of the upper beak, so that it is difficult in some cases to draw a line between exencephaly and hyperencephaly. It is also found accompanied by microphthalmia.

ECTOPIA.—Eversion of portions of the viscera.

PROGNATHISM.—Reduction of the upper or lower beak so that its fellow protrudes. (This does not include the "parrot-beaked" condition found in chondrodystrophy.)

DUPLICITY.—Conjoined monsters exhibiting various degrees of incomplete twinning ranging from chicks with two heads (katadidymus) or two bodies (anadidymus) to those exhibiting only slight fission, usually in the head region.

OTOCEPHALY.—In this class have been included several embryos with the head entirely lacking.

CYCLOPIA.—A syndrome including a single median eye, absence of cerebral hemispheres, reduced mandible and a small proboscis-like structure above the eye.

MALFORMED BEAKS.—Absence of premaxillae or nasal bones or of both.

MALFORMED LIMBS.—Absence of one or both legs, absence of one or more toes, thickened and flattened tarso-metatarsus, or absence of leg muscles.

ACARDIA.—An embryo lacking a heart usually attached parasitically to a normal chick. In one case observed in the chick and one in a turkey only the hind limbs remained of the acardiacus.

UNCLASSIFIED.—Unrecognizable development. Most of these general classes exhibit varying degrees of defective development, so that each might easily be subdivided into smaller classes.

The relative frequency of these monsters as observed in the writer's material is shown in Table I.

TABLE 1. *Frequency of Monsters Observed Among* 17,771 *Dead Embryos.*

Class of Monster.	Edinburgh (1928) 11,797 unhatched eggs.	Minnesota (1929) 5,974 unhatched eggs.
Hyperencephaly	179	8
Microphthalmia	96	76
Exencephaly	100	10
Microphthalmia + Exencephaly ...	29	5
Prognathism	8	4
Ectopia	6	6
Duplicity	7	4
Otocephaly	1	7
Malformed beak	2	1
Cyclopia	2	0
Malformed limbs	2	3
Acardia	1	0
Unclassified	—	2
	433	126

Origin.—Monsters of the types described above, and others, have been produced by a variety of experimental procedures, among which are hybridization, exposure of eggs to the fumes of alcohol, ether or other gases, narcotics, restriction of oxygen supply, excess of oxygen, excess of carbon dioxide and abnormal temperatures. A review of such experimental teratology in the chick has recently been given by Hyman (1927).

From the extensive researches of Stockard (1921) it would appear that the majority of these methods induce abnormal development through a common mechanism, viz., an arrest of development at a critical period. His investigations showed that by chilling the eggs of *Fundulus* prior to gastrulation, or early in that process, he could produce every known type of monster, and that the particular kind of teratism resulting depended upon the stage of development at the time growth was arrested as well as upon the degree of chilling.

Patterson (1909, 1910) has shown that in eggs of the pigeon and of the fowl gastrulation takes place close to the time of laying and that there is considerable variation in the degree of development of embryos in unincubated eggs. If this stage of embryogeny be as critical for birds as for fish embryos it is quite conceivable that some eggs might be chilled at the dangerous stage and that in these there would be produced various kinds of monsters. During the hatching season air temperatures are usually (except in equatorial regions) so low that eggs are chilled below the physiological zero (20° to 21° C.) shortly after laying, and growth of the embryo is not

resumed till incubation is begun. This discontinuous development suggests that chick monsters may easily result from an arrest of growth at a critical period.

During the hatching season of 1929 data were gathered at the University of Minnesota which tend to corroborate the theory that arrested development is responsible for at least a part of the monsters observed in chick embryos. It is obvious that among eggs laid early in the morning there are a certain proportion which were ready for laying at some time during the night, but which have been retained in the uterus because the sensory stimulus necessary for oviposition does not usually occur while the hen is asleep. The embryos in such eggs will be developed further than those in eggs laid as soon as they are ready and retained in the body only for the normal period of 21 to 27 hours. The latter class of eggs would be expected to have a higher proportion of embryos in the critical early stages of gastrulation and should therefore exhibit a greater frequency of teratological abnormalities Such eggs are more likely to be laid in the afternoon. Conversely, the early morning eggs will contain a higher proportion of embryos developed well beyond the dangerous period of early gastrulation and among them abnormalities in development should be fewer.

One would expect, therefore, a higher mortality rate in afternoon than in early morning eggs. In a previous communication (Hutt and Pilkey, 1930) it has been shown that this is actually the case, there being at both University Farm and the Crookston sub-stations a mortality rate among afternoon eggs which was from 10 to 3 per cent. higher than in eggs laid before 9 a.m. Similar results (unpublished) were obtained at the Morris sub-station, where the mortality rate in eggs laid before 8.30 a.m. was approximately 4 per cent. less for the entire season than in eggs laid at later periods of the day.

This difference was most marked early in the season but disappeared entirely after April 15th (at 45° and at 47° 45′ N. latitude). This finding is consistent with that of Hutt and Greenwood (*loc. cit.*) that the incidence of monsters declined as the incubation season advanced. Whether this seasonal decrease in abnormalities and the elimination of the differential mortality rate after April 15th be owing to higher temperatures (and consequently less rapid chilling of newly laid eggs) or to a shorter night decreasing the number of eggs carried past the normal period, cannot be definitely stated. It is commonly observed, however, that as the days get longer the number of eggs laid late in the afternoon decreases, and it is logical to assume, in view of the data presented above, that this automatically reduces the numbers of eggs laid prematurely at critical stages of development.

By actual count the frequency of monsters observed in afternoon eggs at three different stations was twice as great as that in eggs laid before 9 a.m. (Table 2) These cases included only teratological abnormalities which could be detected with the naked eye. It is

certain that many others escaped detection, especially in embryos dying in the first few days of incubation, which were partially decomposed when examined.

TABLE 2. *Frequency* of Observed Teratological Abnormalities at University Farm and at Crookston and Morris sub-stations.*

Period of laying.	University Farm.		Crookston.		Morris.	
	Fertile eggs.	Frequency of Monsters. Per cent.	Fertile eggs.	Frequency of Monsters. Per cent.	Dead Embryos.	Frequency of Monsters. Per cent.
To 9 a.m. ...	1,196	·75	1,907	·26	234	1·71
9.05–12 m. ...	2,345	·94	2,697	·37	751	1·11
12.05–2 p.m. ...	1,148	1·74	1,073	·37	296	2·70
After 2 p.m. ...	1,229	1·46	1,133	·53	355	3·10

* Note that in University Farm and Crookston eggs the frequency of monsters is expressed as a percentage of the *fertile eggs*, which is the most informative way, but that in the Morris material, where the exact number of fertile eggs was not available, it is expressed as a percentage of the *dead embryos*

These findings corroborate the theories that (1) teratological abnormalities result when eggs are chilled at a critical period in embryonic development and (2) that eggs at such a stage are more frequent in afternoon than in early morning eggs.

There is evidence that monsters may also result from unsatisfactory conditions after the eggs are put in the incubator. Alsop (1919) has shown that high temperatures during the first three days of incubation will induce abnormal development. Unfortunately she did not incubate her material longer than three days, so it is impossible to state the extent to which the abnormalities induced were comparable to those described in this paper, which were produced under supposedly optimum conditions of incubation.

The very careful studies of Eycleshymer (1907) indicate that if conditions of natural incubation (which is still superior to man's best efforts) are to be followed, the incubator temperature for the first two days should not be higher than 102° F. at the top of the egg. Miss Alsop found that temperatures higher than 103° during the first 24 hours induced a very high percentage of abnormalities and, although these were not the same as those found in her controls, it seems reasonable to conclude that a certain number of chick monsters may result from eggs being subjected to temperatures of 103° or higher during the first 24 hours of incubation. Even where an attempt is made to maintain a temperature of 101° or 102° at this period some eggs may easily be subjected to 103° or more through variability inside the incubating chamber, unsatisfactory heat regulation or inaccurate thermometers.

In this case abnormalities arise from acceleration of growth beyond the normal rate, but since some eggs are not affected at all it is obvious that, just as in cases of arrested development, there must be a critical period, or periods, at which the embryo is particularly susceptible to any abnormal acceleration in its rate of growth.

Miss Alsop, Riddle (1923) and other earlier workers have shown that low temperatures may also give rise to abnormalities in avian embryos. This method operates in the same way as a complete arrest of growth, *i.e.*, by retarding the development of the primordia of certain parts at a time when their rate of development should be at its maximum.

There is some evidence in the data gathered in 1928 and 1929 that there is a greater frequency of teratisms among eggs set in small single-tray incubators than in the so-called " cabinet " incubators with large capacity and a uniform temperature from top to bottom maintained by forced circulation of air.

In Table 3 are shown the frequencies of monsters in embryos from seven different sources.

TABLE 3. *Frequencies of Monsters observed during* 1928 *and* 1929.

Source.	Number of Fertile Eggs Set.	Number of Dead Embryos.	Frequency of Monsters.	
			In Fertile eggs. Per cent.	In Dead embryos. Per cent.
1928—A	25,215	9,834	1·41	3·63
B	4,229	1,015	1·11	4·63
C	2,255	654	·80	2·75
D	905	294	1·21	3·74
1929—University Farm	5,918	2,007	1·16	3·44
Crookston ...	6,810	2,331	·36	1·07
Morris	—	1,636	—	1·95

At " C," where the incidence of monsters in 1928 was lowest, the majority of the eggs were incubated in large forced-circulation incubators. At A, B, and D all the eggs were incubated in small single tray machines. Similarly in 1929 small machines were used for about half the eggs at University Farm, where the incidence of monsters was greatest, whereas at Crookston and Morris, where all the eggs were incubated in cabinet machines, the frequency of abnormalities was significantly less. It seems probable, in view of Miss Alsop's work, that the difference may be due to the greater variability in temperature of the smaller machines where the thermometer at a central point may read 101° or 102°, while in the corners or under the heating pipes the temperature may be one or two degrees higher. Most of the larger machines are run at 100° and a uniform temperature is maintained in all parts of the incubator by air circulation forced with electric fans. For embryos at stages susceptible to acceleration of development, this would seem somewhat safer during the first two days of incubation.

The results in Table 3, though suggestive, can certainly not be construed as indicating that hatches are better in cabinet incubators than in good single-tray machines when the latter are properly operated.

It is difficult to account for the greater frequency of hyperencephaly and exencephaly in the 1928 material examined in Scotland compared with that of 1929. In both cases White Leghorns provided the majority of the embryos, and in any case no differential production of monsters by any breed or variety was evident. Climate may play a part, but it also seems possible that these brain defects arose from accelerated development in the small incubators from which practically all of them came. On the other hand, the frequency of eye defects was about the same in material from all sources, suggesting that these arise from arrest of development following laying at a critical period and are independent of temperature fluctuations and of different methods of incubation.

Economic Significance.—The frequencies given in Table 3 do not represent accurately the total mortality from teratological abnormalities, since they are based only on monsters detected with the naked eye. Most of the embryos examined were over five days of age. However, Dareste (1891) found that certain types of abnormalities invariably caused death within the first few days of development, and it is therefore quite certain that the loss from teratological development is much greater than the range of from 1·07 to 4·63 per cent. of the total mortality as indicated above. The frequency with which such abnormalities are encountered in embryological laboratories is certainly much greater than these figures indicate, but the only actual data which the writer has been able to find for eggs incubated under normal conditions, and where every egg was examined at an early stage, are those for Miss Alsop's controls, in which 6·43 per cent. of the embryos exhibited abnormal development. Further data on this point are highly desirable.

Stockard's investigations indicate that the actual loss from this kind of abnormal development is really much higher than can be easily detected. Among *Fundulus* embryos from treated eggs were found defects so small that some of them escaped detection until the hatched embryos proved unable to swim. The majority of the monsters described in this paper exhibit gross external defects easily recognized, but other smaller defects may be present in various internal organs which could be detected only by careful dissection and examination of the entire anatomy. Such invisible teratological development may be responsible for the death of many of the chicks found fully formed and apparently quite normal in eggs which have failed to hatch. If this be so it is not improbable that various kinds of abnormal development may be responsible for as much as ten per cent. or more of the total mortality.

Not enough is yet known that much can be done to prevent embryonic mortality of this kind. It is hardly practicable to warm the afternoon eggs for three or four hours in order to carry past the

danger point the small percentage of them which contain embryos at critical stages of development. It may be possible to breed a strain of hens none of which will lay eggs prematurely, but from an economic point of view such a cure, even if it be possible, would probably prove more expensive than the disease, since such hens would not be likely to lay at 24 hour intervals as do our highest producers. Whatever loss there may be from accelerated development can be reduced by maintaining a uniform temperature not higher than 102° F. at the top of the egg during the first two days of incubation and by avoiding temperatures higher than 103° thereafter, except in the final days of the incubation period.

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E. AVIAN PHYSIOLOGY

(mostly endocrinology and physiology of reproduction)

FURTHER EXPERIMENTS IN FEEDING THYROID TO FOWLS

L. J. COLE AND F. B. HUTT

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FURTHER EXPERIMENTS IN FEEDING THYROID TO FOWLS [1]

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In an attempt to learn more of the properties and functions of the thyroid gland, several investigators have tried feeding fresh or desiccated thyroids to fowls. During 1924 and 1925 experiments of this nature were made by the writers. In view of the varying reports of other investigators, our results are considered of sufficient interest to warrant their presentation. They include effects of thyroid feeding on moulting, egg production, weight, plumage color and plumage structure.

MOULTING

Forty White Leghorn hens were divided into two equal lots and fed identical rations. To make the environment natural and equal for both lots, ten birds to receive thyroid and ten controls were put in each of two identical colony houses, both of which had out-door yards.

For a period of six weeks, beginning November 1st, the twenty thyroid-fed hens were given desiccated thyroid (Armour and Co., 0.2% iodine) at the rate of 196 mg. per bird daily. This was equivalent to approximately 59 mg. per pound of live weight. The powder was administered individually in gelatine capsules.

Each bird was leg-banded and checked over weekly. To make observations easier, the primaries and secondaries of one lot were stained with "lichtgrün" and the other with eosin.

At the start of the experiment, several of the hens in both lots had already started to moult and some were practically finished. Of those receiving thyroid, seven had not yet started to moult, and three more had just begun. Of the controls, five showed no sign whatever of moulting, and four others had just started.

As the experiment progressed, seven of the ten thyroid-fed birds which had undergone little or no moult at the start went

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into more or less precipitate moult at various stages of feeding. Three showed decided body moult at the end of the first week, three more at the end of the second week, and one during the third week. These hens seemed to lose their feathers most rapidly from the tracts on the breast and neck. It was sometimes possible to pull a handful of feathers from these regions by the lightest touch. In some cases two or three primaries could be plucked out at once with almost no effort. Three of these ten birds moulted more slowly and were never in a semi-naked or "quilly" condition as were some of the others.

Two of the controls did not moult at all during the six weeks of the experiment, and the others moulted slowly. They grew new feathers in all regions before all the old ones were gone, in contrast to the thyroid-fed birds, which seemed to drop all feathers at once (particularly on the breast and neck) and then grow a new crop uniformly.

This faculty of shedding many feathers simultaneously with a resultant naked condition is not necessarily a distinctive characteristic of thyroid-fed birds, for it is commonly seen in hens which moult late. In this experiment, however, such a condition was not noted among the controls, but was evident in seven of the birds receiving thyroid. It seems reasonable to conclude, therefore, that the thyroid feeding was here responsible for the precipitate moult.

Since this experiment, Giacomini (1924), B. Zavadovsky (1925) and Crew (1925) have reported precipitate moulting in fowls, induced, however, by much larger doses.

WEIGHT

Results of weekly weighings are reported in Table 1.

TABLE 1. AVERAGE WEIGHT PER BIRD IN POUNDS

	Thyroid-Fed	*Controls*
November 1	3.30	3.34
November 8	3.30	
November 15	3.28	3.25
November 21	3.27	3.23
November 27	3.28	3.33
December 4	3.30	3.32
December 12	3.34	3.40

These data indicate that the dosage given in this experiment (59 mg. daily per pound of live weight) had no effect on body

weight. Other workers have reported rapid loss in weight, when much larger doses of raw thyroid were fed.

EGG PRODUCTION

All hens were trap-nested. Egg records for a period of four months from the start of the experiment are shown in Table 2.

TABLE 2. EGGS LAID PER MONTH

	Nov.	*Dec.*	*Jan.*	*Feb.*	*Total*
Thyroid-fed (20)	26	23	36	44	129
Controls (20)	27	19	35	46	127

It seemed possible that an early quick moult, such as is favored by the thyroid feeding, might lead to an earlier increased egg production. There is, however, no evidence that egg production was at all affected by the dosage fed in this experiment. Crew and Huxley (1923) reported similar results with fowls receiving small doses of thyroid. Crew has since found (1925) that senile females were markedly stimulated to egg production by larger doses.

PLUMAGE (COLOR AND STRUCTURE)

Hen feathering in the male fowl has been reported as a result of thyroid-feeding by Torrey and Horning (1922), Cole and Reid (1924) and Crew (1925), and the authors of the last two papers also noted increased melanism in feathers growing in exposed to the influence of the thyroid. Torrey (1926) has noted the same effect in barred feathers of Barred Plymouth Rock males. All of these workers fed desiccated thyroid powder.

In direct contrast to this, Giacomini (1924) reported depigmentation of plumage as a result of feeding raw ox thyroid to fowls. Subsequent experiments of B. Zavadovsky (1925), M. Zavadovsky (1925), Torrey and Horning (1925), Krizenecky (1926a, 1926b), and Podhradsky (1926) confirm the toxic effect of large doses of thyroid in producing abnormal feather structure and decrease of pigmentation. In many cases the feathers grown under these conditions are largely or entirely white.

It seemed possible that there might be some difference in the physiological properties of raw and desiccated thyroid. Accordingly an experiment was planned to find the effects of both on plumage color and structure in males and females of different breeds. Twenty-two birds were given dosages as follows:

Raw pig thyroid—0.42 grams daily per pound of live weight. Males—Barred Rock, Buff Leghorn, Silver Laced Wyandotte, Rhode Island Red, Silver Spangled Hamburg, Golden Sebright. Females—Barred Rock, Buff Leghorn, Ancona, Black Minorca.

Desiccated thyroid—84 mg. daily per pound of live weight. Males—Barred Rock, Buff Leghorn, Silver Laced Wyandotte, Golden Sebright. Females—Barred Rock, Buff Leghorn, Blue Andalusian, Ancona.

Controls—Males, Golden Sebright. Females—Barred Rock, Buff Leghorn, Ancona.

On the assumption that one gram of the desiccated powder (Armour and Co., 0.2% iodine) is equivalent to five grams of fresh normal thyroid gland, the actual doses of active thyroid substance were approximately equal for all birds whether they received raw or desiccated gland.

Feeding began on March 2. On March 11, small areas on the neck, wing bow and saddle of each bird were plucked free of feathers and samples of the latter were saved for future comparison. Weights were taken every two weeks. Feeding was discontinued on May 8th.

No lack of pigmentation was observed except very small white spots in the normally black marginal lacing of new neck and saddle feathers in both thyroid-fed Sebrights. The two Silver Wyandotte males grew distinctly darker feathers, particularly in the neck region. The Silver Spangled Hamburg and Rhode Island Red males showed a slight but perceptible tendency toward increased melanism in new feathers, but no appreciable change in color was evident in any of the females or the other males on either raw or desiccated thyroid. Torrey and Horning (1926) have subsequently noted increased melanism induced in males but not in females by thyroid feeding.

SECONDARY SEX CHARACTERS

The lanceolate, pointed appearance of neck, wing bow and saddle feathers of male fowl is due to a lack of barbules and hooks in the margin of the web. It constitutes a secondary sex character. In this experiment, various degrees of hen-feathering were noted in all males receiving either desiccated or raw thyroid, with the exception of the Rhode Island Red. It was quite evident that after six weeks of feeding the influence

of the thyroid was completely lost, so far as feather structure was concerned. The Hamburg male grew a crop of female-type feathers on his neck and saddle, lost all on his neck by fighting, and subsequently replaced them with feathers of the normal male type. One of the Barred Rock males first grew "henny" feathers on the wing bow, then feathers of intermediate structure on the neck, and finally, after five weeks of feeding, saddle feathers of the normal male type. The other birds showed instances of the same phenomenon. The Rhode Island Red did not grow any new feathers till after six weeks of thyroid feeding, hence they were not henny, as were the earlier feathers of the other males.

The most interesting changes were observed in the Silver Wyandotte male receiving desiccated thyroid. This bird grew female feathers on neck, wing bow and saddle. The new neck feathers tended toward solid black rather than the typical black feather with white lacing. On the wing bow, however, where the male feathers had been white with a black base, there were produced feathers entirely female both in structure and color pattern, viz., white with black lacing. Similarly on the saddle, the new feathers were white with a black lacing one-eighth to one-quarter inch wide where those plucked had been black with a white margin. These effects were also present in the Silver Wyandotte male receiving raw thyroid, but were less distinct because his feathers grew in more slowly and hence partially escaped the influence of the thyroid.

The Silver Wyandotte is a dimorphically colored breed, i. e., the male differs from the female in plumage pattern as well as feather structure. In this experiment both of these secondary sex characters were completely suppressed in the males and altered to the female expression as a result of thyroid feeding.

No change was noticed in feather structure of the Sebright males, a breed in which the male is normally hen feathered.

OTHER EFFECTS

No significant change in weight was noticed in any of the birds.

Giacomini and M. Zavadovsky have noticed a stimulus for

feather growth in birds fed large doses of fresh thyroid. In our experiments, the desiccated thyroid seemed to have a much more stimulating effect on feather growth than had the raw thyroid. The fowls receiving desiccated thyroid were, with only one exception practically full feathered by May 8th, while many of those receiving raw thyroid had made very little feather growth. This difference was more noticeable in the females than in the males. Two of the former had made no feather growth except a few quills on the neck and wing bow by May 8th, and one had not even a new quill. The latter was given her raw thyroid dose till May 26th, at which time she still had not a quill. After feeding was discontinued the other two made a fairly good growth of feathers and were well covered in the neck and wing bow regions by May 26th. The number of birds used was too small to warrant any conclusion regarding this apparent difference between raw and desiccated thyroid.

It was noticeable that in all birds the saddle feathers developed more slowly than those in the neck and wing bow regions.

SUMMARY

1. Hen-feathering was induced in male fowls by feeding both desiccated and raw thyroid.

2. In males of a dimorphically-colored breed the color pattern of the feathers was changed from that of the male to that of the female by thyroid feeding.

3. At our dosage there was no appreciable evidence of the lack of pigmentation in new feathers described by other investigators.

4. Raw thyroid appeared to have less stimulating effect on feather growth than desiccated thyroid at approximately the same level of feeding (based on assumed iodine content).

5. Feather structure in Sebright males was unaffected by thyroid feeding.

6. A daily dose of 59 mg. desiccated thyroid per pound of live weight hastened the normal moult of yearling hens.

7. The same dosage did not have any appreciable effect on body weight or egg production during the period of four months.

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Potentially Fatal Fatigue of Cervical Muscles of the Fowl Resulting from an Excessively Large Comb.

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THERE have recently occurred at this department two cases in which a cock was reduced to the verge of death as a result of an excessively large comb, and was restored to perfect health by the single expedient of cutting off that structure. These cases are unique in the experience of the writer, and are considered worthy of record as illustrating a condition that would seem to be a potential cause of death in the fowl.

The first bird observed was a Single Comb Brown Leghorn cockerel, No. 21 on the records of this department, hatched in May, 1927, and operated upon at six weeks of age, when the left testis and half of the right were removed. The cockerel developed normally, and at eight months was indistinguishable in appearance or behaviour from normal cocks of the same age, except that it had a rather large comb.

On January 1st, 1928, he was put in a small house with six other cockerels of the same breed and about the same age. These seven birds were used for individual matings with selected hens, the object being to determine how No. 21 and other partially castrated males compared in fertility with normal cockerels. No. 21 was the largest of the seven and was recognised by the others as ruler of the roost. By this time his comb had lopped over on one side.

On the morning of February 11th this bird was noticed standing motionless in the pen with his head bent down almost to the floor. The only signs of impaired health previously observed had been a slightly reduced activity on the preceding two days. There was no evidence of fighting or of anything else that might have injured the bird. When placed by himself in a good-sized cage his head, at first erect, slowly but inevitably sagged till his beak rested on the floor. When roused he raised the head slightly, but almost at once it began to sink gradually back to the floor. It seemed as though his head were far too heavy for him to stand erect for any length of time.

Since the bird took no interest whatsoever in food, he was forcibly

fed twice daily with pellets of moist mash. When unassisted, he made no attempt to drink. When his beak was put in water, the patient would gulp several times, but could swallow only when someone lifted his head.

On February 12th, the day after he was first noticed, all power of the flexor muscles seemed entirely gone, and when the head was lifted it dropped straight down again to the floor (Fig. 1). The extensor muscles were able to exert pressure, and did so when the head was lifted too high or pushed back toward the body. The symptoms were not in the least suggestive of botulism or of any injury to the auditory labyrinths, either of which conditions would be accompanied

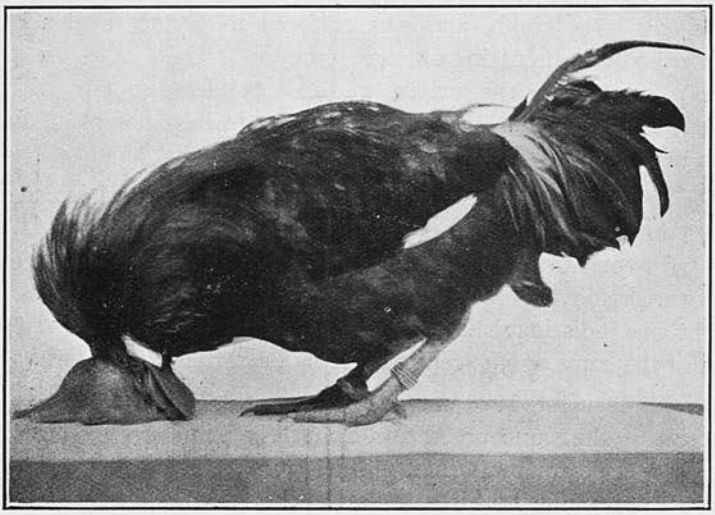

Fig. 1.

by a twisting of the head to one side. Both eyes were bright and showed the usual reflex winking.

On February 14th, No. 21 was distinctly weaker. Though it was recognised that a pathological disturbance in the central nervous system might be one cause of the condition exhibited, it was decided to adopt the view that the condition was one in which the comb had increased in size by virtue of its own developmental impulses to an extent that overtaxed the power of the muscles of the neck to support it. This view could be put to the test of experiment. If the idea were correct, a reduction in the weight of the comb should be followed by an amelioration of the condition. Accordingly, after measurements had been taken, the comb was sliced off with a razor about one half-

inch from the head. In spite of its vascular appearance, the comb of the cock is composed almost entirely of connective tissue. In the present case a few drops of adrenaline stopped bleeding so effectively that not a drop of blood fell from the comb.

Measurements taken prior to its removal showed that No. 21's comb was 14·6 cm. in its longest dimension, 8 cm. high and 2·6 cm. through at its thickest point. The portion cut off weighed 58·7 grm. The body weight was 2,041 grm. on February 7th and only 1,814 grm. on February 11th.

When the bird was placed on the floor he staggered drunkenly

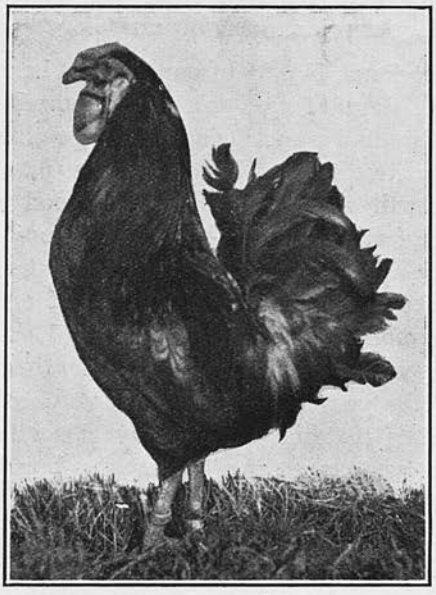

Fig. 2.

right across the room, holding his head about 8 or 9 in. above the floor. When he ceased walking the head again sagged down, but when roused the bird was able to hold his head off the floor for over a minute. On being offered grain and water, the cockerel drank thirstily and ate voluntarily for the first time in three days.

The following morning No. 21 was found in his old position with the head on the floor, but a marked improvement was noticeable in his general demeanour when aroused. Thereafter a gradual daily improvement was noticeable, and on February 19th, five days after removal of the comb, the bird was returned to an outdoor yard, where he celebrated his recovery by lusty crowing (Fig. 2). Matings

were resumed, and the fertility of his hens was soon as good as before he became incapacitated, and equal to that of normal cocks.

The history of the other case, also a Single Comb Brown Leghorn cockerel, was so like that given above that detailed description is unnecessary. In this case 19·9 grm. of wattles and 37·85 grm. of comb were removed on March 13th. Only the posterior half of the comb was removed, and the remainder seemed to weigh downward on the anterior part of the head, causing the beak to be tucked in close to the neck. Recovery was not complete till most of the remaining comb (18·2 grm.) was cut off on April 3rd. Altogether 75·95 grm. of comb and wattles were removed from this cockerel, whose body weight was 1,927 grm. prior to the operation.

This second cockerel, No. 25 of our records, had been completely castrated at nine days of age, and the removed testes implanted subcutaneously one under each wing. It is possible that the interference with the testes may have been in part responsible for an excessive growth of the comb. Greenwood and Crew[1] and Blyth[2] have put forth evidence that the development of the comb is dependent to a considerable extent upon the degree of spermatogenic activity of the testes. No. 21 was found on *post-mortem* examination five months later to have only 5·77 grm. of testis on the left side and a regenerated nodule of ·05 grm. on the right side, but not connected with the vas deferens. It is possible that in the small fragments of testis material retained by this bird the process of spermatogenesis was proceeding during the breeding season at a much faster rate than in normal cocks and that this condition was responsible for excessive comb growth as the hypothesis of the writers mentioned would suggest. No evidence of any gross abnormality of the central nervous system was encountered. No. 25 is still living, and therefore can contribute little to this discussion.

However, several other cockerels similarly operated upon had no trouble with their combs and therefore, in the opinion of the writer, the large combs of these two cases described represent less an abnormal condition resulting from the operations to which the birds were submitted than the natural result of breeding for a large comb. In Great Britain the exhibition standard for Leghorns calls for a much larger comb than is desired in Canada and the United States. The writer has seen combs on normal Leghorn cockerels in poultry shows in this country, which were quite as big as those removed from Nos. 21 and 25. The suggestion is made that this process of breeding for large combs has brought that structure to a size where its wearer may be taxed to the limit to support it. In such exceptional cases as here

described it may even grow beyond the limits of endurance of the supporting muscles and unless the cause is removed the muscle fatigue thus induced may cause death from starvation and exhaustion.

After No. 25 had completely recovered, a circlet of leaden washers weighing 50 grm. was fastened around the stub of comb remaining. This weight he was able to carry quite easily, and did so for a week. Two months later a 76 grm. leaden crown (equal to the weight of comb and wattles removed) was fastened to the top of the cockerel's head. It was not possible to attach this so that its weight acted in the same direction as the removed comb, but after four days' observation, it was evident that the extra weight was reducing the bird to a condition similar to that induced by the comb, and it was therefore removed.

Champy[3] and others have adduced considerable evidence to show that in all probability the disharmonious increase in size of a part in relation to the body as a whole has led to the extinction of species. It is easy to recognise the inevitable end of the Irish Elk, for example, if, as the individuals of that species increased in general body size at a given rate for generation after generation, the antlers, already large, increased disproportionately in size and weight. Ultimately the head would become too heavy for the neck muscles to bear. Such heterogony (i.e. the growth of a part of the body out of proportion to the growth of the organism as a whole) must, indeed, limit the ultimate body size attainable by many species.

It would seem to be the case that there is a limit to the weight of comb which a body of limited size can sustain. If, by selection, the breeders seek to produce a Leghorn with an excessively large comb, they will probably also have to adopt the practice of "dubbing" (i.e. cutting off the comb), which, in turn, would defeat the object of breeding, or else the breed might be led to extinction. The writer does not suggest that the Leghorn fowl as a breed is doomed to such a fate, but the cases described above show that there is a limit beyond which a large comb can so interfere with the normal function of the bird as to render it non-viable.

Another reason for the preference of combs of medium rather than large size is the fact that in areas having cold winter climate loss from frozen combs is a serious problem. In Canada and the United States loss by this cause has been instrumental in establishing a preference for medium-sized combs, and an exhibition standard that would eliminate from show classes some of the large-combed Leghorns which carry off red and blue ribbons in this country.

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On the Relation of Fertility in Fowls to the Amount of Testicular Material and Density of Sperm Suspension.

By F. B. Hutt, B.S.A.

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IX.—On the Relation of Fertility in Fowls to the Amount of Testicular Material and Density of Sperm Suspension.

By **F. B. Hutt**, B.S.A., Animal Breeding Research Department, University of Edinburgh. *Communicated by* Professor F. A. E. Crew.

(MS. received September 26, 1928. Read January 7, 1929.)

Introduction.

Studies on fertility in the domestic fowl so far reported have been concerned more with the influence of the female than with that of the male. At the same time it is common knowledge among poultry breeders that there is often a great difference in the fertility of different cocks of the same breed under the same environmental conditions. Such differences are conceivably dependent upon three different factors, these being (1) the frequency of copulations, (2) the number of spermatozoa ejaculated, and (3) the physiological efficiency of the spermatozoa. The present study was undertaken to determine the influence on fertility of the second of these factors.

In attacking this problem it was assumed that the number of spermatozoa elaborated by different males would be dependent to some extent

upon the amount of testicular tissue present. Presumably the same assumption is the basis of the very general objection on the part of breeders of horses, cattle, and other domestic animals to males with only one testis or having one or both testes undersized. While there is considerable variation in the size of testes among normal cocks of the same breed, such differences could be accurately determined only by post-mortem weighing. If normal birds were used, a great number would be necessary to ensure that among them appreciable differences in the amount of testicular material present would be found upon examination at the conclusion of the experiment. Accordingly it was considered that the most suitable material would be cocks which had been castrated in various degrees.

Material.

The males used in the experiment were eleven Single Comb Brown Leghorns hatched in the spring of 1927. The degree of castration in each is shown in Table I.

Table I.—Males tested.

Bird Number.	Age at Operation. Days.	Tissue removed.
142	7	Left testis.
152	7	„ „
149	7	„ „
157	7	Almost all of left testis.
176	14	Right testis. Removed tissue implanted subcutaneously.
5	7	Left testis and about half of right testis.
21	38	Left testis and about half of right testis.
172	12	Left testis and anterior and posterior ends of right testis. Removed tissue implanted subcutaneously under right wing.
382	control	None.
384	control	„
387	control	„

In cases where part of a testis was removed care was taken at the time of operation not to destroy the epididymal connection. These operations were performed by Dr A. W. Greenwood, and to him are due the writer's sincere thanks for the excellent material provided.

The development and growth of these birds were unexceptional, and at six months of age they were indistinguishable in any way from normal males.

The females to which these cocks were mated were mostly Brown Leghorns, but included also some Rhode Island Reds and cross-bred birds, which

were distributed as equally as possible among the different males. The majority were pullets.

Methods.

(a) *Stud Matings.*—The accommodation available did not permit giving each male a separate pen with a flock of females, and therefore the "stud-mating" system was used for the earlier part of the experiment. The cocks were housed in two separate pens, and kept confined till late in the afternoon. Fifty-eight females were used in the first part of the experiment, these being housed in three separate pens, with continual access to an outdoor yard. All were leg-banded and trap-nested. To each male were assigned the leg-band numbers of from five to seven females, and whenever a hen was taken from a trap-nest she was put in a small breeding-coop along with the male indicated by her number. After a period of fifteen or twenty minutes both hen and cock were returned to their original pens.

In assigning the females to the different males care was taken to see that females of different ages and breeds were distributed as evenly as possible among the ten males used for stud mating. The system is open to the criticism that the hen laying six times a week is mated more often than one laying only twice, but since the hens were assigned regardless of their laying ability, the probability of any one male getting a high proportion of good or poor layers among his five to seven hens was not great. The actual records indicated that the chance distribution of frequent layers and poor layers was fair enough to permit comparable tests of the fertility of the various cocks.

All birds were given a grain ration of wheat, oats, and maize; mash of Sussex ground oats, wheat middlings, bran, maize meal, soy-bean meal, and fish-meal, with cabbage occasionally, and oyster-shell *ad libitum*. Milk was supplied irregularly. Cod-liver oil was fed at a level of two per cent. in the mash.

Before records were begun, the females were isolated from males for a period of two weeks, and those laying were not used till their eggs were found, after incubation, to be infertile. Matings were begun on 1st January 1928, and continued till 8th March. Matings with Cocks 172, 176, 157, 382, and 387 were not begun till 27th January owing to there being insufficient laying hens before that date.

All eggs laid after the beginning of the experiment were incubated and examined for fertility by candling in the usual way at five or six days of incubation. All doubtful eggs were broken and the germinal disc examined under a dissecting microscope.

(b) *Flock Matings.*—In a stud-mating system such as has just been described it is obvious that the sexual activity of all males would be appreciably less than if they were at liberty with a flock of the usual fifteen or twenty hens. It was conceivable, therefore, that while the fertility of partially castrated males might compare favourably with that of normal cocks when each male was given only two to five hens per day and sometimes less, it might be different under conditions where sexual activity was unrestricted. Accordingly, three pens of flock matings were established after the termination of the first series in the early part of March. Cocks 149, 5, and 382 were each given free run of a large yard with nineteen, eighteen, and seventeen females respectively. Male 5 had not been previously tested, but both ♂ 149 and the control ♂ 382 had been used in the stud-mating series.

In these flock matings a period of ten days was allowed for fertility to become established, and then all eggs from each pen over a period of from seven to eleven days were incubated and examined as before for fertility. Some of the hens in this series had also been used for stud matings. However, in view of Crew's (1926) findings that on removal of one male and introduction of a second the influence of the first is lost after seven to ten days, it is fairly certain that the ten-day interval allowed in these experiments was ample to ensure that eggs gathered from the eleventh to the twenty-second day indicated only the fertilising power of the second male.

During the course of the experiment samples of semen were obtained and counts made of the spermatozoa therein. At the conclusion of the experiment all males were killed and weights of testes were determined.

Observations on Fertility.

In the stud-mating series it was necessary to establish some arbitrary standard for the number of potentially fertile eggs. The work of Crew (1926) and Dunn (1927) indicates that in single matings a fertile egg may rarely be obtained on the first day following mating, but that fertility is well established by the second day. In Dunn's cases fertility was complete and maximum forty-eight hours after mating, but Crew found that with some males the time required for the onset of fertility was three and even five days. In these experiments all eggs laid on or after the third day from the first mating were considered as potentially fertile. The duration of fertility after removal of the male has been shown by the writers quoted and others to decrease after the first week. In these records it was considered that all eggs laid up to five days after the last

mating ought to be fertile, but any fertile eggs laid after that were also included. Infertile eggs intervening between the first and the last fertile egg counted for any hen were also considered potentially fertile.

The results observed on this basis, in both stud and flock matings, are shown in Table II.

TABLE II.—FERTILITY OF MALES TESTED.

Cock.	Number of Hens.	Potentially Fertile Eggs.	Fertile Eggs.	Fertility per cent.	Days to last Fertile Egg after removing Male.
			Stud Matings.		
Experimental:					
21	6	127	91	71·65	18
142	5	119	110	92·44	9
152	6	119	86	72·27	10
149	7	118	98	83·05	7
172	6	67	54	80·59	12
176	5	53	35	66·04	7
157	5	58	41	70·69	
			Average*	76·67	
Controls:					
382	6	64	47	73·44	11
387	5	62	48	77·42	13
384	7	100	80	80·00	13
			Average*	76·95	
			Flock Matings.		
Experimental:					
5	18	84	72	85·71	
149	19	126	117	92·85	
Control:					
382	17	47	33	70·21	

* *I.e.* the average fertility of the cocks, *not* of the potentially fertile eggs.

It is evident that in the stud matings the fertility of partially castrated cocks was equally as good as that of normal cocks, the averages for the two groups being, by a coincidence, practically equal. In the flock matings ♂ 149 gave better fertility than when stud-mated, and both his record and that of the previously untested ♂ 5 were excellent. The one control cock gave slightly lower fertility than in stud matings, but this was probably due to a chance difference in the smaller number of eggs tested from his pen. The duration of fertility after removal of the male was on the average practically as long in the experimental group as in the controls.

Table III presents a biometrical analysis of the individual performances of the females in the stud-mating series.

TABLE III.—STATISTICAL CONSTANTS FOR FEMALES IN THE STUD-MATING SERIES.

Class.	Number of Individuals.	Mean Fertility and Probable Error per cent.	Standard Deviation and Probable Error per cent.
Experimental	40	76·45 ± 2·38	22·33 ± 1·68
Control	18	76·22 ± 2·76	17·41 ± 1·95

It is evident that there is no significant difference between the mean fertility in each group. The difference in the standard deviations is 4·93±2·37. Since this difference is only 2·08 times its probable error, it is not statistically significant. It may therefore be concluded that the results are not affected by any undue variability of the females in either group.

SPERMATOZOA COUNTS.

The hypothesis suggested by these results, namely, that fertility of the male is entirely independent of the amount of testicular tissue present, could not be proved until that tissue had been weighed after death. Both Benoit (1925) and Domm and Juhn (1927) found a compensatory hypertrophy of the remaining testis following unilateral castration of young chicks. It was therefore to be expected that compensatory hypertrophy would take place to an unknown extent in some or all of the material used in this experiment. An attempt had been made to prevent such hypertrophy in ♂ 172 by grafting subcutaneously the portions of testis removed at the time of operation. If the compensatory hypertrophy were occasioned by the demand for production of a certain degree of physiological activity of the testis this demand might be met (and hypertrophy prevented) by a functioning testis in any part of the body, while obviously only the testicular material in communication with the vas deferens would be available for reproduction. Nevertheless, the amount of testis in any of the males was entirely unknown. To determine whether or not the density of sperm supension in the semen bore any relation to the size of testis, or could be accepted as a measure of potential fertility in mating, an examination was made of thirty-six samples of semen from ten different males.

(a) *Collection of Semen.*—Determinations of the density of sperm suspension in semen of the fowl have been previously made by Payne (1914) and Craft, M'Elroy, and Penquite (1926). These workers obtained their samples from the cloaca of the hen after coitus, and it is therefore

difficult to see how a certain amount of dilution of semen by fluids in the cloaca could have been avoided. Since this method was hardly accurate, an attempt was made in the present study to secure normal semen from the ejaculate.

It was found that after the cocks had become accustomed to being handled and had been used in stud matings for over a week, they would readily copulate when a willing female was introduced, regardless of the presence of an observer. With a little practice it was possible to intercept the ejaculate and collect it in a watch crystal. By using this method, samples were obtained from the ten cocks stud-mated. Male 5, which was used only for flock matings, could not be induced to copulate when confined in a small coop with an observer present, and therefore no semen was obtained from him.

(b) *Technique of Counting.*—The densities of the sperm suspensions in the semen were determined with a Thoma-Hawksley hæmacytometer having a depth of 0·10 mm. Ringer's solution, to which had been added 3·5 per cent. of formalin, was used as a diluting fluid and gave excellent results. Without any formalin, movement of the spermatozoa was not arrested, so that accurate counting was difficult. More than 3·5 per cent. formalin caused the sperms to curl up and become more difficult to see and to distinguish from minute masses of debris. For each sample counts were made of 160 out of the 400 squares ruled off on the hæmacytometer.

"Student" (1907) has demonstrated that when the technique of dilution and counting is accurate, the distribution of yeast cells on the squares of a hæmacytometer conforms to a Poisson Series. Moreover, Fisher (1925) points out that the standard error of a random sample from such a distribution is $\pm\sqrt{m}$, where m (the number of cells counted) is a large value. The same error should apply to any large hæmacytometer count if the technique be accurate. Two or three counts of the same dilution of semen were compared on several occasions. It was found that the deviations from the mean were within the standard error in most cases, and in no case significantly greater than the limits of that error. It may, therefore, be considered that the technique used was satisfactory, and that the counts reported below are accurate measurements of the density of sperm suspension in the various samples of semen examined.

It was found that the numbers of spermatozoa per cubic millimetre varied somewhat between different individuals, but even more in samples from the same individual secured following various degrees of sexual

activity. Thus, on 30th March the first sample for several days from ♂ 142 contained 5,500,000 sperms per cu. mm. After forty minutes' freedom with a hen, a sample was again obtained which contained 1,690,000 per cu. mm. The cock was left with another hen for forty-five minutes, and a sample secured thereafter had only 277,600 per cu. mm. Accordingly an attempt was made to secure representative samples from each male, *i.e.* after similar degrees of sexual activity. This presented some difficulty because of the unwillingness to copulate on the part of some of the males after one or two previous matings. In most cases this could be overcome by leaving the cock with one hen for an hour or so, then removing her and introducing another hen of a different colour, but with others a similar artifice was unsuccessful (*e.g.* ♂ 149). The data in Table IV give the average number of spermatozoa per cu. mm. in representative samples from each male, including in each case (except ♂ 149) the first ejaculation of a day, and two or more other semen samples taken after various degrees of sexual activity.

TABLE IV.—SPERMATOZOA COUNTS, TESTIS WEIGHTS, AND FERTILITY.

Bird Number.	Body Weight (grams).	Testis Weight (grams).			Ratio Body Wt. / Testis Wt.	Spermatozoa.		Fertility.	
		R.	L.	Total.		Samples.	Av. No. p. cu. mm.	Flock.	Stud.
Experimental:									
21	1927	5·77	...	5·77	333·9	4	1,782,650	...	71·6
142	1843	15·95	0·55	16·50	111·7	7	1,836,650	...	92·4
152	1757	24·00	...	24·00	73·2	3	825,960	...	72·2
149	1786	13·59	...	13·59	131·4	1	5,300,000	92·8	83·0
172	1814	8·98	...	8·98	202·0	2	5,032,500	...	80·0
176	1474	...	10·52	10·52	140·1	5	3,994,380	...	66·0
157	1956	15·55	0·94	16·49	118·6	4	7,328,500	...	70·6
5	1360	1·06	3·51	4·57	297·6	...	...	85·7	...
Average :	1740	...	...	12·55	201·1	...	3,728,663	89·2	76·6
Controls:									
382	1843	9·82	8·54	18·36	100·4	4	3,615,000	70·2	73·4
387	1814	6·07	8·00	14·07	128·9	3	4,848,667	...	77·4
384	1672	6·22	4·83	11·05	151·3	3	5,425,333	...	80·0
Average :	1776	...	...	14·49	126·8	...	4,629,667	...	76·9

Weights of Testicular Material.

In the same table are given the weights of testes found in each fowl at post-mortem examination. Only that tissue is included which was in communication with the vasa deferentia, as evidenced by active sperm being found in these ducts. In one or two cases small nodules of testes had regenerated after the operation but were not in communication with the vas and so are not included.

Interpretation of Results.

(a) *Fertility in Relation to Size of Testis.*—Inspection of Table IV shows that while the testis size and spermatozoa counts of the controls were on the average slightly higher than in the experimental males, the average fertility was practically identical in each group in the stud matings. In the flock matings the two partially castrated cocks excelled the control. The latter bird did no better in flock matings than in stud matings, while ♂ 149 showed considerable improvement.

It is particularly significant that ♂ 5, tested only in flock matings, yielded a fertility of 85·7 per cent., a figure excelled by only two of the whole eleven males, in spite of the fact that he had only 4·57 grams of testis material. Male 172, with 8·98 grams of testis, gave 80 per cent. fertility. In contrast to these, ♂ 152, with 24 grams of testis, gave only 72·2 per cent. fertility. Cock 157, with 16·49 grams of testis, secured only 70·6 per cent. fertility; while ♂ 142, with exactly the same amount of testicular material, induced 92·4 per cent. fertility.

From these data it is reasonably certain that within the ranges covered by the eleven males tested (*i.e.* fertility from 66 per cent. to 92 per cent. and testis from 4·5 to 24 grams) fertility is entirely independent both of the absolute amount of testicular tissue present and of the amount in proportion to body weight.

(b) *Fertility in Relation to Density of Sperm Suspension.*—It is also evident that within the same limits of fertility a density of sperm suspension ranging from 825,960 per cu. mm. to nine times that figure has no relation to the resultant fertility. Thus Cocks 142 and 157 had equal amounts of testicular tissue, but while the former had an average sperm count of 1,836,650 and 92·4 per cent. fertility, the latter had an average count of 7,328,500 and a fertility of only 70·6 per cent. Similarly the fertility of ♂ 152 and of ♂ 382 was practically the same, although the number of sperm calls per cu. mm. was four times as great in the semen of the latter as in the former. The cock giving the lowest fertility in

the stud-mating series had over twice as many sperms per cu. mm. of semen as the cock with the highest fertility in the same series—♂142.

Walton (1927) found that with rabbits, a sperm suspension of less than 1,000,000 in 3 c.c. resulted in reduced fertility, and that below 10,000 in 3 c.c. sterility occurred. Possibly the same dilutions might produce similar results in the fowl if only one insemination were made. However, since for none of the ten cocks examined was the average density of sperm suspension less than *eight hundred times* Walton's critical figure, and in all but one it was *over one thousand times that figure*, it is extremely doubtful if in normal cocks density of sperm suspension is a factor contributing to the differential fertility often observed. Moreover, insemination in the fowl occurs not just once as in rabbits, but is repeated in an irregularly continuous manner often several times daily.

It is quite conceivable, however, that in certain pathological conditions the density of sperm suspension may become so low as to affect fertility. It is quite probable that the partial sterility observed in the first breeding year of Gowen's (1926) case of a fowl with cystic testes, reflected a degree of that occlusion of the vasa deferentia which post-mortem examination revealed had later become complete. In such cases one would expect a sperm suspension low enough to affect fertility even if the sperm were physiologically normal.

The extremely high average sperm count and the low fertility of ♂157 may have both resulted from a mild cloacitis with which he was affected throughout the entire breeding season. It is perhaps possible that the local irritation may have induced hyper-active spermatogenesis. No great difference in motility or appearance of his spermatozoa was evident when compared with those of others.

In view of these findings, it seems reasonable to infer that the differential fertility of cocks is dependent (except in cases of obviously unwilling breeders) upon differences in the physiological efficiency of the spermatozoa. No difference in motility of sperm cells from the ten cocks was noticed, but it was observed that the motility depends to a marked extent upon the temperature of the semen when examined.

Williams and Savage (1925) have shown that even a small proportion of certain types of abnormal sperms in the semen of bulls indicates low fertility. In a later paper Savage, Williams, and Fowler (1927) have also shown that the breeding efficiency of an unsound bull can be detected by measuring the head lengths of a representative sample of spermatozoa, determining the statistical constants for the distribution and the degree

of skewness of the curve of frequency distribution. A coefficient of variation greater than certain physiological limits or a statistically significant skewness indicated a poor breeder. Such results were usually confirmed by cytological and clinical findings, but in some cases of low fertility no evidence of unsoundness was found except by the statistical analysis of head lengths of spermatozoa. This means that a great variation in size of sperm, or a certain proportion of large or small cells, indicates some unknown condition which results in poor breeding efficiency. It is probable that similar conditions apply in the case of the fowl.

(c) *Amount of Testis and Density of Sperm Suspension.*—Contrary to expectation, the density of sperm suspension does not appear to bear any definite relation to the size of testis. In the first ejaculations of the day, or after a rest period of several days, practically no difference was observed between the counts of control and experimental males. It was only after being allowed to copulate several times that variations became marked. Such differences were not necessarily in accord with the amount of testis tissue present, although the decrease was less marked in the control males. For example, the lowest count obtained was one of 18,700 per cu. mm. from ♂ 152 after he had been 1½ hours with a hen. After a longer period at liberty with two different hens ♂ 142 gave a count of 277,600. The latter had 16·5 grams of testes, the former 24. Such a difference may, of course, reflect only different degrees of activity, but in this case both were keen breeders. In general, the slightly lower average counts of experimental cocks from which three or more samples were obtained reflect reduced counts following sexual activity, this reduction being equally apparent in cocks with a large but unilateral testis (*e.g.* ♂ 152) as in those with less testicular tissue.

The data of Craft *et al.* (1926), when re-arranged, also indicate that the density of sperm suspension is not dependent upon the size of testis (Table V). The counts made by these workers are much below those observed by the writer. Nevertheless it is probable that the degree of error in them (*vide infra*) was approximately the same in each case, especially since the number of samples from each pen (of three White Leghorn yearling cocks) ranged from eight to twenty-two, and that therefore the average counts are comparable one with another.

It seems probable that the density of sperm suspension in the semen is a reflection more of the degree of spermatogenic activity than of testis mass, and that at any one season differences in this activity depend upon the individual peculiarities of different birds as well as upon environmental conditions.

TABLE V.—RELATION OF AMOUNT OF TESTICULAR TISSUE TO THE DENSITY OF SPERM SUSPENSION IN THE SEMEN.

(Rearranged from data of Craft, M'Elroy, and Penquite.)

Pen.	No. of Males.	Samples counted.	Average No. Spermatozoa per cu. mm.	Average Total Testis Weight (grams).
1	3	7	105,400	22·30
2	3	20	177,500	10·80
3	3	12	225,300	13·46
4	3	11	224,900	17·01
5	2	8	96,500	12·17
6	3	10	676,000	22·66

Polowzow (1927) has recently shown that the rhythm of sperm production in the horse differs in different individuals besides being influenced by the frequency of breeding and length of rest periods.

(d) *Average Sperm Counts.*—The individual counts observed by Craft, M'Elroy, and Penquite ranged from 2000 to 4,000,000 per cu. mm. The semen was collected from the cloaca of the female *post coitu*, usually from the first matings in the morning. Since, in the present work, samples taken at this time were much higher than those taken after several matings, the writer is of the opinion that the low average counts of Craft *et al.* indicate that their samples were somewhat diluted by fluids of the cloaca. The determinations made in the present experiment ranged from 18,700 to 8,864,000 per cu. mm., with an average of 4,015,088 for 36 counts. The average counts for individual males ranged from 825,000 to 7,328,500, with an average of 3,998,642 for each of the ten cocks tested. Payne (1914) found that the density of sperm suspension in the semen of five different cocks ranged from 1,920,000 to 5,470,000 per cu. mm., with an average of 2,928,000. These figures are in accord with those observed in this experiment.

(e) *Amount of Ejaculate.*—It was found exceedingly difficult so to improve upon the method of collecting semen used in the present study that one could always secure the entire ejaculate. It was also difficult to measure the exact volume of the very small samples invariably obtained; the weight, however, could be accurately determined. The weights of eight samples of semen definitely known to contain the entire ejaculate are given in Table VI.

These data merely indicate that there is considerable variation in the amount of the ejaculate of different individuals and of the same individual

at different times. It is also apparent that the density of sperm suspension is independent of the amount of ejaculate. The available data do not permit of any statement concerning the relation between the total number of sperms ejaculated and fertility.

TABLE VI—WEIGHT OF EJACULATE WITH CORRESPONDING SPERM COUNTS.

♂.	Ejaculate (grams).	Sperm per cu. mm.
152	0·079	787,200
152	0·184	...
157	0·124	8,864,000
176	0·036	5,935,200
142	0·055	564,800
383	0·067	2,720,000
„	0·141	5,962,000
„	0·217	5,864,000
Average:	0·113	

(f) *Compensatory Hypertrophy.*—With regard to compensatory hypertrophy, it is evident (Table IV) that in males 142, 152, 149, and 157 the retained testis had hypertrophied to an extent approximately equivalent to the combined weight of both testes in the three controls. Indeed, in ♂ 152 the remaining testis was larger by 5·64 grams than the total testis material in the largest control pair. In another cock (♂ 147) unilaterally castrated at sixteen days, but untested for fertility owing to shortage of females, the retained right testis was found on autopsy thirteen months after the operation to be 17·1 grams. The results in this case are uniform with those of the other four.

In general these findings agree with those of Benoit (1925) and Domm (1927), but they differ from the latter's results in one important particular. Domm found that unilateral castration of birds 16, 24, or 40 weeks of age resulted in compensatory hypertrophy of the retained gonad whether right or left was removed, but that if one testis were removed from a week-old chick, compensatory hypertrophy resulted only when the left testis was retained and *not when the right one remained.* In all of the five birds mentioned above compensatory hypertrophy of the *right* testis was manifest to an extent approximating the normal weight of two testes.

On the other hand, the right retained testis of ♂ 21, operated on at five weeks of age, did not hypertrophy at all, and represents exactly what was left at the operation, *i.e.* half of a testis. Similarly ♂ 5, from whom

the left testis and half of the right were removed at seven days of age, had only 1·06 grams of testis on the right side at post-mortem but had regenerated a 3·51 nodule of testis tissue on the left. Neither the data now presented nor those of the other two workers afford a satisfactory explanation of why there should not have been hypertrophy in these two cases to the same extent as in the others. The theory suggests itself that mutilation of the surviving testis may prevent its hypertrophy, but the numbers are too small to substantiate the theory.

Cocks 172 and 176 present another aspect of the case. Both these birds were operated upon at twelve to fourteen days (Table I), but the tissue removed was grafted subcutaneously under the wing. These grafts persisted and were present at autopsy over a year after the operations. That of ♂ 172 was 2·41 grams, while ♂ 176's graft was only 0·125 gram. In neither of these cases was there any distinct evidence of hypertrophy (see Table IV). Another cock, untested for fertility (♂ 162), fell in the same class with a retained right testis of 7·58 grams, and an unweighed testis graft 1·9 cm. × 1·5 cm.

While the present data support the previous workers' establishment of the occurrence of compensatory hypertrophy, they are not sufficient to account for that phenomenon. The obvious assumption is that a certain amount of testis tissue is necessary to maintain the balance between different organs of the body. Nevertheless, some of the cases reported above have matured and reproduced in every respect like normal males, yet had only about half of a normal testis. The fact that hypertrophy was not evident in the three birds carrying grafts suggests that the balancing action of the testis calls for production of an optimum amount of testicular hormone, and that if this be in part supplied from a graft the testis retained need not hypertrophy. However, the grafts of these birds, plus retained testis, did not amount to much more than a single normal testis.

Summary.

1. Fertility tests of eight partially castrated cocks and three controls show that within quite wide limits of fertility and of testis size the fertility of the male fowl is not in any way dependent upon size of the testis.

2. The average density of sperm suspension was found to be approximately four million spermatozoa per cubic millimetre for the thirty-six samples examined from ten males.

3. The variations in the average density of sperm suspension from

different birds ranged from 825,000 to over 7,000,000 cells per cu. mm., but within this range the density of sperm suspension bore no relation to fertility.

4. The number of sperm per cubic millimetre of semen appears to be entirely independent of the size of the testes within the ranges covered by this experiment.

5. Compensatory hypertrophy to a degree approximating to the normal weight of both testes was observed in the retained right testes of cocks castrated unilaterally on the left side at one week of age.

6. Exceptions to the rule of compensatory hypertrophy included birds with subcutaneous testis grafts and two in which one whole testis and part of the other had been removed.

7. It is suggested that fertility in the male fowl is dependent upon the physiological efficiency of the spermatozoa rather than upon their quantitative production.

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A NOTE ON THE EFFECTS OF DIFFERENT DOSES OF THYROID ON THE FOWL.

By F. B. HUTT.

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A NOTE ON THE EFFECTS OF DIFFERENT DOSES OF THYROID ON THE FOWL

By F. B. HUTT.

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(With Two Text-figures.)

Reports of the effects of thyroid feeding on fowls published to date by various investigators show some lack of uniformity. Giacomini (1924) and B. Zavadovsky (1925) have noted depigmentation of plumage when fresh thyroid gland was given orally in doses which, though not stated exactly, were relatively large. The latter writer also obtained the same result with dried preparations.

On the other hand, Cole and Reid (1924) found that desiccated thyroid when given to Brown Leghorns at the rate of 84 milligrams per pound of live weight caused a greater production of melanin. A similar result was mentioned by Crew (1925). More recently, Torrey and Horning (1925 *a*) found that feeding thyroid resulted in the production of darker feathers in cockerels and capons but not in hens. The latter workers concluded that the ovary secretes a substance which nullifies the action of the thyroid in normal hens. This view hardly seems consistent with the findings of Giacomini and Zavadovsky, each of whom observed depigmentation in both cocks and hens following administration of thyroid.

Hen feathering in male fowls fed upon desiccated thyroid has been reported by Torrey and Horning (1922 and 1925) and Cole and Reid (1924). A trial by Crew and Huxley (1923) with cockerels on a very low thyroid intake failed to confirm these results, but in later work with adult birds Crew's findings agreed with those of the other observers. Neither Giacomini nor Zavadovsky makes any mention of hen feathering in their papers. It might seem at first glance that these differing results were due to the fact that the latter two investigators used fresh thyroid gland while the other workers used a desiccated product. Zavadovsky used dried glands and Poehl's thyroidin, but so far as can be determined from his paper these substances were fed only to hens and all the cocks used received fresh thyroid. Cole and Hutt (1928), however, obtained hen feathering in males with both fresh gland and Armour's desiccated thyroid.

It seemed possible that the discrepancies in the findings reported above were due to the fact that the thyroid substance was given in different dosages by the several investigators. Cameron and Carmichael (1920) have shown that different rates of thyroid feeding produce decidedly different results in rats, and that comparable data are most readily obtainable by feeding dosages of known amounts of iodine in thyroid combination. Accordingly an experiment was planned to determine the effects of various dosages on feather colour, feather structure, and body weight.

EXPERIMENT.

Ten Black Minorca pullets nine months old and seven cockerels of the same breed and about the same age were given daily doses of desiccated thyroid (Parke, Davis and Co.—0·3 per cent. iodine) in addition to a well balanced ration. A control of each sex (No. 11) received a capsule of beef-scrap daily.

Females 1 to 10 received daily doses of from 4 mg. thyroid iodine per 1000 gm. of body weight to 4 mg. per 10,000 gm. of body weight. The males were numbered 1, 2, 3, 5, 7, 10 and each received a daily dose identical with that given the female of the same number. Thus in each lot No. 5's dose was 4 mg. thyroid iodine per 5000 gm. of body weight, No. 7 received 4 mg. thyroid iodine per 7000 gm. of body weight, and so on.

The two largest doses were administered by mixing the powder with moist mash, shaping the whole to cylindrical pellets and thrusting these down the gullet of the fowl. The smaller doses were given in gelatine capsules. All birds were weighed every three days. Doses for each bird were made up afresh subsequent to each weighing and changed as required to keep the amount given in a constant proportion to the body weight. Since one result of heavy thyroid feeding is a decrease in weight, it is obvious that if the same amount were given throughout the experiment the actual dosage would increase as the body weight decreased.

Feeding was begun on February 4th, 1926, and continued for 31 days.

RESULTS.

Effects of large doses.

The two largest doses, viz. 4 mg. thyroid iodine to 1000 and 2000 gm. body weight, proved lethal to both cockerels and pullets. They caused rapid decline in weight as shown in Figs. 1 and 2. The birds showed evidence of intense thirst and diarrhoea followed by loss of appetite, dullness and lack of co-ordination. The excreta consisted mostly of white urates and mucus. On autopsy the ureters of all four birds were found to be congested with urates through their entire length. Male No. 1 had enormously hypertrophied testes weighing 20·6 and 19·95 gm., whereas 9 or 10 gm. would have been normal for a cockerel of his weight.

Table I.

Effects of large doses of thyroid.

Bird	Original weight (gm.)	Dose of thyroid iodine	Death	Loss in weight (gm.)	Desiccated thyroid consumed (gm.)	Thyroid iodine consumed (gm.)
Male 1	2691	4 mg. 1000 gm.	9th day	182	24·927	·075
„ 2	2598	4 mg. 2000 gm.	14th „	682	14·904	·045
Female 1	2541	4 mg. 1000 gm.	22nd „	781	27·703	·083
„ 2	2364	4 mg. 2000 gm.	22nd „	647	19·731	·056

The two females went into precipitate moult. No. 1 started ten days after feeding began, No. 2 on the thirteenth day. None of the birds were given any thyroid when they had lost their appetites, but feeding was resumed on alternate days when the appetites were regained.

Fig. 1. Changes in body weight of Males.

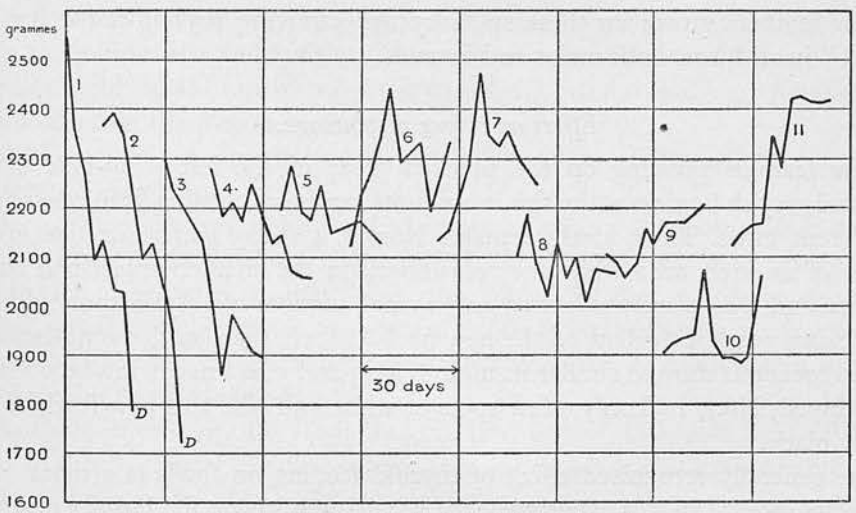

Fig. 2. Changes in body weight of Females.

In the case of both sexes No. 1 received daily 4 mg. thyroid iodine per 1000 gm. of body weight; No. 2, 4 mg. per 2000 gm.; No. 3, 4 mg. per 3000 gm. and so on. No. 11 was given beef-scrap. 4 mg. per 1000 and 2000 gm. proved to be lethal (*D*). 4 mg. per 3000 to 5000 gm. caused loss in weight in both sexes. 4 mg. per 7000 gm. affected the male only. Smaller doses did not affect the weight of either sex.

Table I summarises the effects of the two largest doses. There was not much difference between the sexes in their ability to withstand heavy doses. The females probably lived longer because thyroid feeding was stopped for several days when they lost their appetites. All four birds succumbed to doses which other workers have shown are not lethal for rats.

Effect on body weight.

The changes in body weight are shown graphically in Figs. 1 and 2. There is considerable normal variation in the weights of fowls even when taken at the same hour each day, hence the data do not present smooth curves. It is evident that the female on a dose of 4 mg. thyroid iodine per 3000 gm. lost weight rapidly. No. 4 had a marked decline, No. 5 a slight one and the remainder on the smaller doses were not significantly affected. Of the cockerels Nos. 3, 5 and 7 lost weight steadily, while No. 10 receiving only 4 mg. thyroid iodine per 10,000 gm. of body weight was entirely unaffected. The number of birds used was small, but the results give some indication that the females were better able to withstand the thyroid treatment than were the males.

Effect on feather structure.

One week after feeding started a patch of feathers was plucked from the neck and left wing-bow of every bird.

In male fowls, feathers in these areas have a marginal zone at the distal end in which the barbs lack barbules and hooks. Such feathers appear pointed in contrast with feathers from the same regions in females. This difference constitutes a secondary sex character in the domestic fowl.

New feathers grown on these spots by the surviving thyroid-fed males were "henny" in all birds, both males and females.

Effect on colour of plumage.

New feathers growing on the plucked areas of the left wing-bow showed white and greyish borders at the tips in amounts varying according to the doses given to different birds. Thus, of the females Nos. 3, 4 and 5 had white tips up to a quarter of an inch wide while the remainder, on the smaller doses, had smaller amounts, and Nos. 9 and 10 showed only a small number of greyish spots. White or grey ticking was less evident on the new neck feathers than on the wing-bow.

The cockerels showed similar results. Nos. 3 and 5 had new wing-bow feathers white tipped, No. 7 had only a few spots of white and No. 10's new feathers were entirely black.

One generally recognised effect of thyroid feeding on fowls is a more or less precipitate moult. In this experiment the surviving birds on the heavier doses, viz. females Nos. 3 and 4 and male No. 5, underwent partial moults. New primary feathers on these birds had patches of white ranging from small white tips in male No. 5 to nearly solid white feathers in female No. 3. Female No. 7 also shed two primaries on each wing, but the new feathers growing in were entirely black.

It is of interest to note that among the wing-bow feathers plucked from two of the males were quite a number which were partly red in colour. This is not an uncommon defect in Black Minorcas. The new feathers replacing them, however, were solid black, except for white tips on No. 3.

The degree of depigmentation was difficult to measure exactly, but it was quite evident that the birds receiving daily doses of 4 mg. thyroid iodine to 3000, 4000

and 5000 gm. of body weight showed the most depigmentation. Those receiving smaller doses had very little white in new feathers, while those on doses of 4 mg. to 9000 and 10,000 gm. of body weight produced solid black feathers, only a few of which had (in the females only) small greyish tips.

Since this experiment was done Brambell (1926) has observed corresponding degrees of depigmentation on similar doses. The fact that some of his birds showed less of the effect than others was probably due to differential body weight or differential consumption of the mash containing thyroid, which was fed to the entire flock and not administered individually.

New feathers on the thyroid-fed birds were dull in appearance and somewhat rough, due probably to the partial atrophy of the uropygial gland first noted by Giacomini as a consequence of thyroid feeding. The quilling of the rectrices and remiges observed by Torrey and Horning (1925 *a*) was present to a slight extent in new remiges replacing those shed by the birds on the heavier doses.

SUMMARY.

Daily doses of 4 mg. thyroid iodine per 1000 and 2000 gm. of body weight proved lethal to male and female fowls.

Daily doses of 4 mg. thyroid iodine per 3000 to 5000 gm. body weight caused loss in weight in males and females. The same amount of thyroid iodine to 7000 gm. of body weight caused loss of weight in a cockerel but not in a hen. Smaller doses had no effect on the weight in either sex.

All doses, even 4 mg. thyroid iodine to 10,000 gm. body weight, caused hen feathering in the males.

Depigmentation was quite marked in the case of the heavier doses, but less evident in that of the smaller. In general, depigmentation was most evident in the birds which declined in weight. The minimum daily dose necessary to produce marked depigmentation was 4 mg. thyroid iodine to 5000 gm. of body weight. This explains why several investigators have not obtained the depigmentation described by Giacomini and Zavadovsky.

On doses of 0·8 mg. thyroid iodine per bird or less, Cole and Reid, and Crew observed production of darker feathers. On much larger doses Giacomini, Zavadovsky and the writer observed depigmentation.

It would seem that the smaller doses of thyroid cause increased production of melanin, presumably by the general increase in metabolic processes, but that at a certain stage (which this experiment indicates to be around 4 mg. thyroid iodine per 5000 gm. of body weight) the production of pigment is arrested.

The use of a definite dosage of thyroid iodine based on body weight has led to consistent results and to an explanation of some of the discrepancies which have previously appeared in the literature.

The writer wishes to thank Prof. A. T. Cameron for valuable assistance in planning the experiment described in this paper.

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ON THE FECUNDITY OF PARTIALLY OVARIOTOMIZED FOWLS[1]

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ONE FIGURE

INTRODUCTION

The rate of ovulation in the domestic fowl is an extremely variable physiological function which is subject to modification by numerous genetic and environmental factors. In spite of several attempts to determine its genetic basis, satisfactory evidence has not yet been established for anything more definite than an undetermined number of multiple factors. Much better known are the modifying effects of different environmental conditions, particularly of those which tend to prevent full expression of a fowl's innate capacity to lay. Among the latter may be included insufficient food, unbalanced rations, sudden drops in temperature or prolonged low temperatures, internal and external parasites, chronic infection of the ovary (with Salmonella pullorum), other diseases, sudden fright, changes of quarters, extreme density of the population, and restriction of light.

Apart from avoidance of these undesirable conditions and others not mentioned, ways and means of stimulating fowls to lay up to the maximum possible limits of their genetic capacities, or beyond them, are few in number. It is probable that daily removal of eggs from the nests exerts a psychological effect and induces sustained ovulation in the domestic fowl, as it has been shown to do in wild species. A temporary stimulation results from the increased consumption of food

[1] Paper no. 1169 of the Journal Series of the Minnesota Agricultural Experiment Station.

induced by feeding a moist mash in addition to a dry one, but the effect in such cases is more directly attributable to increased intake of animal protein for which the mash is the vehicle.

A lengthened period of exposure to light is the most effective means of stimulating production, whether it occurs naturally, as in the spring months, or is provided by supplementary artificial lighting during the periods of short days. The remarkable increase in egg yield resulting from such artificial illumination of poultry houses has made its use a general practice on commercial poultry farms during the fall and winter months. The effect here has always been attributed to the increased food consumption made possible by prolongation of the hens' 'working day.' However, in view of the remarkable stimulation of gonadic activity induced in males of Sturnus vulgaris by exposure to white and red light (Bissonnette, '32), it seems probable that the increased ovulation in fowls subjected to artificial lighting may be due, at least in part, to some direct physiological effect of the light.

It has recently been reported in a series of papers by Steggerda ('28, '29 a, '29 b, '31) that partial ovariotomy, or other injury to the ovary, induces in some fowls a higher level of fecundity than that prevailing in birds not so treated and, moreover, causes the fowls thus operated upon to begin laying at an earlier age than controls. The same effects were apparently induced by other injuries to the body, such as the removal of the membrane overlying the ovary and intermittent blood letting at fortnightly intervals.

Inasmuch as these findings suggested physiological reactions hitherto unknown, it seemed desirable to repeat his experiments. The complete results of such an investigation are reported in this paper.

MATERIAL

The experimental and control birds all came from a flock of about 300 Single Comb White Leghorn pullets reared at the North Central Branch Experiment Station, Grand Rapids,

Minnesota, in 1931. All were hatched on March 26th, all came from one flock, and all were reared together on the same range.

METHODS

On May 20th, 21st, and 22nd, 1931, when these chickens were 8 weeks of age, 109 pullets chosen entirely at random were partially ovariotomized by the senior author. Six weeks was considered by Steggerda ('31) as favorable an age for manifestation of the effects as a later one, so that 8 weeks should also have been a suitable age for the operations. Complete ovariotomy in birds is extremely difficult, but partial ovariotomy is a comparatively simple operation. The birds were starved for 16 hours before operations. An incision was made between the last two ribs on the left side. That portion of the ovary closest to the dorsal aorta was raised by inserting under it a fine wire bent at right angles and was cut off with a pair of long-handled, curved scissors with sharp points. Following this, the covering membrane was pulled away and additional tissue was removed with forceps. Every precaution was taken to avoid injury to adjacent membranous tissues, so that the oviduct would not be disturbed. The ovarian tissue was not weighed, but, following Steggerda's technique, an effort was made to take out not more than one-half and not less than one-quarter of the gonad. Operated birds were identified by numbered wing bands.

At the time of the operations a number of the birds in the flock were affected with the first stages of a respiratory disorder which had apparently been precipitated by a sudden onset of extremely cold weather. Resistance to this condition was apparently lowered as a result of the operation, and 25 of the 109 died within 3 days with symptoms resembling those of laryngotracheitis. The remainder ran with the control birds during the summer months.

On August 26th, after 4 or 5 eggs had been found on the range, 50 controls and 50 operated birds were leg-banded and given winter quarters in one large pen. Subsequent egg

records showed that at this date only 3 or 4 of the more than 260 pullets of this age group had actually begun to lay. The egg records are therefore practically complete. On September 4th, 25 more of each class were added to this flock. Trapnest records of individual egg production for these 150 birds were kept from the day of housing.

PRECAUTIONS AGAINST ERROR

Because of the great influence of environmental conditions on egg production, it was essential that the 75 partially ovariotomized and 75 control birds should be kept under identical conditions. This was easily accomplished by housing the whole 150 in one large pen. All had the same well-balanced ration, the same attendant, the same amount of light, and were exposed in equal degree to such other environmental conditions as changes in temperature, disturbances, and respiratory disorders. To insure that stimulation of production beyond the birds' genetic capacities should not be induced by any factor other than that under investigation, no artificial light was used.

With such conditions, and since the whole 150 were of the same age and origin and had been reared under identical conditions, it is evident that the only environmental factor which could influence one group more than the other was the operation at 8 weeks. Genetic variability is inevitable. It could easily obscure any possible effect of experimental procedure in population samples of even thirty birds or more, unless that effect were comparatively great. However, in random samples of seventy-five, such variability is more likely to be normally distributed in both experimental and control animals, and hence to permit manifestation of any other difference between the two groups, even if the effect of that difference be relatively small. The uniformity of the two samples tested in this experiment is indicated by the statistical constants for their body weights, which are given in table 1. The weight of each bird was taken to the nearest tenth of a pound.

Since the difference in neither case is twice its standard error, the two groups are not significantly different in mean body weight or in variability with respect to weight.

TABLE 1

Body weights of experimental and control fowls

	NUMBER OF BIRDS	MEAN WEIGHT IN POUNDS	STANDARD DEVIATION
Partially ovariotomized	75	3.462 ± 0.040	0.350 ± 0.029
Controls	75	3.356 ± 0.044	0.383 ± 0.031
Difference		0.105 ± 0.059	0.003 ± 0.042
$\frac{\text{Difference}}{E_{diff.}}$		1.8	0.07

RESULTS

For purposes of this experiment the egg records from August 26th to April 30th have been analyzed. Since this period is somewhat over 8 months, covers the performance of the birds up to the age of 401 days, and includes the months of March and April when the ovulation rate is at its maximum, it is more than adequate for the manifestation of any stimulation resulting from partial ovariotomy at 8 weeks of age.

Fecundity

Records of birds not laying were included in the determination of the statistical constants. The records for August are those of the 100 birds housed on August 26th. Only eggs laid in trapnests are included. Since the experimental birds were no more likely to lay on the floor than were controls, the inevitable slight error resulting from such unrecorded 'floor' eggs was equally apportioned to the two groups. Eggs laid by birds dying prior to the sixteenth of any month are omitted from that month's figures, but records of those dying after the fifteenth are included. Mean productions for the entire period are based on the numbers which lived on April 30, 1932. The statistical constants for the birds' egg production, which are derived from the usual formulae applicable to a normal distribution, are shown in table 2.

Since the standard error is used throughout this paper, it follows that a difference exceeding twice its error may be considered significant. Table 2 shows that, with the exception of August, when only a few eggs were laid, the fecundity of the normal control pullets exceeded that of the partially ovariotomized birds in every month and was significantly higher in October, November, and December. The mean production for the whole laying period of 248 days was higher

TABLE 2

Fecundity of partially ovariotomized and control pullets

PERIOD	PARTIALLY OVARIOTOMIZED		CONTROL		DIFFERENCE
	Number of birds	Mean eggs per bird	Number of birds	Mean eggs per bird	Diff.
August	50	0.76 ± 0.17	50	0.48 ± 0.14	
September	75	8.41 ± 0.86	75	9.68 ± 0.80	1.1
October	73	11.07 ± 1.11	75	14.57 ± 1.07	2.3[1]
November	72	9.47 ± 1.09	73	14.73 ± 1.03	3.5[1]
December	70	7.27 ± 1.09	72	11.53 ± 1.16	2.7[1]
January	70	6.7 ± 1.09	71	7.80 ± 1.13	0.07
February	70	7.76 ± 1.09	70	9.11 ± 1.06	0.9
March	66	13.60 ± 1.27	69	16.55 ± 1.08	1.8
April	66	15.54 ± 1.28	65	18.71 ± 1.05	1.9
August 26–April 30:					
Mean	66	82.40 ± 6.90	65	103.11 ± 5.80	2.3[1]
Standard deviation	66	56.07 ± 4.88	65	46.79 ± 4.10	1.4

[1] Significant.

in the controls by 20.71 ± 9.01 eggs, which is a statistically significant difference.

On the other hand, the variability, as measured by the standard deviation, is greater among the partially ovariotomized birds, but not by a difference large enough to be statistically significant.

Age at sexual maturity

The age at first egg can be considered for the fifty birds of each group which were housed on August 26th, when exactly 5 months of age. Since only 4 or 5 eggs were found on the range prior to this date, the error arising from some of these

birds having begun to lay before being housed cannot be large. It would have been considerably greater in the birds taken in on September 4th, and, for that reason, the latter are not included in the determination of age at sexual maturity.

To avoid errors from the inclusion of birds possibly pathological, there were excluded from the calculations the records of four fowls which had not yet begun to lay by February 1, 1932, when they were 311 days of age. One of these subsequently laid sixty-four eggs in February, March, and April. Of the other three, one never laid, another was credited (probably erroneously) with one egg in April, and the third was found to be infected with Salmonella pullorum and was removed in March without ever having laid.

TABLE 3

Age at first egg in experimental and control birds

	47 PARTIALLY OVARIOTOMIZED	49 NORMAL	DIFFERENCE / Ediff.
Mean age at first egg	168.95 ± 3.08	171.32 ± 2.65	0.6
Standard deviation	21.04 ± 2.17	18.54 ± 1.87	0.8

Since 3 of these 4 birds were from the partially ovariotomized group, their exclusion is more favorable than otherwise to the records of that group.

The statistical constants for age at first egg (table 3) show clearly that the partially ovariotomized birds and the normal ones do not differ significantly either in mean age at first egg or in variability with respect to this character. Partial ovariotomy has obviously not caused the partially ovariotomized birds to lay any earlier than the untreated fowls.

DISCUSSION

The results of this experiment show:

1. That partial ovariotomy at an early age does not stimulate subsequent fecundity to a level higher than normal, but that, on the contrary, it lowers the mean fecundity of fowls so treated.

2. That the variability of partially ovariotomized fowls with respect to fecundity may be somewhat greater than that of normal birds, but that the difference is apparently too small to be statistically significant in populations of sixty-six birds.

3. That the operation has no effect on the age at which the first egg is laid, or on the variability of the population with respect to that character.

All of these conclusions are directly opposite to those of Steggerda, but are not incompatible with his data. A re-examination of these reveals that Steggerda's material was hardly extensive enough in any of his experiments to permit measurement of any minor effect of partial ovariotomy, or of the other treatments, nor does it present any significant evidence of a major effect. The number of eggs laid by fowls in a year has been known to range from 0 to 358. With such a variability, Steggerda's populations of control and experimental animals (which ranged in number from 7 to 24, but in no case exceeded 25) could not be expected to reveal anything but a very great difference between the two groups.

A second factor leading to Steggerda's erroneous conclusions was his use of the range of the distribution as a measure both of type and of variability. He considered that, because the mean production was apparently the same in his experimental and control groups, whereas the best and poorest layers occurred in the experimental birds, some birds in the latter group must have been stimulated to lay more eggs, others less, than they would have laid without operative interference.

While the range is a suitable measure of dispersion in large populations of equal size, it is totally unsuitable for measuring, in population of less than twenty-five, such a character as egg production, in which one may encounter by chance a range of over 300 eggs between the highest and the lowest of a year's egg records. Moreover, it is axiomatic that the range increases with the size of the population, so that it is hardly satisfactory for a comparison of 5 or 9 experimental

animals with seven controls, or to compare results in three groups A, B, and C, originally of twenty-four birds each, but which suffered mortalities of 7, 4, and 1, respectively, during the test (Steggerda, '29). Much less is it justifiable to lop off the highest eight records in each of the three groups just mentioned and to conclude from their averages of 124.2, 125.8, and 128.5 that "each bird in (series B) the membrane removal experiment lays on an average of 1.5 more eggs than do the controls (series A), and the blood removal (series C) birds 2.5 more eggs than those of series B."

To obtain a more accurate interpretation of Steggerda's results, his data for those experiments in which the number of experimental animals exceeded twelve have been statistically examined by the present writers. Taking the mean as the measure of type and the standard deviation as the measure of variability, the experimental and control animals in four tests have been compared with respect to these statistics. The methods used are those devised by Fisher ('30, p. 107) for determining in small samples the significance of differences between means and (ibid., p. 194) between variances. The results are shown in tables 4 and 5. Statistically significant differences are indicated by the small numeral [1].

In all four trials the mean production of the operated birds is less than that of the controls, in one case by an amount which is statistically significant.

On the other hand, the variability of the experimental birds is significantly greater than that of controls in 3 of the 4 experiments, so that there may possibly be a biological basis for it. The greatest difference is between those birds from which blood was drawn at intervals and their controls. In this case the withdrawal of blood may have somewhat upset the rhythm of production, but it is equally probable that such an effect was produced by the disturbance to the flock associated with their being captured and handled on the five separate occasions when the birds were bled. Steggerda's suggestion that periodic bleeding of fowls raises their vitality is not supported by the finding of Ackert and Porter ('31) that periodic

bleeding reduces the resistance of chickens to the nematode parasite, Ascaridia lineata.

In the more adequate data reported in the writer's experiment (table 2) the partially ovariotomized birds were somewhat more variable in fecundity than were the controls, but the difference is not significant. There is some evidence, however, that the operation may have reduced the number of

TABLE 4

Analysis of mean fecundity. Data of Steggerda

EXPERIMENT	MEAN EGG PRODUCTION		t	n	P
	Experimental	Controls			
S. (1928) Series A	167.39	179.48	0.971	44	0.33
S. (1928) Series B	104.75	104.83	0.005	22	0.99
S. (1929) Membrane removal	95.50	107.23	2.558	35	0.01[1]
S. (1929) Blood letting	90.87	107.23	1.545	38	0.13

[1] Significant.

TABLE 5

Analysis of variability of fecundity. Data of Steggerda

EXPERIMENT	STANDARD DEVIATION		z	n_1	n_2	5 PER CENT POINT	1 PER CENT POINT
	Experimental	Controls					
S. (1928) Series A	51.52	30.11	0.5370[1]	22	22	0.3582	0.5118
S. (1928) Series B	39.98	25.44	0.4517	11	11	0.5151	0.7439
S. (1929) Membrane removal	31.37	17.48	0.5894[1]	19	16	0.4128	0.5931
S. (1929) Blood letting	41.61	17.48	0.8670[1]	22	16	0.4062	0.5841

[1] Significant.

eggs laid by several of the experimental birds. The mean production of the latter was lower than that of controls during every complete month of the experiment. It was significantly lower in 3 of the 8 months and for the entire period. Moreover, in both March and April, when both flocks were in maximum production, the difference was almost great enough to be significant.

A comparison of the distributions of egg production in the two groups suggests the reason for the differences between

them (fig. 1). Such a comparison is valid in these data because of the comparatively large numbers used, and because mortality in the two groups was remarkably uniform and was evenly distributed throughout the year.

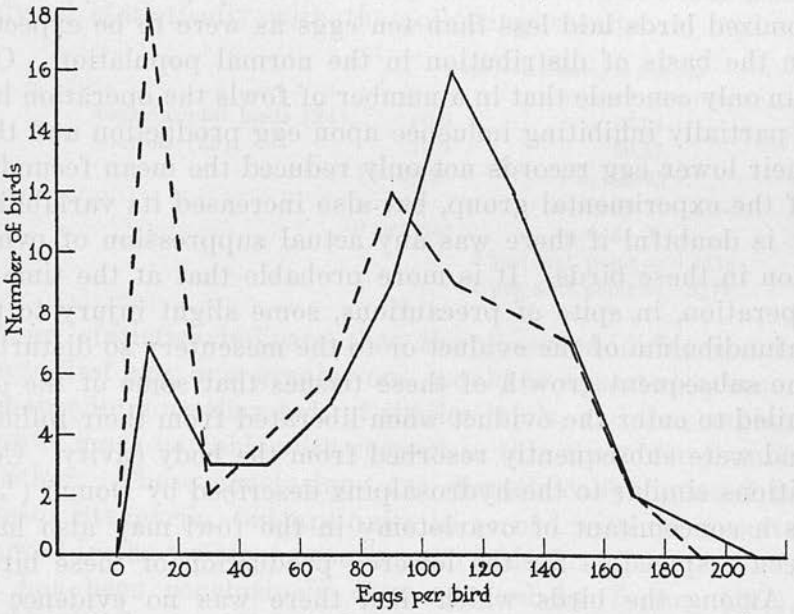

Fig. 1 Distributions of individual egg records up to 401 days in experimental and control groups. Partially ovariotomized ------; normal fowls ———.

Figure 1 shows that, whereas the distribution of egg records of the controls is approximately normal, with the exception of a slight peak in the lowest class, the distribution of records for the partially ovariotomized birds is distinctly bimodal, with eighteen birds laying less than twenty eggs. For the sixty-five controls and sixty-six partially ovariotomized birds which completed the test, the numbers in the high and low classes were as follows:

	Number of birds laying	
	More than 150 eggs	Less than 10 eggs
Partially ovariotomized,	7	17
Controls,	8	4

Contrary to Steggerda's conclusions, the operation has had no effect on the upper portion of the distribution curve. By chance the two highest records—187 and 175 eggs—were made by controls.

On the other hand, four times as many partially ovariotomized birds laid less than ten eggs as were to be expected on the basis of distribution in the normal population. One can only conclude that in a number of fowls the operation had a partially inhibiting influence upon egg production and that their lower egg records not only reduced the mean fecundity of the experimental group, but also increased its variability. It is doubtful if there was any actual suppression of ovulation in these birds. It is more probable that at the time of operation, in spite of precautions, some slight injury to the infundibulum of the oviduct or to the mesentery so disturbed the subsequent growth of these tissues that some of the ova failed to enter the oviduct when liberated from their follicles and were subsequently resorbed from the body cavity. Conditions similar to the hydrosalpinx described by Domm ('33) as a concomitant of ovariotomy in the fowl may also have been responsible for the lowered production of these birds.

Among the birds which died there was no evidence of abdominal yolk concretions, and the fact that there were ten deaths among controls and nine among the experimental birds indicates that the operation had no marked effect on the general health of the birds. Two controls and one operated bird reacted to the test for Salmonella pullorum. There was no evidence of temporary assumption of male secondary sex characters following the operation, nor was any to be expected, in view of the fact that at least half of the ovary was left in every bird.

In his latest paper, Steggerda ('31) supports his earlier conclusion that ovarian injury advances the age of sexual maturity with data purporting to show that partial ovariotomy and injury of the ovary at 6 weeks of age induced birds so treated to lay 4 days earlier than controls. Disregarding the biological errors inherent in this material, be-

cause, 1) the exact ages of the birds at any stage are not given, 2) there is no evidence that they were all the same age and, 3) age at first egg was measured not from date of hatching, or even from date of operation, but only from the date of housing the birds, the records for the two series have been analyzed statistically with the following results:

	Days from housing to first egg	
	Mean	Standard deviation
Experimental birds (24),	66.6	27.04
Controls (22),	70.9	20.27
	$t = 0.606$	$z = 0.2882$
	$n = 44$	$n_1 = 23$
	$P = 0.545$	$n_2 = 21$
		5 per cent point = 0.3618
		1 per cent point = 0.5176

These statistics indicate that the observed difference in days to first egg, or a greater one, would be expected to occur by chance in more than half of similar trials, and that neither group is more variable with respect to this character than is the other. These conclusions are diametrically opposed to those of Steggerda, but are quite in accord with the results observed in the writers' experiment (table 3).

It has been conclusively demonstrated by Domm ('27), Pearl and Schoppe ('21), and others that removal of part of the fowl's ovary is followed by regeneration until the injured gland is restored to its usual mass in proportion to body size. However, Pearl and Schoppe observed that the fecundity of individual fowls bore no relation whatever to the number of their visible oocytes, so that even if regeneration should cause some ovaries to become larger than they might otherwise be, there is no reason to expect such an ovary to produce more eggs. Similarly, Hutt ('29) has shown that in the male fowl the rate of gametogenesis, as measured by the density of sperm suspension, bears no definite relation to the mass of the testis.

The number of eggs laid depends upon the number of oocytes filled up with yolk, and this number is always a mere fraction of the total oocytes available. Fauré-Fremiet and

Kaufman ('28) found the oocytes in the cortical layer of the ovary of 2-day-old chicks to number 3.6×10^6 in White Leghorns and 12.5×10^6 in Rhode Island Reds. At 15 days the number of 'privileged oocytes' apparently capable of further development was 1074 in White Leghorns and 1571 in Rhode Island Reds. Even if the number of eggs laid were limited by the number of 'privileged oocytes,' the latter would apparently greatly exceed the total eggs produced by the majority of fowls. Moreover, it seems probable that the number of oocytes capable of development is greater than the figures of Fauré-Fremiet and Kaufman would indicate. Pearl and Schoppe (loc. cit.) found that in twenty-four mature fowls the mean number of oocytes visible to the naked eye (including eggs laid), was 1906. In one case where the oocytes were counted with the help of a low-power lens, a hen which in 14 months had laid sixty-nine eggs was found to contain 13,476 oocytes. The limiting factor, apart from environmental influences, is thus not the number of oocytes, but the genotype of the bird, which, presumably through the medium of endocrine secretions, determines the rates of formation of yolk at different periods of the year and under varying conditions of environment.

The conclusions reached in this paper are confirmed by the report of Card and Roberts ('31) that scarifying or mutilating the ovaries of senescent fowls which had once been very good layers had no effect on their subsequent production. Thirteen such fowls had an average production in 152 days of only 10.9 eggs, while thirteen controls had an average for the same period of 16.2 eggs.

In view of all these findings, it is obvious that, so far as fecundity is concerned, the effects of partial ovariotomy and injury to the ovary are deleterious in some cases, innocuous in others, but beneficial or stimulatory in none.

SUMMARY

The fecundity of seventy-five White Leghorn pullets which had been partially ovariotomized at 8 weeks of age was compared with that of seventy-five controls up to the age of 401 days.

There was no evidence that the operation exerted any stimulatory effect upon ovulation in these birds.

The mean fecundity of the partially ovariotomized birds was consistently and significantly lower than that of controls.

The variability in fecundity of the partially ovariotomized fowls was greater than that of controls, but not significantly so. While the number of birds laying over 150 eggs was practically equal in the two groups, the number laying less than ten eggs during the test was over four times as great in the experimental birds as in the controls. It is suggested that in some partially ovariotomized birds injury to the reproductive tract at the time of the operation subsequently prevents some ova from entering the oviduct.

The operation had no significant effect upon mean age at sexual maturity as measured by the age at first egg or upon variability of this character.

Steggerda's data bearing on the effects of partial ovariotomy and ovarian injury are reexamined and found to present no evidence of any stimulatory effect of such procedures upon egg production.

It is concluded that the effects of such operations are deleterious in some cases, harmless in others, but beneficial or stimulatory in none.

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IDIOPATHIC HYPOPARATHYROIDISM AND TETANY IN THE FOWL

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IDIOPATHIC HYPOPARATHYROIDISM AND TETANY IN THE FOWL*

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Tetany has been noted in several species of mammals, but, so far as can be ascertained, no account of tetany in birds has hitherto been published. This paper reports a case of tetany in the fowl which apparently resulted from temporary subnormal functioning of the parathyroid glands.

A pullet hatched on May 4, 1933, from a cross of S. C. White Leghorn ♂ x Rhode Island Red ♀, began laying on October 27, 1933, at the age of 175 days, her weight then being 2,220 grams. In the 16 days up to November 12 she laid in trapnests a total of 11 eggs, which was a normal rate of egg production. On November 13 this fowl was apparently normal in every respect when the flock was fed at 7:30 A.M., and also at 9:00 A.M., when the birds were given their daily supply of skimmed milk. At 10:15 A.M. she was found prostrate with the legs paralyzed and both wings braced against the floor. When picked up the wings beat spasmodically, but the legs remained paralyzed. When examined at 3:30 P.M. she again underwent uncontrollable convulsive movements of the wings, quite different from the usual flappings made by a captured bird attempting to escape. These were accompanied by dyspnea and by evacuation of the ureters and intestine. The legs remained paralyzed. The convulsions subsided in less than two minutes, but there was no improvement with respect to the paralysis.

Partial or complete paralysis of the fowl may result from several different causes, chief among which are: (1) *neurolymphomatosis gallinarum,* or so-called "fowl paralysis," a practically incurable disease usually accompanied by lesions in the sciatic nerve, (2) polyneuritis resulting from deficiency of vitamin B, and (3) exhaustion of the bird's storage of vitamin D. None of these conditions seemed to be indicated in this case. The flock to which the pullet belonged had been receiving a normal laying ration consisting of wheat and yellow corn (equal parts) fed night and morning, and a hopper-fed mash containing, among the other usual ingredients, wheat germ meal, cod liver oil and 2 per cent of ground limestone. The birds were also given both skimmed milk and ground limestone (96 per cent $CaCO_3$) *ad libitum.* With this ration it seemed doubtful that there could be any deficiency of vitamins, especially so early in the laying period. Nor did the symptoms resemble those of neurolymphomatosis.

On the contrary, the uncontrollable, convulsive movements of the wings, associated with paresis, indicated a case of tetany. Tetany, in turn,

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may arise from different causes, but the fact that the bird had just recently begun to lay suggested that in this case it had been induced by the drain of calcium associated with the formation of shells for the eleven eggs laid prior to its onset. If that were so, presumably the condition might be overcome as in mammals by injecting a calcium salt. Accordingly this was done. The complete protocol of the case follows:

November 13, 4:00 P.M.—Tetanic spasms, paresis. Blood calcium 9.47 mg. Ten cc. calcium gluconate (20 per cent solution) injected intraperitoneally.

November 14, 11:00 A.M.—No change. Five cc. calcium gluconate given intravenously.

November 15-16—Condition worse, no tetanic spasms, complete paralysis.

November 17, 4:00 P.M.—Comatose. Five-tenths cc. Parathormone (Lilly) given intramuscularly.

November 18, 9:30 A.M.—Marked improvement; prostrate but feeds and drinks and attempts to rise. Weight 1949 gm. Twenty-five-hundredths cc. Parathormone injected intramuscularly.

November 20—Improvement; bird walks, but stiffly.

November 21—Improvement; blood calcium 11.51 mg.

December 5—Laying resumed.

December 6—Weight 2201 gm.; blood calcium 19.18 mg.

February 2—Weight 2393 gm.; has laid 48 eggs in 60 days since December 4. Blood calcium 21.46 mg.

INTERPRETATION

Some points in connection with this case require explanation and interpretation. The level of 9.47 mg. Ca in the blood on the day of onset of tetany is only from one-third to one-half of the normal level for a laying hen. It has been shown by Hughes, Titus and Smits (1927), Macowan (1932) and others that while immature fowls, non-laying females, cocks and capons usually have from 12 to 18 mg. Ca per 100 cc. of blood serum or plasma, hens actively laying have levels ranging from 17 to 35 mg. Similar fluctuations were earlier noted in ring-doves and pigeons by Riddle and Reinhart (1926), although in those species the levels were somewhat lower at all stages. Since the fowl with tetany had laid on the day prior to her collapse, her blood calcium should have been about 20 mg. or more.

The output of calcium in the 11 eggs laid before the onset of tetany, assuming that each of them weighed 48 to 50 grams and contained about 1.8 grams Ca, would be approximately 20 grams. This indicates a rather high rate of calcium metabolism for those 16 days, but one not in any way approaching the limits of the species, as hens have been known to lay daily for over one hundred consecutive days. Ordinarily the calcium loss is recovered from the feed, and one's first interpretation of this case would be that the ration was too low in calcium to permit such recovery. Apart from the fact that milk and ground limestone were constantly available

and that the mash contained 2 per cent of ground limestone, as well as calcium in its meat-scraps and alfalfa meal, this interpretation is shown to be erroneous by the fact that the same ration was satisfactory for over 200 other laying pullets which had been receiving it for a period of two months, and among which there occurred no similar cases. Moreover, after the recovery of the bird in question and an experimental period in isolation, she was returned to the flock, where she received the same ration and laid exceptionally well for a period of six months.

If the hypocalcaemia resulted merely from greater calcium loss than calcium intake, one would expect it to have been overcome, as are similar cases in mammals, as soon as the blood calcium was raised to a normal level. The 10 cc. of calcium gluconate injected intraperitoneally, though ineffectual, was approximately ten times the dose in proportion to body weight that effects complete recovery when given subcutaneously in cases of bovine parturient paresis (250 cc. for a cow of about 1000 pounds). The amount of this intraperitoneal dose absorbed by the blood is unknown, but, even if none of it whatever were utilized, the 5 cc. given intravenously on November 14 was enough to raise the blood calcium to an abnormally high level. The writers have been unable to find any determinations of blood volume in the fowl, but, from the figures for several species of mammals, it seems unlikely that the fowl's blood volume would exceed 7 cc. per 100 grams of body weight. On this basis, and assuming the bird to weigh about the same as when she first laid (2,220 grams), the 5 cc. of 20 per cent calcium gluconate [$Ca(C_6H_{11}O_7)_2$] given intravenously would have added about 93 mg. Ca to about 155 cc. of blood. This is approximately three times as much as was necessary to restore the blood calcium to the normal level for a laying hen.

In spite of this there was no improvement and when the first injection of Parathormone was given, 106 hours after the onset of tetany, the bird was on the point of death. The resultant recovery was phenomenal. Eighteen days later laying was resumed and in 176 days from December 5 to May 31, the hen laid 108 eggs. After the original treatments, no further supply of Parathormone was needed to maintain the bird in perfect health. The eggs laid subsequently attained a maximum weight of over 60 grams. The shells were all examined and weighed up to February 2, and in that period they were normal in every respect.

To the writers the best interpretation of the evidence is that the bird suffered from a temporary subnormal functioning of the parathyroid gland at a time when the demand for calcium was particularly heavy. As a result the blood calcium was lowered so much that tetany ensued.

The difficulty is to explain why recovery was not effected when large amounts of calcium gluconate were injected into the blood stream. Calcium therapy is ordinarily successful in cases of tetany, and Lieberman and Szurek (1931) have found the subcutaneous administration of calcium gluconate effective in overcoming tetany even in dogs apparently completely parathyroidectomized. It is now a standard treatment for parturient

paresis in cattle. In this case calcium therapy was entirely ineffectual, but treatment with parathyroid hormone produced immediate results. It seems probable, therefore, that the original hypocalcaemia and tetany resulted, not from any unusual drain of calcium, but from temporary idiopathic hypoparathyroidism. Instances of this sort are apparently rather rare in man, but Davidson (1925), Liu (1928) and Albright and Ellsworth (1929) have reported chronic cases effectively treated with parathyroid extract.

The effectiveness of Parathormone, when injection of calcium had failed, suggests that its function is not so much to raise the level of total blood calcium as to convert some of the calcium into a form, or forms, indispensable to the bird. Heller, Paul and Thompson (1934) found that the rise in blood calcium associated with egg production results chiefly from a marked increase in the adsorbable non-filterable fraction while the adsorbable filterable calcium decreases and the ionized remainder stays fairly constant. It seems probable that the rôle of the parathyroid hormone in avian reproduction may be to increase the adsorbable non-filterable calcium.

DISCUSSION

In view of the amount of calcium metabolized by the fowl in the formation of egg shells, it is remarkable that cases of hypocalcaemia and tetany are not more common in this species. So far as the writers can ascertain, no such case has been reported in the fowl or in any other bird, except for the not uncommon occurrence of paresis (without tetany) in flocks long maintained in egg production without an adequate supply of vitamin D. Such instances are usually found in February or March in confined flocks not receiving cod liver oil, or its equivalent, and are easily cured by supplying vitamin D. The limits of calcium tolerance (up to 35 mg. per 100 cc. blood serum) are much higher in birds than in mammals and the ability of birds to mobilize calcium very rapidly has been amply demonstrated (Riddle and Reinhart, 1926). Presumably these capacities have been evolved as necessary corollaries to the avian type of reproduction. This same facility in metabolizing calcium may be adequate protection against hypocalcaemia except when the available calcium or vitamin D is restricted (in which cases ovulation is retarded and eventually ceases), or, as in the present case, when the parathyroids fail to function normally.

The present case extends somewhat the previous knowledge of the effects of administering parathyroid extracts to birds. Collip (1931) was unable to demonstrate any effect of the hormone after injection in non-laying hens. Similarly, Macowan (1932) found Parathormone to have no effect on the blood calcium in moulting fowls, even when injected in doses as large as 1 cc. Smaller doses did cause a rise in the blood calcium of immature females. The writers' case demonstrates a marked response to Parathormone in a bird in which the parathyroids were apparently not functioning normally.

SUMMARY

After laying 11 eggs, a young pullet was afflicted with tetany and paresis, and found to have only 9.47 mg. Ca per 100 cc. blood plasma, when 20 mg. would have been normal.

Liberal doses of calcium gluconate given intraperitoneally and intravenously had no effect and the paresis persisted, with tetany succeeded by coma.

Treatment with Parathormone (Lilly) given on the fifth day resulted in complete recovery. Blood calcium was raised, egg production was resumed and the bird laid exceptionally well for a period of six months thereafter, maintaining 21.46 mg. blood calcium 77 days after administration of Parathormone.

The case was diagnosed as one of hypocalcaemia and tetany resulting from temporary idiopathic hypoparathyroidism. It is apparently the first case of tetany reported in birds. It is suggested that the rarity of hypocalcaemia and tetany in birds may result from special facilities for metabolizing calcium evolved as necessary functions in the avian type of reproduction, and that the rôle of the parathyroid hormone in that process is to increase the non-filterable adsorbable calcium in the blood.

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THE INFLUENCE OF ESTROGENS IN EGG YOLK UPON AVIAN BLOOD CALCIUM

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Following the demonstration by Riddle and Reinhart (1) that in pigeons and doves there is a marked increase in blood calcium as the females come into laying, it has been shown by a number of workers that a similar situation prevails in the fowl. Whereas the level of blood calcium in non-laying females and males of that species is usually less than 15 mg. per 100 cc. serum, in laying fowls blood calcium is maintained at from 15 to 33 mg., with even higher levels in exceptional cases.

The experiments reported in this paper were initiated in 1934 to determine the endocrine basis for this remarkable rise in blood calcium. Realizing that the rise occurs while egg yolk is rapidly accumulating in the follicles, and following the lead of Russell, Howard and Hess (2) who showed that the serum calcium rose when an ovum attained a diameter of one centimeter or more, the working hypothesis upon which the investigations were based was that something accumulates in the yolk which, upon reaching a certain amount, has the ability to activate the parathyroids and thus to raise the blood calcium.

Evidence that egg yolk contains estrogenic hormone was found by Fellner (3), who tested a lipoid fraction from egg yolk on rabbits, and by Kopec and Greenwood (4), who were able to feminize growing feathers of a poularde by injecting egg yolk. On the other hand, Allen et al. (5) and Glimm and Wadehn (6) were unable to detect any evidence of sex hormones in yolk or in extracts of whole egg.

METHODS AND MATERIALS[1]

Procedures utilized included *a)* injection of egg yolk into immature females and capons to simulate the laying condition, *b)* injection of purified endocrine preparations and *c)* removal of egg yolk from laying fowls to simulate in part the non-laying condition.

Blood samples were drawn from the vena brachialis and tested for calcium content by the micro-determination method of Shapiro and Zwarenstein (7). According to these authors determinations on the same sera by the original Kramer-Tisdall method and by their modification of it did not differ by more than 0.4 mg. per 100 cc. of serum. Considering the levels of calcium studied in this paper such a small deviation is insignificant. All calcium determinations were made in duplicate.

[1] Grateful acknowledgment is made to the Schering Corporation for their kindness in furnishing progynon-B, to Ayerst, McKenna & Harrison, Ltd., for thyrotropic factor and to Parke, Davis & Co. for the extract of anterior pituitary used and for theelin.

Blood calcium was determined in all experimental birds before treatment, so that each bird served to some extent as its own control. In addition, untreated controls of the same breed and age as the experimental birds were kept in the same pen under identical conditions of management, feeding, light and temperature. All received an adequate diet containing ample cod liver oil.

EFFECTS OF EGG YOLK

Experiment 1. Immature females. Measured quantities of fresh egg yolk were shaken in a sterile container with a little Ringer's solution and injected into the peritoneal cavity of 6 pullets, which at the beginning of the experiment were 63 days old. The dose was 60 cc. of yolk per bird, distributed in 9 injections over 17 days.

TABLE 1. EFFECT OF INJECTION OF EGG YOLK UPON SERUM CALCIUM OF IMMATURE PULLETS 63 DAYS OLD

Group	No. of birds	Average mg. Ca per 100 cc. serum					Change[1] %
		Before test	Days after first injection				
			2	8	14	20	
Injected	6	14.3	16.3	19.2	19.3	14.7	+35.0
Control	6	—	16.6	16.7	15.6	15.1	− 6.02

[1] In tables 1 to 5 the last column gives the maximum deviation from the level before test; control reading on the same day.

Blood calcium of the yolk-injected birds rose steadily and at 8 days after the first injection was 34 per cent higher, at 2 weeks 35 per cent higher, than before injections were begun (table 1). Controls varied only slightly. The decline in blood calcium on the twentieth day is attributed to disturbance of the normal physiology by the mass of injected material. Although the rise induced is highly significant, the maximum level of 19.3 mg. does not equal

TABLE 2. EFFECT OF INJECTION OF EGG YOLK UPON THE BLOOD CALCIUM OF CAPONS

Group	No. of birds	Average mg. Ca per 100 cc. serum							Change %
		Before test	Days after first injection						
			2	5	8	11	14	19	
Injected	3	12.0	15.7	16.8	13.4	13.7	14.2	13.2	+40.0
Control	3	12.1	—	13.1	—	—	13.4	—	+ 8.3

the normal level for laying hens, which would ordinarily lie between 22 and 30 mg. This discrepancy is not surprising since in the normal ripening oöcyte the yolk material is surrounded by a follicle which is very highly vascularized. Any hormones in such yolk material would presumably be much more available and more effective than those in the injected yolk which lacked direct vascular connection.

Experiment 2. Capons. In order to have birds which could take, without ill effects, more yolk than could the immature Leghorn pullets, Barred Rock capons one year of age were used. Each received 100 cc. of yolk intraperitoneally over a period of 19 days. The results (table 2) show an increase of 40 per

cent to a level of 16.8 mg. on the fifth day, with somewhat lower readings thereafter. It is possible that the rather quick relapse of the blood calcium curve and the resumption of normal levels of calcium while injections were still being made in this and in other experiments resulted from the formation of antihormones or antibodies which counteracted the effects of the agent injected.

Experiment 3. Immature, but older, females. A repetition of this experiment, using New Hampshire pullets 100 days of age, yielded similar results

TABLE 3. EFFECT OF INJECTION OF EGG YOLK UPON THE BLOOD CALCIUM OF IMMATURE NEW HAMPSHIRE PULLETS 100 DAYS OLD

Group	No. of birds	Mg. Ca. per 100 cc. serum					Change %
		Before test	Days after first injection				
			12	16	18	23	
Injected	4	10.5	15.0	14.2	14.8	15.1	+43.8
Control	2	10.5	12.4	12.2	13.0	13.0	+23.8

(table 3). Following injection of 50 cc. of yolk per bird over 8 days, there occurred a rise of 43 per cent from 10.5 mg. before the test to 15 mg. on the twelfth day with a slightly higher level as late as the twenty-third day. In these birds the higher levels induced by treatment were maintained over a longer period than in the previous experiments and were consistently higher than those in the controls.

EFFECTS OF ESTROGENIC HORMONE

The assumption that the agent in the egg yolk responsible for the rise in blood calcium was of the nature of the estrogenic hormone led to experiments with two different preparations of that substance.

Estradiol monobenzoate. Three immature White Leghorn females, 96 days

TABLE 4. EFFECT OF INJECTION OF PROGYNON-B ON BLOOD CALCIUM OF IMMATURE WHITE LEGHORN PULLETS 96 DAYS OLD

Group	No. of birds	Mg. Ca per 100 cc. serum				Change %
		Before test	Days after first injection			
			3	7	11	
Injected	3	15.2	19.7	15.2	14.8	+29.6
Control	3	15.8	15.6	—	15.3	− 1.3

of age, were each given intramuscularly 500 R.U. of this substance in oil (progynon-B) over a period of 9 days (table 4).

By the third day after the first injection the average blood-calcium of these 3 birds was 19.7 mg., a figure over 29 per cent higher than the level before treatment. One bird showed an increase to 26.5 mg. Ca, a level that would be normal for a bird in full laying condition. Controls showed no change and in determinations at 7 and 11 days the experimental birds had lapsed back to the level of calcium prevailing before treatment. Effects of the injected

hormone did not last as long in this case as when yolk was injected.

Estrone (theelin). Intramuscular injection of 1400 R.U. of this substance in oil solution per bird over a period of 14 days resulted in an increase of blood calcium on the sixteenth day to 16.8 mg., a level over 64 per cent higher than in the same birds prior to the test.

Since both estrone and estradiol are able to raise blood calcium by significant amounts and since it would be quite natural for some form of the estrogenic hormone to be accumulated in egg yolk, it seems reasonable to conclude that the principle in egg yolk responsible for raising blood calcium is some form of the female sex hormone.

EFFECTS OF ANTERIOR PITUITARY

It has been suggested by some investigators that the anterior pituitary produces a parathyrotropic hormone. Since such a substance is more likely to be found in an extract of the whole gland than in any preparation of specific principles of that gland an extract of whole anterior lobe prepared by Parke, Davis & Company was first tried. When injected intramuscularly into immature White Leghorn females 150 days old at the rate of 4 to 6 cc. per bird over a period of 4 days, this substance was found to have no effect whatever on blood calcium.

Intramuscular injection of the thyrotropic fraction of the anterior lobe (8) in White Leghorn females 150 days old was followed in 5 days by a drop of 31 per cent in the level of blood calcium to 7.63 mg. per 100 cc. of serum. The depressing effect of the thyrotropic principle of the pituitary upon the level of blood calcium observed in these experiments is in agreement with the evidence of Prawochenski and Slizynski (9) that in the fowl there is an antagonistic action between the thyroid and parathyroid glands.

Since in some cases the synergistic action of two endocrine secretions differs from that of either alone, a trial was made to determine the effect of extract of anterior pituitary administered in conjunction with egg yolk. Three White Leghorn females, 150 days old, but not yet in laying condition, were given 4 to 5 cc. of anterior pituitary extract and 30 cc. of egg yolk over a period of 3 days. Serum calcium in these birds and their controls was unusually low before the test began, averaging 8.8 mg. for the experimental birds and 9.1 mg. in controls. The treatment induced a rise of 32 per cent to 11.7 mg. on the second day following the first dosage. In 2 birds which received 5 cc. of the extract, there was an increase of blood calcium to 14.4 mg. per 100 cc. of serum, a rise of over 65 per cent. At the same time the level in controls remained at 10.5 mg.

It is questionable whether the yolk alone could have been the sole cause of the rise, since in two previous experiments (albeit with younger birds) doses of 50 and 60 cc. of yolk alone were unable to induce a rise of more than 35 to 40 per cent. It is possible that the substance affecting blood calcium is more potent in birds 150 days old, and not far from laying, than in younger birds of 100 days or less. Otherwise this experiment would suggest that there is a synergistic action of some fraction of the anterior pituitary and of the principle in egg yolk.

REMOVAL OF EGG YOLK

Following the experiments previously described, evidence of the relation between some principle in egg yolk and the level of blood calcium was sought in a different way. If the injection of egg yolk into non-laying fowls induces a rise in blood calcium, then, conversely, the removal of yolk from females in full-laying condition should be followed by a temporary lowering of blood calcium.

To test this hypothesis 8 fowls in full-laying condition and with levels of blood calcium varying from 23.1 to 34.4 mg. were separated into three different lots and under nembutal anaesthesia operated upon[2] as follows. .

Group A, 3 birds: Yolk was removed from the largest follicles, a different amount in each bird.

Group B, 3 birds: The three largest follicles were ruptured and the contained yolk squeezed into the body cavity.

Group C, 2 birds (controls) : The largest follicles were disturbed but not ruptured and no yolk was removed.

The results of these experiments (fig. 1) show conclusively that some

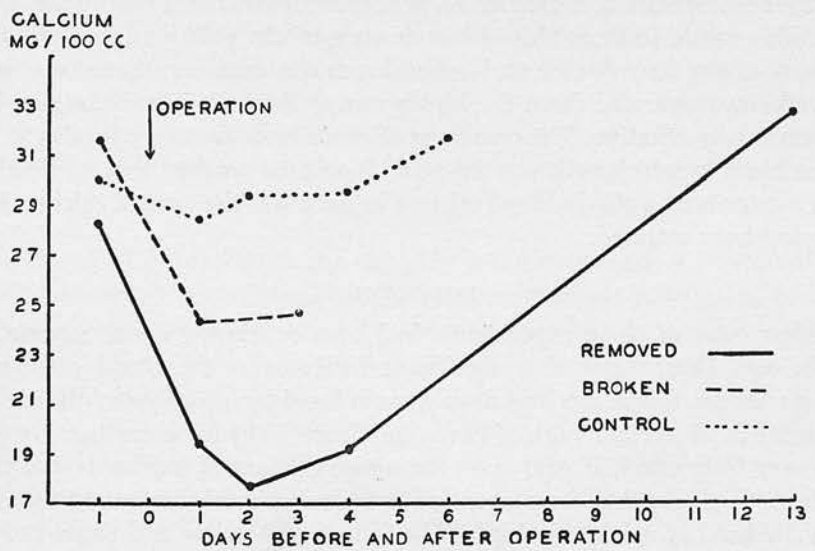

Fig. 1. The effects of removal of yolk and of rupturing mature follicles upon the serum calcium of laying females. The curves show averages for 3 birds in each experimental lot and for 2 in the controls.

substance in the egg yolk is instrumental in the maintenance of the high level of blood calcium characteristic of laying fowls.

Following removal of egg yolk the average blood calcium of the three birds dropped within 24 hours and on the second day was 17.7 mg., a figure 37 per cent lower than their average blood calcium (28.2 mg.) on the day before the operation. Furthermore, in these three birds the decrease was directly proportional to the amount of yolk removed (table 5). By the thirteenth day following the operation the average blood calcium of these three birds was up to 32.8 mg. and laying was resumed at 20, 15 and 16 days.

[2] The authors are indebted to Mr. R. K. Cole for conducting these operations.

Evidence that the lowering of blood calcium was not attributable to adverse effects of the operation is provided by the two control birds, *group C*. In these the level of calcium remained practically unchanged at 29.4 mg. on the second day after the operation and the birds continued to lay.

It is particularly interesting that the effect in *group B,* the birds in which

TABLE 5. EFFECTS ON BLOOD CALCIUM OF REMOVING DIFFERENT AMOUNTS OF YOLK

Bird no.	Yolk removed cc.	Serum Ca, mg. per 100 cc.		Decrease %
		Before operation	Two days after	
C-112	15	23.1	17.4	24.7
C-132	24	31.1	19.8	36.3
C-158	37	30.5	15.8	48.2
Average		28.2	17.7	37.2

yolk was squeezed out of the follicles but left in the body, was intermediate between the normal level maintained by the controls and the extreme reduction experienced in *group A*. The average for this group was 24.31 mg. on the day after the operation, a drop of 23 per cent from the level before the operation. This would indicate that although none of the yolk was removed from the body cavity, its influence on blood calcium was considerably reduced when the yolk was separated from the highly vascularized follicles through which it is ordinarily effective. The condition of these birds was very similar to that of the birds in which yolk was injected. It will be recalled that while all of these experienced a rise in blood calcium in none was the normal calcium level of laying hens attained.

DISCUSSION

After most of these experiments had been concluded it was reported by Riddle and Dotti (10) that significant increases in the blood calcium of pigeons, doves, fowls, rats and dogs were induced by injections of theelin and progynon-B. The recent work of Levin and Smith (11) indicates that estrogens have very little effect, if any, upon the serum calcium of mammals, but there can be little doubt that these agencies have an extremely important influence upon the level of calcium in birds. The failure of Marlow and Koch (12) to find any consistent rise in the blood calcium of their fowls treated with female sex hormones may seem at first sight to conflict with the results presented in this paper, and with those of Riddle and Dotti, but it is evident that the effects of their treatments were obscured by great variations in the blood calcium of their controls and experimental animals. Such variations are inevitable in mature fowls and in those close to laying age. They were reduced to a minimum in the present experiments by the use of birds less than 150 days of age. It is true that the pre-test levels of calcium varied from lot to lot, possibly because the different lots were hatched (and used) at different seasons of the year and following different degrees of confinement. In any one experiment controls and experimental birds differed only very slightly at the beginning of the test.

The object of the present experiments was not merely to find some endocrine preparation which in large doses might affect calcium levels, but rather

to determine how the rise occurs in the fowl under natural conditions. To complete the chain of evidence it was necessary to determine if the estrogenic hormone in the yolk acts directly on the blood calcium or indirectly by stimulating the parathyroids. From histological studies (to be presented elsewhere) of the latter glands in non-laying, laying and yolk-injected fowls it seems certain that the estrogen in the yolk affects the parathyroids, either directly or indirectly, and that their activation results in the higher level of serum calcium characteristic of laying fowls.

This permits reconstruction in part of the major endocrine reactions associated with laying. Presumably at maturity the gonadotropic hormone of the anterior pituitary is produced in greater quantity, thus inducing deposition of yolk in the oöcytes. (This reaction can be accelerated even before maturity by stimulation of the pituitary with artificial light.) Accumulation of yolk is accompanied by accumulation of the estrogenic hormone produced by the ovary and when an adequate amount is available the parathyroids are stimulated to action.

The failure of an extract of anterior pituitary to affect blood calcium in the present experiments may seem in contrast to the results of Shapiro and Zwarenstein (7) and of Friedgood (13). However, the positive effects of these investigators were obtained in amphibia and in mammals, not in birds. As Loeb, Anderson, et al. (14) have shown, species differ in the activities of their anterior pituitaries. The contrasting effects of prolactin in mammals and in birds show that species can also differ in their response to certain hormones.

SUMMARY

Injection of 50 to 100 cc. of egg yolk into the peritoneal cavities of immature female fowls and of capons induced a significant rise in the levels of blood calcium. Similar results were obtained by the use of the estrogenic hormone in the form of progynon-B and as theelin. Removal of 15 to 37 cc. of yolk from laying hens caused a significant drop in the level of blood calcium, the decrease varying from 25 to 48 per cent and being directly proportional in different birds to the amount of yolk removed. Operated controls were unaffected but in birds in which yolk was squeezed from the follicles and left in the body cavity the serum calcium dropped 23 per cent. The thyrotropic principle of the anterior pituitary depressed blood calcium and an extract of whole anterior pituitary had no effect.

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VARIABILITY OF BODY TEMPERATURE IN THE NORMAL CHICK

BY

W. F. LAMOREUX AND F. B. HUTT

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Variability of Body Temperature in the Normal Chick

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IN CONNECTION with other studies involving variations in the temperatures of chicks during the first two weeks of life, it was necessary to determine first the normal variations and environmental conditions affecting them during that period. So far as could be ascertained the only data on this subject obtained with adequate numbers of chicks under normal conditions of brooding and diet are the unpublished figures of Fronda (1922) which indicate that at one and two weeks of age the temperature is about 3°F. higher than at hatching. Card's (1921) unwatered chicks, kept to five days, showed considerable diurnal variation in temperature. The present writers sought to find (1) possible sources of error in determination of temperature, (2) an adequate technic for temperature determination in baby chicks, (3) normal temperature variations in relation to age, weight, sex, and breed of the chick from hatching until it approaches the normal temperature of the adult. Such information is considered essential for many kinds of physiological experiments with chicks.

METHODS

The data reported were obtained by the senior author at Cornell University and by the junior author at the Minnesota Agricultural Experiment Station. Readings of temperatures obtained to establish normal curves were taken at the same time each day to avoid error from diurnal fluctuations. Brooding equipment for all chicks included a heated compartment and one at room temperature, the two together providing a range of temperature adequate to permit the chick to select its own environmental temperature as is customary in ordinary brooding practice.

Thermometers: Clinical rectal thermometers were used for determining the body temperatures. In taking the temperature, the bulb of the thermometer, after being dipped in non-irritant oil to facilitate insertion into the cloaca and rectum, was held in the chick from one to one and one-fourth minutes. The depth of insertion usually varied according to the age of the chick; that is, the older the chick the greater the actual depth of insertion, but an effort was made to insert the thermometer to an approximately uniform depth for any given age and lot of chicks. In certain specified lots the thermometer was banded with several turns of adhesive tape, so placed as to limit the insertion of the thermometer to approximately the desired depth. The increased accuracy obtained with this procedure and its convenience make it desirable for any investigation involving temperature determination of chicks.

In the course of this study a rapid and accurate method of reading the thermometers was devised in which the thermometer rests against a rubber pad on the inclined stage of a microscope. The observer makes the reading through the empty tube of the instrument, thus avoiding error that might otherwise result from refraction of light by the glass if the thermometer were viewed at an acute angle.

Thermocouples mounted in glass tubing and paraffin were compared with mercury thermometers by taking alternate readings with the two instruments upon individual chicks. However, such a thermocouple mounting had sufficient mass to delay recording of constant temperature to a time comparable to that required by the mercury thermometer. Therefore, the instrument was not used extensively and none of the data obtained are reported here.

Satisfactory use of the thermocouple will require refinement of the thermocouple mounting, and perhaps insertion into another part of the body. Such technics have been described by Baldwin and Kendeigh (1932) as used with the House Wren, and by Huggins, Blocksom, and Noonan (1936) as used with rats. However, unless the thermocouple can be shown to be significantly more accurate than mercury thermometers under the conditions of such a study, its extensive use is handicapped by two disadvantages, relatively high cost and inconvenience.

RESULTS OF EXPERIMENTS

A post-hatching rise in the body temperature of chicks is shown by data obtained from studies involving 398 chicks. That such a rise might occur is perhaps obvious from the fact that the temperature of the adult fowl (105-109°F.) is much higher than the incubation temperature of around 100°F. at which the chick hatches. Our data (figs. 1 and 2) show that this rise is most rapid during the first four days. This accounts for the differences between Fronda's (1922) mean temperatures for chicks at one day and at seven days.

Breed differences in body temperature were determined by taking the rectal temperatures of 189 White Leghorns and 162 Rhode Island Reds brooded together in six different lots during six consecutive two-week periods. The same brooder was used for all lots. Temperatures were taken at 1:30 to 4:00 p.m. The differences in temperature between these breeds were at 7 days of age 0.904 ± 0.073°F. and at 10 days of age 0.989 ± 0.079°F., the Leghorns being the higher at both ages (fig. 2). Both these differences are statistically significant when considered in relation to their standard errors. The extensive data of Simpson (1911-12) show no difference between the temperatures of adult Rhode Island Reds and those of five other breeds. Thus, the age at which the differences in body temperature between White Leghorns and Rhode Island Reds cease to be significant has not been established except that it must lie between 10 days of age and maturity.

Sex. The mean temperature of 25 adult females was reported by Löer (1909) to be 0.52°F. higher than that of 25 males. However, Fronda (1922) found "no consistent difference in the body temperatures" between sexes at various ages from "day-old" to 24 weeks. Card (1921) found no evidence that sex could be determined with practical accuracy by taking the temperatures of day-old chicks.

A comparison between the male and female chicks in six lots of White Leghorns (totalling 189) and eight lots of Rhode Island Reds (302 chicks in all) showed that at 1, 2, 4, 5, 6, 7, and 10 days of age, 12 of 16 differences between the sexes were statistically insignificant. Sex differences, therefore, must contribute little or nothing to total variability in temperature. The data for the Rhode Island Reds (Table 1) show that such differences as were found were not consistent. At 1 and 2 days of age the temperature of the males exceeded that of the females, whereas at 7 and 10 days of age the females had the higher temperature.

Differences between lots brooded in the same brooder during consecutive two-week

intervals and cared for by the same attendants were determined by subjecting the data to analysis of variance. For the ages 1, 2, and 4 days two variables were considered, "breeds" and "lots." For the seventh and ninth days "sex" was included. These analyses showed that for each age considered there were significant differences between lots, thus indicating that the mean temperature of one lot of more than 25 chicks may not represent the mean of another group of chicks brooded under comparable conditions. For example, at four days of age the mean temperatures of six lots of Rhode Island Reds varied from 104.79 ± 0.22°F. to 106.41 ± 0.10°F. The difference between these extremes is statistically significant. This occurred in spite of there being 27 chicks in one lot and 29 in the other, and in spite of efforts to ensure identical environment for all lots. Particular caution is therefore essential in comparing the work of different investigators or the results of different experiments.

Body weight in two lots of Rhode Island Reds and Barred Plymouth Rocks (176 chicks) bore no relationship to body temperature at four days of age as indicated by the insignificant coefficients of correlation, 0.118 ± 0.108 and 0.025 ± 0.099.

Stability of body temperature in individual chicks was studied by determining the correlation between the temperatures on the fourth and the fifth days of age in the 176 chicks mentioned above. The correlation coefficients of 0.304 ± 0.009 and 0.005 ± 0.100 obtained indicate that at this age the temperature of individual chicks is not consistent from day to day.

Diurnal variation. To establish a normal curve of the temperature of White Leghorn chicks, without any error that might result from diurnal variation (that is, within a 24-hour day), two groups of 25 and 22 chicks were subjected to temperature determinations at a fixed hour each day. Lot 9 was recorded from 8 to 9 p.m. and lot 10 from 9 to 10 p.m. at intervals from hatching to 13 days of age (fig. 1). At 14 days of age their temperatures were taken from 1:50 to 3:30 p.m. with lot 9 taken first as before. Throughout these experiments the thermometer was taped to obtain uniform depth of insertion.

It is noteworthy that during the first 13 days the mean temperature of lot 9, recorded from 8 to 9 p.m., consistently exceeded that of the other lot, taken one hour later. This indicates that the temperature of the chicks was consistently declining from 8 to 10 p.m. However, at 1:50 to 3:30 p.m. on the fourteenth day, the mean temperatures in lots 9 and 10 were higher by 0.6 and 0.8°F., respectively, than at 8 to 10 p.m. on the previous evening. The relatively high afternoon temperature and

TABLE 1.—*Differences between the mean temperatures of Rhode Island Red males and females at various ages**

Chicks number	Age in days	Mean temperature		Difference
		♂♂	♀♀	♂♂−♀♀
162	1	103.407±0.087	103.168±0.118	0.239±0.147
162	2	104.250±0.077	104.019±0.107	0.231±0.132
302	4	105.776±0.190	105.626±0.187	0.150±0.267
140	5	106.058±0.099	106.001±0.112	0.057±0.149
162	7	105.778±0.079	106.006±0.087	−0.228±0.117
162	10	106.063±0.060	106.232±0.106	−0.169±0.122

* Standard errors are used throughout this paper.

declining evening temperature show that in these young chicks diurnal fluctuation of temperature is much the same as that found in adult fowls by Simpson (1911), Hilden and Stenbäck (1916), and Fronda (1921). The graphs in Figure 1 demonstrate that comparatively small changes, such as those resulting from a difference of one hour in the time of taking temperatures, may be detected and measured consistently when conditions are adequately controlled.

The fact that the temperature of White Leghorns at seven days is shown in Figure 2 (afternoon) to be somewhat higher than in Figure 1 (8-10 p.m.) results in part from normal diurnal variation, which would cause the former to be at the high point for the day, and in part from the depth of insertion of the thermometer being restricted when the readings shown in Figure 1 were taken but not for those in Figure 2.

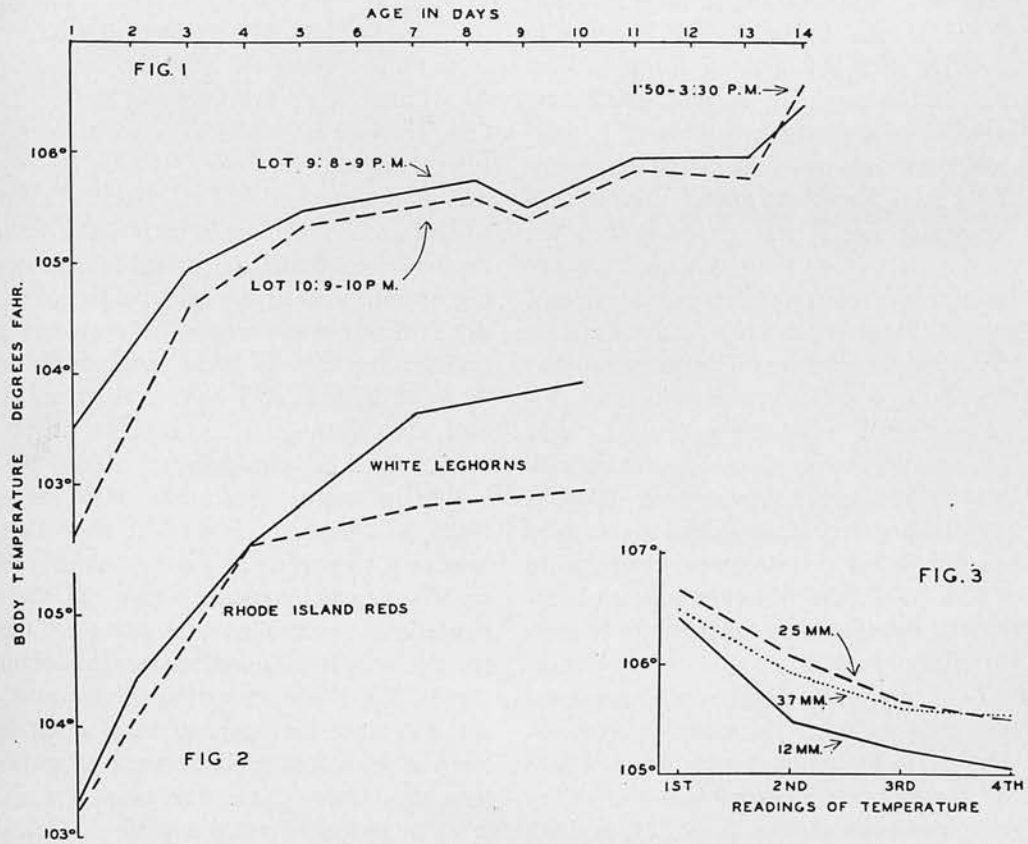

Fig. 1. Difference in temperature resulting solely from a difference of one hour in time.

Fig. 2. Difference between the post-hatching temperatures of White Leghorn and Rhode Island Red chicks.

Fig. 3. Influence of depth of insertion of the thermometer upon the reading of body temperature.

Differences between attendants. Attempts to eliminate errors from diurnal variation by having several persons record temperatures at one time may not be successful, since differences between attendants may also be important sources of error. A comparison of mean temperatures of chicks from the same lots, taken by two different

persons (without tape on their thermometers), showed that in two of three comparisons there were statistically significant differences between the means of the temperatures taken by the two observers. To ensure uniformity, therefore, one individual should, where possible, take all readings of temperature. If large numbers of chicks must be handled, it is desirable to divide them into lots which can be handled within an hour, thereby facilitating correction for diurnal variation.

Temperature gradient becomes a relatively more important source of error with decreasing size of an animal, that is the change from internal to external temperatures involves a shorter distance in the small animal. Some difficulty is encountered in the introduction of recording instruments to the depth of constant temperature in animals as small as a newly-hatched chick. The temperature gradient is steepest with low environmental temperature (Bazett, 1927). However, since chicks are usually brooded in an environmental temperature of 90 to 95°F., the difference between internal and external body temperature is comparatively small.

To determine the influence of temperature gradient upon the recorded temperatures of chicks, readings were obtained with the thermometer inserted into the chicks to approximate depths of 12, 25, and 37 mm. as controlled by several turns of adhesive tape around the stem of the thermometer. Three tested thermometers were inserted consecutively for one minute in the same chick to depths of 12, 25, and 37 mm., after which the thermometers were read. This procedure was carried out four times, giving 12 readings, from each of six chicks. The means for six chicks of the comparable readings at each depth showed that in every case the least depth of insertion gave the lowest mean temperature (fig. 3). The consistency of this difference proves that the temperature recorded depends upon the depth of insertion of the thermometer and that failure to control this source of error may seriously affect the accuracy of the readings.

While insertion of the thermometer to 25 mm. results in higher readings than at 12 mm., it does not follow that insertion to 37 mm. would give readings any higher or any more accurate. Actually, insertions to 37 mm., after previous readings at 25 mm., resulted in somewhat lower temperatures being recorded (fig. 3). This fact, as well as the gradual decline in temperature with successive readings at each of the three depths of insertion, is attributed to the cooling of adjacent tissue by repeated insertion of the thermometers, which were cooled between readings to room temperature of 75 to 80°F. It is obvious that the mass of cool glass introduced is greatest at the deepest level of insertion.

Elimination of error that might arise from the temperature gradient may thus involve (1) insertion of the thermometer to an adequate and constant depth, (2) thermostatically controlled preheating of the thermometer in oil to reduce to a minimum the cooling of tissues by the thermometer, and (3) since the temperature gradient is steepest with low environmental temperature, error from this source may in some cases be reduced by maintaining as high a brooding and room temperature as is feasible.

SUMMARY

In studies involving 580 chicks several factors contributing to the variability of temperatures in normal chicks were investigated.

There is a pronounced rise in the body temperature of the chick during the first week after hatching, particularly during the first four days. There is a significant difference between the mean temperatures of White Leghorn and Rhode Island Red

chicks at 7 and 10 days of age. No consistent relationships were found between body temperature and sex, between temperature and body weight, or between temperatures at four and five days of age. It has been shown that diurnal variation and temperature gradient may be consistent and important sources of error. Differences found between attendants in their ability to record temperatures accurately and between lots of comparable chicks, brooded under comparable conditions but at different times, indicate the necessity of carefully standardized technic and of caution in comparing results of different experiments.

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F. ORNITHOLOGY

[From ' The Auk,' Vol. XLIX, No. 2, April, 1932.]

BIRDS OBSERVED FROM SHIPBOARD IN CROSSING THE NORTH ATLANTIC.

BY F. B. HUTT.

BIRDS OBSERVED FROM SHIPBOARD IN CROSSING THE NORTH ATLANTIC.

BY F. B. HUTT.

DURING four crossings of the North Atlantic Ocean made in the past four years, the writer has made many notes upon the birds which could be seen from the ship. A few of these are familiar to most Canadian and American ornithologists but most of them are seldom seen except by dwellers along the rocky coast of the continent and still others are found only in European waters. It is hoped that the notes below will give the intending ocean traveller some idea of what species are likely to be seen by careful observation from the deck, and of the regions in which each should be sought.

The sailing dates were as follows:

Eastbound, leaving Montreal, Sept. 16, 1927.
Westbound, leaving Glasgow, Sept. 29, 1928.
Eastbound, leaving Montreal, July 12, 1930.
Westbound, leaving Glasgow, Aug. 31, 1930.

It is evident that the observations hereinafter reported are limited to the summer and early autumn months.

The route followed was the same on all four crossings, namely, along the St. Lawrence River, north of the Island of Anticosti in the Gulf of St. Lawrence, through the Straits of Belle Isle (between Newfoundland and Labrador), across the Atlantic from approximately Lat. N. 52° to Lat. N. 56°, around the north of Ireland and up the Firth of Clyde, west of the island Ailsa Craig to Greenock. One's ticket is made out with Glasgow as the destination, but few large ocean boats go further up the Clyde than Greenock, and passengers are conveyed the last eighteen miles by train. The present notes are confined to "deep water" birds and do not include those seen further inland than Greenock on the Clyde, or Quebec on the St. Lawrence.

The first voyage served chiefly as an introduction to species which were practically all new to the writer, but on subsequent crossings identifications were made much more easily. These

were facilitated in part by consultation of standard works of reference, but somewhat more by inspection between voyages of specimens in the Royal Scottish Museum, Edinburgh, the Victoria Memorial Museum, Ottawa, and the Field Museum of Natural History, Chicago. With the exception of the first voyage, all observations were made with good field glasses. In the following list of the birds seen, only those are included which were positively identified. Except when there was too much fog or rain to see very far, at least ninety minutes of each morning and of each afternoon were spent on the watch so that the list below should represent fairly well the species most likely to be seen.

The nomenclature follows that of the new A. O. U. 'Check-List' but I have (usually) deliberately avoided sub-specific names because it would be impossible to assign one by any criterion other than range and I do not consider that a sound practice.

In the case of the Shag and some of the vernacular names of European races that do not occur on the American coast I have followed the British List.

Alca torda. RAZOR-BILLED AUK.—On September 4, 1930, our boat (westbound) passed Belle Isle between 9 and 10 P. M. On this same day eight small flocks and strings of birds, each numbering from four to twenty-five and believed to be mostly (if not all) Razorbills were seen between 1 and 2 P. M. at a distance estimated to be 150 miles off-shore. Others were noted closer to land. On the previous west-bound voyage, in October, Razorbills were almost abundant in the Gulf of St. Lawrence up to some sixty miles beyond the western end of Anticosti.

It was often impossible to distinguish between the Razorbill and Brünnich's Murre when the birds were at such a distance that their bills were not easily seen. This was the case with large flocks of stolid birds lining the edges of several of some forty icebergs encountered in the Straits of Belle Isle in July, 1930. Those perching were accompanied by a milling guard of birds on the wing. They were evidently Alcidae, and were most likely Razorbills, but distance prevented definite identification.

In European waters, Razorbills were seen 135 miles off the Irish coast, and on all four trips were frequently seen in the Clyde.

Uria aalge. ATLANTIC MURRE OR GUILLEMOT.—This species was seen in the Firth of Clyde on all four voyages. The birds are often deployed in long lines, one of which, seen just outside of Belfast Lough, had over fifty birds in it. The Guillemot is common right up to Greenock. It was not positively identified on the Canadian coast.

Uria lomvia. BRÜNNICH'S MURRE.—Fairly common in the Gulf and

in the Straits of Belle Isle. From the deck it is impossible to say whether one sees this species or the one above, but Brünnich's Murre is common on the American coast and rare in European waters, whereas *U. aalge* is almost abundant off the Scottish coast and *U. lomvia* is there exceedingly rare.

Alle alle. DOVEKIE or LITTLE AUK.—The Dovekie was seen on only one voyage, in October, 1928, when several score were noted in the Gulf of St. Lawrence and others outside the Straits of Belle Isle. Although I have handled a live Dovekie, I found it difficult to distinguish at a distance between this species and other Alcidae. The differences in size are less obvious when the birds are partially submerged. The Dovekie seemed more inclined to patter away from the boat with its wings before diving, whereas the Razorbills and Puffins usually dived as soon as they became alarmed. This species is likely to be seen (on either side of the Atlantic) only in the late fall and winter months.

Cepphus grylle. BLACK GUILLEMOT.—Only one specimen was seen and that northeast of Anticosti in July, 1930.

Fratercula arctica. PUFFIN.—This bird was seen on both sides of the Atlantic on all four trips. In October, 1928, Puffins were fairly common from Belle Isle through the Gulf up till about four hours after passing the western end of Anticosti. In September, 1930, one was noted approximately forty-five miles out in the Atlantic from Belle Isle. In the Clyde the Puffin is most common near the rocky island, Ailsa Craig, where so many sea birds nest.

Puffinus puffinus. MANX SHEARWATER.—On the one occasion when the writer stayed on the boat till it reached Liverpool, the Manx Shearwater was found to be quite common in the Irish Sea, particularly between the Isle of Man and Liverpool. It is easily recognized by its entirely black upper parts, with white color below.

Puffinus griseus. SOOTY SHEARWATER.—A single representative of this species was seen about eighty miles out from Belle Isle on September 4, 1930.

Puffinus gravis. GREATER SHEARWATER.—This species was common in the open sea in July and in early September, 1930, but I have no mention of it in notes of my first voyage eastbound in late September, 1927, and saw only three specimens on the return trip in early October, 1928. In July and early September it was usual to see from five to thirty Shearwaters in an hour's watch, and single flocks including up to thirty-four were noted.

The rather erratic flight, just skimming the waves and turning first on one wing, then on the other, reminded me of the somewhat similar flight of the Night-hawk.

Fulmarus glacialis. ATLANTIC FULMAR.—This is the most common bird of the North Atlantic. On every crossing it was noted in abundance almost every day on which we were out of sight of land, with the exception that in mid-Atlantic one might see only two or three, sometimes none, in a watch of over an hour.

On July 16, 1930, when about 700 miles out (eastbound) from Belle Isle, over fifty Fulmars were following the boat at 5 P. M. The next day in mid ocean not more than three were visible at one time till 7 P. M., when eight were following the boat, but on July 18 there were over sixty in our wake most of the day.

It is quite usual to have a flock of Herring Gulls following the vessel as night falls on the first day out from either Canadian or Scottish coasts. When one goes out for his before-breakfast stroll the next morning, the gulls are apparently still there, till a second look shows that the crowd behind is composed entirely of Fulmars with perhaps one or two Kittiwakes, but that there is not a Herring Gull in the lot. Few of the passengers recognize that there has been a change. The Fulmar is not unlike a short-necked gull with the front edge of the wing rounded rather than angular, but its sweeping glides and soaring flight distinguish it from any gull. As one looks back at forty of fifty following Fulmars, their white heads stand out against the water almost like polka dots on dark green cloth.

I noticed in October, 1928, when our westbound boat approached land in daylight, that the Fulmars deserted the boat before land was sighted. It is well known that these birds are less common offshore than in the open sea, but the interesting problem is to find out why they turn back even before land is visible. The same behavior was repeated as we approached the Irish coast in July, 1930. Although visibility was very poor, the Fulmars began to drop astern thirty-five miles out from the point where land was first visible from the deck. My notes on this occasion read as follows:

July 19, 1930.

2 P. M. In sight: 50–80 Fulmars, 13 Kittiwakes, 4 Gannets (including one young bird).

2:20. No decrease in the number of Fulmars.

2:30. A Lesser Black-back and 2 Herring Gulls have joined us.

2:40. Only 5 Fulmars left.

4:00. Many Herring and Black-backed Gulls behind. No Fulmars.

4:15. Land sighted.

From this it was concluded that the signal to return to the open sea was given when the Black-backed and Herring Gulls arrived on the scene. There was no evidence that the gulls were molesting the Fulmars, but the latter seemed to realize that they had reached the limit of their usual range as soon as the coastal gulls appeared on the scene. On both the other voyages the boat came to land during the night and the desertion of the Fulmars could not be studied.

A small proportion of the Fulmars seen were in the dark phase. Many of those observed in July were apparently molting, having lost the inner primaries of both wings.

Oceanodroma leucorhoa. LEACH'S PETREL.

Oceanites oceanicus. WILSON'S PETREL.—It is fairly certain that both of these species were among the petrels commonly seen as much as ten hours out from Belle Isle in September and October. In early September, 1930, one could usually see from one to five in a ten minute watch. One of these which flew up over the deck was identified as Wilson's from its lack of distinct fork in the tail, but I was unable to distinguish between the two species at a distance.

Moris bassana. GANNET.—These great birds were fairly common in the Gulf of St. Lawrence, especially off the western end of Anticosti, and were also seen in the Straits of Belle Isle. On the European side of the ocean Gannets were quite common off the north of Ireland and up the Clyde. One has an opportunity to see one of their few known nesting places as the boat passes that round dome of granite called Ailsa Craig, in the Firth of Clyde. In the summer months there is usually a swarm of sea birds around it and Gannets can be seen headed for the rock from various directions.

Phalacrocorax carbo. EUROPEAN CORMORANT.—The Common Cormorant was seen in the Firth of Clyde on all four voyages but was not identified off the Canadian shore.

Phalacrocorax auritus. DOUBLE-CRESTED CORMORANT.—This species was noted, but not frequently, in the Gulf of St. Lawrence on every trip.

Phalacrocorax graculus. EUROPEAN SHAG.—From the boat it was possible to distinguish this species from *P. carbo* but, had the range of the former overlapped that of *P. auritus*, it would have been impossible to differentiate in the flying bird between these two species. However, since the European Shag is not found in North America, its identification was simplified. It is fairly common in the Firth of Clyde and in the Irish Sea.

Stercorarius pomarinus. POMARINE JAEGER.

Stercorarius parasiticus. PARASITIC JAEGER or ARTIC JAEGER.—Both of these Skuas were noted with varying frequency at different times all the way across the ocean, the Pomarine being the commoner of the two. Both species were more abundant on the western side of the Atlantic than on the eastern, and less common in July (when only two were seen) than in September and October. The inverted V formed by the projecting central tail feathers of the Pomarine Jaegar is not always visible, but one can more often see whether these feathers are broad and rounded, indicating *S. pomarinus*, or narrow and pointed as is typical in *S. parasiticus*.

Two unidentified jaegers were seen at the same time as the Great Skua noted above. The next day, in the course of a two-hour watch in the vicinity of Lat. N. 55° 29′ and Long. W. 39° 54′, a total of forty-two jaegers was counted, including one flight of fifteen. The majority of these were flying south. Most of those identified were the Pomarine, but both species were present. Several of these were in the light color phase. A jaeger was

seen as far inland as Father Point, about 156 miles east from the city of Quebec.

Stercorarius longicaudus. BUFFON'S SKUA or LONG-TAILED JAEGER.—The long, pointed, central tail-feathers, projecting more than twice as far as those of *S. pomarinus* and *S. parasiticus*, made it quite easy to identify the only Buffon's Skua seen in all four crossings. In this case the distinguishing characteristic was visible even without field glasses. The bird was noted on September 3, 1930, at approximately Latitude N. 55° and Longitude W. 42°, a distance of about 500 miles from the Labrador coast.

Catharacta skua. NORTHERN or GREAT SKUA.—Only a single representative of this species was seen and that at 8 P. M. on the second day out from Greenock, westbound, September 2, 1930. From the ship's positions taken on the bridge at noon on September 2 and 3, and the fact that a steady speed was maintained for the twenty-five hour interval between those readings, it has been possible to determine fairly accurately by interpolation that this Great Skua was seen at Lat. N. 56°, 08′, Long. W. 36°, 57′, or practically in mid ocean.

Larus marinus. GREAT BLACK-BACKED GULL.—On every crossing this gull was noted on both the Canadian and Scottish coasts. It was not uncommon to see four at once in the Gulf of St. Lawrence.

Larus fuscus. LESSER BLACK-BACKED GULL.—This European species is distinguished from *L. marinus* by its smaller size (being slightly smaller than the Herring Gull) and by having yellow legs in contrast to the flesh-colored shanks of the larger gull. It is abundant in the Firth of Clyde and off the north of Ireland, making up almost half of the crowds of gulls which follow boats in those waters.

Larus argentatus. HERRING GULL.—This familiar gull was as abundant in the Firth of Clyde as in the St. Lawrence where crowds of fifty or more might be seen following the boat anywhere between Quebec and Anticosti.

Larus delawarensis. RING-BILLED GULL.—A few of these were seen in the St. Lawrence River.

Larus canus. COMMON GULL.—The name of this European bird is a misnomer for it is decidedly less common than several others of the same genus. A few representatives were usually seen in the upper part of the Clyde but the birds did not follow the boat to deep water. The Common Gull somewhat resembles the Kittiwake in size, but is built more heavily and has white spots on the tips of the primaries, which the Kittiwake lacks.

Larus ridibundus. BLACK-HEADED GULL.—In the Tail-o'-the-Bank at Greenock, in Belfast Lough, and in the harbour at Liverpool, the little Black-headed Gull was seen in swarms. It is a versatile scavenger but does not follow the boats out of the harbor as do some of the larger gulls. I have frequently seen it following the plough in Scotland just as its cousin, Franklin's Gull, does in Manitoba.

Rissa tridactyla. ATLANTIC KITTIWAKE.—This gull is the first of its

family to greet the traveller to either shore of the Atlantic, and is quite common on both coasts. On October 4, 1928, at a point forty miles out in the ocean from Belle Isle, nearly 300 Kittiwakes were observed milling about in a dense swarm almost like gnats. Many were in the so-called "Tarrock" plumage of immature birds in which there is a black terminal tail band, a thin, dark mark making an incomplete collar, and a black border on the anterior margin of the wing. In September, 1930, two Kittiwakes in Tarrock plumage were seen twenty-nine hours before reaching Belle Isle, at a distance estimated to be 490 miles from land. Others were seen over ninety miles from the Irish coast, and in July, 1930, at fifty miles from this same coast the ship had a convoy of fourteen Kittiwakes.

This bird is more a lover of the open sea than any of the gulls listed below. On both eastward voyages the Kittiwakes picked up at sea followed the ship up the Clyde well past Ailsa Craig, but on neither westbound journey were any Kittiwakes seen till the second day out from port.

Oenanthe oenanthe leucorhoa. GREENLAND WHEATEAR.—In September, 1927, at about four hundred miles east of Belle Isle, an unrecognized Passerine bird spent most of the day on the boat. It was suspected of being the Greenland Wheatear and this identification was later confirmed by comparison of my notes with descriptions of the bird and by acquaintance with *O. oenanthe* in Scotland. Four others, believed to be of this species but not positively identified, flew around the boat when twenty miles from Belle Isle in October, 1928. The Greenland Wheatear is known to migrate through Europe to Africa and might easily be seen in the autumn months if it is accustomed to using passing boats as resting places.

The above list of twenty-eight species identified from the decks of liners does not exhaust the possibilities afforded the ornithologist by a crossing of the North Atlantic. A few ducks were seen but were not identified. Small flocks of swiftly flying birds thought to be Red Phalaropes were seen in the Gulf of St. Lawrence. A pair of Juncos which came aboard in the St. Lawrence are not included. A great gull which accompanied an outbound vessel met some eighty miles off Belle Isle was possibly a Glaucous Gull, but positive identification was not possible. This species should be seen in these waters by more fortunate observers. Storm Petrels are frequently noted near the European side of the Atlantic, but in spite of careful watch, I was unable to find any in four crossings. These few species and others not mentioned are quite likely to be seen by observers more experienced, more fortunate, and more persistent than the present writer.

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NUMBER OF FEATHERS AND BODY SIZE IN PASSERINE BIRDS

BY F. B. HUTT AND LELAH BALL

NUMBER OF FEATHERS AND BODY SIZE IN PASSERINE BIRDS

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OTHER things being equal, the amount of heat lost by a warm-blooded animal is directly proportional to the surface area of that animal. The surface area, however, is not directly proportional to the weight, but varies approximately as does weight$^{2/3}$. This means that the surface area per unit of weight is much greater in the small animal than in the large one, and *ipso facto*, that in homoiothermic animals the problem of maintaining body temperature above that of the environment is more difficult for small individuals than for large ones. Kleiber (1932) calculated that if a mouse and a steer had the same heat production per gram of body weight, and if both were required to maintain the same temperature, the mouse would need a specific insulation twenty times that of the steer.

The extensive data of Wetmore (1921), including 1,558 records of body temperature for 327 species of birds, indicate that small birds maintain temperatures just as high as those of larger species. For example, the mean temperature in ten species of Paridae (tits and chickadees) was 107.9° F., exactly the same as for fourteen species of Corvidae (jays, magpies and crows).

Determinations of the metabolic rates of forty-five birds representing thirty-two different species ranging in size from 0.02 to 17.6 kilograms, made by Benedict, Giaja, Terroine and others, as summarized by Brody (1932, pp. 89–97), show that in birds, as in mammals, the metabolism per unit of body weight is highest in the smallest species and decreases with increasing body size. This indicates that maintenance of high temperatures by small birds is accomplished in part by an increase in the metabolic rate. However, the amount of insulation may also be an important factor. If this be so, the greater insulation needed by the smaller birds to maintain temperatures the same as those of large ones, which are from 3° to 12° F. higher than those of mammals, might be obtained by increased length of the feathers, but the resultant disproportion between size of body and length of feathers would probably interfere with flight and hence have little 'survival value.' Since the type of feather changes little within any one order, or group of birds, it is probable that any additional insulation needed by small birds is provided by an increase in the number of feathers per unit of body surface. On theoretical grounds, therefore, there should be many more feathers *per unit of area* in the Chickadee weighing eight to ten grams than in the Blue Jay of about ten times the latter weight. It follows that, since the surface area per unit of weight increases with diminishing body

size, there should be a still greater difference between the Chickadee and the Blue Jay, with respect to the number of feathers *per unit of body weight.* On this basis a fairly consistent increase in the number of feathers per gram of body weight is to be expected in progressing from the larger to the smaller species within a group of related birds.

MATERIAL

Data have recently been provided by Wetmore (1936) with which the validity of this hypothesis can be tested. Actual counts of contour feathers are given for 152 birds, and for 101 of these there is also given the weight of the bird on the day it was collected. All but four of these 101 belong to the Passeriformes. This one order thus provides for analysis 97 determinations on a group of birds similar in form and homogeneous with respect to many characteristics, but with a range in size from 5.5 grams in a Golden-crowned Kinglet (*Regulus satrapa*) to 117.7 grams in a Purple Grackle (*Quiscalus quiscula*).

Since sex dimorphism in size is not extreme in this order, little error is incurred by disregarding sex in the analysis. Wetmore points out that counts of feathers were in general lower in late spring and early summer than at other seasons, but owing to the comparatively small number of determinations available for analysis and the fact that these were distributed from February to October, it has not been possible in this study to correct for changes with season. This source of error would be most serious if large birds were collected at one season and small species at another. This was not the case. The determinations were well distributed throughout the year, not only in the whole series, but also in the many species having feather counts for several birds.

ANALYSES

A. Passeriformes

No attempt has been made to relate the number of feathers to surface area by using the von Meeh formula for determining surface from weight. Brody (1932, p. 11) showed that determinations of surface area for one species by different investigators may differ by more than sixty per cent. Since the birds considered here vary greatly in size it is doubtful if any one value for the species constant (k) in the formula would be justifiable. Giaja (1925) determined k as 7.4 for a finch, 8.69 for a shrike, and from 6.54 to 11.5 for chickens of different sizes. For these reasons the numbers of feathers in birds of different sizes have been considered in relation to body weight.

On plotting the data it was found that they conform to the general equation $y = ax^n$, where y = number of feathers, x = body weight in grams

and a and n are constants. After fitting the data to this equation by the method of least squares, the values of these constants were found to be $a = 910.17$ and $n = 0.185$. The curve for the distribution of feathers in relation to body weight is therefore:

$$y = 910.17\ x^{0.185}.$$

For twenty-nine warblers (Mniotiltidae) representing twenty species, the distribution follows the curve $y = 861.54\ x^{0.2361}$ and for twenty-four sparrows (Fringillidae) of twenty species the corresponding curve is $y = 747.86\ x^{0.236}$. The warblers evidently have denser plumage than the sparrows but the change with size of bird, as indicated by the exponent, is apparently very similar in both families.

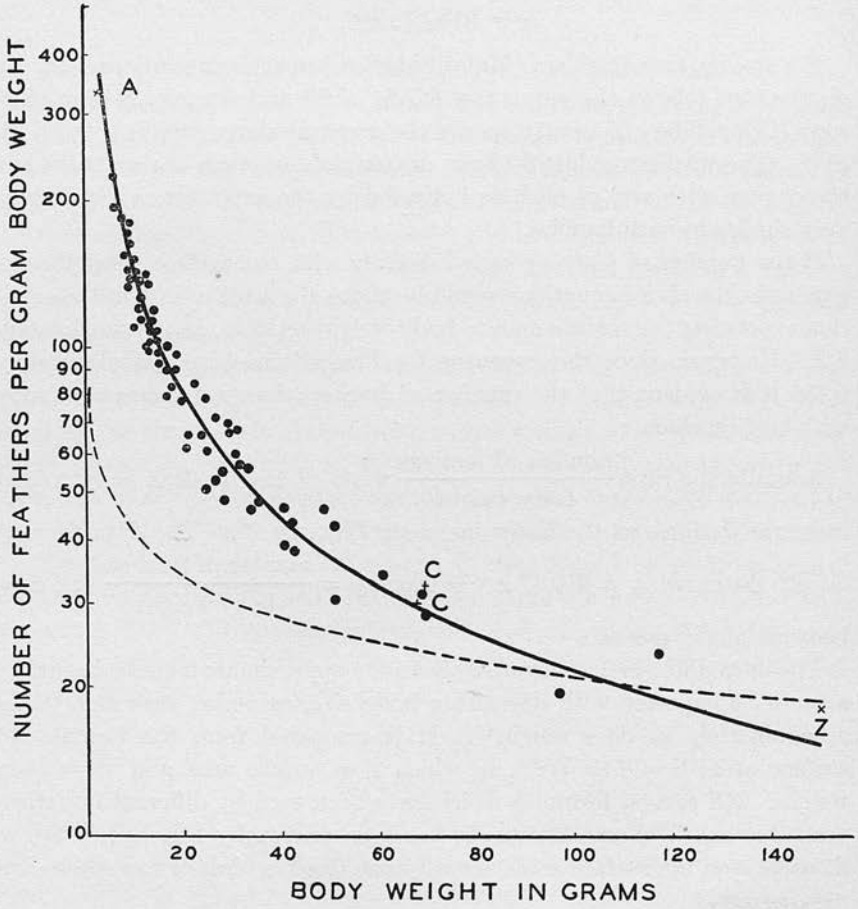
If the number of feathers varied directly with the surface, then the exponent in the above equations would be about the same as it is in the equation expressing the surface area to body-weight relation, i.e., approximately 2/3. However, since the exponent for Passeriformes as a whole is only 0.185 it is evident that the number of feathers does not increase directly with body surface.

Actually the ratio $\frac{\text{number of feathers}}{\text{body weight}}$ when plotted against body weight in grams declines as the latter increases (Text-fig. 1). The data fit very closely the curve: $y = 910.17\ x^{-.815}$ where $y = \frac{\text{number of feathers}}{\text{body weight}}$ and $x =$ body weight in grams.

The dotted line in Text-figure 1 shows the approximate increase in surface area to be expected with decreasing body size, assuming that area varies approximately as does weight$^{2/3}$. It is computed from the formula for surface area, $S = 8.19\ W^{.705}$, in which S = surface area and W = body weight. (Of several formulae which have been used by different investigators, this one is chosen because it has been proven by Mitchell (1930) to fit quite well the surface areas actually measured in birds of one species but varying greatly in size.)

If the increase in number of feathers per gram of body weight with decreasing size were attributable solely to the associated and inevitable increase of surface area (with number of feathers per unit of area remaining constant) then Wetmore's feather counts should be grouped along a curve of the shape of the dotted line in Text-figure 1. Actually the number of feathers per gram of body weight increases much more rapidly than surface area. In only five cases out of 97 does the ratio deviate by more than 20 per cent from the theoretical expectation based on the equation for the solid line, given above. It is evident, therefore, that in the Passeriformes the increase in the number of feathers per unit of body weight is much greater

than could result solely from the increase in surface area per unit of weight and that there must be a consistent increase in the number of feathers per unit of area with decreasing body size. This probably indicates an adaptation for maintenance of high temperature in the smaller birds.



TEXT-FIG. 1.—Relations between body size and (a) number of feathers (solid line) and (b) surface area (broken line) per unit of body weight in the Passeriformes, both plotted on a semi-logarithmic grid. Each dot represents a count of feathers on one bird except in species smaller than 20 grams where, to avoid crowding, one dot shows the average for one to four individuals. The scale on the ordinate for the broken line must be divided by 10 to show the number of square centimeters per gram of body weight. Non-passerine birds are shown at A (*Archilochus colubris*), C (*Chordeiles minor*) and Z (*Zenaidura macroura*).

B. Other Orders

Some indications that this principle might apply equally well to some non-passerine species is provided by the four such birds for which Wetmore gives both feather count and body weight, and also to a lesser degree by

Ammann's (1937) swan (Table 1). For these birds the expected ratios of feathers/weight have been calculated by determining for the given body weight the corresponding value of y in the equation used for the Passeriformes, $y = 910.17\ x^{-.815}$. The actual determinations, with the exception of that for the swan, are shown in Text-figure 1 at A, C, and Z. It is quite evident that, with the exception of the swan, these birds conform closely to the curve established for the Passeriformes. This is all the more remarkable because one would not expect single determinations, like those for the hummingbird and Mourning Dove, to conform to the rule as closely as would the average of several determinations for a species.

TABLE 1

Application of the equation for the feathers/weight ratio in Passeriformes to birds of other orders

Order and Species	Feathers No.	Body weight grams	Ratio: No. of feathers / Body weight Observed	Expected
MICROPODIFORMES				
Ruby-throated Hummingbird, *Archilochus colubris*	940	2.8	335.71	393.23
CAPRIMULGIFORMES				
Eastern Night-hawk, *Chordeiles minor*	2265	69.3	32.68	28.76
Eastern Night-hawk, *Chordeiles minor*	2034	67.9	29.96	29.24
COLUMBIFORMES				
Mourning Dove, *Zenaidura macroura*	2635	152.7	17.26	15.03
ANSERIFORMES				
Whistling Swan, *Cygnus columbianus*	25216	6123.0	4.12	0.75

The excess of feathers above the theoretically expected number in the swan is not surprising because of the elongation of the neck, a densely feathered region in this species. Ammann's data show that 80 per cent of the swan's feathers are on the head and neck. Presumably the values of k and n in this equation should be somewhat different in Anseriformes from those applicable in Passeriformes and other birds like them.

DISCUSSION

Wetmore (1936) points out that the hummingbird had the smallest number of feathers of all the species counted and that such a condition was to be expected, since it was the smallest of all birds examined. It seems equally noteworthy that in this diminutive species the number of feathers per gram of body weight (and presumably, therefore, the insulation) is greater than in any other of the sixty-six species for which data are available. It would be of interest to know if the northward-ranging Ruby-throated Hummingbird carries more feathers per unit of weight than those members of its family which remain in the tropics. On theoretical grounds one would expect fewer feathers in the latter.

In the curve expressing for Passeriformes the change in the ratio feathers/weight, the exponent is −.815. This curve is satisfactory for representation of three other Orders. In the corresponding curves for warblers and for sparrows the exponent is −.764. It seems justifiable to formulate the following general rule:

The number of contour feathers per unit of body weight (y) increases with decreasing body weight (x) according to the relation $y = ax^{-n}$, in which the values of n thus far determined are of the order of 0.8.

The finding that the number of feathers per unit of body weight increases with decreasing body weight is in accordance with the original hypothesis that small birds need more insulation than large ones if both groups are to maintain the same body temperature. It would appear to indicate a special adaptation for conservation of heat in the smaller species. However, since the general tendency is for the smaller birds to have higher rates of metabolism, it is probable that both adaptations contribute to the maintenance of high temperatures.

It is beyond the scope of this paper to determine the relative importance of these two influences, but the importance of the plumage and retention of the heat produced are indicated by recent studies of the physiology of birds lacking normal plumage. It was shown by Hutt (1930) that fowls homozygous for the frizzling mutation have defective plumage and may become practically bare when defective feathers break off. According to Benedict, Landauer and Fox (1932) such birds have abnormally high rates of metabolism, and, associated with it, a lack of fat deposits, enlarged thyroids, increased heart rate, hypertrophy of the heart, decrease of hemoglobin and frequent sterility. These conditions are decidedly not conducive to the survival of a species so affected, either in domestication or in Nature. It seems probable, therefore, that while the temperature of the smaller birds is maintained in part by an increase in the rate of metabolism, any adaptation for more efficient retention of the heat produced is equally important, if not more so. The inverse relationship between the number of feathers and body size provides exactly such an adaptation.

It would be of interest to know how closely this rule applies to variations in size within a species such as the Domestic Fowl, where mature males may weigh, according to the senior author's determinations, from 550 to 4970 grams in different breeds. Equally interesting would be tests of its validity in aquatic species and in the Ratites which, because of lack of barbules, have a somewhat unusual type of plumage.

SUMMARY

Analysis of Wetmore's counts of contour feathers in ninety-seven birds of the order Passeriformes shows that the number of such feathers per unit of

body weight (v) increases with decreasing body weight (x), according to the relation $y = ax^{-n}$, where $a = 910$ and n approximately 0.8. In spite of seasonal variation, in only five cases out of ninety-seven did the number of feathers per gram of body weight deviate by more than 20 per cent from the numbers calculated from body weight with this equation. In warblers (Mniotiltidae) the plumage was slightly denser than in sparrows (Fringillidae). Feather counts for representatives of three other orders fitted closely to the numbers computed from the equation for Passeriformes. It is concluded that while the increased metabolism of smaller birds is instrumental in their maintenance of high temperatures, the rapid increase in the number of feathers per unit of body weight with decreasing size of bird is an adaptation for retention of the heat produced.

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G. REVIEWS AND GENERAL

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PARADOXICAL TERMINOLOGY IN GENETICS

THE increasing use by geneticists of the term "recombination" prompts the writer to point out that in the majority of cases the meaning now implied by the geneticist using the word is directly opposite to its true and generally accepted interpretation.

When the two members of any pair of factors segregate in the germ cells and maternal and paternal factors are subsequently brought together in pairs in the zygote, it is quite correct to refer to the latter process as recombination, in the sense that units occurring first in pairs and then separately are finally again combined in pairs. It is therefore correct to say that Mendel's laws depend upon segregation and recombination at random. It was in this sense that the latter term was first used in genetics. However, when one considers the genes introduced in a cross by both parents, it is not correct to refer to all the genotypes or phenotypes obtained in backcross or in the F_2 generation as recombinations, since some of them consist of entirely different combinations of genes and phenotypes from those of either parent, *i.e.*, they are *new combinations*. Paradoxically, it is just these new combinations which are being labelled by many geneticists as "recombinations" when the latter term is really applicable only to the genotypes and phenotypes which are the same as those of either parent.

The error is best illustrated by an example. One may take the coupling phase of a cross in which a new recessive mutation, *aa*, is being tested for linkage with the dominant character represented by the gene *B*. If the F_1 be back-crossed to the double recessive homozygote, one obtains the condition listed below.

Parental genotypes;	$AABB \times aabb$
Parental phenotypes:	$AB \quad ab$
F_1 genotype:	$AaBb$
F_1 gametes:	$AB, \quad Ab, \quad aB, \quad ab$
Phenotypes in back-cross:	$AB, \quad Ab, * aB, \quad ab$

Of these four phenotypes obtained in the back-cross, two, *AB* and *ab*, are referred to as "parental combinations." Since they demonstrate the production by the F_1 of gametes containing the

same combination of genes as was contributed in the gametes of either original parent, this terminology is quite correct. On the other hand, the two phenotypes *Ab* and *aB* have not previously been encountered in the cross under consideration. They indicate the production of F_1 gametes containing respectively the gene combinations *Ab* and *aB*. These are strictly new combinations and not "recombinations" in any sense of the word.

When a deficiency of these new combinations below the 50 per cent. expected on the basis of random assortment indicates linkage of *A* and *B*, the gametes *Ab* and *aB* are then referred to as cross-over gametes, or, more commonly, the phenotypes *Ab* and *aB* are simply called cross-overs. However, when there is independent assortment instead of linkage, it is incorrect to refer to the new combinations as cross-overs, since there has been no crossing-over. It has become common usage with some geneticists to label all new combinations as "recombinations" and then to state that their frequency indicates either independence or a certain percentage of crossing over.

Concerning the prefix "re," Webster's Dictionary (1930) says:

> A prefix denoting:
> 1. *Back,* esp. *back to an original or former state or position;* backwards; . . .
> 2. Again;—used chiefly to form words, esp. verbs of action, denoting in general *repetition* (of the action or of the verb), or *restoration* (to a previous state); as in *re*join, to join again, *re*iterate, to iterate again, *re*new, to make new again, . . . etc.

No special meaning is given for "recombination" as is done for numerous other words of which the exact shade of meaning is not clear from the prefix "re" and the root.

With this in mind, whenever no discrimination is made between different combinations of genes, one may say that in a general way all the genotypes and phenotypes obtained in an F_2 or backcross represent recombinations of factors which segregated in the F_1 germ cells. However, when, as in a linkage study, these same genotypes or phenotypes are differentiated into two classes, in one of which the combinations of characters are exactly the same as those found in the parents, while in the other the combinations of characters differ from those of both parents, then, from this aspect, only the former class contains recombinations, whereas the latter class obviously consists of entirely new combinations.

Inspection of the example given above shows that the only recombinations are *AB* and *ab*, *i.e.*, the parental combinations. To be accurate, since "recombination" implies reunion after separation, it is really applicable to the parental combinations only when linkage has not occurred and when there has therefore been in the F_1 germ cells independent segregation of the chromosomes bearing the genes *A* or *a* and *B* or *b*. It is not strictly correct to label as recombinations those cases in which *A* and *B* or *a* and *b* are linked and remain together throughout the cross, but the term "parental combinations" is appropriate in cases both of linkage and of independence.

To designate the phenotypes *Ab* and *aB*, which are unequivocally " new combinations," as "recombinations" is not merely making the terminology ambiguous; it is a direct subversion of fact. Geneticists who use the term "recombinations" really mean "new combinations." To them the two expressions seem synonymous, but in actual meanings each is the very antithesis of the other. For this reason, no one but a geneticist could easily understand the paradoxical statement in the second sentence of the following quotation from a leading text-book:[1]

> The two large classes contain the factors in the same combinations in which they occurred in the parents and are, therefore, called parental combinations. The two smaller classes represent combinations of the factors different from those of the parents and are, therefore, called recombinations.

Similar inaccuracy is to be found in many recent papers reporting linkage studies.

Equally undesirable is the use of the term "recombination" in place of "crossing-over," as it is employed in the text just quoted (pp. 136–137) and in other writings on genetics. Crossing-over is a distinct phenomenon in genetics and it is therefore highly desirable to retain for it this accurate and descriptive term by which it is usually designated. Since the process forms new combinations of genes, and not recombinations, it can not with accuracy be referred to as a process of recombination.

In the interest of a comprehensible terminology, the writer suggests that geneticists refrain from using the words "recombinations" and " recombination" in these senses and stick to the terms "new combinations" and "crossing-over." Where the frequency of new combinations indicates linkage, such combina-

[1] E. B. Babcock and R. E. Clausen. "Genetics in Relation to Agriculture." Second ed., p. 126, McGraw-Hill. 1927.

tions may correctly be referred to as cross-overs, but the general term suggested will cover all cases.

Some readers of this note will counter the suggestion with the reply that no up-to-date geneticist who can read English will be confused by the continued use of "recombinations." This may be correct, but it does not exempt us from the mental anathema of the German, Russian or Japanese student who may find it difficult to translate correctly the paradoxical terminology of the American geneticist. After all, since science is exact knowledge, should this knowledge not be expressed in exact and accurate terms?

The intricacies of genetics are not easy to master. The adoption of accurate terminology should make them slightly less recondite to the beginning student, to the foreigner struggling with a strange language and to the worker in other fields who may try to acquire a nodding acquaintanceship with the youngest of biological sciences.

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RESEARCH WITH A HEN

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RESEARCH WITH A HEN[1]

THE subject of this address was unwittingly suggested by a member of the board of governors of one of the largest universities in the United States. This institution employs upwards of two hundred graduate students as assistants in research and in teaching, but when the board was requested a few years ago to appoint a research assistant in the Department of Poultry Husbandry, it was confronted with a situation quite unprecedented in all the years of the university's existence. It was not surprising, therefore, that one member of that body should have expressed his doubt about the desirability of the proposed new appointment, but it was surprising that he should do so by asking the very pointed question, "What research can you do with a hen?"

What research can you do with a hen? Among the members of this society there are over a hundred who could answer this question by quoting investigations now under way in the various universities and experiment stations which they represent, and concerned with the nutrition, genetics, physiology, embryology, pathology, parasitology or psychology of the fowl. These experiments are planned in nearly every case to discover new facts and to add to our store of knowledge. It is because such investigations have been carried on for many years that we now have a sufficiently great accumulation of facts to justify the designation of this branch of knowledge as poultry science.

To discuss research intelligently we must have an understanding of what the word implies, but, in attempting to define it, no one realizes better than I the difficulty of giving any arbitrary and fully satisfactory definition. As every director of an experiment station knows, there are investigations of divers kinds, not all of which are entitled to be desig-

[1] Address of the president at the twenty-fifth annual meeting of the Poultry Science Association, East Lansing, Michigan, August 2 to 5, 1933.

nated as research. Some are merely *re*-search, but, in so far as they improve upon previous methods and by so doing establish new facts or provide more accurate interpretations, such investigations may fairly be classified as research. This can hardly be said, however, for the all-too-common repetition of experiments in which the results have been so conclusive that additional substantiation is entirely unnecessary. Nor can we classify as research those investigations in which sources of error and of environmental modification of results have been so ill-controlled that no definite answer to the original problem is provided by the experiment. Fortunately such experiments are becoming fewer.

The majority of our experiments, however, are set up to determine new facts about the hen and about other domesticated birds. Except for studies of costs of production, which are largely economical rather than biological in nature, our investigations are concerned with the hen's nutritional requirements, her physiology, her genes and the characters they produce, her embryonic and postnatal growth, the parasites that beset her, the bacteria that invade her and, withal, the mental processes that are responsible for her often inexplicable peculiarities of behavior. In pursuing these researches we usually apply already well-known principles of nutrition, physiology, genetics, embryology, parasitology, bacteriology and animal psychology. We are not concerned primarily with extending the boundaries of these fields of science, but rather with utilizing them to give us more information about the fowl. However, if an investigation adds one small fact to the already large amount of knowledge about the hen, that investigation is undoubtedly research so far as poultry science is concerned. It may also be called research in nutrition, genetics or any of the other sciences, if it extends to a new species the application of a principle not yet known to have universal application.

But there is still another kind of research with a hen, a kind which has not been adequately appre-

ciated even by members of this association, and certainly not by the gentleman who asked, "What research can you do with a hen?" I refer to those investigations in which the hen has been used, not for the application of existing principles, but for the discovery of entirely new concepts in biological science.

To begin with, our whole knowledge of early embryology of vertebrates rests largely upon the great body of facts which has been accumulated about the development of the chick. As early as the fifth century B. C., Hippocrates, the father of medicine, was advising his students to verify his teachings of human embryology by daily examination of incubating eggs from the second day to hatching and assuring them that therein "you will find everything as I say, in so far as a bird can resemble a man." Twenty-three hundred years later I send many dozens of eggs each year to the modern disciples of Hippocrates at our university in order that they may do just what he directed.

A century after Hippocrates, Aristotle wrote a description of the development of the chick embryo which was so remarkably accurate that little improvement was made upon it until the work of Fabricius ab Aquapendente early in the seventeenth century. Nevertheless, up to the time of William Harvey (1578–1657) there was no clear conception of where or from what materials the embryo of either birds or mammals began its development. It remained for that scientist to show that in the hen's egg the little light spot on the yolk, which he called the cicatrula, and which we call the germinal disk, or blastoderm, was the original center of growth. It was not a far cry from this discovery to his all-important doctrine of *ex ovo omnia*—"all things come from eggs." It is interesting to recall that Harvey, whose demonstration of the circulation of the blood marked perhaps the greatest single advance that physiology has ever known, was a keen student of the embryology of the chick, and that his "Anatomical Exercitations on the

Generation of Animals" was based almost entirely on his observations of chick embryos.

A little later, under the microscopes of Malpighi, the chick afforded to man his first view of the neural groove, the somites and the optic vesicles, characteristics of all vertebrates at early stages of their development. Malpighi's studies, published in 1672, may be said to have established the chick as the animal *par excellence* for both microscopical and macroscopical studies in embryology, and, in succeeding years, with its aid, Wolff, von Baer, Balfour and others laid the foundations of our present detailed knowledge of embryonic development A glance through any current journal of zoology or anatomy will show that the chick's usefulness in this field is by no means ended.

If the chick has been of service in revealing the processes of normal development, it has been of equal value in the experimental study of teratology or abnormal development. The difficulties of experimental manipulation of mammalian embryos are obvious. On the other hand, the chick embryo is ideal for this purpose, because, in the words of Dareste, "the fertilized ovum is endowed by fertilization with the capacity for development, it is separated completely from the maternal organism; it results therefrom that the egg which contains it can be submitted to all the influences which we believe capable of modifying its development." It is not surprising, therefore, that the chick embryo should have been utilized by Geoffroy Saint-Hilaire, one of the founders of teratology, or that Dareste should have devoted practically his whole life to the experimental study of abnormal development in the same species. To-day the more recent science of experimental embryology, the "Entwicklungsmechanik" of Roux, counts the chick embryo among its most useful subjects for research.

Man's knowledge of the physiology of reproduction was greatly clarified by two important advances at the close of the seventeenth century. One of these was the discovery of mammalian ova by De Graaf in

1672; the other occurred two years later, when a Dutch medical student, Ham, saw for the first time the myriads of minute animalculae which we now know as spermatozoa. Professor Punnett quotes Fridericus Shrader as authority for the statement that these were first detected by Ham in the semen of a male fowl. This seems not unlikely, for in 1677 in one of his numerous letters to the Royal Society of London, Leeuwenhoek, to whom Ham had revealed his discovery, stated that he had been able to find in the semen of a cock a "huge number of little snakes or eels," which were not to be found in the material from hens. Ham's discovery was the result of a decidedly valuable research in which the fowl proved to be a most useful animal.

The contributions of the fowl to science thus far mentioned were mostly made before the eighteenth century. To show you that the hen is still in the rank as a laboratory animal, I should like to describe a few more recent important advances in which she has played a part.

It is doubtful if the world has seen any more dramatic demonstration of a scientific fact than that given by Pasteur in 1882 at the farm of Pouilly le Fort, near Melun in France. The great scientist had offered to convince a skeptical world that he could make animals resistant to anthrax, a disease which up to that time was rightly considered as one of the worst scourges afflicting domestic animals. Twenty-five sheep were inoculated twice with attenuated organisms of the kind causing anthrax, and twenty-five others were set aside as controls. Subsequently, all sheep in both lots were given a virulent dose of the organism. Within five days every control sheep was dead, but of the twenty-five that had been inoculated all were in perfect health except one that died from conditions associated with pregnancy. The potentialities of vaccination were established.

Let us remember, however, that this dramatic and convincing experiment would not have been possible if Pasteur had not first discovered that the deadly

organisms could be attenuated, or weakened, until they were capable of causing only a mild infection to which the body could speedily build up a resistance. This fundamental discovery was made not with *Bacillus anthracis,* but with the organism causing fowl cholera. It was the discovery that aged cultures of this bacterium produced only mild and temporary symptoms of the disease that led Pasteur to the successful use of attenuated organisms as a means of building up resistance to anthrax and, later, to other diseases. Research with a hen has not been barren in the field of bacteriology.

Let us consider endocrinology, probably of all the biological sciences the field of greatest activity at the present time. It is well known that the secondary sex characters of the fowl are easily modified by subjecting them to the influence of hormones of the male or female gonads, or by complete gonadectomy. Moreover, the feather follicle can be used to excellent advantage as an indicator of thyroxine. For these reasons the fowl has been selected as a favorable subject for the study of sex and secondary sex characters by research laboratories in Chicago, Edinburgh, Strasbourg and Moscow, as well as by numerous individual workers. Apart from all the present activities, however, let us keep in mind that the first actual demonstration that there was any such thing as an endocrine secretion was made by Berthold in 1849, when his transplantation of testes of the fowl was followed by typical male-like growth of the comb in the recipients. It is true that Berthold, like many another investigator, did not fully appreciate the significance of his discovery at the time, but that does not vitiate the importance of his research as the first step in the now tremendously complicated field of endocrinology.

The Dutch physician, Eijkman, is justly famous for his experiments in 1897 in the etiology of beriberi. It should be remembered that these investigations were prompted by his discovery that fowls fed the garbage from the hospital of the Javanese prison,

at which he was medical officer, contracted a paralysis very similar to that afflicting his patients. This led him to conduct experiments in which some fowls and pigeons received rice with the outer layer left upon it, while others were given only polished rice. When the latter group contracted the degeneration of peripheral nerves, which Eijkman designated as *polyneuritis gallinarum,* it was the first time in history that a disease of dietary origin had been experimentally produced. It is little wonder that Eijkman's findings should be hailed by Dr A. V. McCollum as "the most remarkable observation in the history of beri-beri, and the one which inaugurated the modern era of investigations in the field of nutrition." Obviously, research with a hen has been decidedly worth while in that branch of science.

In the field of genetics, the fowl has been so extensively studied that more is known about its inheritance than that of any other domestic animal. This is not surprising, partly because of its more rapid rate of reproduction, but also because the domestic fowl was the first species to bear witness that Mendel's laws apply to the animal kingdom.

When William Bateson presented to the Evolution Committee of the Royal Society his initial report on "experimental studies in the physiology of heredity," covering extensive researches with four species of plants and with poultry, that document included sufficient data to show conclusively that rose comb, pea comb and the dominant white of White Leghorns were simple dominant characters, segregating in a 3:1 ratio in the F_2 generation and in a 1:1 ratio in back-crosses. It is important to remember that this report was presented on December 17, 1901, and that the experiments were begun in 1898. It is difficult to escape the conviction that, if Mendel's laws had not been rediscovered in 1900 by De Vries, Correns and von Tschermak, they would have been worked out independently by William Bateson. It has well been said that, were it not for Mendel, Bateson might be sleeping in Westminster Abbey.

From this auspicious beginning the fowl has continued to provide valuable material to the geneticist, including one of the first cases known of sex linkage, the first experimental demonstration in domestic animals of the possibilities of reducing losses from disease by breeding resistant strains, unrivaled evidence of the value of the progeny test, information about the phenomenon of crossing-over in females having only one sex-chromosome, to say nothing of the variety of morphological characters, which, I venture to predict, will some day be utilized to find out how the gene gives rise to the character, a field of genetics as yet practically unexplored.

More of these examples might be given, but I have cited enough to show that the hen has a right to stalk with pride through almost any biological laboratory, whatever its particular fields of activity may be. Nor has she shown the narrow specialization of Drosophila, of the albino rat, or the guinea-pig, whose spheres of usefulness are largely confined to the researches of the geneticist, the nutrition specialist or the bacteriologist, respectively. She is, withal, a lass of many parts.

As poultrymen we respect the hen as being of all domestic animals the most efficient converter of raw materials into edible food stuffs. We respect her as one of the most profitable sources of farm revenue, the mainstay of many a farm home where crops have not yielded the promise of spring. As biologists, let us also respect her as one to whom science owes no inconsiderable debt of gratitude. It is fitting that we should draw on all the resources of science to keep her in a state of maximum efficiency, to prolong her useful life, to prevent the ills to which she is subject and to raise her progeny with a minimum of loss. By so doing we make some slight return for the contributions to knowledge which have resulted from "research with a hen."

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Inherited Lethal Characters in Domestic Animals

The author would appreciate being advised of the occurrence of any abnormalities similar to those described in this paper.

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INHERITED LETHAL CHARACTERS IN DOMESTIC ANIMALS.[1]

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The suggestion has sometimes been made in the past that the hereditary variations which most closely obey Mendel's laws of inheritance have little more than an academic importance because they appear to affect only such comparatively unimportant characters as hair color and structure, body pigments, unimportant bones in the hand or foot, plumage patterns, feather structure and the comb of fowls. This idea was proven to be quite erroneous when the multiple-factor hypothesis brought into line such important physiological characters as body size, milking-capacity, egg production and resistance to disease, and showed that they too conformed to Mendel's laws although not in the same simple way as did the various types of peas responsible for the formulation of these laws.

The old idea has been still further refuted by the discovery that in nearly every species adequately investigated there are found hereditary defects so serious as to make the animals affected by them completely non-viable. To the geneticist these are known as *lethal characters*.

Within the past decade genetic investigations have brought to light a considerable number of these lethal characters which affect various domestic animals. Most of this research has been done in Europe. In this review the writer aims to bring to the attention of American veterinarians the lethal characters of domestic animals which have thus far been shown to be hereditary. In so doing, it is hoped not only to facilitate their recognition, if they should occur in this country, and thus

1. Paper No. 1244 of the Journal Series of the Minnesota Agricutural Experiment station.

to retard their spreading, but also to enlist the aid of the veterinary profession in detecting other lethal characters not yet recognized as being genetic in origin.

Persons unfamiliar with the comparatively new science of genetics sometimes fail to understand how an abnormality can be perpetuated in the germ-plasm of a species when that abnormality is so severe as to cause the death of the affected animal. In some cases, as in Huntingdon's chorea and polycystic kidneys, hereditary diseases in man, the lethal effect is not exerted until after the affected individual has become old enough to have offspring. In the majority, however, the character is lethal either during gestation or shortly after birth. It is perpetuated by animals apparently normal in every respect but actually carrying a *gene*, or hereditary determiner for the character. When two such "carriers" are mated together, the types of offspring resulting are approximately as follows:

1 normal, viable, not showing the lethal character, or carrying it.

2 normal, viable, not showing the lethal character but carrying a gene for it. Such animals are said to be *heterozygous* for the character in question.

1 abnormal, non-viable, *homozygous* for the character and dying because of it.

It is obvious that when carriers of a lethal gene are mated together approximately one-quarter of the offspring are lost, and two-thirds of the normal progeny are carriers of the gene, which they, in turn, transmit to the next generation.

In explanation of this mode of inheritance, it will suffice here to say that the homozygous animal receives a gene for the lethal character from both sire and dam, while the heterozygote receives that gene from only one parent. The great majority of lethal characters are *recessive* in nature, exerting no readily perceptible effect on the heterozygous carriers. A few exceptions are mentioned below.

In the following account there are included only those conditions which, unless otherwise stated, have been definitely proven to be hereditary and serious enough to cause death in the majority of animals so affected. By some geneticists the term "lethal" is restricted to those genes and characters causing death before birth, while those operative at a later age, even if immediately after birth, are called "sub-lethal." To the writer such a distinction seems undesirable and accordingly all

hereditary conditions causing premature death are classified as lethal regardless of the age at which they are effective. In most cases descriptions are given with enough detail to permit ready recognition of the character, while the genetic evidence is greatly reduced or entirely omitted. Such omission is not to be construed as indicating any lack of such evidence. The latter may be found in detail in the papers cited.

CATTLE

1. *Achondroplasia.* (Figure 1) The "bull-dog" calf of Dexter cattle has been shown by Crew (1923) to exhibit pathological conditions identical with those of achondroplasia in man. The skull is vaulted and much rounder than normal. With flattened nose, split upper lip, protruding lower jaw, and protruding swollen tongue thrust out, the head of the abnormal calf bears a resemblance to that of the bull-dog. The

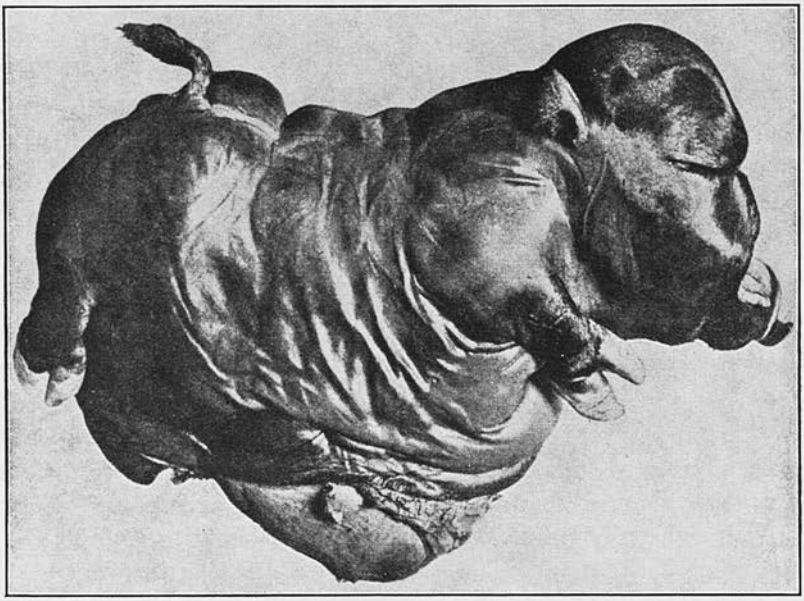

Figure 1.[2] A bull dog calf of the Dexter breed. (*From Crew.*)

limbs are so extremely short that sometimes only the digits (widely separated) appear to protrude from the body. This effect is heightened by the swelling of the body tissues result-

2. The illustrations are reproduced by courtesy of the following journals: Fig. 1, *Proc. Roy. Soc. Med.;* Fig. 2, *Wis. Bull. 86;* Fig. 3, *Naturens Verden;* Figs. 4 and 5, *Journ. Genet.;* Fig. 6, *Der Züchter;* Fig. 8, *Journ. Hered.*

ing from the presence of much subcutaneous fat and from generalized anasarca. This swelling gives the tail-head an appearance of being seated far up the back. Umbilical hernia is usual.

Extreme bull-dog calves are aborted from the third to the eighth month, delivery at the latter stage being particularly difficult because of the abnormally large head. A "bull-dog" pregnancy is usually associated with recurrent *hydrops amnii,* the fluid sometimes being lost several times in one gestation.

Extreme bull-dog calves are produced by the short-legged, broad-headed Dexter cattle originated in Ireland but latterly somewhat more popular in England. Approximately one-quarter of the calves born from matings of pure bred Dexter cattle are "bull-dogs," but these same matings also produce Kerry-type cattle with longer legs and narrower heads than the Dexters. From the evidence of Seligmann (1904) and of Crew (1923) it would appear that the correct Dexter type is produced by the presence in the heterozygous state of a partially dominant gene which causes the short legs and brachycephaly considered good type in that breed. Segregation of genes produces the bull-dog calf, homozygous for the character, and the Kerry-type animal which lacks the gene entirely. This interpretation is confirmed by the fact that when Dexters are crossed with the longer-legged Kerry (from which breed they originated) no bull-dog calves are produced.

2. *Recessive achondroplasia.* A condition similar to that described above but less extreme in nature has been observed by Wriedt (1925) and Mohr (1926) in the Telemark breed in Norway. These calves are carried to full term, but, with one exception which survived to three months, those observed were unable to stand and died within a few days of birth from inability to breathe properly. Since the parents are normal the character is here a recessive. Calves exhibiting varying degrees of achondroplasia have also been encountered by Weinkopf in Holsteins in Germany (quoted from Mohr, 1929) by Downs (1928) in a calf of three-quarters Holstein blood, and quite recently Carmichael (1933) has reported the occurrence of 12 typical bull-dog calves in a herd of Nganda cattle in Uganda. Obviously the condition is one that may be encountered in any breed, but the more extreme abnormality is not likely to be encountered except in the progeny of short-legged, brachycephalic animals.

3. *Epitheliogenesis imperfecta.* (Figure 2) In this condition, definitely shown by Hadley and Cole (1928) to be a simple recessive character, the calves are born alive at full term but have such serious defects in the skin and mucous membranes that bacterial invasion cannot be averted and the calves die from the resultant septicaemia. The lesions are most common in the extremities of the limbs, the muzzle, nostrils, tongue and ears. The latter are deformed as a result of adhesions of defective areas on the inner surfaces. There are one or more defective claws. Up to 1928, 55 calves thus afflicted had been encountered in 17 Holstein-Friesian herds in Wisconsin and one elsewhere. The same lethal character has been observed in Holland in animals of apparently similar ancestry.

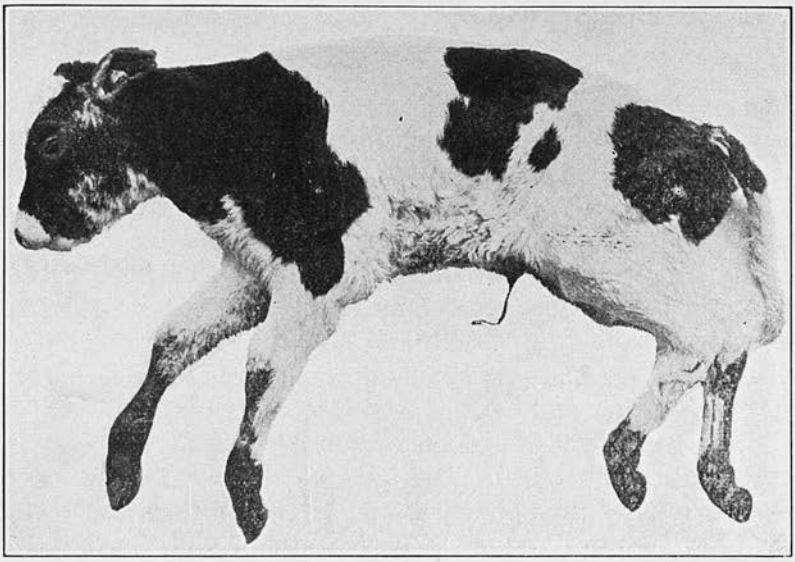

Figure 2. Epithelial defects. Raw areas are seen on the extremities of the limbs. (*From Hadley and Cole.*)

4. *Hypotrichosis congenita.* Varying degrees of hypotrichosis are not necessarily lethal in swine, man, mice and rabbits, but in Swedish Holstein cattle Mohr and Wriedt (1928) found an almost complete hairlessness which causes the death of affected calves within a few minutes after birth. Except for a few hairs on the muzzle, eye-lids, ears, horn processes, pasterns and the distal part of the tail, the entire skin is completely hairless. The teeth and hoofs are normal. On histological examination the hair follicles of the hairless areas were

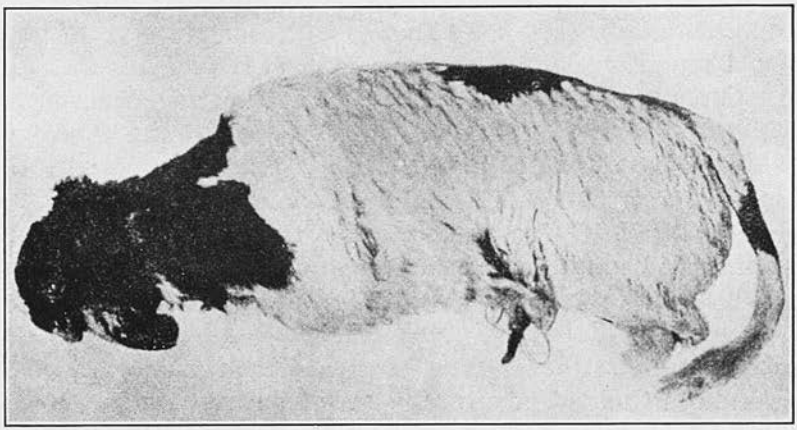

Figure 3. Hereditary amputation of the limbs. (*From Mohr.*)

found to be retarded in development, presenting at birth the same appearance as is normal in early embryos. On the other hand, the sebaceous glands had undergone premature development and exhibited cystic enlargement. Skin of the few areas bearing hair is normal. Twelve such calves all traced to one imported sire. The character is a simple recessive.

5. *Acroteriasis congenita.* (Figure 3) Thirteen calves, all traced back to the most famous bull in the Swedish Holsteins, were found by Wriedt and Mohr (1928) to be afflicted with hereditary malformations of a most remarkable nature. They were either still born or died soon after birth.

The eyes protrude and the eyelids are small. The ears are short and asymmetrical. The face is deformed by the downward turning of the upper jaw and by the rudimentary condition of the lower jaw which lacks most of the teeeth. The palate is cleft. The tongue is normal and protrudes pendulantly. Most remarkable of all is the fact that the forelegs are terminated at the elbow and the hind limbs at the hock joint. The ends of all four are bluntly rounded and covered with hairy skin. The viscera are normal, as are the pelvis and the axial skeleton.

This abnormality has very fittingly been called "amputated".

6. *Short spine.* (Figue 4) In the Oplandske breed of Norwegian Mountain cattle Mohr and Wriedt (1930) found 11 calves from a single sire which exhibited in remarkably uniform fashion a peculiar hereditary abnormality. The head,

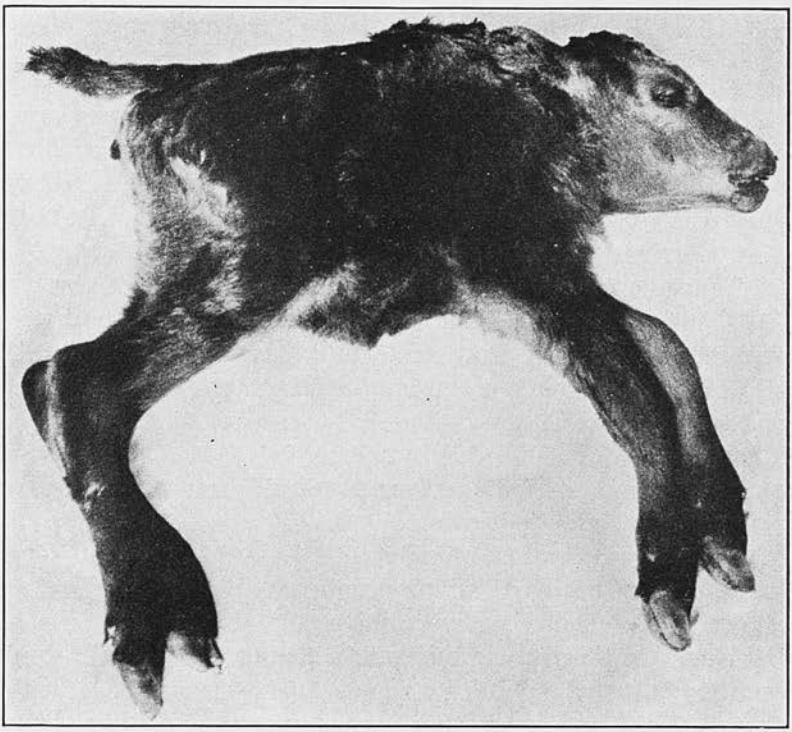

Figure 4. Short spine. Prior to genetic investigation the appearance of such a calf led farmers to believe that it had been sired by an elk. (*From Mohr and Wriedt.*)

teeth, limbs and hoofs are entirely normal but the whole vertebral column is shortened. The neck is so short as to be barely evident and in dissected specimens all the cervical vertebrae except the atlas were solidly jammed together. The tail is very short, measuring in one specimen only 12 cm., and the shortening of the spinal column gives it the appearance of having an abnormally high insertion. Only 5 vertebrae could be recognized in the thoracic region, where 13 is the normal number. There is a very slight curvature of the ribs.

Affected calves are born at full term, but if not still born, die almost immediately, death being due to suffocation during parturition. The latter process is very difficult.

7. *Mummified foetuses.* Loje (1930) has reported an unusually high incidence of mummified foetuses in certain matings of Red Danish dairy cattle. Most of the calves apparently died during the eighth month of gestation, but were carried to full term. Those aborted earlier showed less evidence of mum-

mification. Since the latter condition is the result of death and not the cause of it, it can not be considered as the hereditary character.

The visible departures from normality were fewer than in other lethal characters but the neck was short, the legs stiff and the joints prominent. The hoofs were well developed. *Hydrops amnii* was constantly associated with the condition. In many cases veterinary aid was necessary to overcome dystocia.

Ten such foetuses were produced by daughters of the bull, Oluf Godthaab, and all ten were sired by one or another of three descendants of that same bull. In these matings the ratio of normal calves to mummified foetuses was 22:10, the expectation in this case being 28:4 (see p. 21). By other bulls these cows produced only normal calves and similarly the sires of the mummified foetuses produced only normal calves when bred to unrelated cows.

8. *Lameness in the hind limbs.* Loje (1930) also found Red Danish calves born alive and apparently normal in every respect, except that they were incapable of standing on their hind legs. One such calf was fixed in a sling for some time, but, even though it thrived and grew, the lameness was not lessened. All such calves had to be slaughtered, since they could not have survived without very much assistance.

This defect appeared at about the same time on three different farms where nothing of the sort had previously been noticed. In one series of matings of somewhat inbred animals a ratio of 14 normal to 10 lame was found. In another there were 30 normal to 4 lame. The material is not suitable for the calculation of expected ratios, but all the evidence together justifies Loje's assumption that the condition is a simple recessive character.

9. *Muscle contractures.* Mohr (1930) has briefly mentioned the occurrence of congenital muscle contractures in cattle in Norway. An apparently identical condition is reported from Minnesota by Hutt (1934). In the latter instance the affected calves were alive at full term but were so abnormal that three of five cases had to be dismembered to overcome dystocia and the other two died during or after birth. The head was drawn up toward the back and the neck was extremely rigid. The fore and hind limbs were folded and almost wrapped around the body. They were extremely rigid, there being

practically no movement at the joints. All five calves were the same. All were sired by the same bull; in three cases the cows producing them were his daughters and another was a half-sister. The character is apparently a simple recessive. It occurred in Holsteins.

This lethal is an unusually serious one because it is fatal, not only to the homozygous calves, but also, in some cases, to the cows which carry them. One heifer was fatally injured in delivering the abnormal calf and another was subsequently sterile.

10. *Ljutikow's lethal.* A great deal of data has been presented by Ljutikow (1932) indicating that in a Swiss breed of cattle (presumably Brown Swiss) in Russia there occurs a lethal character different from any of those described above. Unfortunately very little description is given but it would appear to be characterized by short legs and under-developed toes. There is almost no indication of two separate digits in some cases and only a slight division of the hoof in others. The character is not achondroplasia. In most cases the calves are aborted prematurely but no trace of the Bang organism was found in one specimen examined bacteriologically.

When animals which had produced the abnormal calves were crossed with local Caucasian cattle only normal calves were produced. Other data, more extensive than are usually available in investigations with cattle, show clearly that the character is a simple recessive. One series of heterozygous cows bred to heterozygous sires yielded 150 normal: 58 abnormal, a very close fit to the expected 3:1 ratio. It was also shown by breeding tests that when heterozygous sires were mated with normal cows which had not produced the abnormality, half of the daughters received the lethal gene from the sire. Extensive data on bovine abortion at other experiment stations are given by Ljutikow, but it is not clear how much of the loss was attributable to the lethal character found in Swiss cattle and how much to other causes.

11. *Ankylosis of the lower jaw.* Mohr (1930) has briefly described and illustrated a recessive character in which the lower jaw is greatly shortened and completely ankylosed. The abnormality appeared in the progeny of one bull of the Norwegian Lyngdal cattle, but genetic data concerning it have not yet been reported.

and paralyzed pigs in the ratio of 71:25, indicating it to be de-

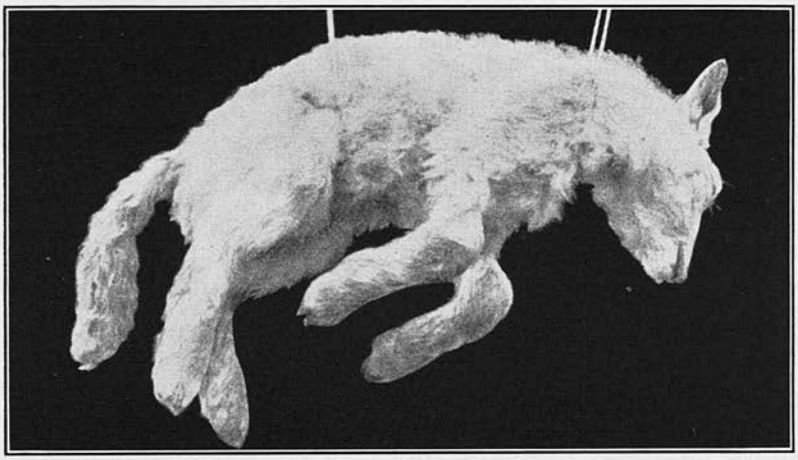

Figure 5. Muscle contracture in the fore limbs. The lamb is suspended by cords to show that the limbs cannot straighten out. (*From Roberts.*)

SHEEP

1. *Muscle contractures.* (Figure 5) A condition in which the limbs are rigidly fixed, permitting only very slight movement at the joints, has been studied in detail by Roberts (1929). Prior to birth of an affected lamb there is marked *hydrops amnii.* Wryneck is usual. Sometimes all four limbs are rigid but in other cases only one pair of limbs, usually the front ones, are affected. In three specimens affected with contractures which were examined in great detail by Mr. Wm. Miller, the hip joints were quite free and the shoulder joints were free in two cases. All lower joints of the limbs were rigid except in one specimen in which the fetlocks of the hind limbs were free. Parturition is difficult, the removal of a limb being sometimes necessary, and a certain proportion of the ewes die as a result.

Only one of over 50 cases investigated was born alive, and it lived only three days. The genetic evidence clearly showed this condition to result from a single recessive gene. It was first noticed in Welsh Mountain sheep but was later found in a Longwool breed and in a Down breed, as well as in another variety of Mountain sheep.

2. *Rigid fetlocks.* Zophoniasson (1929) has described an hereditary abnormality encountered in Iceland. The body of affected lambs is short and thick and the skull unusually large, with the upper jaw compressed and short, and the lower jaw always shorter than the upper. The wool is short and free

from crimp. A strip from brisket to anus is devoid of wool. Behind the navel is a small hernia in which part of the intestine lies. The legs are bent at the fetlocks and cannot be straightened. Nearly all lambs are born alive but, though they creep about on their knees, they cannot stand and eventually die without having suckled the ewes.

Two "carrier" rams mated to daughters of carrier rams produced 77 normal to 17 abnormal lambs. The expectation (7:1) for a simple recessive in this type of mating is 82:12, so that the agreement is quite close. Other confirmatory evidence was also established.

3. *Paralysis of the hind limbs.* Zophoniasson (1929) has also reported a condition of lameness in lambs very much like that observed by Loje in Red Danish calves and by Mohr in swine. The lambs are born alive but with the hind limbs partially paralyzed, so that they can hardly stand to nurse the ewe. Sometimes they die, but, if carefully handled, some will live, although they always remain partially paralyzed. They appear to be mentally subnormal. Since such lambs could never survive in nature, the condition is a lethal character. When one ram was mated for three years to his own daughters, there were produced 102 normal: 16 partially paralyzed, almost a perfect fit to the 7:1 expectation for a simple recessive gene.

4. *Homozygous grey.* In the Tzourcana sheep of Roumania are found black, white, spotted, and grey animals, the fleece of the latter being a mixture of black and white. In genetic studies of these colors, Constantinescu (1932) found that while blacks breed true, greys do not. Seven grey rams mated with grey ewes, all produced one or more black lambs and of 36 such ewes in these matings 25 produced black lambs. The other 11 were not adequately tested but were assumed to be also heterozygous for grey. The proportion of grey to black offspring was 78:35, which is closer to a 2:1 ratio than the 3:1 expected if the greying condition were caused by a simple dominant factor. The shortage of grey offspring and the failure to find a homozygous grey animal led Constantinescu to conclude that homozygosity for grey is a lethal condition. This might mean either that the greying gene is lethal when homozygous, or that some other lethal gene is so closely linked with that for grey that the two act as one.

There is ample precedent for such an association between a color and a lethal condition. It has been shown conclusively

that the homozygous yellow mouse and the homozygous white canary perish at early stages of development and that only heterozygotes survive. Similarly mice homozygous for dominant white spotting perish soon after birth from anæmia.

While the evidence for the lethal nature of the homozygous grey condition is good, it might be confirmed by analyses of the breeding records. If one-quarter of the offspring of these matings perish, the number of lambs per ewe should be considerably less in matings of grey x grey than in crosses of grey x black or of black x black. However, if the lethal effect were exerted very early in gestation, the ewes might be bred again, in which case the grey x grey matings should show a higher proportion of ewes coming back to the rams than is found in other matings.

Constantinescu suggests that the same condition is found in the Shiraz or grey Karacul sheep.

5. *Mohr's lethal.* Pending a detailed description this designation is applied to an abnormality briefly reported from Norway by Mohr (1929). The syndrome includes earlessness, cleft palate, shortened and ankylosed lower jaw and usually tripartite claws. The character is somewhat variable in its expression. No genetic data have yet been given but the abnormality is said to be a simple recessive.

6. *Amputations.* Kroon and Van der Plank (1931) have reported the occurrence in related stock of 5 lambs with limbs amputated at the fetlocks, so that digits are lacking on all four feet. The condition is somewhat similar to that in the "amputated" calves studied by Mohr. No genetic data have yet been reported.

SWINE

While several investigators have shown that there is a rather high rate of foetal mortality in swine, and a number of abnormalities have been reported, few of the latter have yet been shown to be definite genetic entities. Those which have been adequately investigated are listed below.

1. *Paralysis of the hind limbs.* Mohr (1930) reports an hereditary condition in which the homozygous piglings have complete paralysis of the hind limbs. Since they could not be raised except by special treatment, the character is definitely a lethal one. Heterozygotes mated together produced normal

See last line p. 9.

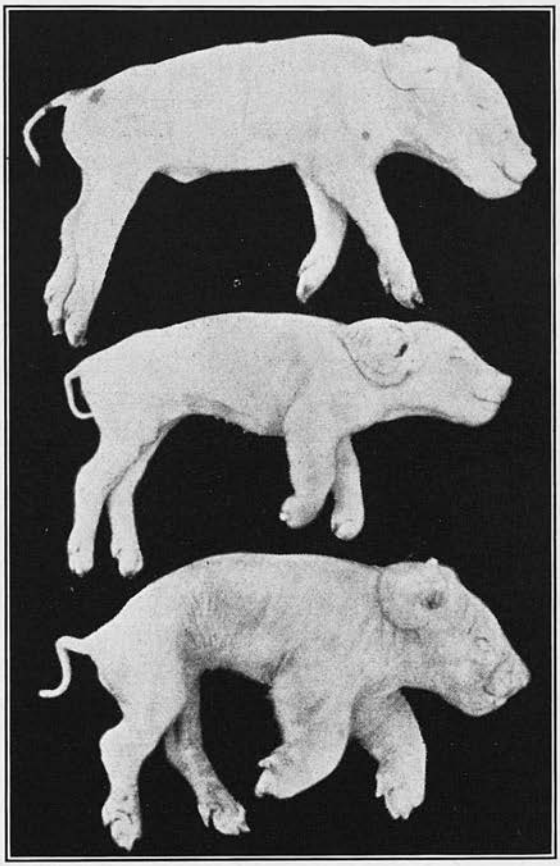

Figure 6. Thick fore limbs. Of these three litter-mates the upper is normal and the two lower show different degrees of the lethal abnormality. (*From Walther et al.*)

pendent on a single gene.

2. *Thick fore-limbs.* (Figure 6) Walther, Prufer and Carstens (1932) have studied an abnormality in which the fore-limbs are greatly swollen. The underlying cause is a displacement of muscle fibres by a gelatinous infiltration of the connective tissue, particularly in flesh closest to the bones. The latter are also somewhat thickened. The character is extremely variable but is sharply differentiated from the normal condition.

Affected animals are born at the same time as the normal pigs in the litter but die shortly after birth. Only one lived as long as 48 hours. In 5 litters from heterozygous animals the ratio of normal to abnormal pigs was 51:13, the theoretical expectation being 48:16. The character is evidently a simple recessive.

3. *Muscle contracture.* In a Swedish breed resembling the Yorkshire, Hallquist (1933) has thoroughly studied an hereditary abnormality similar to the muscle contractures occurring in sheep and cattle. Usually only the fore-limbs are affected and these are quite rigid. In exceptional cases only one is affected. The variability of the condition is very slight. Affected piglings are usually born dead and the few born alive die within one or two hours. Apart from the rigidity of the limbs, the affected animals are apparently normal.

All carriers of the gene for this character were descended from an outstanding sire whose blood-lines had been spread to almost all Swedish breeding herds. From experimental matings of carriers together, there were produced 220 normal piglings and 46 with rigid limbs. The deficiency of the latter below the 25 per cent. expected for a simple recessive character may indicate that some of the affected foetuses died at earlier stages and were resorbed. In matings of heterozygous animals to unrelated stock there were produced 222 pigs, not one of which exhibited the abnormality.

4. *Atresia ani.* Experiments of Kinzelbach (1931) show that there is a hereditary basis for this abnormality in swine, but that it does not segregate as a simple recessive character. The condition can be overcome by operation to make an artificial anus, but otherwise it is fatal. Kinzelbach found it occurring with unusual frequency in litters sired by related boars, and from critical matings in which both boar and sow had originally had *atresia ani* there were obtained 7 normal and 4 with the abnormality. In various matings of these progeny, there were obtained 37 piglings of which 9 had *atresia ani.* In 93 other cases of the abnormality, only 17 per cent were females. There is evidently a genetic basis, albeit an irregular one, for the abnormality.

THE HORSE

1. *Atresia coli.* In the descendants of a Percheron stallion, Superb, imported into Japan from Ohio in the latter part of the 19th century, Yamane (1927) has traced a lethal character in which death results from complete closure of the ascending colon in the region of the pelvic flexure. Affected foals are born alive but usually cannot stand. They show symptoms of colic about an hour after birth and all die within four days. The contents of the rectum, often exuded from the

anus, are quite different from meconium. The swollen colon can be detected by palpation of the left flank. On post-mortem examination, the caecum and lower colon are found full of meconium, while the upper colon and rectum are quite empty. Cerebral glioma is present in some cases and hydrocephaly in others. Following inbreeding to Superb and his offspring, the character appeared in frequencies indicating it to be a simple recessive.

Three cases of *atresia coli* in the progeny of one stallion were also reported from Germany by Nusshag (1925).

2. *Sex-linked lethal.* It is easier to detect lethal genes which exert an effect late in embryonic life, or after birth, than those causing the death of an embryo at very early stages. Some representatives of the latter class are recognized by reason of their being located in the same chromosome as some "marker" such as a gene for a certain color, pattern, or morphological character. Any deficiency of these "marker" characters suggests the operation of a lethal gene. Examples of this type are the lethal apparently linked with grey in sheep, (p. 11) and Dunn's lethal linked with recessive white in the fowl, (p. 17). Similarly, when a lethal gene is located in a sex-chromosome, its presence can be detected by a marked deficiency of one sex. In the fruit fly, Drosophila, whose inheritance has been studied more completely than that of any other animal, sex-linked lethals are more abundant than those in any other chromosome. It is to be expected, therefore, that they should be not uncommon in other species.

Evidence that one of these occurs in the horse has been found by Kisslowsky (1932) in examination of the stud-books of the Oldenburger breed. In the first four generations of descendants of the mare, Jelka, there was a sex ratio of 55 males: 90 females, a significant deviation from the usual equality.

The chromosomes of the horse (and of other mammals) are such that a sex-linked lethal would be expected to destroy half the male progeny of a female carrying it. Female offspring would be unaffected, but half of them would receive the lethal gene and would, in turn, give ratios of approximately 1 male: 2 females regardless of the sire, while half would not receive the lethal gene and would give only normal sex ratios.

These conditions were realized in the descendants of Jelka. Moreover, Kisslowsky was able to show that, in the lines of

descent believed to carry the lethal gene, the reproductive efficiency, (taking one foal per mare per year as 100 per cent.) was only 68 per cent., whereas in the lines presumably not carrying the gene it was 73.8 per cent. The difference supports the other evidence that a lethal gene was operating in this line but is smaller than was expected. However, if the gene were operative at a very early stage, for example, before implantation of the fertilized ovum, the mares need not miss the whole breeding year, but could be gotten in foal at a later date. The interval between foaling and being settled should then be somewhat longer in the lines carrying the lethal than in their normal relatives, and this was found to be the case.

Although herd-book data are not the most desirable materials for the study of sex ratios or of lethal genes, Kisslowsky's findings are quite in accord with expectations for a sex-linked gene in this species.

DOGS

1. *Cleft palate.* A strain of bull dogs producing puppies with cleft palates was studied by Wriedt (1925). The defect permitted the mother's milk to run out through the nose of the nursing puppies. Since most of them starved to death during the nursing period, the character may properly be classed as lethal. Six matings together of animals carrying the defect yielded 24 normal and 9 with cleft palate, an almost exact fit to the expectation for a simple recessive character.

Wriedt has also described an association between the dappled-white color in Norwegian Dunkerhounds and various physiological deficiencies, none of which is extreme enough to cause death *per se.*

2. *Hairlessness.* The so-called hairless breeds of dogs—Mexican, Turkish and Chinese—are not completely hairless, since short and fine hair is usually found on the head, tail and feet. The dentition is faulty. From a study of such animals Letard (1930) confirmed previous indications that hairless dogs are heterozygous for this characteristic and, when bred to normal dogs, produce approximately equal numbers of hairless and normally-haired offspring.

Although the data were not extensive, there was some evidence that the homozygous hairless puppy is either born dead, in which case it lacks external ears and exhibits gross abnormalities of the buccal cavity, or is born alive but perishes

in a few days because of complete occlusion of the lower part of the œsophagus.

THE FOWL

1. *Recessive white lethal.* A great number of lethal genes exert their effects at such early stages of development that the dead embryos are resorbed and no abnormality is noted other than a rather high rate of embryonic mortality. Such is the case with a lethal gene found by Dunn (1923) to be linked with the gene for the recessive white of White Wyandottes. It was detected only as a result of males heterozygous for black and for white being back-crossed to related White Wyandotte females. Some of them yielded such a marked deficiency of white offspring, where equal numbers of black and white should have been obtained, that it was obvious that approximately half of the white birds had died.

This is an interesting example of how a lethal gene may be detected, though its effects are not visible, by its linkage with another gene. If in this case only White Wyandottes had been mated together, there would have been a rather high early embryonic mortality, but none of it could have been traced to the single gene linked with recessive white.

2. *Congenital loco.* (Figure 7) In this condition the chicks are unable to stand when hatched. The head is drawn over the back, with the beak pointing upward, usually on one side. Eventually the chick falls over and lies on its back or side, with its feet in the air until, after a rest, it rights itself and the whole performance is repeated. The basis for the abnormality has not yet been determined.

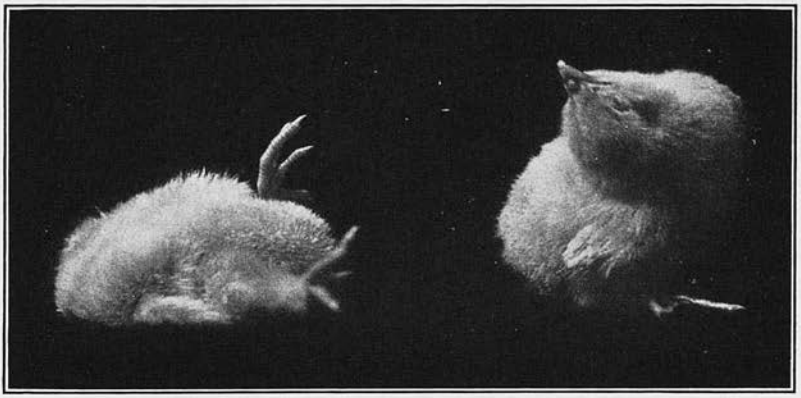

Figure 7. Congenital loco. The chick on its back has gone through the stage exhibited by the other and has then lost its balance completely. (*Original*)

Knowlton (1929) who designated the condition "congenital loco" proved conclusively that it is a single recessive character. Fowls heterozygous for the character are normal in every respect, but from matings of such fowls together there were produced 146 "loco" chicks in a total of 607.

3. *Creeper.* (Figure 8) The Creeper or Scots Dumpie fowl is characterized by extremely short legs, all bones of which are reduced in size, particularly the tibia. The fibula is more developed than in normal fowls and is firmly attached to the tibia near the distal end of the latter. When Creepers are mated together, the progeny consist of Creepers and normal-legged birds in the proportion of 2:1.

In what is undoubtedly the most exhaustive investigation yet made of a lethal gene in vertebrates, Landauer and Dunn (1930), with a total of 4500 chicks and embryos, have conclusively demonstrated that all Creepers are heterozygous and that the homozygotes all perish during incubation, the great majority doing so on the fourth day after incubation has begun.

The creeper character is obviously due to a dominant gene which is lethal in the homozygous condition. Strictly speaking, the gene is only partially dominant, since the deviations from normality found in the heterozygote are relatively slight compared with those in the homozygote. The few of these that survive to the end of the incubation period exhibit extreme phokomelia—the humeri and femora are lacking or nearly so, and other bones of the limbs are so shortened that the feet seem attached to the body without legs.

4. *Sticky embryos.* Byerly and Jull (1932) have studied a hereditary abnormality fatal to chick embryos, usually during the last three days of incubation. The amniotic and allantoic fluids are unabsorbed and are viscous and sticky. Oedema is general but variable in degree. The embryos are somewhat smaller than normal and when the unutilized yolk is drawn into the abdomen, the latter appears much more distended than in normal chicks. The bones are soft and rubbery, being markedly deficient in calcium. The tibiae are usually bent. Genetic investigation showed that it is caused by a single recessive gene.

5. *Congenital palsy.* Hutt (1932) has described a peculiar condition of palsy, or tremor, in newly-hatched chicks. In extreme cases the bird is unable to stand, but in others the affected chicks appear normal except for a tremor, which is

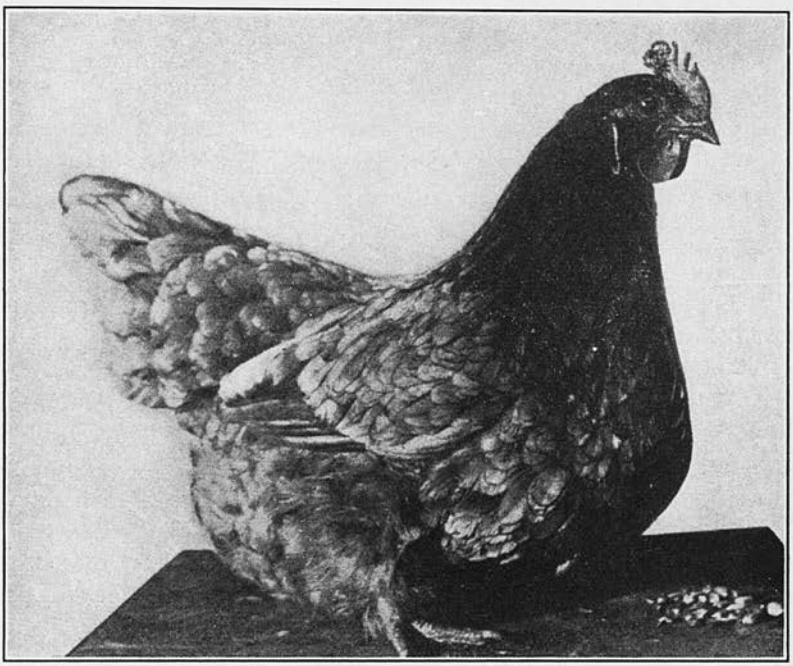

Figure 8. Creeper. All such birds are heterozygous for the creeper gene and none can breed true. (*From Cutler.*)

evident as long as the bird stands. Extremely palsied chicks die within a week of hatching; few live longer than three months, but, to date, two have been raised to maturity, so that the character is not lethal in every case. It appears to be a simple recessive.

THE DUCK

Crest. From the studies of Rüst (1932) it would appear that embryos homozygous for crest perish when almost ready to hatch because of extreme cerebral hernia. The upper beak is reduced to a nubbin. Embryos carrying a single gene have normal viability and about half of them are crested, the remainder showing no signs of it whatever. Avian embryos occasionally exhibit teratological malformations similar to those described, without any apparent genetic basis for their occurence, but the frequency with which these conditions occurred in progeny of crested ducks was too great for any other interpretation than that they were hereditary. Since the inherited character is not merely the crest, but an occipital encephalocoel which gives rise to the crest, Rüst's interpretation seems a very likeley one, though further genetic investigation is necessary.

GENERAL CONSIDERATIONS

There have been listed in this review 31 hereditary characters in domestic animals, 25 of which may be classed as fully lethal, two are semi-lethal and four are apparently lethal but need further investigation. The genes for these characters fall into the following four categories:

Class 1. Dominant, or partially dominant genes, exerting a visible effect upon the heterozygote and lethal to the homozygote, e. g. dominant achondroplasia in cattle, hairlessness in dogs, the creeper character of fowls.

Class 2. Recessive genes, not visibly affecting the "carrier" but lethal to the homozygote and recognizable because the lethal effect is exerted late in foetal life or after birth, e. g. hairlessness and recessive achondroplasia in cattle, muscle contractures in cattle, sheep and swine.

Class 3. Recessive genes whose effects are exerted so early in embryonic life that their existence is detected only by virtue of their being linked with a gene for some morphological or color character, e. g. the lethals linked with recessive white in the fowl and the greying gene in sheep.

Class 4. Sex-linked lethals, recessive in action, detected either by a deficiency of one sex (if operative early in ontogeny) or by an abnormality associated with one sex, if the gene exerts its effect at later stages.

Since all of these lethal genes are responsible for a considerable reduction in reproductive efficiency, and consequently (with an exception to be noted below) for no small economic loss, it is essential to consider how to prevent their spreading.

Loss from lethals of Class 1 can be easily avoided by not breeding from those animals obviously carrying a gene for the lethal. If breeders wish to practise pure-breeding with Dexter cattle and to stand a 25 per cent. loss of calves, as well as the loss from the 25 per cent. long-legged off-type animals produced, that is their expensive privilege. Otherwise, the obvious alternatives are either to discard the Dexters or to breed them, as was done in Ireland, to Kerry cattle. From the latter mating no bull-dog calves result, half the progeny are Dexters and half are Kerry type. Similarly, creeper fowls can be perpetuated by crosses with normal ones.

Loss from lethals of Class 3 can be eliminated by avoiding matings involving the tell-tale marker character. If the

latter be a particularly desirable breed characteristic it would be worthwhile to examine the possibility of obtaining a cross-over, or separation of the lethal gene from its marker. This would be possible if the two were not located too closely together in the chromosome.

The great majority of lethal genes fall in Class 2. Here the heterozygous carrier can only be detected by suitable breeding tests. Such a test is most rapidly made by mating the suspect to one or more animals known to be heterozygous for the lethal suspected. The difficulty is that no breeder is likely, knowingly, to retain in his herd an animal proven to be the carrier of a recognized lethal character, any more than Holstein breeders will now retain animals which have produced red calves. Moreover, even where a simple recessive character is concerned, although the probability of a sire's being free from a certain lethal gene increases with every normal offspring (from heterozygous females), one could not be reasonably sure that he lacked the undesirable gene until 10 or more normal progeny had been born without the appearance of the lethal character. If only two or three animals known to be carriers of the lethal were available for such a test, the male would be almost past the period of his maximum efficiency before it could be made unless, by chance, the lethal character were to appear early in the tests. The probability of getting a recessive in any single offspring of a mating of two carriers is 1 in 4.

If known heterozygous females are not available for such a test of a suspected male, the alternative is to mate him to his own daughters. If a study of the pedigree reveals that the lethal gene was carried by the suspect's sire, then half-sisters on the sire's side can be used for the test as well as the suspect's daughters, and similarly if the sire's dam were a carrier, half-sisters on the dam's side can be utilized. The expectation in such matings is that half of the females will have received the lethal gene and half will not. If the male is free from the taint no abnormal progeny whatever will appear, but if he is heterozygous for the gene, the lethal character may be expected in 1 out of 7 offspring. Obviously about 20 offspring would be necessary to answer the question.

However, once such a test has been made, and a sire proven not to be a carrier of the suspected lethal gene, his *male progeny can be utilized* without further test and with full confidence that none of them can transmit the undesirable gene to his offspring.

Breeding trials of this nature can be carried out fairly quickly with swine and with smaller animals. They would not be too slow to be effective with sheep. It is doubtful, however, if individual breeders of cattle and horses are likely to stand the expense and time necessary to purge a herd of its defects in this manner. The loss of a colt or of a calf of beef breeds is undoubtedly serious, but to some breeders of dairy cattle, the loss of a few calves might be relatively unimportant. The latter viewpoint would certainly not be entertained by breeders of pure-bred stock commanding good prices and for such persons it would be almost imperative to eliminate hereditary defects from their herds. Failing private enterprise, this objective might possibly be attained by cooperation of breeders and the state, with resultant maintenance of a herd of "carriers" to be used for the testing of suspected sires in lines of breeding too valuable to be sacrificed. It is not inconceivable that some of the obvious difficulties confronting such a project might be overcome by the use of artificial insemination, the technique of which has been greatly improved in recent years by investigators in Russia.

DETECTION OF LETHAL CHARACTERS

The fact that the great majority of known lethal characters have only been discovered within the last six or eight years, suggests that many more will be identified in the future. It is hoped that the foregoing discussion will suggest to veterinarians ways and means by which genetic abnormalities may be distinguished from the non-genetic teratological conditions not infrequently encountered in practice. The occurrence of two cases of *Schistosomus reflexus* in one herd does not prove that it is hereditary, and some conditions are known (e.g. rumplessness in the fowl) in which a hereditary abnormality is almost indistinguishable from a similar condition resulting from an accident during development. The ear-marks of a recessive lethal character may be listed as follows:

1. Recurrent similar abnormalities in the progeny of related animals.
2. Descent of "carriers" from a common ancestor.
3. A 3:1 ratio of normal to abnormal offspring when carriers are mated together.
4. A 7:1 ratio of normal to abnormal offspring when a carrier is mated to his daughters.

It is evident that lethal characters are most likely to appear following inbreeding. Those best studied in cattle and the one in the horse have been brought to light as a result of varying degrees of inbreeding to some outstanding sire. Similarly the inbreeding resulting from geographical isolation has been instrumental in revealing lethal genes in such different regions as the mountain valleys of Norway, the island of Iceland, and a frontier agricultural area in Minnesota.

DESIRABLE LETHALS

While pratically all lethal genes in domestic animals cause considerable financial loss, there is one case in which that loss might be more than compensated for by attendant benefits. If a sex-linked lethal, operative at a very early stage of development, could be found in any of our breeds of dairy cattle, it would probably be welcomed by most breeders of such animals.

The effect of such a gene would be to cause the females carrying it to produce approximately twice as many heifer calves as bull calves. Such a desirable accomplishment would be offset by the fact that approximately once in every four gestation periods there would be some delay in getting the cow in calf. Half of the female progeny would carry the lethal gene and breeding tests would reveal which daughters should be used to perpetuate the line.

To the writer's knowledge such a gene has not yet been found in cattle but from what is known of the other species there is every reason to suppose that some day sex-linked lethal genes will be discovered in cattle and used extensively in dairy herds to reduce the number of unwanted bull calves. A gene having the same effect would be welcomed by nearly all breeders of Leghorn fowls, but, unfortunately, the chromosomes of birds are such that a sex-linked lethal gene would kill half of the more desirable sex—the females.

COMPARATIVE GENETICS

It is worth noting that hereditary defects in one species are likely to occur in others belonging to the same taxonomic group. For example, in three domesticated representatives of the Artiodactyla—cattle, sheep and swine—there have been found recessive lethal genes for paralysis of the hind limbs, and for rigidity of one or more limbs apparently resulting from muscle contractures. Hereditary amputation of the limbs

has been encountered in cattle and sheep, both members of the family Bovidae. (It is also a hereditary character in man.) It is not unlikely, therefore, that some of these lethal characters thus far known only in one species may subsequently be found, with or without modification, in others.

SUMMARY

An annotated list is given of hereditary lethal abnormalities in domestic animals. Of these there are 11 in cattle, 6 in sheep, 4 in swine, 2 in horses, 2 in dogs, 5 in the fowl and one in the duck. Ways and means of detecting such characters and of preventing their dissemination are discussed.

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THE GENETICIST'S OBJECTIVES IN POULTRY IMPROVEMENT

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THE GENETICIST'S OBJECTIVES IN POULTRY IMPROVEMENT[1]

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THE average producer of poultry and eggs would probably be satisfied if all members of his flock could mature in less than seven months and convert in a year about 95 pounds of feed into 200 eggs with an average weight of two ounces each, if mortality in that period were less than 10 per cent., if two thirds of the flock were worth keeping a second year and if his breeders could reproduce from 85 per cent. of their fertile eggs chicks capable of repeating the whole performance. To these desiderata may be added, as minor considerations, the special demands of the New York customer for white eggs, of Bostonians for brown ones, and similar local whims elsewhere, as well as the special needs of these poultrymen who produce the heavy fowls which poultry markets prefer.

These objectives, though modest, are much more easily stated than attained. In the first decade of the present century the economic needs of the poultryman were much simpler and the chief objective of those then seeking to breed better poultry was to produce fowls capable of laying more and still more eggs. This view-point has changed gradually with the realization that the market would pay a premium for large eggs, that enormous losses resulted annually from the failure to hatch of 35 per cent. or more of the eggs incubated, and, in later years, that mortality of pullets during their first laying year might run from 20 to 80 per cent.

Attainment of the geneticist's objectives in poultry improvement is complicated by two serious obstacles. In the first place nearly everything that he desires is dependent upon multiple factors rather than upon easily

[1] Read at a symposium on "Breeding to Meet Economic Needs" before the Genetics Society of America, American Association for the Advancement of Science, Indianapolis, Ind., December 30, 1937.

manipulated single genes. A slow process of progeny-testing is inevitable. Secondly, the attainment of any one of his several objectives is of little value without most of the others. A strain capable of high egg-production is undesirable if it can produce only 50-gram eggs for a market demanding a 24-ounce dozen. Of several different lines of Leghorns in the Cornell flock, that with the greatest viability during the past two years is utterly worthless economically because it has been so consistently bred for low fecundity that few of its members now produce as many as 100 eggs in a laying year. However, keeping before him the encouraging example of those plant breeders who have produced spring wheats not only resistant to many physiologic forms of stem rust, but also with stiff straw, high yield and good milling quality, the poultry geneticist struggles onward toward his *ultima Thule*.

To reach his objectives efficiently the geneticist really needs a great deal more information than is now available about those same objectives. This means that he must carry out two different programs. One of these seeks to sift the germplasm of his fowls as rapidly as possible through the sieve of the progeny test, to accumulate valuable genes and to eliminate those undesirable. The other program is concerned with the discovery of genes affecting the phenotypes desired and of means for determining more accurately the true genotype behind the observed performance. It seeks more light on the interaction of genes, on their manifold effects, their interactions with different environments and to find those outward and visible signs of inward and physiological grace to which the geneticist refers as an association between morphological and physiological characters.

In the following exposition of some of the more important objectives an attempt is made to show the way in which the straightforward work of progeny testing is interrelated with attempts to extend what is now known about the genetics of the fowl.

VIABILITY

While extensive losses occur during embryonic development and in the period of growth, nothing exceeds in importance at the present time the need of reducing by every means possible the prevailing high mortality among pullets successfully reared to breeding age.

Most of the figures quoted as measures of mortality in farm flocks are misleading because they omit from consideration birds culled as unprofitable producers, a class in which mortality is known to be much higher than among good layers. A better measure of mortality is obtained from the official laying tests, where birds from different sources are compared under uniform environment, and where all birds entered are kept for 51 weeks. Only carefully selected birds are sent by the breeders to these laying trials. They are kept in small flocks of 13 to 26 birds, under conditions in which epornithic outbreaks of disease are rare or absent, and where the social pressure on the weaker birds is much less than in large flocks.

Tabulation of results at thirteen such tests scattered in this country from Maine to Arizona and from Illinois to Florida shows that among 10,239 pullets mortality during 51 weeks in 1936–37 was 24.2 per cent. Losses in commercial flocks are considerably higher, and at few agricultural experiment stations does the mortality fall below 40 per cent. during the first laying year in unselected flocks not bred for viability.

The economic loss is not limited to the market value of the birds which die. Most of them are unprofitable layers for several months before they die (Harris, 1927, 1928). Loss of half the flock means that buildings and labor are not used with maximum efficiency and there is the additional cost of rearing more chickens each year than would be needed if mortality were negligible.

Of what do these birds die? Since the inception in 1931 of two New York State laying tests under the supervision of Cornell University, all birds dying at both have been sent to the New York State Veterinary College for diag-

nosis. An analysis by the writer of six-years' data from the annual reports of these tests reveals the distribution of causes of death shown in Table 1.

TABLE 1

CAUSES OF DEATH IN 1922 FOWLS (EXCLUDING 290 NOT DIAGNOSED) DYING AT TWO NEW YORK LAYING TESTS, OVER THE SIX YEARS 1931–37; 9,893 BIRDS ENTERED, MORTALITY 22.4 PER CENT

Causes of death	Incidence in deaths diagnosed
	per cent.
Neoplasms	38.4
Disorders of reproduction	25.3
Prolapse of oviduct	11.2
Kidney diseases	5.9
Impactions, alimentary tract	3.4
Internal hemorrhage	2.4
Other causes	13.4
	100.0

In this summary the term neoplasms includes tumors, neurolymphomatosis, leucosis, lymphocytoma and similar conditions. Disorders of reproduction include internal laying, ruptured yolks and impactions of the oviduct. Under "other causes" are included a miscellaneous assortment of conditions none of which by itself caused as much as two per cent. of the total mortality.

The significant point is that the conditions apart from "other causes" account for 87 per cent. of the mortality in 9,893 representative birds entered at these tests. The geneticist would feel less responsibility in the matter of reducing these losses if the orthodox techniques of the veterinarians, *i.e.*, sanitation, immunization and elimination of carriers and exposed birds, were as effective against those conditions now most serious as they have been in temporarily controlling fowl pox and pullorum disease. But this they are not. For none of the diseases causing 87 per cent. of the mortality at these tests are known to have any satisfactory preventive measures whatever, apart from breeding.

The rise in mortality is thought by some to have resulted in some way from a general rise in the productivity of fowls which is commonly believed to have occurred over the past twenty years. Conclusive proof for or against

this hypothesis has not yet been established. Unfortunately, the published data on the productivity of fowls in the laying tests of this country are not accurate measures of production because up to 1938 the published figures for average production have been based on the records of only the ten highest birds out of 13 entered per pen.[2]

However, figures from the English National Laying Trials at the Harper Adams Agricultural College show that, while the mortality rate there has risen steadily from 5.2 per cent. in 1925–26 to more than four times that figure in 1936–37, the average egg-production per bird has been slightly lower in the last seven years than in the period 1925–28. This does not prove anything except that a rise

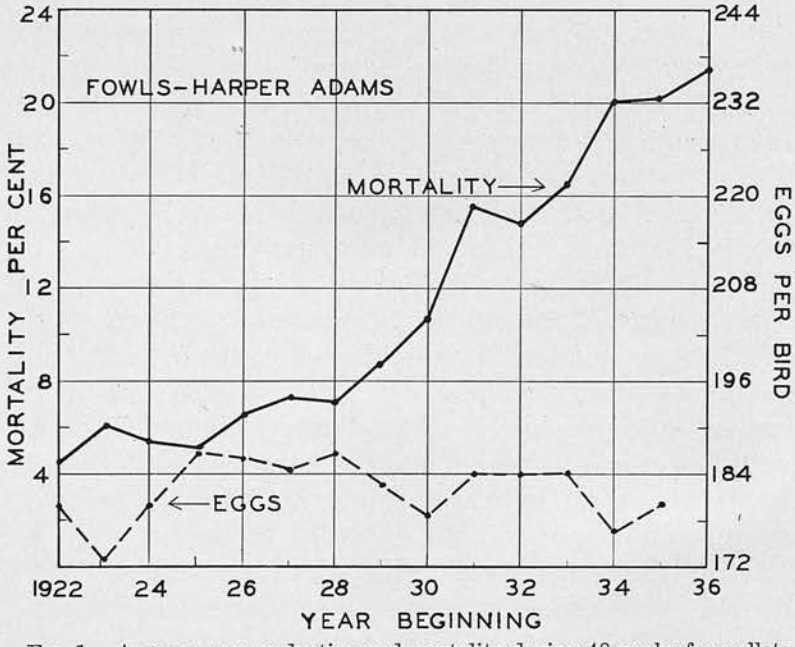

FIG. 1. Average egg production and mortality during 48 weeks for pullets entered in the Laying Trials at Harper Adams Agricultural College, Newport, Salop, England. The data are taken from volumes 8 to 22 of the *Harper Adams Utility Poultry Journal* and cover the laying years from 1922–23 to 1936–37.

[2] These efforts to gild the lily have fortunately been abandoned and standard laying tests will hereafter base their averages on the number of birds entered.

in mortality is not necessarily associated with higher egg yield by survivors. Autopsy records show that deaths in this English trial result from much the same causes as are chiefly responsible at the New York laying test. The rise in the frequency of neoplasms and of disorders associated with egg-laying is by no means a localized condition.

Fortunately evidence is accumulating to show that by proper breeding methods there can be established not only strains of fowls resistant to specific diseases, but also others with marked resistance to disease in general. In five generations of breeding for resistance to fowl typhoid, Lambert (1932) established a strain in which mortality during three weeks following inoculation was only 9.4 per cent., while in controls similarly treated 85 per cent. of the chicks died. Strains with marked resistance to *Salmonella pullorum* have been established by Roberts and Card (1935). While these experiments have been valuable in demonstrating the possibilities of genetic attack on poultry diseases, geneticists are still confronted with the task of producing strains of birds resistant under field conditions to the neoplasms and disorders of reproduction responsible for most of the mortality among adult birds.

How is this to be done? Controlled experiments in which successive generations are treated with standardized doses of a causative agent are desirable, but now almost impossible because of the wide divergence of opinions among poultry pathologists concerning the diagnosis, homologies and transmissibility of the various conditions here considered under the general heading of neoplasms. Pending the establishment of some order out of the existing chaos and the standardization of laboratory techniques, the geneticist must proceed under field conditions. This means careful progeny testing, maintenance of uniform environment for populations under test and such precautions against isolation and excessive sanitation as are necessary to ensure some exposure to whatever causative agents there may be.

Is such a breeding program likely to be effective? The

indications are that it will be. Extensive investigations have shown that in mice strains may be developed which differ significantly in susceptibility to lung tumors, others in susceptibility to mammary tumors, to leukemia or to neoplasms of other kinds. Similar data are not yet available for the fowl, partly because it is only in the last ten years that the seriousness of neoplasms in this species has been recognized. However, experiments seeking to establish genetic resistance to disease have recently been started in at least four state experiment stations and, although detailed reports have not yet been published, the indications thus far have been that mortality is markedly reduced even in the second selected generation. In 1936 the writer began breeding resistant and susceptible strains from a stock in which the mortality of adult pullets had exceeded 50 per cent. in each of the previous three years. In the second generation, hatched in 1937, the mortality among unselected pullets from 160 days of age up to November 28th was already 20 per cent. in the susceptible line but only 8 per cent. in the more resistant line. The latter figure is considerably below that for the corresponding period in the first generation. Both lots have been intermixed at random in the incubators and brooders, on the rearing range and in the laying pens.

The foregoing statement of the poultry geneticist's major problem raises many questions to which answers are not yet available. Are fowls resistant to neurolymphomatosis resistant also to lymphomatosis of the liver? If so, are they resistant to disorders of reproduction, or must we build a complex of many specific resistances, as seems probable from Gowen's (1933) demonstration that strains of mice highly resistant to one disease may be extremely susceptible to another? Lynch (1926) has shown that mice highly susceptible to lung tumors may be resistant to mammary tumors, and *vice versa*. Does this mean that specific resistances must be established for several different organs in the fowl? Under natural exposure will the spontaneous appearance of neoplasms and

other diseases prove adequate to make progeny testing effective, or will the incidence of these diseases be too low to permit differentiation of resistant and susceptible families? Is high egg-production partially incompatible with viability? These and many other problems require the cooperation of geneticists and pathologists for their solution.

Extension of Productive Life

While the most important immediate objective is that of keeping fowls alive for a year's production (*i.e.*, to about 18 months of age), from the long-time view-point extension of the productive life of fowls to three or four years or more is equally important, or more so. Among the survivors of the first laying year, productivity declines rapidly in succeeding years. Hall and Marble (1931) report that for 1,867 Leghorns kept for three laying years the production in their first, second and third years was 169, 146 and 124 eggs respectively. In their fifth year 450 survivors of these same birds, probably retained as superior stock, had an average production of only 96.5 eggs.

The majority of fowls are not kept for egg production beyond their first laying year. This means that the poultryman must bear annually the cost of renewing the flock, made excessively high by losses from infertile eggs, embryonic mortality and deaths during the period of growth.

A few fowls have shown exceptional capacity to maintain profitable production for several years. The most remarkable of these was a Leghorn reported by Hall (1938) which laid over 200 eggs annually (altogether 886) in her first four years, 188, 152, 175 and 114 eggs in succeeding years and died in her ninth with a total of 1,515 eggs to her credit. If the poultryman could only get a flock of such birds, and not have to renew his flock more than once in three or four years, the whole structure of the poultry industry would be altered and eggs could profitably be sold at much less than the prices now prevailing. This will not happen soon. The difficulty of

breeding for any character in which even the phenotype is unknown till the animal is from three to five years old is aggravated by the fact that the reproductive efficiency of fowls apparently declines somewhat after three or four years. These same conditions make difficult any application of the progeny test in breeding for sustained productivity and, if mass selection alone be utilized, progress toward the objectives will be slow. Greenwood (1937) has reported results of breeding which indicate that this very desirable character is amenable to selection and that the obstacles mentioned above are not insurmountable.

Information is urgently needed about the extent to which modification of certain environmental factors, now considered optimum, may influence longevity. The evidence of McCay (1934) and of McCay, Crowell and Maynard (1935) that length of life is extended in the rat by reducing the intake of feed suggests that the present common practice of keeping feed constantly before fowls at all stages of their lives may have an undesirable effect upon viability. There is no critical evidence to support the common belief among investigators in nutrition that the maximum rate of growth is optimum (except where early attainment of market weight is desired) and yet diets during growth are evaluated for farm animals according to their ability to promote rapid growth. Interesting in this connection are the experiments of Dove (1935) which indicate that neither the fastest nor the slowest rates of growth are compatible with maximum viability in the fowl, as measured by survival to one year of age, and that in between the extremes lies the optimum condition.

To encourage breeding for sustained production, less importance should be attached to records of first-year egg production, and more to the performance over a period of three or four years. Poultrymen, as well as breeders of dairy cattle, might well take a leaf from the book of the breeders of East Friesian cattle in Germany, who, according to Köppe (1936), have abandoned records of

performance for a registry of cows which have produced not less than 1000 kg. butter fat and five living calves by the end of their ninth year.

Efficient Reproduction

Estimates of the annual loss in the United States from failure of eggs to hatch vary from 20 to 35 million dollars. In the opinion of the writer this loss is not likely to be reduced by improvement in the environment during incubation or by changes in the mechanical operation of incubators. When these conditions are so satisfactory that 80 per cent. of the fertile eggs may hatch, and when some hens hatch over 95 per cent. of their fertile eggs, it seems likely that more benefit will result from improvement of the viability of the embryos. That this may depend somewhat upon improving the diets for the breeding stock is attested by the adverse effects upon hatchability when vitamins D, E or B_2 (riboflavin) are reduced to abnormally low levels in the diet of breeders, when essential minerals such as calcium and manganese are supplied at abnormal levels, or when a toxicant, such as selenium, is included in the diet.

It is probable, however, that the elimination of lethal genes from the breeding stock will prove the most effective means of raising hatchability. The marked rise in the viability of embryos and hatched chicks which results from suitable crosses (Warren, 1927) suggests that within any one breed lethal genes are to a considerable extent responsible for embryonic mortality. Five genes lethal to the homozygous embryo have thus far been demonstrated and it is certain that more will be found in the future. A recently-discovered sex-linked mutation, "naked," is lethal to about three quarters of the affected chicks (Hutt, 1938). Less is known about genes responsible for mortality after hatching but it seems probable that hereditary conditions, of which "congenital loco" (Knowlton, 1929) and congenital tremor (Hutt and Child, 1934) are examples, are to some extent responsible for the peak of mortality which occurs within two weeks of hatching.

Discovery of lethal genes and the studies necessary to establish criteria for their identification and differentiation, so that they may be eliminated from strains otherwise superior, constitute one of the more important objectives of the geneticist seeking to improve the domestic fowl.

EFFICIENT UTILIZATION OF FEED

Undoubtedly fowls differ, as other animals do, in the degrees of efficiency with which their feed is utilized for growth maintenance and production. The finding of Morris, Palmer and Kennedy (1933) that in nine generations of selective breeding they could establish two strains of rats, in one of which the utilization of feed was only 56 per cent. as efficient as in the other, suggests that similar breeding with fowls might have extremely valuable results. Such work has not yet been done with respect to total intake of feed but there is ample evidence of genetic bases for differential requirements of such limiting constituents of the diet as vitamins and certain minerals.

Geneticists and poultrymen have thus far failed to appreciate the significance of the fact that diets for fowls are now so formulated as to provide protection against shortage of any constituent known to be essential, no matter how extreme the variation among individuals may be with respect to their requirement of that constituent. In other words, the diet is made to protect the weakest and least fit bird in the flock. This is merely enhancing the usual tendency of domestication to permit much greater variation in a domesticated animal than is possible in the same species in nature.

The case may be illustrated by the conclusion of Ringrose and Norris (1936) that the minimum requirement of vitamin A for Leghorn chicks during the first eight weeks of life is about 150 U.S.P. units of that vitamin per 100 grams of feed. In their experiments 50 chicks receiving only two thirds of that amount were slightly lower in weight than those receiving 150 units, though not significantly so, but only one out of 50 exhibited any symptoms

of deficiency of vitamin A. While the protection of this one chick is undoubtedly desirable from the view-points of both the feed-manufacturer and the poultryman, it is obviously preferable from the ethnocentric standpoint to maintain the level of vitamin A at 100 units and pass along to posterity strains of fowls from which the extreme variants in the direction of unfitness have been systematically eliminated. It would seem, therefore, of considerable economic importance for the geneticist to establish breeding stock in which requirements of some of these essentials is reduced to a minimum, particularly with respect to expensive supplements like riboflavin and vitamin D.

This task is already partly done. Ample evidence has been adduced to prove that in ability to tolerate suboptimal levels of essential nutrients the Leghorns are markedly superior to the so-called heavy breeds. Nichita and his co-workers (1934) have shown that on diets deficient in vitamin B_1 adult Rhode Island Reds died of polyneuritis in from 9 to 21 days, while White Leghorns were extremely resistant, with some birds entirely unaffected after 78 days on such a diet. Lamoreux and Hutt (1937) using chicks of these same breeds and of Barred Rocks also found that Leghorns are markedly superior to heavy breeds in their ability to withstand a deficiency of this vitamin.

Slipped tendon of fowls, or perosis, is a condition in which at from 3 to 8 weeks the tibio-tarsal joint becomes enlarged and the tendon from the gastrocnemius muscle frequently slips out of the intercondylar groove. The fact that most cases are prevented by adding manganese to the diet indicates that deficiency of this element is largely responsible for the syndrome (Wilgus, Norris and Heuser, 1937). It is well known, however, that on ordinary diets White Leghorns seldom manifest the condition while in heavy breeds its occurrence is much more frequent. Serfontein and Payne (1934) found that perosis occurred in 14 per cent. of their Rhode Island Reds but in only 0.7 per cent. of the White Leghorns receiving the same diet. Moreover, within the more susceptible breed

those birds which had demonstrated resistance to perosis while on a deficient diet conducive to its causation produced offspring markedly resistant to the deficiency while breeders which, though susceptible, had survived produced chicks of which one half were also susceptible. There is evidently some genetic character associated with those constituting the White Leghorn breed which permits tolerance of a level of manganese too low to be adequate for heavy breeds, but it is significant that tolerance in the latter can be raised by selective breeding.

In the northern part of the United States and in Canada, a supplement of vitamin D is considered indispensable in winter, and, for confined birds, in summer also. It is usually supplied in cod liver oil. Olsson (1936) found that even as far north as southern Sweden, some hens are able to maintain satisfactory egg-production and hatchability without any supplement of vitamin D whatever. He aptly points out that if this attribute should prove to be hereditary, the selection of breeders should be made from flocks on diets with minimal rather than optimal levels of vitamin D.

These supplements and others are more important in the diet of the fowl than in larger animals because the storage in the smaller bird is more quickly exhausted, especially in those laying steadily. Some essentials, like vitamin B_1, are abundantly supplied in ordinary feeds, but others have to be added and some, like riboflavin, which is best obtained from milk, are among the most expensive constituents of the ration. It is time for geneticists to consider the selection of breeding stock from fowls whose variability from the mean requirements of these supplements is in the direction of tolerance of sub-optimal levels, and the elimination of those variants requiring the most protection in the diet.

Association of Morphological and Physiological Characters

Most animal breeders seek, whether consciously or otherwise, to discover some association between an easily

seen external character and a capacity to produce milk, eggs, meat, work or other things of economic value. A common poultryman's question of to-day "Which is the best breed?" was evidently equally prevalent nineteen centuries ago when Columella advised farmers that the most prolific fowls were likely to be red, with black wings, white ear lobes, five-toed and without spurs (in females). It is true that Columella would have considerable difficulty in proving his points but we do know of several very important associations of the kind to which he refers. Most of these are physiological differences between breeds.

White Leghorns are not only more resistant to some nutritional deficiencies but they also excel the heavy breeds in resistance to *Salmonella pullorum,* an attribute of great economic value. The writer has shown (Hutt, 1935) that White Leghorn chicks differ from Rhode Island Reds in the rate at which their temperatures rise in the first nine days after hatching and also in their erythrocyte and leukocyte counts. The Leghorns possess the sex-linked genes for rapid feathering, not found in many heavy breeds. This is a most interesting and exceptional case of a single gene determining a physiological character of considerable economic value.

As an offset to their superiority in many respects there is something about Leghorns which, according to Ackert (1935), makes them more susceptible than heavy breeds to parasitism by the nematode, *Ascaridia lineata.* Breeds also differ in capacity for reproduction. It is probable that the somewhat lower hatchability of eggs characteristic of White Wyandottes has been responsible for the decline in favor of this once popular breed.

In all of these cases, except that of rapid feathering, the physiological characters are ones that could not have been intentionally included in the establishment of the breeds, nor is it evident that they could be easily attained by artificial selection. Extension of the chromosome map may reveal that genetic linkages are in part responsible, and for that reason and others it is highly desirable to extend

our present knowledge of linkage in the fowl. By so doing we may expedite the incorporation of desirable physiological characters in our commercial breeds and the elimination of characters incompatible with economical productivity.

ADAPTATION TO ENVIRONMENT

Strains of the fowl with high viability in one region may be unsatisfactory in another. This is most marked when European or American stock is imported in tropical countries, but Hinshaw and Asmundson (1936) have shown that within the state of California turkeys susceptible to the contraction of pendulous crop, a condition with a genetic basis but much affected by environment, are much more likely to be affected in the hot valleys of the interior than along the coast. During an unusually hot week at Ithaca in July, 1936, the mortality among heavy breeds was about four times as great as among White Leghorns. Obviously genes are important in establishing stocks resistant to differing climatic conditions.

Apart from this the diseases prevalent in one locality may be somewhat different from those in another so that a strain with high viability in one region may be much less viable in another or even on another farm. For these reasons the geneticist's objectives may well include study of the relation of different environmental factors to viability and performance.

OTHER OBJECTIVES

It is understood that in striving to attain the desiderata discussed above the geneticist will not forget the importance of maintaining a profitable level of egg-production. In the opinion of the writer, if the other objectives are attained, higher flocks averages will follow, though the proportion of outstanding individuals may not be increased. It is doubtful if present techniques for analyzing characters dependent upon multiple factors are adequate to justify further attempts to assign a genetic basis for egg production, or for other quantitative characters, but

any new means of increasing the effectiveness of the progeny test are greatly to be desired.

Space prevents detailed discussion of other important objectives. Economic needs may well be met by a greater utilization of hybrid vigor and studies are desirable to determine to what extent that valuable condition may be effective in reducing mortality of embryos, chicks and adults or in extending productive life. Inbred strains are desirable for obtaining the maximum hybrid vigor, and, apart from immediate economic needs, they are urgently needed for studies in nutrition.

Even though improved strains should be attained without too much difficulty, the geneticist then has the problem of maintaining a number of quantitative characters in a sexually-reproducing organism, a feat much more difficult than the maintenance of improved varieties in most species of cultivated plants. Ways and means of doing this must be found.

Finally, the poultry geneticist must produce not only better fowls but also better poultrymen. Demonstration that some of the objectives discussed are not unattainable is more likely than anything else to encourage the better breeders in the task of establishing and maintaining superior germplasm in the domestic fowl.

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