GENETIC STUDIES OF THE HOUSE MOUSE.

A dissertation in candidature for the D.Sc. degree of the University of Edinburgh

By

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INTRODUCTION.

Soon after the rediscovery of Mendel's work, in 1900, it was realised that the domesticated varieties of Mus musculus offered opportunities for research in hybridisation which were then unparalleled. Accordingly breeding experiments with mice were carried out during the first decade of the century by Lucien Cuénot in France, by A.D. Darbishire and Florence Durham in Great Britain and by W.E. Castle in the United States. The results did much to help establish the generality of Mendel's laws and for this reason their importance should not be underestimated.

During the second decade of the century, however, Drosophila melanogaster supplanted the mouse as the chosen species for research in formal genetics. In the hands of T.H. Morgan, A.H. Sturtevant, H.J. Muller, C.B. Bridges and others of the Columbia University genetics school it acquired an importance in genetical research which is still unequalled; and it is only in the last twenty years that the need for material with a still shorter generation period and larger number of progeny per generation has led to the introduction of micro-organisms such as Neurospora, Aspergillus and Paramecium.

Nevertheless, for some genetical purposes the mouse has remained pre-eminent. Where it is essential to work with a mammal, because the experiment is intended to reflect conditions in human or farm animal populations, it is difficult to find better experimental material than the mouse. It breeds rapidly, an average female producing her first litter when about ten weeks old. It is prolific, an average female raising more than fifty young in a breeding lifetime. It is small, and therefore requires relatively little animal house space. It is tractable. It is easily reared. It is one of the very few mammals for which inbred (and therefore genetically fairly homogeneous) strains are available. It has been extensively used in biological laboratories, and there is a wealth of information available about its physiology, morphology, histology, cytology, pharmacological reactions, embryology and genetics. In short, the mouse makes an excellent small-scale genetic model of man or the large domestic mammals, provided always that the experimenter does not hope to draw quantitative conclusions from his experiments and apply them without further check to human or agricultural problems.

One branch of human medicine in which work on the mouse promises to be particularly valuable is the study of congenital abnormalities (vide Grüneberg, 1947). About twenty mouse mutants are discovered each year, and many of them have morphological, neurological, endocrinological and other effects closely paralleling those seen in various human conditions. The aetiology of a rare abnormality in man, and its/

its prenatal development, are usually very difficult to study; the early stages are rarely seen or, if seen, difficult to identify with certainty. In contrast with this, a similar condition in the mouse, if genetically controlled, can be repeated indefinitely through selective breeding; and it can be identified in embryos with certainty, per continuitatem, back to the earliest morphologically recognisable stages. Spina bifida, hydrocephalus, renal agenesis, microphthalmia, syndactyly, polydactyly, hemimelia - these and many other conditions occur, fortunately, only rarely in man; but for this very reason their study and prevention present difficulties not met with commoner conditions. In the mouse, on the other hand, it has been possible by selective mating to reproduce and study these conditions at all morphologically recognisable stages of their development. Furthermore, there is now reason to hope that, with improvement in the techniques of immunology, cytochemistry and chromatography, the study of some may be carried back to embryonic stages before the establishment of morphological differences.

A more recent development in the study of congenital abnormalities is the demonstration that exposure of a pregnant mammalian female to abnormal environmental conditions may also affect the development of her embryos. The possibility of this phenomenon was visualised thousands of years ago by the writers of the Pentateuch (vide Genesis 30); but it is only within the last twenty years that its existence has been firmly demonstrated. First it was shewn (Kaven, 1938) that there is a high proportion of congenital abnormalities among newborn mice which had been exposed as embryos to ionising radiations; and tragic confirmation that this is true also of the human embryo was found from a study of children exposed as early embryos to the atomic explosion radiations at Hiroshima and Nagasaki (Plummer, 1952). The demonstration that there is a raised incidence of congenital defect among the children of mothers who had suffered from an attack of rubella during the early stages of pregnancy (Gregg, 1941) was followed by much experimental work on laboratory rodents. It was found that congenital defects could be induced in rats by maintaining the female before and during pregnancy on a deficient diet (Warkany & Nelson, 1942), by injecting her with trypan blue (Gillman, Gilbert & Gillman, 1948) or with thyroxin (Giroud & de Rothschild, 1951). In all this work the teratogenic agent was non-genetic, but the response was shewn in some cases to be under genetic control. Thus Hamburgh (1952), who injected trypan blue into mice of the BALB/c inbred strain on the eighth day of pregnancy, found a high proportion of pseudencephaly among the embryos; Waddington & Carter (1952), who injected the same dose into inbred CBA females at the same stage of pregnancy, found many other abnormalities but no pseudencephaly. Murakami (1952), who worked with two other strains of mice, found significant differences between them/

them in the response of their embryos to similar maternal doses of trypan blue.

Another field in which the mouse is of great value as an experimental model of man is radiobiology. With the introduction of radioactive materials into cancer therapy and heavy engineering, their growing use in biological and physical research, and the promise of atomic energy for industrial purposes, there has come a pressing demand for research into the nature and magnitude of the biological hazard of nuclear radiations to human populations. This hazard is both somatic and genetic. The somatic effects are now fairly well understood; but even the nature of the genetic hazard is only dimly perceived, and of its magnitude almost nothing is known. There can be no direct experimentation on human populations; it is therefore necessary, faute de mieux, to use a great diversity of genetic material, including species phylogenetically as close as possible to man, and thus to attempt to make some reasoned judgment* about the human hazard. Extensive studies of mutation rates of mammals under conditions of chronic irradiation are thus essential; and the mouse and rat are the only laboratory mammals of which the very large numbers at present required for such work could be raised in any reasonable length of time and with reasonable experimental resources.

The mouse thus constitutes good material for the study of congenital abnormalities and the best available mammalian material for mutation rate studies. For either purpose, however, it is beset with difficulties which might be considerably eased if its genetic constitution were better understood. Thus mutation rate studies might be very greatly simplified if there were stocks of mice with genetically marked chromosomes in which crossing-over is suppressed, as in the Muller-5 stock of Drosophila melanogaster. Again, the maintenance of a recessive pathological mutant with a sterile or inviable homozygote is made very much simpler if another gene is known which is closely linked to it. The mutant in question may then be maintained in a balanced system, carriers being recognisable with a degree of accuracy dependent only on the closeness of the linkage. Linkage detection and measurement thus form a preliminary to the study of a genetically-controlled abnormal condition which, if not essential, may at least be very helpful. Thus, though the mouse has for forty years been surpassed as material for the study of formal genetics per se, formal genetical studies in the/

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* Pithily described by the current aeronautical americanism gues**s**timate.

the mouse should nevertheless still be an ancillary to any studies of genetically controlled abnormalities. They may make the investigation much easier; and they cannot fail, on the average, to ease the problems of stock maintenance.

Much progress has been made with the construction of a genetic map in the forty years since the first linkage was found in the mouse (Haldane, Sprunt & Haldane 1915); and the probability of success in a linkage detection test is now well over one half. However, there remains a number of problems of linkage and independence which may prove to be incapable of solution, or nearly so, by the breeding techniques of formal genetics. The mouse has a high chiasma frequency and, as a result, linkage between the two ends of one chromosome may be genetically indistinguishable from chromosomal independence unless there is a series of marker genes known along the chromosome. An alternative approach to the problems of linkage and independence lies in the induction by radiations of chromosomal structural changes, followed by genetic detection of the linkage groups and cytological detection of the chromosomes involved. This by its nature is a slow and long-term process, and its success depends on the development of efficient cytological as well as genetical techniques; but, unlike a pure linkage study, it has the potentiality for providing a positively negative answer. A negative linkage test merely fails to find linkage; but if two translocations are chromosomally independent, the associated linkage groups are proven unlinked. Independence studies based on the use of chromosome structural changes, supplemented by the measurement of linkages where detected, thus promise to form a foundation on which to build up a sound knowledge of the genetic constitution of the mouse.

The body of this thesis consists of papers in three fields of genetical research: congenital defect, radiation hazard and formal genetics. Some of the papers on congenital defect include formal genetical results, where these were obtained as an ancillary to the study of the defect in question. The third category, however, includes a number of papers on the theory of recombination and linkage detection, and descriptions of experiments in this field, which have no direct relation to any inherited abnormality except insofar as it can be used as a chromosome marker. The experiments with genetically tagged chromosome translocations probably represent the first systematic attempt to establish the chromosomal independence of mouse linkage groups by this method.

Many of the papers were written jointly; the co-authors were Dr. D.S. Falconer, Dr. H. Grüneberg, Dr. M.F. Lyon/

Lyon, Miss R.J.S. Phillips, Dr. A. Robertson and Professor C.H. Waddington, F.R.S. Acknowledgment of technical assistance is made, where applicable, within the bodies of the papers. The work described in two papers, I and II in the series on The genetics of luxate mice, formed the substance of a thesis submitted in 1949 in candidature for the Ph.D. degree of Cambridge University; reprints are included here for the sake of completeness.

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THE GENETICS OF LUXATE MICE

I. MORPHOLOGICAL ABNORMALITIES OF HETEROZYGOTES AND HOMOZYGOTES

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(With Plate 12 and Twelve Text-figures)

Introduction

The tetrapod limb has been the subject of many comparative studies in the fields of anatomy and palaeontology; and it has long been recognized that the limbs found throughout the higher vertebrates, though they are often extensively adapted to particular habits, may be considered as derived from a single basic pattern, the cheiropterygium (vide Goodrich, 1930; Romer, 1945).

The comparative study of the differences between individuals within a species received less scientific attention before the present century. The more striking variant forms have long attracted popular attention—the two-legged dog was always worth a place in a fair-booth, together with the two-headed calf and any other viable monsters—but in general they attracted little scientific study, especially when the anomaly was slight, and they rarely received detailed examination. After the publication, in 1836, of the system of classification of terata put forward by Isidore Geoffroy-St-Hilaire, little more was done in this field, apart from brief descriptions in the medical literature of individual cases, until the present century.

The hereditary nature of many abnormal conditions such as hexadactyly in man has probably been recognized for thousands of years (Chuangtse, quoted by Goldschmidt, 1945), and with the rediscovery of Mendel's work attention was focused on them and particularly on their modes of inheritance. Human pedigrees were published indicating the Mendelian inheritance of such anomalies as brachydactyly, polydactyly and lobster-claw; and there followed numerous reports of the Mendelian inheritance of limb abnormalities in poultry, cattle, cats, dogs, guinea-pigs, mice and many other species of vertebrate (vide R. R. Gates, 1946).

Inherited abnormalities of the limbs of the house mouse (*Mus musculus* L.) have been known since 1914, and the list of them is now a long one. Even when those conditions are omitted in which the limb is abnormal only in the course of a generalized abnormality of the whole body, such as the failure of secondary bone absorption (Grüneberg, 1936), there still remains a formidable list. It includes syndactyly (Hertwig, 1942), oligodactyly (Hertwig, 1942), duplication of the hind limbs (Danforth, 1930), postaxial polydactyly of the forelimbs (Strong, 1934), clubfoot (Kobozieff & Pomriaskinsky-Kobozieff, 1946), various foot anomalies associated with myelencephalic blebs (Bagg, 1929), preaxial polydactyly of the hind limbs (Murray, 1932; Fortuyn, 1939; Grüneberg, 1943; Holt, 1945; Chase, 1946; Kobozieff & Pomriaskinsky-Kobozieff, 1946), brachypody (Landauer, quoted by Law, 1948), and absence of the tibia (Rabaud, 1914).

Rabaud's mice with absence of the tibia, *souris luxées*, received much detailed study.

Their formal genetics was studied by Rabaud (1914, 1917), their morphology by Hovelacque (1920) and their embryology by Hovelacque & Noël (1923). Rabaud found that the condition was due to a single recessive mutant gene with variable expression. He also found individuals showing polydactyly, hyperphalangy and other digital anomalies in his stock, both among the mice lacking the tibia and among those in which it was present; but the nature of the relationship, if any, between the digital and the tibial abnormalities was never elucidated. The stock is now extinct.

Hovelacque's (1920) studies covered the skeletal and muscular abnormalities of the hind limbs of Rabaud's *souris luxées* and the corresponding parts of normal mice. In dissections of preserved material he found that the abnormalities of the *souris luxées* were confined to the distal end of the femur, the leg and the foot. The main feature of the condition was the replacement of the tibia, more or less completely, by a ligament. The fibula was hypertrophied and bowed towards the tibia. The proximal end of the tibia was always ossified to some extent, and the knee-joint usually consisted of a ligamentous sheath which bound the rudiment of the tibia to the modified femoral condyles. The foot was modified by fusions between the tarsal elements and by an accentuation of the transverse arch, so that the first and fifth digits were almost opposed.

The object of the present paper is to report the results of morphological work on a new stock of luxate mice. The origin of the stock has been reported elsewhere (Carter, 1948); the condition is due to a single mutant gene which has been shown (Carter, 1949) to be situated in the third linkage group, near the locus of macrocytic anaemia, **W^v**, and remote from that of piebald spotting, **s**. The mutant has been called 'luxate', symbol **lx**, by reason of the phenotypic similarity between **lxlx** homozygotes and Rabaud's *souris luxées*. A luxate homozygote is shown in Pl. 12A.

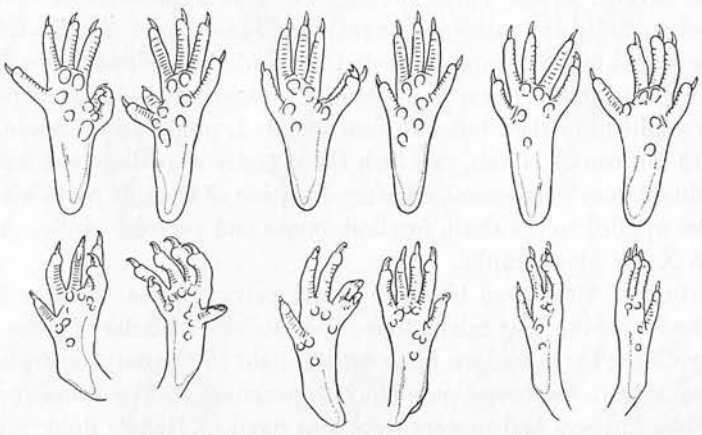
The external appearance of luxate heterozygotes and homozygotes

It is an over-simplification to describe **lx** as a mutant which when heterozygous tends to cause preaxial polydactyly of the hind feet and which when homozygous causes absence of the tibia. Nevertheless, these are the most noticeable features of the expression of the gene in most genetic milieux and may conveniently be used as a basis for describing the anatomical abnormalities associated with it.

A typical heterozygote appears on external examination either to be fully normal or to have supernumerary elements on the preaxial side of one or both hind feet (Text-fig. 1, upper row). All five digits may be three-jointed, or there may be an additional digit or digits, or both. When there is only one additional digit (prehallux), it may have one, two or three phalanges. When there are two additional digits, the first always has three phalanges, and the second, the more medial, is always represented at the level of the third (ungual) phalanx, even though its proximal phalanges may be absent; in such cases the ungual phalanx is attached to the medial side of the prehallux by soft tissues. When the second additional digit is complete, it is always three-jointed. The abnormality is often unilateral, the right side being more often affected; it is rarely exactly symmetrical, the right side usually showing the stronger expression.

A typical homozygote appears on external examination to be rather smaller than its sibs but otherwise normal except for its hind limbs. The thigh appears to be normal, but the leg below the knee is often twisted, so that the foot may take up a grotesque attitude giving the appearance of luxation to which the condition owes its name (see Pl. 12A).

The feet (Text-fig. 1, lower row) also look abnormally long, but this is an illusion due to their exposed position; the feet of luxate mice are, in fact, slightly shorter than those of their like-sexed non-luxate litter-sibs. The transverse arch of the foot is accentuated, so that the most medial digit is almost opposed to digit V; and there is a deviation at the level of the tarso-metatarsal joint in a morphologically mesiad direction. With rare exceptions there is no normal two-jointed hallux, the tibial-sided digits all being triphalangous; there may be additional preaxial digits, up to a total of seven toes; or there may be a loss of toes until only three remain, the fibular-sided III, IV and V. Abnormalities of the hind feet are almost always bilateral in homozygotes, though usually asymmetrical; as a rule the right side is the more strongly affected. At least one leg is usually non-functional, though animals with bilaterally functional legs are not rare; when only one leg is non-functional, it is usually the right. Luxate homozygotes, even with bilaterally non-functional legs, can both walk and swim, though more slowly than their normal sibs; they climb



Text-fig. 1. Plantar views of the hind feet of luxate heterozygotes (above) and homozygotes (below). The left foot of the second heterozygote is normal.

surprisingly well; but if they fall, they are unable to control their rotation and do not, like normal mice, land on their feet.

The expression of **lx** is markedly subject to modification by the genetic milieu, and what may be the heterozygous expression in one family is the homozygous expression in another; there is a continuous spectrum of expression from the normal to the most extreme homozygous form. Nevertheless, within any one family heterozygous and homozygous expressions do not overlap and errors of classification are rare. The expressivity, heterozygous penetrance and lateral asymmetries of **lx** will be described in greater detail elsewhere.

The abnormalities of luxate heterozygotes and homozygotes are recognizable without difficulty in the newborn.

MATERIAL AND METHODS

The first pair of luxate-producing mice were a phenotypically normal male and his polydactylous sister; and their descendants were used to introduce **lx** into five families, thereby making it possible to study the effects of the mutant against five different genetic backgrounds. Three of these families (I, III and IV) were genetically heterogeneous, with several marker mutants segregating; the remaining two (II and V) were made as homo-

geneous as possible, the former by repeated sib-mating with forced segregation of **lx**, the latter by repeated backcrossing on to an inbred strain (Strong *CBA*). The expression of **lx** was weak in family III, very strong in families II and V, intermediate in families I and IV. **lx** was kept segregating in all families, so that it was usually possible to make comparisons within a trio consisting of a normal, a polydactylous and a luxate mouse, all three being like-sexed, from the same litter and of the same age when sacrificed. The five families totalled over four thousand mice.

The systems chosen for detailed study were those which were found, from a preliminary survey, to be most affected by the mutant, namely, the hind-limb skeleton and musculature and the urogenital system.

The methods of investigation were varied as much as possible in the hope that anomalies missed by one method should be brought to light by another. In the first investigations 180 cleared *in toto* preparations of the skeleton were made, using the alizarin red-S staining technique (vide Evans, 1948). These brought to light abnormalities of the hind-limb skeleton, the pelvic girdle and sacrolumbar region of luxate mice (see Pl. 12C); the rest of the skeleton appeared to be normal. Selected parts of the skeleton were next studied in greater detail in dismembered alizarin preparations, especially the regions just mentioned; they were also studied in dried, but not disarticulated, preparations made by controlled maceration with hot caustic alkali, in which the muscles were dissolved but not the ligaments. Individual bones were examined after digestion of the soft parts with papain; this method was also applied to the skull, forelimb bones and pectoral girdle. A few skeletons were studied in X-ray photographs.

The musculature of fifty-seven hind limbs and pelvic girdles, fresh or formalin-fixed, was dissected under a binocular microscope. The intrinsic muscles of some feet were also checked by examining their tendons by polarized light in cleared preparations.

The urogenital system was investigated in 272 specimens by dissection under a binocular microscope. Some kidneys and ureters were also fixed in Helly's fluid, sectioned at $8\,\mu$, stained with Delafield's haematoxylin and eosin and examined histologically.

Anatomical nomenclature

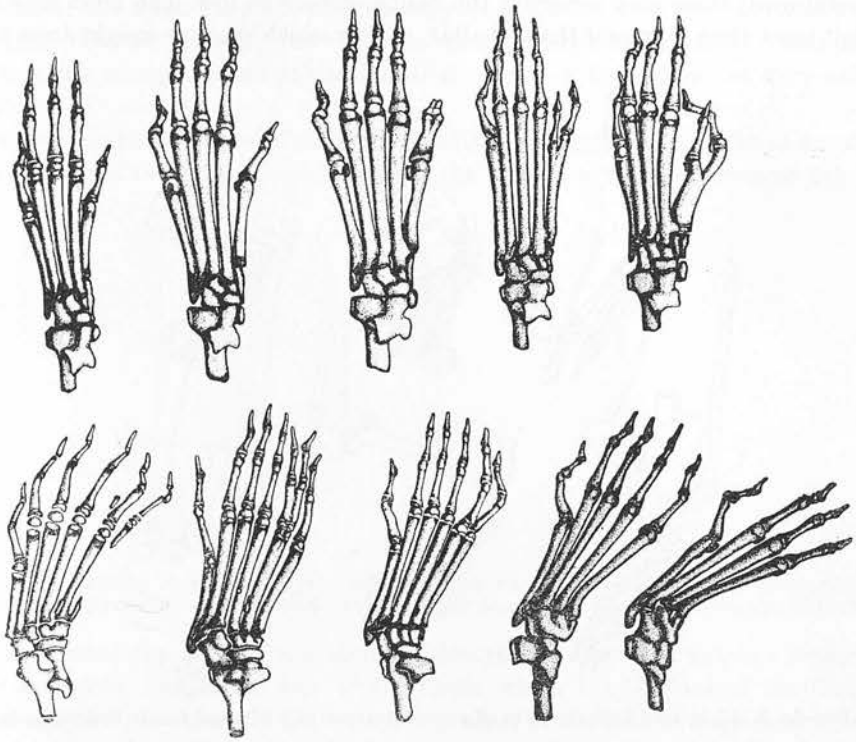
There do not appear to be any detailed descriptions of the normal anatomy of *Mus musculus*, though many limited regions have been described, usually in order to provide a basis for comparison with some mutant form; thus MacDowell, Potter, Laanes & Ward (1942) described the lumbar and sacral vertebrae. In the present work Eunice C. Greene's (1935) description of the anatomy of the Wistar rat was used as a basis, supplemented by Hovelacque's (1920) description of the hind limbs and pelvic girdle of the mouse. Greene's example has been followed in adopting the B.N.A. system of nomenclature with 'some terms anglicized in accordance with the majority of standard English and American anatomical texts'. Certain departures were, however, found necessary. The term m. flexor fibularis has been used to describe the deep extrinsic (perforating) flexor of the toes, thereby avoiding the misleading name m. flexor hallucis longus; and similarly the term m. flexor tibialis, rather than m. flexor digitorum longus, has been applied to the small, deep flexor muscle which originates on the posterior side of the heads of the tibia and fibula and inserts on the medial side of the plantar aponeurosis at mid-metatarsal level. Greene's example has been followed in describing as os tibiale the medial element of the proximal row of tarsal bones, i.e. the element associated with the insertion of m. tibialis posterior;

this bone is sometimes regarded as a sesamoid and the term os tibiale applied to the intermediate element, os talus (=astragalus).

SKELETAL ABNORMALITIES IN LUXATE HETEROZYGOTES AND HOMOZYGOTES

(i) *Hind-foot skeleton.* The skeleton of the hind foot of the normal mouse (Text-fig. 2) is similar to that of the rat as described by Greene.

There are three bones in the proximal row of the tarsus, namely, the lateral calcaneus which forms the heel, the intermediate talus which takes part in the ankle-joint, and the small, medial tibiale. The distal part of the tarsus is represented on the lateral side only by the cuboid, which articulates with the calcaneus



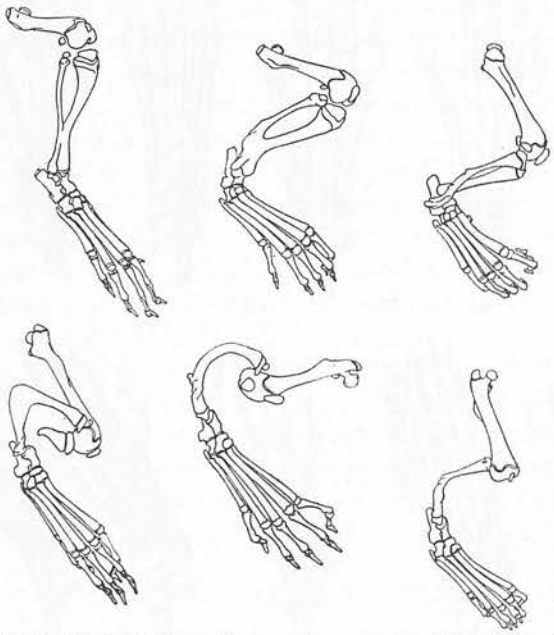
Text-fig. 2. Dorsal views of the skeletons of the *left* hind feet of a normal mouse (top left), luxate heterozygotes (above) and homozygotes (below).

and with which the fourth and fifth metatarsals articulate; on the medial side the navicular articulates with the talus and the three cuneiforms with the navicular, each cuneiform articulating distally with one of the first three metatarsals. There are also cross-articulations between elements in the same row. The first cuneiform is long and articulates with the medial side, rather than the distal end, of the navicular; the second cuneiform is shorter than the first and third, so that the second metatarsal is stepped back into the tarsus, as in man.

The metatarsus is remarkable chiefly for the mechanism which interlocks the three fibular-sided metatarsals; a spur on the postaxial side of the base of the third metatarsal fits under the base of the fourth, and a similar spur on the fourth fits into a notch in the fifth. As a result, abduction of the fifth metatarsal leads to abduction of all three. Metatarsals II–V have a distal epiphysis; the epiphysis of metatarsal I is at its proximal end, like those of the phalanges. The first is the shortest metatarsal, but it extends farther from the heel than does the fifth, by reason of the great length of the first cuneiform bone; the metatarsal formula is III > IV > II > I > V. The phalanges do not show any remarkable features; the triphalangous fifth digit is longer than the diphalangous hallux, so that the digital formula is III > IV > II > V > I.

Luxate heterozygotes show comparatively little change in the foot skeleton; this is seen from the upper row of drawings in Text-fig. 2. The normal foot (upper left) is followed by stages in which first the metatarsal of the hallux is shortened and later the hallux splits. Metatarsal I retains its proximal epiphysis, but acquires also a distal epiphysis in its preaxial branch when the latter articulates with a triphalangous prehallux. Changes in the tarsus involve only the cuneiforms.

The feet shown in the lower row of Text-fig. 2 are of luxate homozygotes. The first drawing shows a bony peg buried in the soft tissues of the lateral side of the first phalanx of the most medial complete digit and a small bony element on the lateral side of the base of its metatarsal; these may represent the hallux, otherwise lost. The most medial complete digit must then represent the prehallux, a view which receives weight from the fact



Text-fig. 3. Right hind limb skeletons of a normal mouse (top left) and luxate homozygotes.

that its metatarsal has a distal epiphysis. The number of digits increases to a total of seven and then regresses until only the fibular-sided II, III, IV and V remain; a further stage is sometimes seen from which II is also absent, leaving a tridactylous foot (Pl. 12 C). Changes in the tarsus include fusion of adjacent elements and losses; in the tridactylous form all the preaxial elements of the tarsus are missing (tibiale, talus, navicular, cuneiforms I and II). There is a progressive mesiad deviation of the metatarsus with respect to the tarsus.

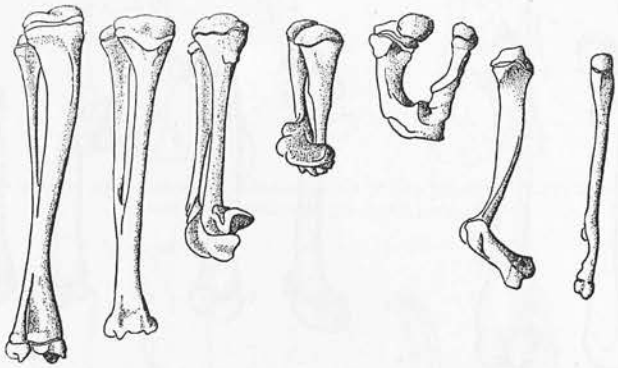
(ii) *Leg and thigh skeleton.* The skeleton of the right hind limb of a normal mouse is illustrated in the first drawing in Text-fig. 3.

Below the knee the leg skeleton consists of the two usual elements, the large medial tibia and the thin lateral fibula; the latter, however, is somewhat displaced posteriorly with respect to the former. The knee articulation, unlike the ankle joint, does not involve the fibula. The two leg bones are separate throughout the proximal two-thirds of their length, but they are fused together along the distal third of their diaphyses. The distal epiphyses and the distal ends of the diaphyses form the medial (tibial) and lateral (fibular) malleoli, which act as pulleys for the tendons of various extrinsic muscles of the foot.

The leg skeleton of a polydactylous heterozygote is similar.

The remaining five drawings in Text-fig. 3 are of the limb skeletons of luxate homozygotes, arranged in increasing order of severity of the abnormality. In each the foot is seen from approximately the same dorsal and lateral aspect as the normal foot. They show the positions which the leg and femur take up in consequence of the changes in the tibia and fibula and, conversely, the positions which the foot must occupy when the femur is in the normal position. The abnormal postures of the hind limbs found in luxate mice can be attributed chiefly to the distortion of the leg segment which is consequent upon a progressive distoproximal reduction in the tibia, unaccompanied by any such reduction in the fibula; the latter becomes progressively more bowed (and simultaneously thicker) as the tibia becomes shorter; but once the disappearance of the tibia is complete, the fibula becomes straighter and thinner until it reaches a final state not very unlike the normal.

These changes in the bones of the leg segments of various right hind limbs are shown in greater detail in Text-fig. 4, which illustrates the fibula and tibia (where present) of seven



Text-fig. 4. Anterior views of the right fibula, and tibia where present, of a normal mouse (left) and luxate homozygotes, showing the fibular changes which accompany progressive reduction of the tibia.

right hind limbs; the head of the fibula is always seen from the anterior aspect. Deep grooves appear in the lateral side of the fibula where the tendons of the four lateral peroneal muscles pass over it; they are not present in the first two specimens, in which there has been little bending of the leg bones, but they deeply insect the distal part of the fibular diaphysis in the next four specimens; they are absent from the seventh, in which the fibula is nearly normal.

The femur of the normal mouse presents no remarkable features; the first two illustrations in Text-fig. 5 show the medial and posterior views of a normal left femur.

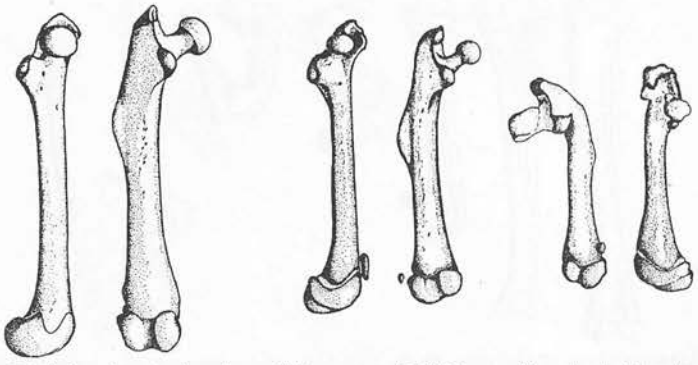
The shaft is long and almost straight; at the distal end it terminates in an epiphysis which forms the two condyles of the knee-joint, the medial being slightly larger than the lateral condyle; at the proximal end it carries two epiphyseal bodies, the greater and lesser trochanters, on to which various muscles insert; between them is the base of the neck, which is set at an angle of about 40° to the shaft and which carries the head. Along the lateral side of the proximal third of the shaft is a low crest, the third trochanter, which also provides an insertion for various muscles.

The femur of a luxate heterozygote is similar to that of a normal mouse.

The other four drawings in Text-fig. 5 illustrate the femora of a luxate homozygote; between them they cover the greater part of the range of variation found. There is a marked asymmetry, the right femur being shorter. The left femur shows only slight

deviations from the state in the normal mouse. The most noticeable feature is the reduction in the size of the medial condyle, which is here smaller than the lateral condyle; there is a corresponding reduction in the depth of the intercondylar fossa. There is no appreciable change in the shaft, but the lesser trochanter arises more sharply from its proximal end. The head is somewhat reduced. In the right femur these changes have occurred to a more advanced degree. The medial condyle is very small and the intercondylar fossa is only a slight depression on the surface of the epiphysis. The shaft is reduced in length by about a fifth, the third trochanter is very much reduced, and the whole of the proximal end is bent over towards the medial side so that the neck makes an angle of about 90° with the shaft. This has been accompanied by changes in the shape of the articular surface of the head, which is no longer rounded.

More extreme changes in the femur are found only in families in which expression in the heterozygote is very strong; examples are illustrated in Text-fig. 6 and the cleared specimen on the right of Pl. 12 C. The proximal end of the diaphysis disappears so that the femur breaks up into three pieces representing the distal part, the greater trochanter and the



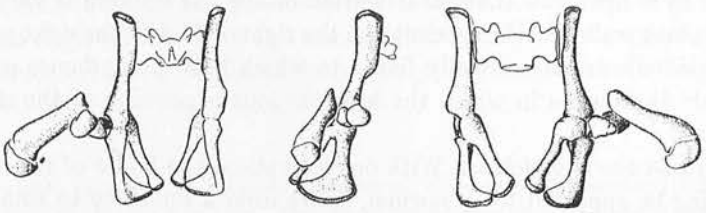
Text-fig. 5. Medial and posterior views of (*a*) a normal left femur, (*b*) a typical luxate left femur, (*c*) a luxate right femur.

lesser trochanter; the latter sometimes carries part of the head; sometimes this third ossicle is missing entirely. The distal part is recognizable without difficulty from its relationship to the fibula and from the muscles which originate on it; the two sesamoid bones in the tendons of origin of m. gastrocnemius, the medial and lateral fabellae, do not appear to be much reduced in size. The greater trochanter is recognizable from the fact that it carries the insertions of m. gluteus medius and m. gluteus minimus. The lesser trochanter is recognizable from the fact that it still carries the insertion of m. quadratus femoris. The two proximal elements are held closely together and to the pelvic girdle by a sheath of fascia; the distal element is floating and has a high degree of mobility with respect to the girdle, but is held closely to the head of the fibula by ligaments.

(iii) *Pelvic girdle.* Abnormalities of the pelvic girdle appear to be of two types. First there are shape changes which may be secondary and are associated with the abnormal directions of the action of certain muscles, themselves associated with skeletal changes in the leg; and secondly there are dystrophies in certain parts of the girdle which appear to be of the same type as those in the limb. This second type of change is found only in homozygotes with very heavy expression in the limb, whereas the former type may occur whenever the leg skeleton is sufficiently abnormal; however, it is not always possible to

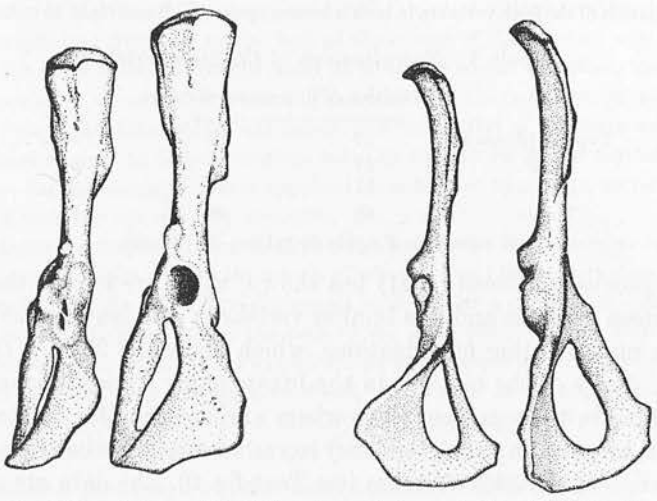
draw a clear line of distinction between the two types. It is also necessary to distinguish between the changes associated with the luxate condition and those which are merely sex differences such as have been described by Gardner (1936).

Text-fig. 8 shows two views of the left os innominatum of two male mice, litter-sibs, one luxate and one normal. Apart from the reduced overall size, the luxate bone shows



Text-fig. 6. Dorsal, left lateral and ventral views of the pelvis and left femur of a luxate homozygote, showing fragmentation of the femur.

Text-fig. 7. Ventral, right lateral and dorsal views of the pelvis of a luxate homozygote, showing reduction of the right pubis.



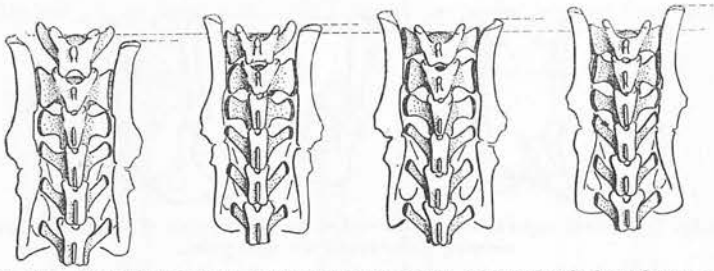
Text-fig. 8. Lateral and dorsal views of the left os innominatum of a luxate (left) and a normal mouse.

four typical abnormalities; there is a strong laterad deviation of the dorsal part of the ischium, a similar deviation of the superior ventral spine of the ilium, the walls of the acetabulum have broken down and the pubis is thin and twisted. The first two changes can probably be attributed to changes of muscular action: the angle of the ischium is the field of origin of the larger head of m. biceps femoris and of m. semimembranosus, both of which insert in the knee region and show an abnormal range of action in the luxate mouse; the superior ventral spine of the ilium is the field of origin of m. gluteus medius

and m. iliacus, both of which have trochanteric insertions and likewise show an abnormal range of action in the luxate mouse.

Text-fig. 7 shows three views of a pelvic girdle with a more extreme form of abnormality. The right pubic bone has split into a small proximal portion, which forms part of the abortive acetabulum, and a small floating distal portion; the missing intermediate part is represented by a ligament. Lateral deviation of the left ischium is very marked, and both acetabula lack walls. In the specimen on the right of Pl. 12 C the right pubis is missing entirely. Individuals are occasionally found in which both pubic bones are absent, but they occur only in families in which the heterozygous expression of the mutant is very strong.

(iv) *Lumbar and sacral vertebrae.* With one exception, the bones of the axial skeletons of mice carrying **lx** appeared to be normal, apart from a tendency to some reduction of size in homozygotes. The exception was the 26th vertebra. In most mice there are seven cervical, thirteen thoracic and six lumbar vertebrae, so that the first sacral vertebra is the



Text-fig. 9. Sacralization of the 26th vertebra in luxate homozygotes. Left and right have been reversed optically.

Table 1. *Sacralization of the 26th vertebra*

	Position of first sacral vertebra		
Type of progeny	27	26	Totals
N + P	44	4	48
X	11	16	27
Totals	55	20	75

N = normal; P = polydactylous; X = luxate.

27th. In some stocks, as Green (1941) has shown, there are twelve thoracic and seven lumbar, or fourteen thoracic and five lumbar vertebrae, still leaving the 27th as the first sacral; or there may be other combinations, which make the 26th or the 28th vertebra the first sacral. Many of the matings in the luxate stock showed a constant number of presacral vertebrae in their progeny; but where variation in this feature coexisted with segregation of **lx**, there was a strong tendency towards association between homozygosity in **lx** and sacralization of the 26th vertebra (see Text-fig. 9). The data are given in Table 1; the association is highly significant ($\chi^2 > 22$ for 1 degree of freedom; $P < 0{\cdot}0001$). In nine individuals the sacralization was unilateral, and in seven of these it was the right lateral process which was of the sacral form.

Abnormalities of the muscles, nerves and vessels of luxate heterozygotes and homozygotes

Interest in the soft tissues of luxate mice was secondary to interest in the skeletal abnormalities; being more variable structures, they were thought to be unlikely to yield much information about the nature of the abnormality which could not be obtained from a study

of the skeleton. In two ways, however, study of the muscles promised to be of interest. First, the identification of an abnormal bony rudiment might be made more positive by a knowledge of the muscular attachments which it carried; and secondly, there was an intrinsic interest in discovering the fate of muscles whose fields of origin or insertion disappeared and, conversely, in investigating the muscle supply of new bony structures.

A certain number of discrepancies were found between the muscles of the hind limbs of normal mice and those described by Hovelacque (1920), but the descriptions of the Wistar rat given by Greene (1935) were found to be completely applicable to the mouse except in a few detailed points.

These were:

(i) *M. peroneus digiti quarti and m. peroneus digiti quinti.* Greene (p. 59) describes the former as arising from the head of the fibula, the latter from the shaft; the reverse condition was found in all the mice dissected, also in the rats dissected as a check.

(ii) *M. flexor tibialis.* Greene (p. 59) says that its tendon, after passing under the medial malleolus, '...gives off a tendon which joins a corresponding one from the flexor hallucis longus to form the flexor tendon of the hallux, then becomes blended with the flexor hallucis longus...'. This situation was found in the rats dissected but not in the normal mice; in them the tendon (which was very fine) passed under the medial malleolus, then crossed the medial side of the foot to gain the plantar aponeurosis and inserted widely on to the fascia in the region of the proximal medial plantar pad.

(iii) *M. adductor brevis.* Greene (p. 54) describes this muscle as originating '...from the ascending ramus of the pubis, from the symphysis pubis...and from the medial half of the ramus of the ischium...' and inserting '...into the third trochanter and into the flexor surface of the distal half of the shaft of the femur...'. In the normal mice dissected there were two distinct and separate muscles with these insertions and origins (see Text-fig. 11). One, which corresponds with the *grand adducteur* of Hovelacque, is a short, very thick muscle originating from the medial half of the ramus of the ischium and inserting into the posterior side of the third trochanter. It is here called m. adductor brevis. The other, the *moyen adducteur* of Hovelacque, originates on the ascending ramus of the pubis and the pubic symphysis and inserts on to the whole posterior face of the distal half of the femur; it is here called m. adductor medius. In the rats dissected the condition seemed to be intermediate between that found in the normal mouse and that described by Greene; her m. adductor brevis appeared to consist of two parts, corresponding with the two muscles in the mouse, but not so easily separable.

(iv) *M. extensor digitorum longus.* Greene describes the tendons of this muscle as inserting on to the base of the third phalanx of digits 2–5. In the mice and rats examined they inserted on to the base of the second phalanx; extension at the distal interphalangeal joint was by a dorsal elastic ligament which ran from the proximal interphalangeal joint to the base of the third phalanx. This system held the distal interphalangeal joint in a state of extension except during active contraction of the long flexor muscle; a direct consequence appeared to be the position of rest, wherein the metatarsophalangeal and proximal interphalangeal joints are lightly flexed but not the distal interphalangeal joint; the claw is therefore held in its normal, somewhat retracted position.

The differences between Hovelacque's (1920) descriptions of the normal mouse and the normal mice dissected in the present work were:

(i) *M. gluteus minimus.* Hovelacque considers this as two muscles, his *petit fessier* and *scansorius*; they were not separable in the present series of dissections, though there are two main tendons.

(ii) *M. gemellus superior, m. gemellus inferior, m. obturator internus, m. obturator externus, m. quadratus femoris, m. adductor longus.* All were present, though the first-named is usually very small.

(iii) *M. adductor magnus.* Hovelacque's use of the term *droit interne* seemed to cover Greene's m. gracilis anticus, m. gracilis posticus and m. adductor magnus. All three originate on the pelvis and insert on the medial side of the knee region; the former two are very thin but separable.

(iv) *M. flexor tibialis.* This is inserted as already described above.

(v) *M. peroneus digiti quarti and m. peroneus digiti quinti.* These were always present; their tendons are

best seen where they radiate out from the lateral malleolus across the dorsum of the foot in company with the two tendons of m. extensor digitorum brevis.

(vi) *M. extensor digitorum brevis.* This muscle gave rise to two tendons, not four; the presence of the adjacent two tendons of the peroneal muscles of the fourth and fifth digits made it appear as though the short extensor gave rise to four tendons.

(vii) *M. quadratus plantae.* This was always present, though thin.

The differences between the findings in the luxate stock and those in Greene's were therefore slight; and some of them could be attributed to interspecific differences. There were rather more differences between the present findings and Hovelacque's, but all of a comparatively minor nature.

In polydactylous luxate heterozygotes no muscular abnormalities were found except in the foot. The most interesting feature of these mice lies in the fact that the prehallux receives no extensor muscle supply though it does receive flexor tendons. This is presumably the reason why the prehallux does not appear to be used and is held half flexed with its distal end lying under the sole of the foot. The prehallux receives slips from both the deep flexor tendon (m. flexor fibularis) and the interosseous short flexor (m. flexor hallucis brevis); like the hallux, it receives nothing from the plantar short flexor (m. flexor digitorum brevis), nor from the lumbrical muscles. A single slip leaves the main deep flexor tendon and runs towards the hallux and prehallux; at mid-metatarsal level it splits into two parts which insert, appropriately, on to the base of the distal phalanx of the hallux and prehallux respectively. The interosseous short flexor divides into two from its origin and inserts on to the medial side of the base of the proximal phalanx of the hallux and prehallux. The tendon of m. tibialis anterior, which normally inserts on to the medial side of the base of the first metatarsal, splits at ankle level into three or four branches which immediately insert widely on to the dorsum and medial side of the tarsus and base of the metatarsus.

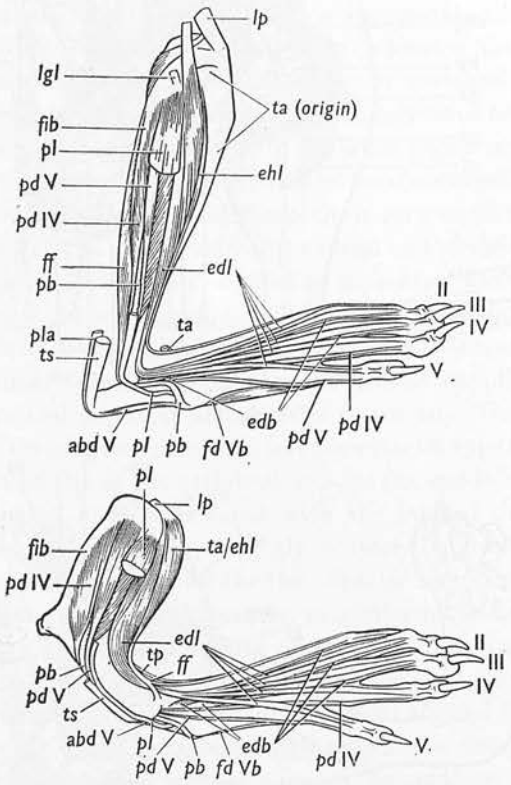
The muscle relationships in luxate homozygotes are very variable, but in general the changes in the leg and thigh are such as might be expected if the muscles maintained their positions with respect to the changed skeletal structures. At the level of the foot the muscles of the luxate mouse are characterized by a lack of specialization on the preaxial side. The preaxial digits (i.e. all digits medial to the medius) acquire a muscle supply strictly similar to that of the normal second digit; this applies to the interosseous muscles, lumbricals, short extensors, long extensors, short flexors and deep flexors. The most medial digit, however, does not usually receive any muscle supply from the distal lumbrical series; the tendon from the plantar flexor to the most medial digit also usually shows an abortive perforation, only the postaxial slip inserting on the base of the second phalanx; the preaxial slip inserts on to the synovial sheath of the deep (perforating) flexor tendon at metatarsophalangeal level. There is also an interosseous adductor muscle, having the same origin as the normal m. adductor indicis, supplying each digit preaxial to the index.

The extrinsic foot muscles of luxate mice (see Text-fig. 10) may be divided into two classes: in one class, of fibular or femoral origin, they are changed only in so far as the skeletal changes dictate; in the other class, of tibial origin, they are abnormal in size or insertion.

The muscles of femoral origin, which are relatively unchanged, are m. extensor digitorum longus, m. gastrocnemius and m. plantaris; m. popliteus, always small, disappears when its insertion (on the upper part of the tibia) is absent. The muscles of fibular origin, which

are also little changed, are m. peroneus longus, m. peroneus brevis, m. peroneus digiti quarti, m. peroneus digiti quinti, m. soleus and m. flexor fibularis. M. quadratus plantae, which inserts at tarsal level on to the tendon of m. flexor fibularis, is usually hyperplastic; it partly takes over the function of retaining the deep flexor tendon which in the normal mouse is performed by the medial malleolus (of the tibia).

The muscles of tibial origin are progressively reduced as the tibia is reduced; their tendons may acquire abnormal insertions. Thus m. tibialis anterior first breaks up into four separate lobes, which insert on to the medial side of the dorsum of the tarsus (navicular

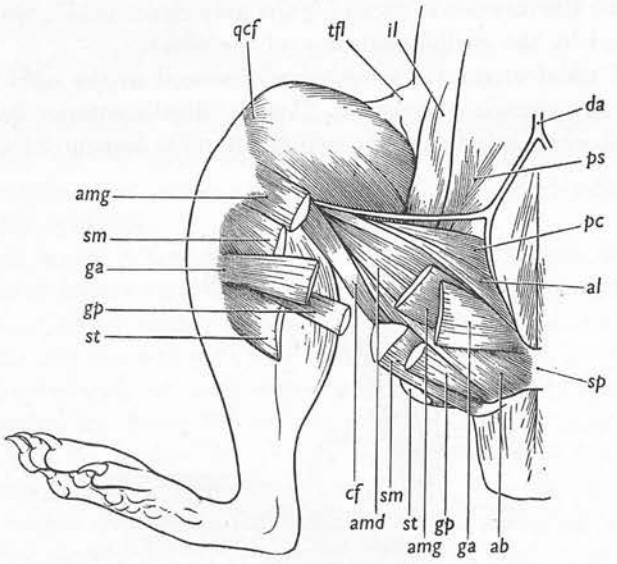


Text-fig. 10. The lateral leg muscles of a normal mouse (above) and a luxate homozygote. The calf muscles have been removed from both and m. tibialis anterior from the normal leg. M. peroneus longus (*pl*) has been cut. *abd* V = m. abductor digiti quinti. *edb* = m. extensor digitorum brevis. *edl* = m. extensor digitorum longus. *ehl* = m. extensor hallucis longus. *fd Vb* = m. flexor digiti quinti brevis. *ff* = m. flexor fibularis. *fib* = os fibula. *lgl* = ligamentum collaterale laterale. *lp* = ligamentum patellae. *pb* = m. peroneus brevis. *pd* IV = m. peroneus digiti quarti. *pd* V = m. peroneus digiti quinti. *pl* = m. peroneus longus. *pla* = m. plantaris. *ta* = m. tibialis anterior. *tp* = m. tibialis posterior. *ts* = m. triceps surae. II, III, IV, V, digits.

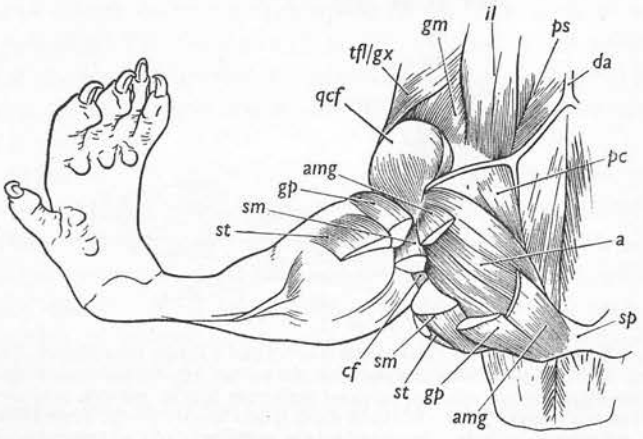
and cuneiform bones and metatarsals), then with further reduction of the tibia it disappears. M. extensor hallucis longus is also reduced; its tendon does not insert as an extensor of a preaxial digit, but joins with the tendons of m. tibialis anterior to insert on the tarsus. M. tibialis posterior, which is of joint tibial and fibular origin in the mouse, is not reduced, but its tendon acquires a new insertion; it joins the tendon of m. flexor fibularis at mid-metatarsal level. So also does the tendon of m. flexor tibialis when this muscle is present.

The extrinsic muscles of the leg and the thigh muscles in general correspond broadly with the descriptions given by Hovelacque; the description below applies to the extreme form, not encountered by Hovelacque, in which the femur has split into a distal element

and two proximal elements and the pubis is represented by a ligament. Text-fig. 12 illustrates a less extreme form, in which the pubis is still ossified but the femur has already broken up.



Text-fig. 11.



Text-fig. 12.

Text-figs. 11, 12. The second layer of muscles of the medial side of the right thigh of a normal mouse (above) and a luxate mouse. Expression is strong in the latter, since the femoral diaphysis is missing, but not extreme, since the os pubis and associated muscles are present. *a* = mm. adductores. *ab* = m. adductor brevis. *al* = m. adductor longus. *amd* = m. adductor medius. *amg* = m. adductor magnus. *cf* = m. caudofemoralis. *da* = dorsal aorta. *ga* = m. gracilis anticus. *gm* = m. gluteus medius. *gp* = m. gracilis posticus. *gx* = m. gluteus maximus. *il* = m. iliacus. *pc* = m. pectineus. *ps* = m. psoas. *qcf* = m. quadriceps femoris. *sm* = m. semimembranosus. *sp* = symphysis pubis. *st* = m. semitendinosus. *tfl* = m. tensor fasciae latae.

In the extreme form the thigh muscles consist of two groups, namely, a deep group inserting on the proximal parts of the femur and a superficial group inserting on to the distal part of the femur or on to the leg. The muscles which normally originate on the pubis are absent. The muscles which insert on the proximal parts of the femur are all well developed and some are even larger than usual; they are m. psoas, m. iliacus, m. gluteus

minimus, m. gluteus medius, m. gluteus maximus, m. piriformis (abnormally large), m. gemellus superior, m. gemellus inferior, m. obturator internus (abnormally large), m. obturator externus, m. quadratus femoris; these radiate out in all directions from the fascia which covers the vestige of the proximal end of the femur. The missing muscles, normally of pubic origin, are the adductor group, m. adductor brevis, m. adductor medius, m. adductor magnus, m. adductor longus, m. pectineus and m. gracilis anticus. It is notable that though these muscles normally all originate on the pubis, their insertions vary widely from the lesser trochanter of the femur (m. pectineus) to the upper third of the tibia (m. gracilis anticus); the points of insertion appropriate to some are present (e.g. the distal end of the femur, normal insertion of m. adductor medius). The superficial muscles which are present are those of ischiatic, iliac or vertebral origin. All are much shorter than in the normal mouse, but they are not thinner than usual; some are thicker. Inserting on to the fascia on the medial side of the knee region are m. gracilis posticus, m. semitendinosus and m. semimembranosus: the first two fuse together near their insertion and the third is abnormally thick. M. caudofemoralis is very much thicker than usual and inserts, appropriately, on to the medial side of the distal end of the distal femoral vestige. M. quadriceps femoris is short, thin, and cannot be separated into its components. Both parts of m. biceps femoris are well developed. This superficial group of muscles holds the distal femoral vestige, with leg attached, as though it were balanced on top of the mound composed of the proximal femoral vestige and its muscle supplies. Between the two groups the sciatic nerve and popliteal vessels wind tortuously. There is a high degree of mobility possible to the distal femoral vestige, but the muscles appear to have little control over the leg. The increased size of the vertebral muscles (m. caudofemoralis and m. biceps femoris especially) appears to be correlated with the laterad distortion and ventrad migration of the ischium and the correspondingly increased volume of the region between the dorsal ischiatic border and the base of the tail. Similar remarks apply to the increased size of some muscles of the deep group, namely, m. piriformis and m. obturator internus.

The muscular changes in luxate mice may therefore be summarized in the following sentences. First, all muscles tend to be present and to have their normal origins and insertions notwithstanding that they may have lost their normal functions as a result of skeletal changes. Secondly, when the skeletal part on which a muscle normally originates is not ossified, that muscle is absent; when it is present but reduced, the muscle is reduced. Thirdly, when the skeletal part on which a muscle normally inserts is absent, that muscle inserts on to the nearest appropriate skeletal element; 'appropriate' here means that a flexor muscle retains a flexor function, an extensor muscle an extensor function. Fourthly, when preaxial digits fail to show the specialization of the skeleton normally seen in the hallux, they also fail to show specialization of the muscle supply; the latter takes up the generalized, serial nature seen in the unspecialized digits. Finally, the leg and thigh muscles of the more extreme forms of luxate mice seem to be less firm than the corresponding muscles of normal mice; they readily break up into their component fibres and are easily damaged at dissection.

The nerves and vessels were not examined in any great detail, except where it was desirable to obtain additional evidence on the homology of certain muscles, notably m. tibialis anterior and m. tibialis posterior. They appeared in general to follow the muscular changes, maintaining their expected relationships to the muscles. The only exception to this rule was the popliteal artery in the knee region. In the normal mouse it passes lateral

to the insertion of m. caudofemoralis on the medial femoral epicondyle; in about half of the luxate homozygotes the muscle divided into two equal parts before inserting, and the popliteal artery passed between them.

Visceral abnormalities in luxate heterozygotes and homozygotes

Some luxate homozygotes, particularly those with very heavy expression, tended to waste and die in the nest; others, though they survived the weaning period, grew very slowly and failed to mature. This occurred to some extent in all families, but it was common only in family II.

At autopsy it was found that there were severe abnormalities of the urinary system; one or both kidneys and their ureters were enormously distended, and the other abdominal viscera were pushed up against the diaphragm; the vital capacity was correspondingly reduced. A large number of routine dissections was therefore undertaken in order to discover whether this were yet another effect of the luxate gene or whether it were coincidentally present in the stock.

Four types of visceral abnormality were found in the course of these dissections, all of them involving the urogenital system: they were hydronephrosis (including hydroureter), horseshoe kidneys, renal agenesia and genital agenesia; the commonest was hydronephrosis, and it occurred sometimes in combination with horseshoe kidneys. The relative frequencies of these types are shown in Table 2; this table includes only data from matings which segregated in **lx** and also produced at least one case of visceral abnormality.

Table 2. *Abnormalities of the urogenital system*

	Condition of the urogenital system											
	N		H		HF		F		R		G	
Type of progeny	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
N+P	63	101	—	1	—	—	4	3	1	1	—	—
X	26	16	17	23	3	4	3	3	—	1	—	2
Totals	89	117	17	24	3	4	7	6	1	2	—	2

N = normal; P = polydactylous; X = luxate; H = hydronephrotic; F = fused (horseshoe) kidneys; R = renal agenesia; G = genital agenesia.

Hydronephrosis (see Pl. 12B) was unilateral or bilateral and was found in both sexes. In its mildest form there is a slight increase in the size of one kidney, which becomes translucent in the region adjacent to the renal pelvis; the blood vessels inside the kidney become visible from the exterior. At this stage there is no great distension of the ureter, but in newly dead material waves of peristalsis, succeeding one another at intervals of about 1 sec., may be seen forming at the boundary of the pelvis and moving towards and rapidly down the ureter. The bladder may be empty, and the impression is of a blockage of the ureter close to the point where it joins the bladder.

In more severe cases the whole kidney and ureter may be distended to many times the normal volume; the kidney wall becomes very thin and transparent and internal compartments are visible. The ureter does not become tortuous. The kidney presses against the dorsal, lateral and ventral body wall and the gut is pushed to one side. Nevertheless, when the hydronephrosis is unilateral, it is possible for a female to become pregnant in the contra-lateral horn of the uterus; a female in this condition is shown in Pl. 12B.

Bilaterally affected hydronephrotics remain viable until there is apparently only a very small amount of excretory tissue left. Even in these cases there is a suggestion that death

may be due to mechanical interference with the alimentary tract; the lower part of the rectum, squeezed between the two kidneys and ureters, is found at necropsy to be empty, while a mass of faecal material has collected immediately above this level.

Experimental puncture confirmed that in the majority of cases there was no blockage of the ureter above a point close to the bladder; it led to the complete draining of the kidney and ureter above the puncture. A few ureters were therefore examined histologically in serial transverse sections of the region close to the bladder. It was found that a localized proliferation of the epithelium had partially or completely filled the lumen of the ureter.

Some hydronephrotic kidneys were also examined histologically. Despite the extremely thin, balloon-like nature of the kidneys, the presence of numbers of glomeruli was immediately recognizable; there was little of the tubule structure typical of renal medulla.

Table 2 shows that hydronephrosis was, with one exception, found only in luxate homozygotes; the association between hydronephrosis and the homozygous state is statistically highly significant.

Horseshoe kidney was found only in the descendants of one male; after the instances reported in Table 2 had been found, a selection line was set up in which the incidence was increased. These investigations are not yet complete.

The other types of urogenital abnormality show no association with the homozygous luxate state. Renal agenesia was unilateral in two instances, in each of which the left kidney and ureter were missing; in a third individual, which died shortly after birth, both kidneys and ureters were absent. Genital agenesia was unilateral in both cases and involved only the testis; epidymis and other organs were present.

DISCUSSION

(i) *Postaxial side of the limb relatively unaffected*

One of the most remarkable features of the luxate mutant is the constancy with which it affects only one side of one pair of limbs, the preaxial side of the hind limbs. Among many hundreds of luxate individuals examined, only one showed an abnormality of digits III IV or V; this exceptional animal, which lacked digit V of one hind foot, died before it could be used for breeding. In all the other mice which carried **lx**, both heterozygotes and homozygotes, the usual complement of skeletal elements of the postaxial half of the limb was present; and where morphological abnormalities were seen in these elements, they were always such as to appear to result from the abnormalities of the preaxial elements. Thus a mesiad deviation at the tarsometatarsal joint was only found when the distal end of the tibia was absent; it appeared to be due to the abnormal direction of the pull of the deep flexor tendon, itself a consequence of the absence of the tibial malleolus. Likewise a heavily bowed fibula was always associated with a reduced (but not absent) tibia, and therefore with an abnormal m. tibialis anterior, so placed as to tend to pull the tarsus towards the knee: the bowing of the fibula was absent when the tibia was completely absent and, with it, m. tibialis anterior.

This limitation of an abnormality to one side of a limb, preaxial or postaxial, has frequently been found in other species. Mutants involving only the preaxial side of a limb include the polydactyly of Houdan poultry (vide Landauer, 1948); 'duplicate', another poultry polydactyly mutant (Warren, 1941); many human pedigrees of polydactyly, brachydactyly, radial and tibial hemimelia (vide R. R. Gates, 1946); polydactyly in dogs

(Stockard, 1930; Gueirrero, 1947), in cats (Danforth, 1947) and in mice (Murray, 1932; Fortuyn, 1939; Grüneberg 1943; Chase, 1946; Kobozieff & Pomriaskinsky-Kobozieff, 1946). Mutants involving only the postaxial side include ulnar and fibular hemimelia in man (vide R. R. Gates, 1946), polydactyly in mice (Strong, 1934) and oligodactyly in mice (Hertwig, 1942). There are some types of inherited polydactyly in which both sides of the limb are affected, but they occur especially in species which have lost both preaxial and postaxial digits during evolution; thus Stockard (1930) described a guinea-pig which had a pollex on one forelimb as well as a minimus on each hind foot; and Wright (1935) described a guinea-pig mutant, the heterozygous expression of which commonly includes the restitution of both the pollex and the minimus of the pes. Holt (1945) found two individuals with postaxial polydactyly among mice related to the stock in which she found preaxial polydactyly of the hind feet, but she did not encounter both forms in one individual.

(ii) *Forelimbs unaffected*

The limitation of the abnormalities associated with **lx** to one pair of limbs is not so widely paralleled in other species, though it is commonly found that one pair of limbs is more strongly or more often affected than the other. Polydactyly occurred both on the forelimbs and on the hindlimbs of Danforth's (1947) cats, but expression tended to be stronger and more frequent on the forelimbs; polydactylous forelimbs were often associated with normal hind limbs, but the converse condition was never encountered. The polydactyly of adult Houdan poultry, by contrast, tends to affect the hind limbs more often and more strongly than the wings (Baumann & Landauer, 1944). Inherited polydactylism in mice appears to be limited, with very few exceptions, to one pair of limbs or the other. Apart from the few exceptional indivuals found by Holt and mentioned above, postaxial polydactylism has been found only on the fore feet (Strong, 1934) and preaxial polydactylism only on the hind feet.

(iii) *Continuous spectrum of gene exhibition*

A further feature of the expression of **lx** is the continuity of the region of involvement in the skeletal changes; no instance was encountered of two abnormal regions being separated by a normal region. Thus when the foot and the femur are abnormal, so also is the tibia. The only exceptions to this rule occurred where there were deformations of the pelvis, such as a deflected ischium, which could be attributed to changes of muscular action, themselves consequent upon changes in the leg skeleton. Changes in the pelvis which were interpreted as being more fundamental, e.g. loss or severe reduction of the pubic bone, were found only in those individuals which showed a reduction of the femur, thus conforming with the rule of the continuity of region of involvement.

Abnormalities of the lowest grade occur in the region of the first metatarsal, which may be shortened or displaced so that it lies somewhat transversely, without any external appearance of abnormality. With stronger exhibition the region of involvement spreads distally to the phalanges of the hallux, which split longitudinally. This is followed by a proximal spread from the first metatarsal, involving progressively all the preaxial skeletal elements of the limb and its girdle. The continuous spectrum of exhibition of the mutant can thus be divided into two parts, namely, (i) the low-grade end of the spectrum, in which only the foot is affected and where there is a progressive increase in the number of

preaxial skeletal elements, culminating in a form with seven digits; (ii) the high-grade end of the spectrum, in which the preaxial skeletal elements of the limb are progressively diminished, again starting at the metatarsal level and spreading both distally to the phalanges and proximally to the tarsus, tibia, femur and finally to the pubis.

A change which occurs in the lower end of the spectrum is the loss of the specialization of the first digit, the hallux. When the phalanges of the first digit have split, so that the first metatarsal articulates with two rows of phalanges, the preaxial row tends to show all the features of an unspecialized digit; it usually has three phalanges, and the branch of the metatarsal with which it articulates bears a distal epiphysis. The postaxial row, by contrast, either consists of two phalanges and shows the specialization usually seen in the hallux or else it is suppressed more or less completely. The two phalanges, or one, either articulate with a part of the metatarsal which has no distal epiphysis, or else there is no articulation. Whether *all* instances of apparent triphalangy of the first digit should be looked upon as the result of a simultaneous complete suppression of the hallux and development of a prehallux, is not clear; experience with luxate mice underwrites Danforth's (1947) view that '. . . some of the classical concepts of homology seem to be misleading, or of little value, for purposes of interpreting polydactyly. . . '.

Whatever the condition of metatarsal I and the phalanges which articulate with it, it was invariably found that any further preaxial digits were of an unspecialized form, though often incomplete. An incomplete digit was always represented at the level of the distal phalanx, usually also at the base of the metatarsal; the last parts to appear were the proximal phalanx and the middle of the metatarsal. A result of the unspecialized nature of the supernumerary preaxial digits is an appearance as of abortive mirror-image duplication in those feet in which some remnant of hallux specialization coexists with two supernumerary digits; but it appears only in this one type of expression and does not extend to the soft tissues, since the supernumerary digits may carry the insertions of interosseous adductor muscles which co-originate with m. adductor indicis; these muscles are therefore lying transversely and in the opposite sense to that required if mirror-imaging had occurred.

A result of the successive increase and decrease, with gene exhibition, of the number of preaxial digital elements is the possibility that one digital state may sometimes correspond with two grades of exhibition, one low and one high. Thus five triphalangous digits may coexist with a fully normal tibia or with a tibia reduced to about a third of its normal length. The former state occurs commonly in heterozygotes and is a prelude to a six-toed normal-legged form; the latter occurs almost exlusively in homozygotes and foreshadows a four-toed tibia-less form. The number of digital elements therefore does not provide a good measure of the grade of gene exhibition; the state of the leg is a better indication. It seems possible that an explanation in such terms may lie behind Taylor & Gunns's (1947) observation, in lethal polydactylous ('diplopodia') poultry, that 'in five-sixths of the cases the right foot possessed more toes than the left', in contrast with the preponderance of left-sided heterodactyls found by most workers in Houdan stocks (see Landauer, 1948). It might be that diplopods, like Houdan polydactyls, usually have stronger gene exhibition in the left leg; but since the whole level of exhibition is much higher in diplopods, stronger exhibition in the left leg may consist, in them, of fewer rather than more digits compared with the right side.

Luxate mice resemble diplopods in many respects. Besides the additional preaxial digits

and metatarsals, Taylor & Gunns (1947) reported suppression of the hallux, shortening and mesiad bowing of the leg, dystrophies of the femur and other anomalies. The postaxial side of the diplopod foot is unaffected; the fifth digit, not normally present in poultry, is still absent in diplopods. The two conditions differ, however, in the fact that diplopodia is lethal and involves not only the hind limbs but also the wings; the maxilla may be shortened. Guinea-pigs homozygous for Wright's (1935) mutant **Px**, which were described in detail by Scott (1937), likewise show many points of resemblance with luxate mice, including the presence of supernumerary preaxial digits and great reduction of the tibia; they differ from luxate mice, but resemble diplopod poultry, in having abnormal forelimbs and a shortened maxilla and in their inviability; in addition, they show several other features, including shortening of the mandible, microphthalmia and abnormalities of the central nervous system.

(iv) *Concomitant visceral abnormalities*

A tendency to association between abnormalities of the limbs and anomalies of the viscera has long been recognized; it was well known to Isidore Geoffroy-St-Hilaire (1836). Where the hind limbs are affected, it is most commonly the urogenital system which is abnormal, though there may also be abnormalities of the gut, especially *atresia ani* (vide R. R. Gates, 1946).

In mice anomalies of the urogenital system, associated with limb defects, including polydactylism, were found in his posterior reduplication stock by Danforth (1930). Gluecksohn-Schoenheimer & Dunn (1945) found that the urogenital system was completely absent from the symmelic mice which occurred sporadically in their tail mutant stocks; there was also *atresia ani*. Extensive abnormalities of the urogenital system have been reported (Scott, 1937) in homozygous **PxPx** guinea-pigs.

A feature of the urogenital anomalies of luxate mice which finds a ready parallel in Danforth's short-tailed mice is the lack of correlation between the severity of the visceral and of the skeletal abnormalities (Dunn & Gluecksohn-Schoenheimer, 1945). In luxate mice each appears to be dependent on the residual genotype; but though severely affected limbs may in some stocks be associated with a high incidence of hydronephrosis, other stocks show a low level of limb expression with a high incidence of renal abnormality or the converse.

(v) *Comparison with Rabaud's* souris luxées

Prof. Rabaud has informed me (1948) that the *souris luxées* are now extinct; tests of the genetic identity of the mutants in the *souris luxées* and luxate mice cannot therefore be made, and comparisons between the two conditions must be based on the phenotype and the mode of inheritance. The latter will be considered elsewhere.

A number of abnormalities were found in luxate mice which were not reported by Hovelacque (1920) as occurring in the *souris luxées*; they include all the abnormalities of the more extreme forms of expression, such as absence of the pubis, shortening of the femur and total absence of the tibia; nor were abnormalities of the urogenital system found in the *souris luxées*. However, neither of these negative observations disproves the identity of Rabaud's mutant with **lx**; his stocks, though founded by a cross between a wild mouse and a tame albino, were thereafter maintained by inbreeding; the luxate stocks were extensively outcrossed. The more extreme forms of expression were not found in every

family of luxate mice, nor were the urogenital anomalies; it is therefore possible that the genetic milieu of Rabaud's stocks, fixed by inbreeding, was one in which the pelvic, femoral and urogenital anomalies would not have occurred. It is also possible that the urogenital anomalies existed but that Hovelacque's material, which was preserved, had also been eviscerated.

A further difference lies in Hovelacque's statement that triphalangy of the medial digit occurred infrequently in the *souris luxées*; in luxate mice with reduction of the tibia (corresponding with Hovelacque's usage of the term *souris luxées*) it is an invariable rule that the two-jointed hallux is absent, all complete digits being three-jointed. All the illustrations of *souris luxées* in Hovelacque's paper, however, show the fifth as the shortest digit, whereas a two-jointed hallux is shorter than the fifth digit.

Hovelacque also described the feet of the *souris luxées* as being longer than the normal in the ratio 3 to 2. This is not true of luxate mice; their feet are shorter than those of their non-luxate litter sibs, but this is a fact which requires measurement for its demonstration since the unaided eye judges the more exposed luxate foot to be the longer.

While it would be improper to conclude that the same mutant was concerned in both stocks, mimic genes being well known in the mouse, it is safe to say that there are no phenotypic differences between luxate mice and *souris luxées* which cannot be attributed to differences of residual genotype or observational technique.

SUMMARY

Luxate, **lx**, is a mutant gene in the house mouse which typically tends to cause preaxial polydactyly (including hyperphalangy of the first digit) in the hind feet of heterozygotes and which causes widespread abnormalities of the hind limbs of typical homozygotes, including reduction of the tibia. Homozygotes may also show preaxial polydactyly or oligodactyly, loss of part of the femur and pubis, sacralization of the 26th vertebra and anomalies of the urogenital system, especially hydronephrosis and hydroureter. The forelimbs are unaffected.

These abnormalities have been studied in a large stock of mice carrying **lx**, and it was found that the skeletal abnormalities occur primarily in the preaxial elements of the limbs, the few abnormalities of the postaxial elements being secondary. Changes in the soft tissues follow the skeletal changes, muscles generally retaining their expected relationships to the changed skeletal elements, nerves and vessels to the muscles. It was also found that in luxate mice a muscle disappears if its origin is unossified; it inserts on the nearest appropriate part if its normal field of insertion is absent; and a supernumerary unspecialized digit tends to acquire a normal, unspecialized muscle supply. The severity of the renal abnormalities was not necessarily correlated with the severity of the limb abnormalities.

The mutant is compared with other limb mutants, especially polydactyly in cats (Danforth), diplopodia in poultry (Taylor & Gunns), polydactyly in guinea-pigs (Sewall Wright) and congenital absence of the tibia in mice (Rabaud). It may be a recurrence of the last-named.

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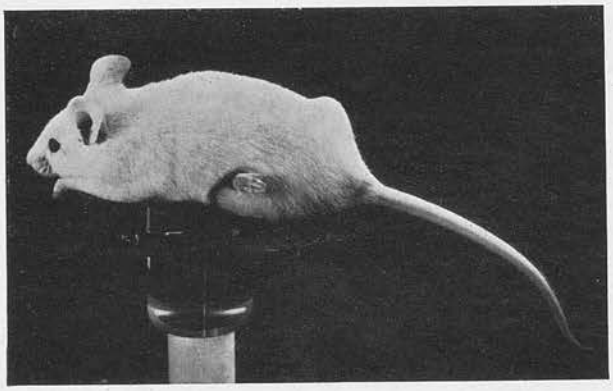
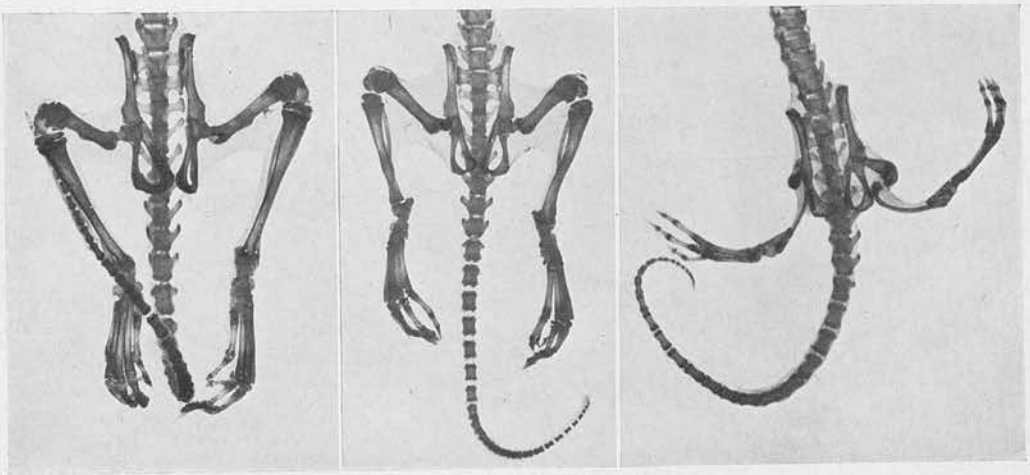


A

B



C

EXPLANATION OF PLATE 12

A. A luxate homozygote.

B. A luxate homozygote with hydronephrosis of the right kidney and a gravid left uterine horn.

C. Alizarin-stained preparations of the pelvic limbs and girdle of a normal mouse (left); a polydactylous luxate heterozygote, showing triphalangy of the right first digit and partial duplication of the left first digit (centre); and a luxate homozygote showing the extreme form of expression with a tridactylous right foot, absence of both tibiae, severe dystrophy of both femora, and absence of the right pubis.

Note added in proof. Hydronephrosis may also occur in homozygotes for the short-ear mutant **se** (personal observation; see also Green, E. L. & Green, Margaret C. (1948), *Genetics* **33**, 106, where the kidneys are described as 'cystic'). The mouse illustrated in Pl. 12 B was homozygous for **se** as well as **lx**, a fact which may account for the unusually advanced stage of the hydronephrosis.

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Position of 'Luxate' in the Third Linkage Group of the House Mouse

THE occurrence of a new strain of mice (*Mus musculus*, L.) resembling the extinct *souris luxées* of Rabaud[1] has been reported[2]; these mice show extensive abnormalities of the hind limbs, including absence of the tibia (hemimelia). The condition was found to be due to the presence in homozygous form of a single mutant gene which was called 'luxate', symbol *lx*. This has been found to be in the linkage group designated as the third by Dunn, Grüneberg and Snell[3], closely linked to macrocytic anæmia, W^v [4]. The other markers known to be in this group are recessive spotting, *s*, hairlessness, *hr* [5], and pirouette, *pi* [6]; *s*, *hr* and W^v are known to lie in that order[7].

Linkage tests now completed between *lx* and *s* indicate a recombination fraction of 50·5 ± 2·5 per cent (standard error); the lx/W^v recombination fraction is 16·0 ± 1·5 per cent. These establish that *s* and *lx* are on opposite sides of W^v and that *lx* is therefore now an end marker, the order being *s*, *hr*, W^v, *lx*.

The conclusion that *lx* lies trans-*W* with respect to *s* does not rest on a claim that the s/lx recombination fraction (50·5 ± 2·5 per cent) is greater than that of s/W; the latter has been estimated to be 46·6 ± 1·1 per cent[8] and, therefore, does not differ significantly from the former. The establishment of the trans-*W* position of *lx* rests on the fact that a hypothetical cis-*W* position is incompatible with the data on the linkages of *hr*. If *lx* were in the cis-*W* position, the s/lx recombination fraction would have to be smaller than that of hr/W; this is a necessary consequence of the fact that the s/hr recombination fraction (8·4 ± 1·9 per cent)[5,8] is significantly smaller than that of lx/W^v (16·0 ± 1·5 per cent). The s/lx recombination fraction (50·5 ± 2·5 per cent) is, in fact, significantly greater than that of hr/W (42·1 ± 2·0 per cent)[7,8]; the cis-*W* position for *lx* is therefore excluded and the trans-*W* position must be accepted.

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THE GENETICS OF LUXATE MICE

II. LINKAGE AND INDEPENDENCE

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Introduction

Luxate, **lx**, is an incompletely recessive mutant gene in *Mus musculus* which affects the hind limbs and urogenital system. A detailed description has already been given (Carter, 1950) of the anatomical abnormalities found in homozygotes and heterozygotes; the following brief description outlines only the salient points which have a bearing on the use of **lx** as a marker gene.

In most genetic milieux the homozygote resembles the *souris luxées* described by Rabaud (1914); it has twisted and non-functional hind limbs, due to partial or total absence of the tibia, and abnormalities of the toes. Expression in the homozygote is variable, but it does not overlap normal. Expression in the heterozygote, which is also variable, most commonly consists of preaxial polydactyly or hyperphalangy of one or both hind feet; it frequently overlaps normal. In any one family the ranges of homozygous and heterozygous expression do not overlap, but the homozygous range of one family may overlap the heterozygous range of another. Some homozygous males are unable to breed, probably for mechanical reasons; most homozygous females are fertile, but their litters tend to be abnormally small. Heterozygotes are fully fertile.

In an attempt to locate **lx** on the genetic map of the mouse a number of systematic tests for linkage were carried out; they led to the conclusion, which has been briefly reported elsewhere (Carter, 1949), that the locus of **lx** is at one end of the presently known map of the third linkage group. The object of this paper is to present the data which led to that conclusion, together with confirmatory data obtained subsequently and the data which establish that **lx** is not linked—unless it be very loosely—to any of the twenty-three other loci tested.

Snell (1931) reported the original finding of linkage in the third group, between hairlessness, **hr**, and the recessive piebald spotting mutant, **s**; recombination was 8%. Gates & Pullig (1945) next found that **hr** is also loosely linked to 'dominant spotting' (macrocytic anaemia, **W**); recombination was about 42%. This proved that **W** must be linked to **s**, despite earlier assertions to the contrary. Fisher (1946) analysed all the published data on the segregation of **W** and **s**, together with some new data; he showed that there was significant evidence of very loose linkage, with $46{\cdot}63 \pm 1{\cdot}14\%$ recombination, and confirmed that the gene order is **W**, **hr**, **s**. **W** and **s**, being the two end loci, were therefore chosen for testing against **lx**. While these tests were in progress, Dickie & Woolley (1946) reported that the waltzer-like mutant pirouette, **pi**, is closely linked to **W**, with 7·2% recombination. Next it became apparent that **lx** is fairly closely linked to the locus of **W**; but **pi** could not be used to determine the position of **lx** with respect to the other markers, as its own position was not known. Therefore **s** or **hr** had to be used; **s** was chosen because independence tests with this locus were already under way. Some con-

firmatory tests with **hr** were made later. Dickie & Woolley subsequently (1948) published data which indicate $35{\cdot}6 \pm 6{\cdot}2\%$ recombination between **W** and **pi**.

MATERIAL, METHODS AND RESULTS

(i) *Tests of independence.* The aim of the linkage test plan was to dispose the available cage-space in such a way as to maximize the tested portion of the mouse's total genetic map. This called for tests with (*a*) as many independent markers as possible, in preference to more extensive tests with fewer markers, (*b*) the two end-markers of each long linkage group, (*c*) only one end-marker of each short linkage group (Carter & Falconer, 1950). However, it was undesirable to use any but 'good' genes as markers; incomplete penetrance and low viability lead to loss of efficiency of testing; and linkage with a gene requiring special methods of classification (e.g. rodless retina), even if found, is of doubtful practical value.

These considerations led to the conclusion that **lx** should be tested against twenty-three of the twenty-five markers listed in Tables 1 and 2; the remaining two markers, **si** and **U**, which require special genetic backgrounds for their classification, were included only because they happened by chance to be present and with the requisite background. Of the twenty-five markers, twenty are in the thirteen known linkage groups and no linkage is known for the remaining five; it is therefore possible that eighteen of the twenty (haploid) chromosomes were involved in the tests.

No special linkage-test stocks carrying the requisite marker genes are in existence and therefore they had to be introduced in whatever combinations happened to be available; consequently dominant and recessive markers were sometimes introduced by the same mouse and therefore the linkage-test matings could not always be of the most efficient type. **lx** was treated as a recessive throughout, since the homozygous penetrance is complete and it is not known whether the heterozygous penetrance, which is incomplete, is affected by the segregation of other marker genes. Most of the test matings were therefore intercrosses; backcrosses were not often used, by reason of the comparatively poor breeding performance of many luxate homozygotes.

The target set for each test was 180 units of statistical information, calculated at 50% recombination, corresponding with a 35% linkage fiducial limit for a $2\frac{1}{2}\%$ significance level; the target was reached in all the tests and considerably exceeded in those with several of the commoner marker genes.

The data, other than those from tests with Linkage Group III markers, are given in Table 1. Single-factor segregations were tested by the maximum likelihood procedure described by Mather (1937); deviations from Mendelian expectation and heterogeneity between bodies of data were not encountered more frequently than would be expected by chance in an experiment of this size.

The data were tested for linkage by the maximum-likelihood scoring technique described by Finney (1943); none of the markers listed in Table 1 showed significant evidence (5% level) of linkage with **lx**.

(ii) *Measurement of linkage.* The non-lethal allele $\mathbf{W^v}$ (Little & Cloudman, 1937) was used for all except the earliest tests with the **W** locus; they indicated fairly close linkage and so established that **lx** is in the third linkage group. It therefore became necessary (*a*) to expand these tests, in order to obtain a more precise estimate of the $\mathbf{lx/W^v}$ recombination fraction, and (*b*) to measure the recombination fraction between **lx** and **s** with sufficient

Table 1. *Independence of* **lx**

Marker, **M** or **m**	Mating type and phase	Phenotype of progeny				Recombination (%)	
		M+	**Mlx**	**m+**	**mlx**	*p*	*s*
a	BR	3	4	8	3		
	MC	109	31	125	40		
	MR	167	53	171	43		
	IC	52	12	19	6		
	IR	103	30	32	16	48·7	±2·6
b	MC	53	12	54	17		
	IC	198	61	80	25		
	IR	137	50	44	14		
	PC	9	6	3	4	48·3	±2·8
bt	BR	7	3	3	6		
	IR	59	26	14	7	55·6	±6·2
c, c^{ch}	BC	13	8	9	6		
	BR	4	5	7	7		
	MC	66	21	71	28		
	IC	87	29	31	18		
	IR	144	40	60	18	46·4	±2·8
Ca	MC	42	16	39	20		
	PC	7	6	4	3	45·3	±7·4
d	MC	1	3	6	—		
	MR	23	8	27	11		
	IC	105	30	29	9		
	IR	137	52	45	15		
	PR	14	12	6	3	49·5	±3·3
f	IC	11	4	3	1		
	IR	108	34	26	14		
	PR	11	5	2	2	57·4	±5·1
Fu	MC	46	22	33	16		
	MR	46	9	58	12	50·0	±5·3
fz	IR	69	27	24	8	47·7	±6·6
je	IR	54	22	10	5		
	PR	9	10	3	2	49·3	±7·2
ln	BC	—	6	4	5		
	MC	8	5	12	7		
	IC	37	19	14	6		
	IR	7	6	6	—	49·8	±6·1
p	BR	1	2	—	1		
	IC	118	43	41	9		
	IR	216	51	66	23		
	PC	16	15	4	5		
	PR	29	22	12	9	54·4	±2·9
pa	IR	109	54	37	12	43·4	±5·2
Re	BR	5	5	3	3		
	MC	36	19	35	16		
	MR	61	22	64	10		
	IC	10	2	1	1		
	IR	69	18	24	5	45·3	±3·9
ru	BR	5	2	3	5		
	IR	50	19	14	8	59·1	±6·7
Sd	BC	20	13	11	11	43·6	±6·7
si	IC	85	25	32	13	45·0	±6·0
U	BR	5	2	2	2		
	IR	57	15	11	5	58·3	±7·1
v	IC	28	14	10	2		
	IR	75	20	17	5		
	PC	8	8	2	1	55·3	±5·5
Va	BC	17	14	18	21	45·7	±6·0
wa-1	IR	72	27	35	7	41·0	±6·3
wa-2	IR	80	26	26	7		
	PC	3	5	—	1	47·5	±6·2
♂	BC	64	52	60	39		
	BR	37	56	51	43		
	MC	226	82	180	73		
	MR	626	205	627	208	49·1	±1·4

Note (i) B = double backcross; C = coupling; I = double intercross; M = mixed cross; P = partial backcross; R = repulsion.
(ii) M and P are formally similar, being a backcross for one factor and in intercross for the other; **lx** is intercrossed in M, backcrossed in P.
(iii) **U** treated as a recessive, ♂ as a dominant.

Table 2. *Segregation of* **lx** *with* **W^v**, **hr** *and* **s**

*Body of data	Genotype of mates ♂	♀	Phenotype of progeny							
			W^{v}W^v+	**W^{v}W^{v}lx**	**W^v+**	**++**	**W^{v}lx**	**+lx**		
1	**W^v+/+lx**	**×W^v+/+lx**	105	4	213	33	36	82	—	—
2	**W^v+/+lx**	**× +lx/+lx**	—	—	40	4	5	29	—	—
3	**+lx/+lx**	**×W^v+/+lx**	—	—	43	9	6	48	—	—
4	**W^v+/+lx**	**× + +/+lx**	—	—	35	27	6	16	—	—
					24		16			
5	**W^v+/+lx**	**×W^v+/+lx**	6	—					—	—
6	**W^{v}lx/+ +**	**× + +/+lx**	—	—	25	58	24	9	—	—
7	**+ +/+lx**	**×W^{v}lx/+ +**	—	—	27	40	18	4	—	—
8	**W^{v}lx + +**	**× +lx/+lx**	—	—	6	25	16	2	—	—
9	**+lx/+lx**	**×W^{v}lx/+ +**	—	—	5	28	21	6	—	—
			W^v+ +	**+ + +**	**W^{v}lx+**	**+lx+**	**W^v+s**	**+ +s**	**W^{v}lx s**	**+lxs**
10	**W^{v}lx+/+ +s**	**× +lxs/+lxs**	12	30	27	7	6	34	34	9
11	**+lxs/+lxs**	**×W^{v}lx+/+ +s**	3	18	11	2	7	15	21	5
			+ +	**+lx**	**+s**	**slx**				
12	**+ +/lxs**	**×lxs/lxs**	28	25	24	18	—	—	—	—
13	**lx+/+s**	**×lxs/lxs**	20	12	16	22	—	—	—	—
14	**lxs/lxs**	**×lx+/+s**	7	7	4	6	—	—	—	—
15	**+ +/lxs**	**× + +/lxs**	143	36	38	13	—	—	—	—
16	**lx+/+s**	**×lx+/+s**	148	43	30	7	—	—	—	—
17	**+s/lxs**	**× + +/lxs**	7	6	12	5	—	—	—	—
18	**+s/lxs**	**×lx+/+s**	16	10	19	11	—	—	—	—
			+ + +	**+hr+**	**+ +lx**	**+hr lx**	**s+ +**	**shr+**	**s+lx**	**shr lx**
19	**lx+s/+hr+**	**×lx+s/+hr+**	130	76	39	18	57	1	22	—
20	**lxhr+/+ +s**	**×lxhr+/+ +s**	11	13	8	2	6	—	—	1
21	**lxhrs/+ + +**	**×lxhrs/+ + +**	4	—	3	—	1	1	1	—

* The recombination fractions quoted by Carter (1949) were based on bodies of data 1–5 and 12–18.

Table 3. *Analysis of linkage of* **lx** *with* **W^v**, **hr** *and* **s**

	lx and **W^v**				Body of	**lx** and **hr**				**lx** and **s**			
Body of data	D_{17}	D_{18}	I	χ^2	data	D_{47}	D_{48}	I	χ^2	D_{51}	D_{52}	I	χ^2
1	− 6·492	−33·247	2675·5	0·413	10	—	—	—	—	+15·646	+ 9·295	635·1	0·385
2	−30·191	−34·146	395·5	2·948	11	—	—	—	—	+20·728	+17·468	326·0	1·318
3	−21·403	−27·642	623·9	1·225	12	—	—	—	—	+ 2·201	− 1·603	380·3	0·013
4	+19·968	+17·472	249·7	1·223	13	—	—	—	—	+25·210	+22·436	277·4	2·291
5	− 3·681	− 3·934	25·3	0·611	14	—	—	—	—	+ 3·041	+ 2·083	95·8	0·097
6	+13·699	+10·050	364·9	0·277	15	—	—	—	—	−20·187	−23·408	322·1	1·265
7	+ 3·062	+ 1·174	188·7	0·007	16	—	—	—	—	− 6·391	− 9·940	294·9	0·138
8	− 2·339	− 5·556	321·7	0·096	17	—	—	—	—	+ 4·810	+ 4·287	52·3	0·442
9	+ 5·670	+ 1·355	431·5	0·004	18	—	—	—	—	− 0·995	− 1·991	99·6	0·010
10	+49·398	+36·450	1294·8	1·026	19	−10·756	−16·654	589·8	0·470	−31·608	−36·881	527·3	1·894
11	+21·687	+15·176	651·1	0·354	20	+11·734	+10·609	112·6	1·000	− 7·304	− 7·967	66·3	0·805
—	—	—	—	—	21	+ 5·047	+ 4·837	21·0	1·115	+ 0·617	+ 0·349	26·8	0·014
Sum	—	—	—	8·184	—	—	—	—	2·585	—	—	—	8·673
Deviation	+49·377	−22·848	7222·5	0·072	—	+ 6·025	− 1·208	723·3	0·002	+ 5·768	−25·273	3104·0	0·011
Heterogeneity	—	—	—	8·112	—	—	—	—	2·583	—	—	—	8·662
Recombination		17·68±1·18%					47·83±3·72%				51·19±1·80%		

precision to discriminate between the two possible positions of **lx**, on either side of the locus of **W**. This called for careful balancing of coupling and repulsion data, in order to minimize possible errors arising from differential viability; but the use of intercross matings was permissible since the recombination fraction was unlikely to differ greatly from 50%.

The results of tests of **lx** with $\mathbf{W^v}$ and **s** are shown in Table 2 and analysed in Table 3. The single-factor segregations are Mendelian and homogeneous. The recombination fractions have been estimated by the joint maximum-likelihood method described by Mather (1935); the **lx**/$\mathbf{W^v}$ recombination fraction is $17{\cdot}7 \pm 1{\cdot}2\%$ and the **lx**/**s** recombination fraction $51{\cdot}2 \pm 1{\cdot}8\%$. Though the latter does not significantly exceed Fisher's (1946) estimate of the **W**/**s** recombination fraction, $46{\cdot}63 \pm 1{\cdot}14\%$, the data establish the gene order as **lx**, **W**, **s** by the following argument. Snell's (1931) data indicate that the **hr**/**s** linkage is closer than the value found here for the **lx**/$\mathbf{W^v}$ linkage; then if **lx** and **s** were on the same side of **W**, the **lx**/**s** linkage would have to be closer than the **W**/**hr** linkage. But the **lx**/**s** linkage, $51{\cdot}2 \pm 1{\cdot}8\%$, is in fact significantly looser than the **W**/**hr** linkage, $42{\cdot}15 \pm 2{\cdot}02\%$ (Gates & Pullig, 1945); hence **lx** and **s** cannot be on the same side of **W** and the order must be **lx**, **W**, **s**.

Table 2 also gives some data on the simultaneous segregation of **lx** and **hr**, but coupling and repulsion are not balanced and therefore the estimate of the recombination fraction is open to errors due to viability disturbances. The indicated **lx**/**hr** recombination fraction, $47{\cdot}8 \pm 3{\cdot}7\%$, is in agreement with expectation based on the gene order **lx**, **W**, **hr**, **s**.

SUMMARY

This paper gives new data on the simultaneous segregation of the mutant luxate, **lx**, in the house mouse, with marker genes at twenty-six other loci. There is no significant evidence of linkage with **a**, **b**, **bt**, **c**, **Ca**, **d**, **f**, **Fu**, **fz**, **je**, **ln**, **p**, **pa**, **Re**, **ru**, **Sd**, **si**, **U**, **v**, **Va**, **wa-1**, **wa-2** or sex. Linkage is found between **lx** and the third linkage group mutant $\mathbf{W^v}$, with recombination estimated at $17{\cdot}7 \pm 1{\cdot}2\%$ (standard error); recombination between **lx** and two other markers in this group, **hr** and **s**, is estimated at $47{\cdot}8 \pm 3{\cdot}7\%$ and $51{\cdot}2 \pm 1{\cdot}8\%$ respectively. The order of the loci is **lx**, **W**, **hr**, **s**.

The work here reported was started in 1946 at the Department of Genetics, Cambridge University, and was completed during 1947–9 at the University Department of Genetics, Edinburgh. I am indebted to Prof. C. H. Waddington, F.R.S., and M. S. Pease, Esq., for their help and advice. Mice carrying the following mutants were kindly given by Prof. R. A. Fisher, F.R.S.: **a**, **b**, $\mathbf{c^{ch}}$, **d**, **Fu**, **ln**, **lx**, **p**, **pa**, **Re**, **si**, **v**; Dr D. S. Falconer kindly gave mice carrying **bt**, **Ca**, **f**, **fz**, **hr**, **je**, **ru**, **Sd**, **sh-2**, **U**, **Va**, $\mathbf{W^v}$, **wa-1** and **wa-2**; **W** and **s** were obtained from a dealer in Cambridge.

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THE GENETICS OF LUXATE MICE

III. HORSESHOE KIDNEY, HYDRONEPHROSIS AND LUMBAR REDUCTION*

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(With Plate 12 and Eight Text-figures)

Introduction

A detailed description has already been given of the abnormalities of hind-limb skeleton and musculature found in mice carrying the mutant gene *luxate*, **lx** (Carter, 1951); the most extreme condition, found in some homozygotes, includes complete absence of the preaxial side of the hind foot and leg, dystrophy of the femur and reduction of part of the pelvic girdle. Some heterozygotes show preaxial hyperphalangy or polydactyly of one or both hind feet, but **lx** is incompletely penetrant in this expression; the presence of two normal feet does not necessarily imply the absence of **lx**. A brief description was also given of other abnormalities found in some stocks carrying **lx**; they included a reduction by one in the number of presacral vertebrae, hydronephrosis, horseshoe kidney, ureteric and renal agenesis and absence of a gonad.

Absence of a kidney, ureter or gonad was found in only five individuals among almost three hundred dissected, and it was not possible to establish whether these were rare effects of the gene **lx** or merely coincidentally present. Hydronephrosis and reduction in the number of presacral vertebrae both showed a strong statistical association with the presence of **lx** in the homozygous state and were interpreted as being irregular expressions of the gene.

Horseshoe kidney was not clearly shown to be related to the presence of **lx**. Its incidence was greater than that of genital or renal agenesis, twenty cases being found; but it was not confined to **lxlx** homozygotes, and the data were not sufficient even to demonstrate a significantly raised incidence in homozygotes.

An important objective of present-day genetical research, especially with mammals, is the analysis of genetically abnormal development, in the hope that causal relationships in normal development may be revealed thereby. Before the development of a syndrome of defects can be successfully investigated, however, it is first necessary to establish the extent of the syndrome, to distinguish between those effects which are causally related to the underlying gene change and those which are merely present by chance in the material used. This is all the more necessary in view of the wide acceptance of the Principle of the Unity of Gene Action (Grüneberg, 1943), which states that the primary effect of a gene is always unitary and implies that one aim of any developmental study of a syndrome should be to trace the manifold outward effects back to a common cause. There is no immediately obvious developmental connexion between horseshoe kidney and the preaxial polydactyly caused by **lx**; it is therefore necessary, before embryological studies are undertaken, to establish beyond doubt whether or not horseshoe kidney is to take its place beside the limb defects, presacral reduction and hydronephrosis as a part of the *luxate* syndrome.

* A report to the Medical Research Council.

This alone would have been sufficient reason for undertaking the work described in this paper; but there was a second reason. Horseshoe kidney has been recognized for thousands of years as a not very rare anomaly in man and the domestic animals; yet knowledge of its origins and development is still largely conjectural. Jazuta (1924) held that horseshoe kidney was due partly to excessive formation of nephrogenic blastema and partly to the morphological instability of the whole lumbar region; this in turn he attributed to a tendency for the pelvic girdle to be displaced craniad. Another mechanism was suggested by Lewis & Papez (1915), who thought that horseshoe kidney might arise through a disturbance of the ontogenetic movement of the kidneys, the disturbance caused, perhaps, by abnormal relationships of the umbilical arteries, which form a girdle through which the migrating kidneys have to pass; but examination of numerous pig embryos failed to provide evidence in support of this hypothesis. There is nothing to contradict it in the very few human embryos in which horseshoe kidney has been found (Boyden, 1932), but they constitute isolated examples rather than a regular series on which a detailed developmental analysis could be based.

There do not appear to have been any reports of the inheritance of horseshoe kidney, the accepted view being that it represents an 'accident of development'. Dunn & Gluecksohn-Schoenheimer (1947), however, found 'a single, median, horseshoe-shaped kidney' in several of fifty-nine young mice carrying both the *tailless* (**Tt^{o}**) and the *uro-recto-caudal syndrome* (**urur**) mutants; this suggests that in their stock it may have had a genetic basis. No further descriptions of their cases have been published.

There is, therefore, an intrinsic interest in an inherited and reproducible horseshoe kidney condition in mice, whether or not it is due to **lx**; and here also morphological studies of postnatal material form a natural prelude to embryological studies. The work described in this paper consisted of two parts. The first was primarily an investigation of the genetics of horseshoe kidney, including its relationships to the limb defects, presacral reduction and hydronephrosis caused by **lx**; this led to the conclusion that horseshoe kidney is, in fact, a further part of the *luxate* syndrome. The second part was primarily an investigation into the morphology of horseshoe kidney, and especially the relationships of the kidney to the abdominal blood vessels. This may be expected to give valuable information about the temporal sequences in the development of horseshoe kidney, since some of the abdominal vessels undergo a caudad migration while the kidneys migrate craniad in normal ontogeny (cf. Hamilton, Boyd & Mossman, 1945).

The opportunity was also taken to make a further study of the hydronephrosis associated with **lx**. This pathological condition, which is well known in man, is believed to be caused by the rise of intrarenal pressure which follows obstruction of the urinary flow, either in the ureter (when the hydronephrosis is unilateral) or in the urethra (when it is bilateral). It is known to occur in mice, having been found in individuals carrying the *myelencephalic blebs* mutant (Bagg, 1929; Brown, 1931), in homozygotes for the *uro-recto-caudal syndrome* mutant (Dunn & Gluecksohn-Schoenheimer, 1947; Grüneberg, 1952) and in *short-ear* homozygotes (Green, 1951).

In the course of this work a further defect was seen in some of the kidneys, namely, a cystic condition. This is broadly similar to hydronephrosis, but the hydropic* condition

* The term *hydrotic* is sometimes applied to hydronephrotic kidneys. The usage in this paper, *hydropic*, follows the *Oxford English Dictionary* and leaves the word *hydrotic* (=*hidrotic*) with its common meaning, sudorific.

is localized within the kidney. In man it is believed to be caused by the presence of glomeruli with obstructed or missing tubules; the glomerular products are therefore unable to escape, thus giving rise to fluid-filled vesicles. Cystic kidney is a disease of age in some strains of mice (Andervont, 1938; Gorer, 1940) and also occurs in *uro-recto-caudal syndrome* homozygotes (Dunn & Gluecksohn-Schoenheimer, 1947).

Finally, an examination was made of the simultaneous distribution of the kidney abnormalities and the number of presacral vertebrae. Studies of the adult condition may point to ontogenetic causal relationships, but can never provide positive proof of them; on the other hand, they can provide positive disproof. For example, if a single individual were found with horseshoe kidney accompanied by the usual number of presacral vertebrae, it would be established that horseshoe kidney is not developmentally dependent on a reduction in the number of presacral vertebrae.

GENETICS

This section is devoted to the presentation of new data on the occurrence of horseshoe kidney, hydronephrosis and a reduced number of lumbar vertebrae in stocks carrying **lx**; from a critical examination of the evidence, it is concluded that they are all effects of **lx**.

(i) *Genetic methods and material.* The question whether horseshoe kidney is an additional effect of **lx** was studied in two ways, namely, (*a*) by observing the association between horseshoe kidney and limb defects in progenies segregating in **lx**; and (*b*) by seeing whether the two could be separated by selection.

Horseshoe kidney was originally found in the descendants of a single **+lx** male which had been mated to a piebald female obtained from a dealer. (It was subsequently found in other stocks carrying **lx** and was not, therefore, a separate genetic entity introduced by the piebald female.) The progeny of this pair were sib-mated and four parallel inbred lines were set up, with selection by progeny test for high incidence of horseshoe kidney. The animals chosen for breeding were usually a polydactyl (**+lx**) and a normal-toed sib (**+ +** or **+lx**); several pairs were mated in each line and each generation and their progeny were autopsied, the pair chosen as line parents for the next generation being those whose progeny showed the highest incidence of horseshoe kidney, usually taken over the first two litters.

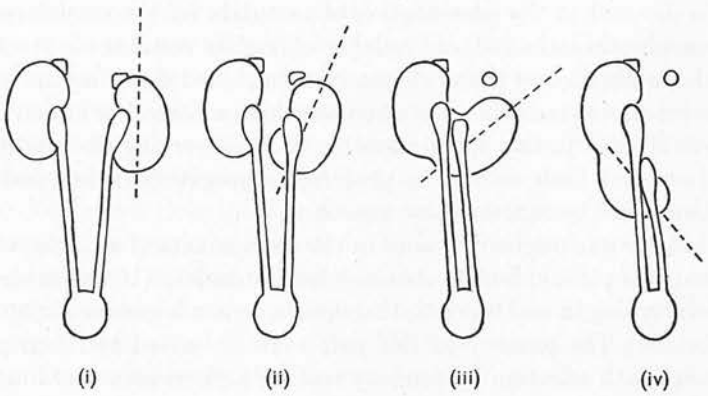
If horseshoe kidney were an expression of **lx**, such a stock should have the following characteristics. (*a*) Among the progeny of intercrosses (**+lx** × **+lx**) the incidence of horseshoe kidney should be higher in hemimelics, all of which carry **lx**, than in non-hemimelics, some of which are **+ +**^{lx}. (*b*) For each parental mating type, the horseshoe kidney incidence in the non-hemimelic young should be in proportion to the expected frequency of **+lx** heterozygotes. (*c*) If, in addition, **lx** were incompletely dominant in its horseshoe kidney expression, the average grade of horseshoe kidney should be higher in hemimelics (**lxlx**) than in non-hemimelics.

Selection against **lx**, by mating together normal-toed pairs, was started after four generations of sib-mating. If horseshoe kidney were an expression of **lx**, the elimination of **lx** from the stock should be accompanied by the elimination of horseshoe kidney also. When a pair failed to produce hemimelic young, indicating that one mate (at least) was **+ +**^{lx}, they were separated and subsequently tested for **lx**, by mating to **lxlx** homozygotes; an animal under test was considered proven free of **lx** if of six or more young none was classified as **lxlx**. After test it was autopsied for examination of the urogenital system.

After six generations of sib-mating, a polydactylous **+lx** heterozygote was outcrossed to an unrelated normal stock and the association between horseshoe kidney and limb defects was again observed, in the inbred descendants of this outcross. This stock was maintained without selection for horseshoe kidney, all matings being between untested polydactylous **+lx** heterozygotes.

(ii) *Routine autopsy methods.* All animals in the stock were autopsied and the state of the urogenital system examined. Exceptions were those, chiefly homozygotes still in the nest, which died and were eaten. Animals for routine autopsy were usually killed at weaning age. Those for detailed morphological study were usually killed either soon after birth, when the complete urogenital system was of a size suitable for sectioning, or as adults after use for breeding.

At routine autopsy only a gross examination was made of the state of the urogenital system, either with the naked eye or under the low power of a binocular dissecting



Text-fig. 1. Kidneys, ureters, bladder and adrenal glands. The broken line indicates the long axis of the left kidney. (i) Normal. (ii) Low-grade horseshoe kidney. (iii) High-grade horseshoe kidney. (iv) Double right kidney.

microscope. The kidneys were classified as 'normal', 'low-grade horseshoe', 'high-grade horseshoe' or 'double right kidney'. Kidneys were considered normal when they were separate organs lying to each side of the aorta in the anterior part of the lumbar region, the right slightly anterior to the left, each ureter passing along the medial side of the posterior half of its kidney (see Text-fig. 1). 'Low-grade horseshoe' referred to cases in which the posterior pole of the left kidney approached that of the right, but without actual fusion. 'High-grade horseshoe' referred to cases in which the posterior pole of the left kidney was fused to that of the right. In the 'double right kidney' condition the left ureter crossed the midline and drained the posterior half of a long double kidney lying in the right side of the abdomen.

A number of complete young litters from the unselected stock, after the outcross, were examined for the simultaneous distribution of limb type, kidney type and number of presacral vertebrae. For this purpose autopsy specimens were skinned and eviscerated (excepting the urogenital system), fixed in 70 % alcohol, dehydrated and cleared in xylene. This procedure allowed satisfactory counting of the number of vertebrae in each group; it had the advantage of being cheaper and quicker than the alizarin-glycerol clearance method.

High-grade hydronephrosis and hydroureter were obvious on dissection; they could usually be determined during life by palpation. The presence of low-grade hydronephrosis was determined by viewing the kidneys against a light; a hydropic kidney is abnormally translucent.

(iii) *Results and conclusions.* Table 1 gives the limb and kidney type classifications of 597 mice from the selected stock; Table 2 refers to a further 180 mice from the unselected stock, after outcross. These are additional to the 272 autopsies reported previously (Carter, 1951). Table 3 gives the full classification, for limb type, kidney type and number of presacral vertebrae, of a number of litters included in Table 2.

Table 1. *Frequencies of renal abnormalities in the selected stock*

Figures in italics refer to hydropic cases; others non-hydropic.

		Kidney type of progeny			
Limb type of progeny	Mating type of parents	Separate kidneys	Low-grade horseshoe	High-grade horseshoe	Double right
Normal	++ × ++	18	0	0	0
	++ × +lx	36+*1*	9	3	1
	++ × lxlx	11	7	6	0
	+lx × +lx	*214+1*	33	53	2
	+lx × lxlx	10	1	1+*1*	0
Polydactyl	++ × ++	0	0	0	0
	++ × +lx	16	2	3	0
	++ × lxlx	6	3	2	0
	+lx × +lx	32	8	16+*1*	0
	+lx × lxlx	2	1	0	0
Hemimelic	++ × ++	0	0	0	0
	++ × +lx	0	0	0	0
	++ × lxlx	0	0	0	0
	+lx × +lx	13	8+*1*	42+*17*	2+*1*
	+lx × lxlx	3+*3*	0	2+*4*	1

* Includes one case of left ureteric and renal agenesis.

Table 2. *Frequencies of renal abnormalities in the unselected stock after outcross*

Figures in italics refer to hydropic cases; others non-hydropic.

		Kidney type of progeny			
Limb type of progeny	Mating type of parents	Separate	Low-grade horseshoe	High-grade horseshoe	Double right
Normal	+lx × +lx	*75	22	5	0
Polydactyl	+lx × +lx	12	7	4	0
Hemimelic	+lx × +lx	12+*1*	7+*1*	26+*3*	4+*1*

* Includes one case of left ureteric and renal agenesis.

(*a*) The incidence of horseshoe kidney among the progeny of intercross matings (+lx × +lx) was higher in the hemimelics (lxlx) than in the non-hemimelics (+ + and +lx), as would be expected if horseshoe kidney were an expression of lx. This is shown in Table 4. It is true of both the selected stock and the unselected stock after outcross.

(*b*) Within the selected stock there were differences between parental mating types in the incidence of horseshoe kidney among the non-hemimelic (+ + or +lx) progeny, as would be expected if horseshoe kidney were an expression of lx (see Table 1). Furthermore, the incidence of horseshoe kidney among the non-hemimelic progeny of each mating type was in proportion to the expected frequency of +lx heterozygotes; for the four mating types + + × +lx, +lx × +lx, + + × lxlx and +lx × lxlx, the horseshoe kidney incidences were in the ratios $\frac{1}{2}:\frac{2}{3}:1:1$. This is shown in Table 5, where the data of

Table 1 are analysed to yield a joint maximum likelihood estimate of the penetrance of heterozygous **lx**, as expressed in horseshoe kidney. There is homogeneity between the mating types, indicating that the data support the hypothesis that horseshoe kidney was an expression of **lx**.

No evidence was found of any maternal effect on the incidence of horseshoe kidney. Matings of the type **lxlx**♀ × **+ +**♂ did not differ significantly, in the horseshoe kidney incidence of their progeny, from matings of the type **+ +**♀ × **lxlx**♂; they are lumped in

Table 3. *Simultaneous distribution of limb type, kidney type and number of presacral vertebrae*

	26 presacral vertebrae			25 presacral vertebrae		
Limb type	Normal kidneys	Low-grade horseshoe	High-grade horseshoe	Normal kidneys	Low-grade horseshoe	High-grade horseshoe
Normal	13	7	—	1	3	2
Polydactyl	—	5	—	1	—	1
Hemimelic	—	2	3	—	6	*4

* Includes one case of double right kidney.

Table 4. *Frequencies of horseshoe kidney (percentage) in the progeny of intercross matings* (**+lx** × **+lx**)

	Limb type of progeny	
Stock	Non-hemimelic	Hemimelic
Selected	31·4	84·5
Unselected	30·4	76·4

Table 5. *Penetrance of heterozygous* **lx**, *estimated from the kidney defect data of Table* 1

	Kidney form of progeny				
	Separate kidneys		Horseshoe, all grades		
Mating type of parents	Obs.	Exp.	Obs.	Exp.	χ^2
+ + × +lx	53	54·42	18	16·58	0·159
+ + × lxlx	17	18·65	18	16·35	0·314
+lx × +lx	247	247·92	113	112·08	0·011
+lx × lxlx	12	8·53	4	7·47	3·027
					3·511

$P > 0{\cdot}3$ for three degrees of freedom.

Note. The maximum-likelihood estimate of the penetrance p of **lx**, as expressed in kidney defects, is given by $\frac{153}{p} - \frac{29}{1-p} - \frac{247}{3/2-p} - \frac{53}{2-p} = 0$, whence $p = 0{\cdot}467$.

Table 1. The same is true of the reciprocal crosses **+lx** × **+ +** and **+lx** × **lxlx**. These bodies of data are rather small, however, and would not suffice to demonstrate any but a strong maternal effect.

(*c*) The average grade of horseshoe kidney was higher in **lxlx** homozygotes (hemimelics) than in putative heterozygotes. Table 6 shows that there was a correlation between the severity of the limb defects and the grade of horseshoe kidney. Normal kidneys were predominant in the normal limb class, of which they constituted over 70 %; low-grade horseshoe kidney occurred in 15 % of the polydactylous **+lx** heterozygotes, but in only 12 and 9 % respectively of the mice with normal and hemimelic limbs; high-grade horseshoe kidney was predominant among hemimelics.

(*d*) Ten normal-toed mice of the selected stock were proven, by direct genetic test, to be $+ +^{lx}$ homozygotes; all were found to have normal urogenital systems. Two of them had been mated together and produced eighteen young; all of these also had normal urogenital systems. Horseshoe kidney occurred in rather more than a quarter (117/408) of the normal-toed mice in the selected stock; the probability is less than 1 in 10,000 that a random sample of twenty-eight should, by chance, fail to include one with horseshoe kidney; it must therefore be concluded that the sample was not a random one with respect to horseshoe kidney. As the sample had been selected only with respect to genotype at the locus of **lx**, it follows that the occurrence of horseshoe kidney was related to the presence of **lx**.

(*e*) The association between horseshoe kidney and the limb defects was not destroyed by outcrossing (see Table 2). An unexpected result was that the incidence of horseshoe kidney fell only slightly after the outcross (see Table 4).

Table 6. *Relationship between limb and kidney defects; data of Table* 1

	Kidney type		
Limb type	Separate kidneys	Low-grade horseshoe	High-grade horseshoe and double right kidney
Normal	291	50	67
	71·4%	12·2%	16·4%
Polydactyl	56	14	22
	60·9%	15·2%	23·9%
Hemimelic	19	9	69
	19·6%	9·3%	71·1%

Table 7. *Genotype at the locus of* **lx** *and number of presacral vertebrae; based on the data of Table* 3

	Presacral vertebrae	
Genotype	*26*	*25*
++	13 –	1 –
+lx	12 +	7 +
lxlx	5	10

(*f*) Horseshoe kidney coexisted with twenty-six presacral vertebrae as well as twenty-five, and with normal and polydactylous as well as hemimelic limbs. Table 3 also shows that all but fourteen of the forty-eight mice classified for number of presacral vertebrae had abnormal limbs or kidneys or both. These mice all came from intercross matings, **+lx** × **+lx**; a sample of forty-eight would therefore be expected to include about twelve $+ +^{lx}$ homozygotes. Assuming that horseshoe kidney and limb defects both constitute evidence of the presence of **lx**, most (or perhaps all) of the fourteen mice with neither type of abnormality must have been the expected $+ +^{lx}$ homozygotes. Of the rest, fifteen were hemimelic **lxlx** homozygotes; the remainder, all with defects of the limbs or kidneys or both, were putative heterozygotes. Table 7 shows the distribution of the number of presacral vertebrae in these three groups. It appears that **lx** in the heterozygous as well as the homozygous state tended to reduce the number of presacral vertebrae, though the effect was more marked in homozygotes. All had seven cervical and thirteen thoracic vertebrae, so the presacral reduction was expressed entirely in the lumbar region.

(*g*) Hydronephrosis occurred in 27 % of the **lxlx** homozygotes and 1% of the heterozygotes in the selected stock; it showed a strong association with horseshoe kidney, the

great majority of the hydropic kidneys being also horseshoe. In the unselected stock its incidence was only 11%, but it was still associated with horseshoe kidney.

(*h*) There were two cases of unilateral ureteric and renal agenesis; in both the genotype at the **lx** locus was ambiguous, as both were normal-toed males, the progeny of intercross matings. Three cases of ureteric and renal agenesis were reported previously (Carter, 1951), two of them unilateral; in all four unilateral cases the left side was affected. There is still not enough evidence to decide whether this defect was associated with the presence of **lx**, but it is notable that only one of the five affected individuals was a homozygote.

(*i*) There was no further case of genital agenesis.

All the evidence on the inheritance of horseshoe kidney in these stocks suggests that it was an effect of **lx**, with a lower manifestation in heterozygotes than in homozygotes. Thus in its action on the limbs **lx** is an incomplete dominant with full penetrance when homozygous; in its action on the lumbar vertebrae and in horseshoe kidney it is an incomplete dominant which overlaps normal, both when heterozygous and when homozygous; in its hydronephrosis expression it is almost completely recessive and overlaps normal extensively.

Morphology

The chief aim of this work was to provide morphological information on which to base embryological studies. In horseshoe kidney cases special attention was therefore paid to the relationships of the kidney, especially to the adrenal glands, the abdominal branches of the aorta and posterior vena cava, and to the ureters, and of the ureters one to another. In cases of hydronephrosis special attention was paid to finding if there was any blockage of the excretory ducts which might have caused the hydropic condition.

(i) *Material and methods.* The material used consisted of selected specimens from the autopsy series. The methods included dissection of fresh material under a binocular microscope, examination of cleared specimens and histological sectioning. Dissection was not altogether satisfactory as a method of investigating the renal vessels in horseshoe kidney cases, as they were usually buried in a mass of fatty and connective tissue which was difficult to remove without damage to the vessels. Unstained juvenile specimens, cleared *in toto* with methyl benzoate, were useful for investigating the relationships of the abdominal blood vessels. Kidney architecture was studied in adult material which had been embedded in paraffin wax, shaved away on one side with a microtome and cleared in xylol. Serial sections of complete urogenital systems, especially of young mice, were used to locate ureteric blocks, to find the detailed relationships of blood vessels and to examine kidney structure. Kidneys were difficult to section after Bouin fixation (cf. Carleton, 1938); less hardening occurred after alcohol-acetic acid fixation. Sections were cut at 10 μ and stained with Delafield's haematoxylin and eosin.

(ii) *Horseshoe kidney.* The gross morphological features of the various grades of horseshoe kidney have been outlined above (see Text-fig. 1). All the cases examined fell into one or other of the four grades, though it was sometimes difficult to allocate a borderline case. Despite the variation, horseshoe kidney cases presented a number of constant features. (*a*) There were always two ureters (save in the two cases of unilateral ureteric agenesis). (*b*) The right ureter always lay wholly on the right side and drained the anterior of the two kidney elements. This element always lay approximately in the position, relative to the right adrenal gland, appropriate to the right kidney. Kidney

fusion always involved its posterior pole. (*c*) The left ureter always lay wholly to the left of the right ureter and drained the posterior of the two kidney elements. This element lay abnormally far caudad, often reaching the level of the aortic bifurcation. Its renal pelvis always lay on the medioventral or anteroventral, never the lateral or posteroventral margin. (*d*) The ureters were always abnormally close together. (*e*) The aorta and posterior vena cava always lay dorsal to the region of kidney fusion.

Variation chiefly concerned the position of the left kidney element and the relations of the left ureter and abdominal blood vessels. In low-grade horseshoe kidney cases the left kidney was almost in its normal position, but its long axis was displaced obliquely, so that the posterior pole lay rather closer than usual to the midline and rather further caudad: the left ureter passed along its ventral instead of its medial surface. In high-grade cases the long axis of the left kidney element was further displaced, even transverse, its right pole being fused to the posterior pole of the right kidney element; both ureters crossed the ventral surface of the fused region. In double right kidney cases the region of fusion was in the middle of the kidney; both renal pelves and ureters lay on the left of the ventral surface of the duplex organ, the right ureter remaining to the right of the left ureter.

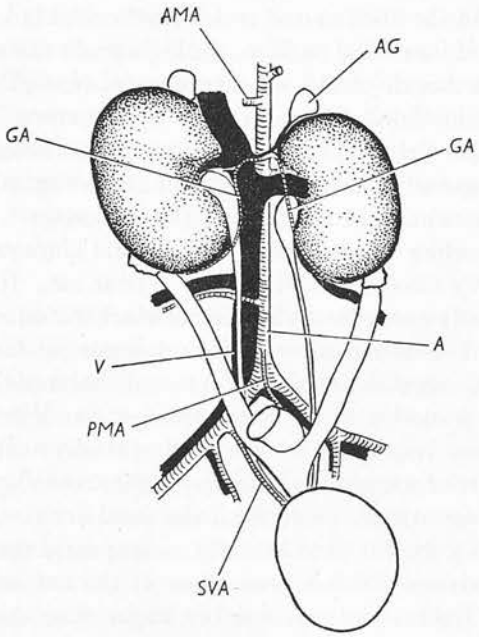
The abdominal aortic branches and corresponding veins of mice with normal kidneys generally agreed with the descriptions given by Greene (1935) for the Wistar rat. In particular, the posterior mesenteric artery usually arose from the aorta a short distance anterior to the aortic bifurcation; in a few individuals it arose farther anterior, as far even as the level of the renal vessels. The gonadic arteries (ovarian or internal spermatic) usually arose from the aorta a short distance posterior to the renal arteries; the right gonadic artery crossed ventrally over the posterior vena cava; each crossed ventrally over the corresponding ureter and followed the posterior margin of the kidney before reaching the appropriate gonad. Occasionally the gonadic arteries arose from the renal arteries, or from a common stem anterior to them (Text-fig. 2). The gonadic veins accompanied the corresponding arteries. The superior vesical arteries differed from those of the rat, as described by Greene; in juvenile material the right vessel was notably larger than the left; this was associated in early postnatal material with a great disparity between their umbilical branches, the right umbilical artery usually being much larger than the left.

There was also a trivial divergence from Greene's descriptions in the range of variation of the left iliolumbar vein; it often drained into the left renal vein instead of the posterior vena cava. Both adrenal glands were wedge-shaped, conforming with the anteromedial margin of the kidney.

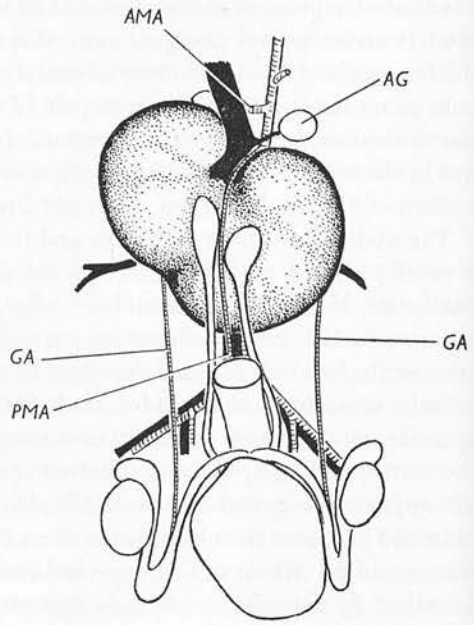
The abdominal vessels of low-grade horseshoe kidney cases, in which there was approximation without actual fusion of the kidneys, were generally similar to those of normal mice. The posterior mesenteric artery, however, was very variable, often originating at mid-renal level. The left gonadic vessels, like the left ureter, crossed over the ventral instead of the medial surface of the left kidney.

In high-grade horseshoe kidney cases the posterior mesenteric artery invariably arose anterior to the fused kidney region and passed caudad over its ventral surface, between the renal pelves and ureters. The gonadic arteries arose from the renal arteries, or from the aorta close to them, and usually accompanied the posterior mesenteric artery caudad over the kidney before crossing ventrally over the ureters to reach the gonads. Occasionally the gonadic arteries arose anterior to the renal arteries and passed along the anterior and lateral margins of the kidney (Text-fig. 3). In some females there was an intermediate

condition (Text-fig. 4), in which the left gonadic artery divided near its origin into ovarian and uterine branches; the uterine branch followed the usual course over the midventral surface of the kidney, while the ovarian branch passed laterally over the left kidney element to reach the gonad. In males the testicular veins usually joined at about the level of the aortic bifurcation and the common testicular vein passed craniad, between the ureters and ventrally over the kidney, to reach the posterior vena cava (Pl. 12). It was accompanied by the internal spermatic and posterior mesenteric arteries, the five vessels forming a compact bundle. In the bundle the posterior mesenteric artery lay dorsal to



Text-fig. 2.



Text-fig. 3.

Text-fig. 2. The normal relationships of the kidneys and ureters to the abdominal aortic branches and the posterior vena cava. Ventral aspect. *A*, dorsal aorta; *AG*, adrenal gland; *AMA*, anterior mesenteric artery; *GA*, gonadic artery; *PMA*, posterior mesenteric artery; *SVA*, superior vesical artery; *V*, posterior vena cava.

Text-fig. 3. Relations of a high-grade horseshoe kidney and ureters to the abdominal aortic branches and posterior vena cava. Ventral aspect. *AG*, adrenal gland; *AMA*, anterior mesenteric artery; *GA*, gonadic artery; *PMA*, posterior mesenteric artery.

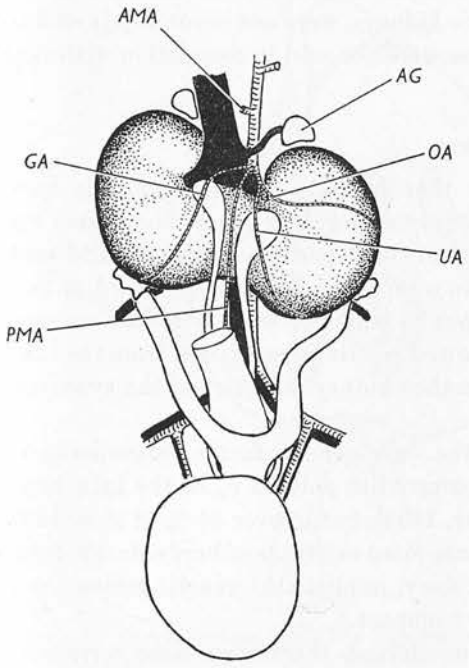
the common testicular vein; it emerged from the bundle between the arms of the inverted Y formed by the paired and common testicular veins, before crossing the mesentery to the colon. The left superior vesical artery and umbilical branch were usually larger than the right, the reverse of the normal condition. The left adrenal gland was a rounded body lying well away from the kidney (Pl. 12).

In double right kidney cases the gonadic arteries arose at mid-renal level; the left artery ran directly to the appropriate gonad. The posterior mesenteric artery arose near the gonadic arteries.

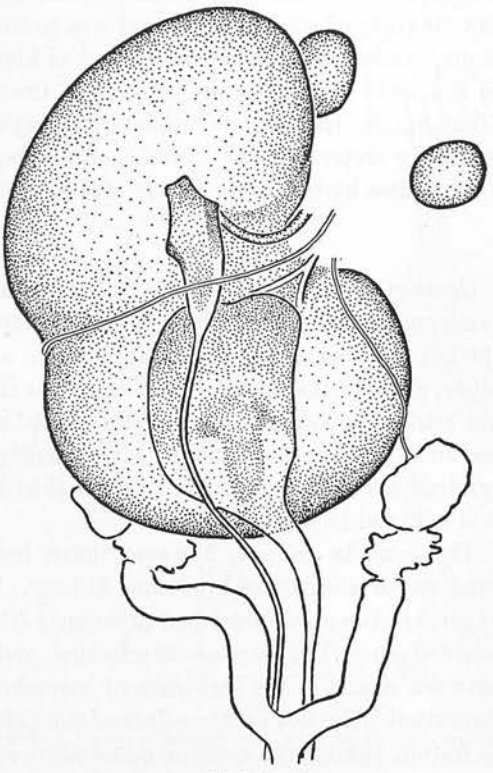
The line of fusion between the kidneys was always quite clear in sections (Pl. 12), though it was not always well marked on the surface.

(iii) *Hydronephrosis and renal cysts.* In advanced hydronephrosis the kidneys some-

times reached an enormous size, distending the abdomen widely; such a kidney felt quite hard on palpation of the live animal and was obviously under pressure. Adhesions between the renal capsule and the lateral or ventral body wall, ileum or liver were common. The ureter was rarely tortuous and gross examination failed to reveal any obvious blockage; when it was cut, the kidney collapsed and drained completely, releasing a quantity of clear fluid. Waves of ureteric peristalsis were often seen in freshly killed



Text-fig. 4.



Text-fig. 5.

Text-fig. 4. Relations of a high-grade horseshoe kidney and ureter to the abdominal aortic branches and posterior vena cava. Ventral aspect. *AG*, adrenal gland; *AMA*, anterior mesenteric artery; *GA*, gonadic artery; *OA*, ovarian artery; *PMA*, posterior mesenteric artery; *UA*, uterine artery.

Text-fig. 5. High-grade horseshoe kidney, with low-grade hydronephrosis of the left element. The left ureter is abnormally wide, the left kidney element large, thin-walled and filled with clear fluid; in this specimen the papilla of the hydropic element has not split up.

material in which hydronephrosis was not very advanced; they formed at the edge of the renal pelvis and moved slowly across it, then rapidly down the ureter.*

In serial sections it was usually found that the ureter draining a hydropic kidney had one or more kinks, close to the vesico-ureteral opening and within the musculature of the bladder wall (see Pl. 12). Above this level it was unobstructed. The ureter usually had an abnormally large cross-section, even when it had appeared grossly normal at dissection. In advanced hydronephrosis the kidney became a hollow, balloon-like structure which

* In the preliminary report (Carter, 1951) it was stated that in hydronephrosis occlusion of the ureter near the bladder was due to localized proliferation of the epithelium. Only a part of the original material is still available; re-examination of this has shown that the cause of the occlusion should be reinterpreted as a small kink in the ureter.

appeared to consist chiefly of cortical elements, built up round a framework of the larger internal blood vessels. The renal papilla, normally single in the mouse, was often replaced by two or more small papillae, and in very advanced cases it tended to disappear more or less completely.

Hydronephrosis was seen in some new-born specimens.

Renal cysts were not uncommon. They were found chiefly in adult specimens, were few in number, often single, and sometimes large, so that it was not always clear at gross examination whether a renal cyst was present, or hydronephrosis. Large cysts occurred almost exclusively in the left element of high-grade horseshoe kidneys and were confined to the right side of its posterior pole, near the region of fusion with the right kidney (Text-fig. 6). Renal cysts, unlike hydronephrotic kidneys, were not immediately drained when the ureter was cut. Multiple renal papillae were not seen in association with renal cysts unless hydronephrosis was also present.

Discussion

Genetics. The data exclude the possibility that horseshoe kidney and the *luxate* syndrome were governed by separate, independently segregating genes. They agree with the hypothesis that horseshoe kidney was a part of the *luxate* syndrome. A third possibility, namely that horseshoe kidney was due to a separate gene closely linked to **lx**, is not critically excluded. In the absence of a stock in which crossing over had occurred, and in which horseshoe kidney consequently showed positive dissociation from the *luxate* syndrome, the simplest hypothesis is that horseshoe kidney is a part of the syndrome; it is adopted in what follows.

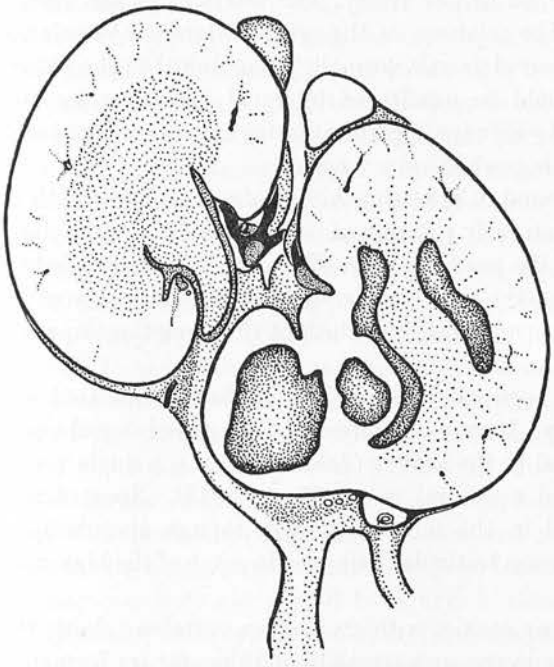
The gene **lx** cannot, however, have been the only genetic factor determining the presence or absence of horseshoe kidney. It occurred in only 13 % of the **lxlx** homozygotes in the stock described previously (Carter, 1951), but in over 84 % of those in the selected stock. The response to selection, and consequent existence of heritable differences between stocks in the incidence of horseshoe kidney, implies that genetic milieu has an important influence on the effect of the primary mutant.

Within the stocks examined, selected and unselected, there were clear correlations between the four main parts of the syndrome. The association of hydronephrosis with hemimelia and horseshoe kidney was very strong; of thirty-six mice with hydronephrosis, twenty-eight showed both hemimelia and horseshoe kidney, a further six hemimelia or horseshoe kidney, and only two had neither abnormality. The association between horseshoe kidney and hemimelia was not so strong, but was still very clear; likewise that between hemimelia and a reduced number of lumbar vertebrae.

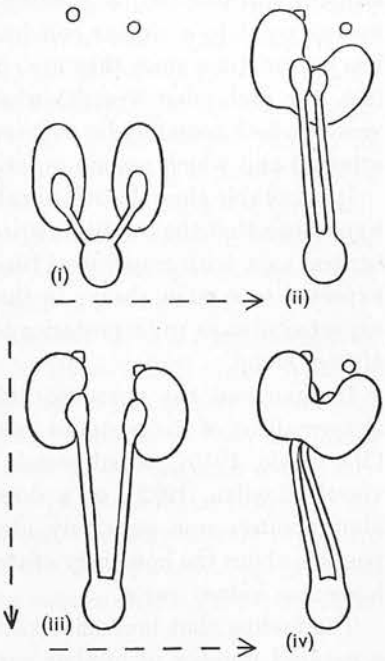
There was also a clear association, within the two stocks, between the occurrence of renal defects and the presence of five lumbar vertebrae, rather than six. When comparisons are made with other stocks, however, a paradox appears. Within families I–IV of the previous paper, as within the present two stocks, the modal number of lumbar vertebrae was six and the presence of **lx** tended to reduce it to five. In all of them renal defects were found. In family V there are commonly only five lumbar vertebrae, so one might expect the incidence of renal defects to be very high; but in fact they were not found. This paradox is resolved if one supposes that the occurrence of renal defects is related to a reduction by **lx** of the number of lumbar vertebrae, rather than to any particular vertebral number; thus in family V, where there are commonly only five

lumbar vertebrae, **lx** does not reduce this number further, nor does it cause renal defects. In the other stocks, where there are usually six lumbar vertebrae, **lx** tends both to reduce this number and also to cause renal abnormalities.

Morphology. The horseshoe kidneys examined in the present work could be arranged in a continuous series in which the position of the right kidney element was nearly constant with respect to the right adrenal gland and aorta, but the left element was progressively rotated about a dorsoventral axis. In low-grade cases the rotation was not enough to



Text-fig. 6.



Text-fig. 7.

Text-fig. 6. Frontal section through a high-grade horseshoe kidney, showing two large renal cysts in the isthmus between left and right elements. (The left element has been cut somewhat obliquely and the section passes through the renal papilla and calyx dorsal and ventral to it.)

Text-fig. 7. Kidneys, ureters, bladder and adrenal glands. (i) Embryonic condition, with the renal pelves occupying the ventral kidney surfaces. (ii) Horseshoe kidney condition, as seen in mice carrying **lx**, reached from (i) by rotation of the left kidney about a dorsoventral axis; the renal pelves are still ventral. (iii) Normal adult condition, reached from (i) by rotation of both kidneys through a right angle about their long axes; the renal pelves are now medial. (iv) Condition reached from (iii) by rotation of the left kidney about a dorsoventral axis: this condition has not been found in mice carrying **lx**.

bring the left kidney into contact with the right; in high-grade cases the rotation led to posterior contact and fusion; in double right kidney cases the rotation approached two right angles, so that the primitively anterior pole of the left kidney became the posterior pole of the duplex organ. The fact that the left kidney is involved in the rotation, while the right is almost unaffected, is remarkable; in its manifestations in the limbs **lx** usually affects the right side more strongly than the left, and almost invariably so when **lx** is homozygous.

This rotation about a dorsoventral axis is to be distinguished from the rotation through a right angle about its long axis which each kidney undergoes in normal ontogeny, and which leads to the renal pelvis lying on the medial surface of the kidney instead of its primitive, ventral position (see Text-fig. 7). This relationship is not seen in high-grade

horseshoe kidney cases; in them the renal pelves always occupy the ventral surface of the kidney. This suggests that the dorsoventral-axis rotation and consequent kidney fusion has occurred before the time at which long-axis rotation normally occurs, and that the latter is prevented as a consequence of the fusion.

The relationship of the posterior mesenteric artery to the kidney in high-grade horseshoe kidney cases likewise throws light on the time of fusion. The fact that the origin of this artery was always anterior to the renal isthmus shows that at the time of fusion the kidneys still occupied a position posterior to the artery. The relations of the gonadic vessels point to a similar conclusion. The relations of the other abdominal vessels give less information, since they are either paired vessels normally lying dorsal to the kidneys (e.g. the iliolumbar vessels) which would be unaffected by renal fusion, or unpaired vessels which normally lie anterior to the kidneys (e.g. the anterior mesenteric and coeliac arteries) and which remain anterior in horseshoe kidney cases.

It is notable that the relationships found in horseshoe kidney cases all agree with the hypothesis that the condition arises by an early rotation of the left kidney about a dorsoventral axis, with consequent fusion of the posterior poles. Thus the left ureter would be expected to remain always to the left of the right ureter; and the left kidney would be expected always to lie posterior to its normal position. Both of these relationships were always found.

In man and the domestic animals horseshoe kidney is sometimes associated with abnormalities of the posterior vena cava. It may be represented by paired dorsal vessels (Marzynski, 1915), paired vessels ventral to the kidney (Johnson, 1914), a single ventral vessel (Boyden, 1932), or a dorsal and a ventral vessel (Story, 1943). None of these abnormalities was positively identified in the mice examined, though speculation is possible about the homology of the common testicular vein seen in some of the high-grade horseshoe kidney cases.

The finding that horseshoe kidney may coexist with six lumbar vertebrae shows that a reduced number of lumbar vertebrae is not an essential condition for its formation. It leaves open, however, the possibility that horseshoe kidney and a reduced number of lumbar vertebrae may both be dependent, in part at least, on a general shortening of the lumbar region. It is unnecessary to suppose that this would lead to a complete one-to-one correlation between the number of lumbar vertebrae and the occurrence of horseshoe kidney: vertebral number is a discontinuous variable, while lumbar length is presumably continuous; likewise the dichotomy into normal and horseshoe kidneys suggests an underlying discontinuity where none in fact exists in the series of morphological states between the normal and the double right kidney condition. It is hardly to be expected that the threshold for the vertebral change should always occur at exactly the same point, on the scale of lumbar length, as the threshold for renal displacement: therefore a complete correlation would not be expected, though some degree of association should occur. The finding that **lx** fails to give rise to renal defects in family V, in which the number of lumbar vertebrae is already reduced, gives unexpected support to the idea that horseshoe kidney may be causally related to lumbar shortening.

The presence of ureteric kinks in mice carrying **lx** implies that in them the ureter is longer than the region into which it has to fit. The urogenital system is bounded ventrally and caudally by the pubic bones and abdominal wall, which are in contact with the bladder; dorsally and cranially by the adrenal glands, at the level of the first lumbar

vertebra, which limit the craniad migration of the kidneys in ontogeny. If hydronephrosis is evidence of ureteric occlusion by a kink, then kinks occurred almost exclusively among **lxlx** homozygotes (see Table 1), and especially among high-grade horseshoe kidney cases. But homozygosis in **lx** is associated with a short lumbar region; and high-grade horseshoe kidney is associated with a caudad displacement of at least one kidney, the left, relative to the lumbar region. Therefore the coincidence of homozygosis in **lx** and the presence of horseshoe kidney are just the conditions which would be expected to reduce to a minimum the distance between the posterior surface of the kidney and the base of the bladder, this being the region into which the ureter has to fit. Moreover, the ventral rather than medial positions of the renal pelves would tend to reduce still further the length available to the ureters. Why the growth of the ureters should not be adapted to the reduction in the available length is not clear; a hint may perhaps be found in the work of Gruenwald (1943), who gave reasons for believing that migration of the kidneys is a purely passive process, a result of the straightening of the embryo. If the ureteric kinks are the result of prematurely limited kidney migration, they too may have their origin in changes of embryonic form external to the ureters. It is certain that the hydronephrosis associated with **lx** may originate during prenatal life, since it was seen in the newborn.

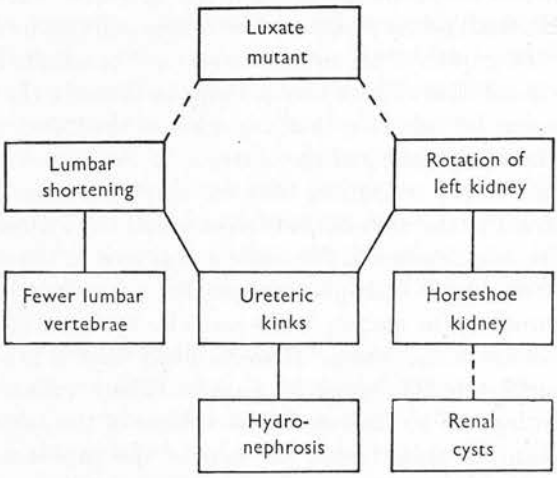
The occurrence of double or multiple renal papillae appears to be a characteristic of advanced hydronephrosis in the mouse; it was seen also by Green (1951) in the hydropic kidneys of some *short-ear* homozygotes. It seems likely that it is a purely mechanical result of the hydronephrosis. The great increase in kidney volume in hydronephrosis appears to be due entirely to an increase in the volume of the calyces, and not to any increase in the amount of renal tissue; the base of the papilla is thus progressively stretched as the hydronephrosis advances, and multiple papillae may merely represent the parts of a single papilla which has been stretched to the point of splitting. Multiple renal papillae were also found by Dunn & Gluecksohn-Schoenheimer (1947) in **urur** homozygotes, but in cystic, not hydropic kidneys.

Renal cysts cannot be attributed to ureteric occlusion, since the cysts were not in communication with the renal pelvis and ureter. It is probably significant that large cysts occurred almost exclusively in association with high-grade horseshoe kidney, and only in the interrenal isthmus. This region is morphologically part of the left kidney, but it is a part which reaches over to the right of the centre line and shows the greatest deviations from normal kidney architecture; if imperfect organization of the glomeruli and tubules were to occur anywhere, this is the region where it might be expected to appear.

Ureteric agenesis was always found to be accompanied by renal agenesis in mice homozygous for the *myelencephalic blebs* mutant (Brown, 1931), in those carrying *Danforth's short-tail* (Gluecksohn-Schoenheimer, 1945) and in *uro-recto-caudal syndrome* homozygotes (Dunn & Gluecksohn-Schoenheimer, 1947). It has been suggested (Gluecksohn-Waelsch, 1951) that this relationship, also found in human cases of ureteric agenesis, is evidence that there might be in mammals an inductive relationship between the ureter and the metanephrogenic blastema similar to that experimentally shown to exist in the chick. The few cases of ureteric agenesis found in the present work do not contradict this.

The many effects of **lx**, on limbs, pelvic girdle, lumbar vertebrae, kidneys and ureters constitute an example of pleiotropism which has many parallels in vertebrate genetics. A complete analysis of its manifold effects into a 'pedigree of causes' (Grüneberg, 1943)

must clearly be based on a study of the development of the complete syndrome, both at morphological and at biochemical levels. Nevertheless, it is possible at this stage to construct a provisional, partial pedigree of causes, based on a study of the adult condition, relating the renal defects and reduced number of lumbar vertebrae to two intermediate causes, namely lumbar shortening and rotation of the left kidney (Text-fig. 8). The objects of further studies with the *luxate* mutant will be to test this provisional pedigree of causes against embryological findings, to attempt to identify the cause of rotation of the left kidney, and to relate them both to a pedigree of causes of the limb defects.



Text-fig. 8. Provisional pedigree of causes of renal and lumbar defects caused by **lx.**

SUMMARY

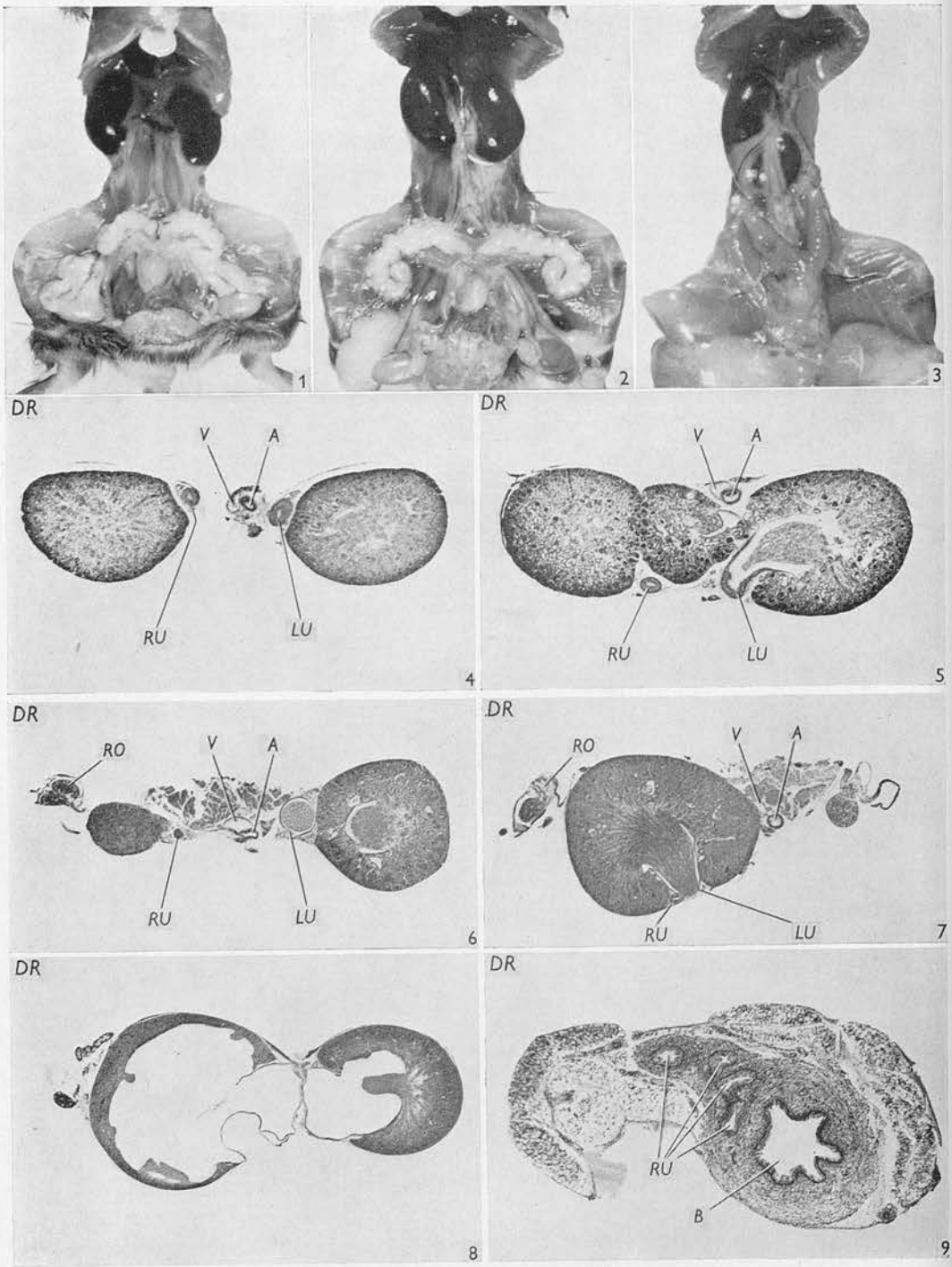
The gene *luxate*, **lx**, of the house mouse regularly causes widespread defects in the hind limbs of homozygotes. A tendency to a reduced number of presacral vertebrae, hydronephrosis and horseshoe kidney also occur in some stocks carrying **lx**. These have been investigated in a selected stock which included more than two hundred horseshoe kidney cases. The results show that all three effects are expressions of **lx**.

In horseshoe kidney cases there were always two ureters and the renal fusion was always posterior. They varied from low-grade cases, in which the kidneys were abnormally close but not fused, to very high-grade cases in which there was a double kidney on the right side and none on the left. The right kidney was relatively little affected, most of the variation occurring in the position of the left kidney. Renal cysts occurred especially in high-grade horseshoe kidneys, but large cysts were confined to the left kidney near the region of fusion.

Hydronephrosis was associated with kinks in the ureter, close to the vesical opening and within the muscular wall of the bladder.

The presence of renal abnormalities was correlated with a reduction in the number of lumbar vertebrae from six to five.

It is suggested that these defects may stem from two underlying causes, (*a*) disproportionate reduction in the length of the lumbar region, leading to reduced vertebral number and ureteric kinks, whence hydronephrosis; (*b*) an unidentified disturbance of kidney migration, leading to rotation of the left kidney about a dorsoventral axis, whence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horseshoe kidney. Elucidation of their causal relationships to the limb defects will require embryological analysis.

I wish to acknowledge my indebtedness to Miss Esmé Mavor for her assistance in looking after the mice and with the histological work; and to Mr D. Pinkney, who took the photographs.

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EXPLANATION OF PLATE

Fig. 1. Normal adult male mouse dissected to show the urogenital system.

Fig. 2. Horseshoe kidney in an adult **+lx** male. The testicular veins join to form a common median vein at the level of aortic bifurcation.

Fig. 3. High-grade horseshoe kidney, approaching the double right kidney condition, in an adult **lxlx** female. Both ureters and the right ovarian vessels can be seen crossing the ventral surface of the kidney.

Fig. 4. For comparison with fig. 5. Transverse section through the kidneys of a normal mouse, near the posterior pole. The ureters (*LU* and *RU*) occupy a mediodorsal position. *A*, dorsal aorta; *DR*, dorsal, right; *V*, posterior vena cava. (×14.)

Fig. 5. For comparison with fig. 4. Transverse section through the horseshoe kidney of a sib, at the same level. The kidney occupies a more caudal position and so presents a larger cross-section. The left kidney element is cut through the renal papilla and the interrenal isthmus. Both ureters lie ventral to the kidney. (×14.)

Fig. 6. For comparison with Fig. 7. Transverse section through low-grade horseshoe kidneys at the level of the right ovary (*RO*). *A*, dorsal aorta; *DR*, dorsal, right; *LU*, *RU*, ureters; *V*, posterior vena cava. (×14.)

Fig. 7. For comparison with fig. 6. Transverse section through the double right kidney of a sib, at the same level. (×14.)

Fig. 8. Transverse section, at the level of the right ovary, through the kidneys in a case of bilateral hydronephrosis; it is more advanced on the right side. The kidneys have collapsed somewhat during fixation. (×6½.)

Fig. 9. For comparison with fig. 8. Section through the base of the bladder (*B*) and the right ureter (*RU*) of the same specimen. The ureter is heavily kinked near its opening into the bladder and appears in the section four times. *DR*, dorsal, right. (×26.)

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THE GENETICS OF LUXATE MICE

IV. EMBRYOLOGY*

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(With Plates 1 and 2 and Twenty-one Text-figures)

Introduction

The *luxate* syndrome in the house mouse comprises a number of congenital defects of the hind limbs, lumbar vertebrae, urogenital system and abdominal arteries (Carter, 1951*a*, 1953). Hind-limb defects vary from partial duplication of the hallux to preaxial hemimelia, with absence of the tibia and severe dystrophy of the femur and pubis. The lumbar vertebrae may be reduced from six to five. Hydronephrosis may be present in one or both kidneys, the corresponding ureter being occluded by kinks close to its opening into the bladder. The kidneys may be fused together at their posterior poles, forming a single horseshoe-shaped organ; in such cases there is always an anomalous origin of the posterior mesenteric artery, anterior to the kidney. In newborn mice the left may be the dominant umbilical artery, a reversal of the normal condition.

The evidence presented previously (Carter, 1951*b*, 1953) established that the occurrence of the *luxate* syndrome is conditional on the presence of a single major mutant gene, **lx**. Its expression is powerfully influenced by the background genotype, and so the syndrome is very variable; but there are some fairly constant features. Tibial hemimelia is characteristic of homozygotes. Preaxial polydactyly (including hyperphalangy of the hallux) also occurs in many homozygotes, but it is typical rather of the heterozygote; most heterozygotes, however, have normal limbs. The urogenital defects occur only when the genetic background is favourable: horseshoe kidney is seen in both heterozygotes and homozygotes, but with a higher frequency and a higher grade of expression in homozygotes; hydronephrosis is found almost exclusively in homozygotes. Lumbar reduction is seen only in stocks in which the normal number of lumbar vertebrae exceeds five; reduction is found in heterozygotes as well as homozygotes, but with a higher frequency in homozygotes.

The expression of **lx** is rarely symmetrical, but the asymmetry shows some constant features. Tibial hemimelia is almost always more severe in the right leg. Unilateral preaxial polydactyly in heterozygotes may appear on either foot, but it is on the right side in about three-quarters of all cases. Sacralization of the twenty-sixth vertebra is often one-sided, the right lateral process showing the sacral form in about three-quarters of all unilateral cases. In horseshoe kidney, on the other hand, it is always the left element which is dystopic, the right being in its normal position relative to the right adrenal body.

* An early draft of this paper is referred to as paper III of the series by H. Grüneberg in *The Genetics of the Mouse* (second edition, 1952).

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The *luxate* gene is thus one of the large number of mammalian mutants with variable and pleiotropic effects. As such it is a challenge to the geneticist, in the light of the Principle of the Unity of Gene Action (Grüneberg, 1943*a*). This principle provides a guiding rule for investigations in developmental genetics; it supposes that the primary action of a gene is always unitary, that true pleiotropism does not exist, and that all cases of apparent pleiotropism should be capable of analysis into a 'pedigree of causes', the various parts of a genetic syndrome being all causally descended from a single primary action of the gene. This paper describes an investigation into the development of the *luxate* syndrome, and especially a search for connecting links between its various, widely separated parts.

In order to discover the developmental abnormalities of mice carrying **lx**, it was first necessary to study the development of normal mouse embryos. There are as yet no general descriptions of the development of the mouse in the second half of intra-uterine life, but the normal development of several selected regions has been described, usually in relation to some particular mutant form. Thus the development of the hind limbs of embryos of 12½ days and later was described by Chang (1939), who investigated the development of polydactyly in Fortuyn's (1939) mice. The development of the ureters and kidneys in embryos of 10½ days and later was described by Brown (1931), who investigated the renal agenesis in Little & Bagg's (1924) mice. More recently Ludwig (1947) has investigated the kidney primordia of 10½-day embryos. These, together with Grüneberg's (1943*b*) descriptions of the development of the external appearance of mouse embryos of 9½ days and more, formed a basis for the investigations described below.

In the course of the work it appeared that abnormalities of the umbilical artery probably occupy an important position in the development of the syndrome, and may form the connecting link between the limb and urogenital defects. Between 10½ and 11½ days of embryonic life this artery undergoes a sudden change of course. In the 10½-day embryo it originates by paired roots from the ventral floor of the dorsal aorta, at the level of the hind-limb primordia; they pass medially between the Wolffian ducts, diverge round the gut and come together in the posterior wall of the coelom before passing out of the embryo to the placenta. In the 11½-day embryo the umbilical artery arises by paired roots from the lateral walls of the aorta; they pass dorsally and laterally round the metanephric blastemata and Wolffian ducts, joining the primitive roots of the artery lateral to the gut. They thus form a girdle through which the kidneys have to migrate to reach their definitive positions. The change from the 10½-day to the 11½-day course of the umbilical artery is associated with great morphogenetic activity in the hind-limb buds, which draw their blood supply from the new umbilical girdle. In embryos carrying **lx** the development of the girdle was often found to be abnormal and was associated with an upset of kidney migration which is thought to be the cause of horseshoe kidney.

This suggested that the occurrence of horseshoe kidney was secondary to the arterial defects; but it left unanswered the question whether the arterial defects were themselves secondary to the limb defects or vice versa, and what relationship they bore to the lumbar reduction. This problem was investigated in 10½-day embryos, in which the lateral umbilical arterial roots have not yet appeared and the hind limbs are represented only by low ridges at the level of the primitive umbilical roots. It was found that in this material the genotype **lxlx** shifted the hind-limb region towards the head by almost the

width of one somite. Abnormalities of the hind-limb buds were first seen in early 11½-day embryos, abnormalities of the umbilical artery in later 11½-day embryos; polydactyly was first detected in early 12½-day embryos, horseshoe kidney in later 12½-day material, ureteric kinks at 14½ days and hydronephrosis at 15½ days. The order in which the various parts of the syndrome become visible provides valuable clues about their possible causal relationships.

STOCKS

Four stocks of mice were used.

The *CBA* wild-type inbred strain was used for studying the development of the hind limbs and associated structures of normal embryos of 10½–12½ days' gestation; for later stages of normal development *CBA* embryos were supplemented by normal embryos from the other stocks.

A *CBA*-**lx** substrain, isogenic with *CBA* save that **lx** had been introduced by six or more backcrosses, was used for investigating lumbar reduction and the development of limb and umbilical arterial defects in 10½–12½-day embryos. It was supplemented by the *CK* and *Z* stocks* for later stages. The matings used to provide embryos were all intercrosses of polydactylous **+lx** heterozygotes, so that the embryos were expected to comprise mutant homozygotes, heterozygotes and type homozygotes (i.e. standard *CBA* embryos) in the ratio 1:2:1. Expressivity of the limb defects is high on this genetic background, but kidney defects are not seen.

The *CK* stock was used for investigating kidney defects and the later stages of limb defects; it is a selected stock in which kidney defects have a high incidence (Carter, 1953), but expressivity of the limb defects is not extreme. All matings in this stock also were of polydactylous **+lx** heterozygotes.

The cross-bred *Z* stock was used in the early part of the work for producing embryos of the 12½-day and later stages. Most matings were between polydactylous **+lx** females and **lxlx** males; the embryos thus consisted of homozygotes and heterozygotes, the latter including phenotypically normal specimens. This material suffered from the disadvantage that the embryos were of necessity sired by a male in whom the homozygous expression of **lx** was weak; consequently expressivity in the embryos also tended to be weak, an undesirable feature where the whole range of defects was to be examined. A few intercross matings were used.

METHODS

Timing embryos. Embryos were normally timed by the copulation plug method. A male was placed with non-suckling females in the evening (5 p.m.); the next morning (9 a.m.) he was removed and the females were examined for plugs, the vagina being gently opened with blunt forceps. Pregnancy occasionally occurred where no plug had been seen, and in these cases the date of coitus was determined from the known dates on which the male was present, together with the 'external landmarks' age of the embryos (Grüneberg, 1943*b*). The latter method was always used as a check, even when the copulation plug age was known; good agreement was found with Grüneberg's descriptions, save that the inbred *CBA* and *CBA*-**lx** embryo tended to be retarded by rather less than a day. It was assumed that copula-

* The crossbred stock here called the *Z* stock is to be distinguished from the inbred *Z* strain of Gowen, and also from the inbred *Z* strain (=*C3H*) of Bittner (see the Report of the Committee on Standardised Nomenclature for Inbred Strains of Mice, 1952). The stock here designated as *CK* is likewise to be distinguished from the *CK* stock of Grüneberg.

tion occurred before midnight: embryos were dissected out between 9 a.m. and midday, so embryos removed, for example, 10 days after a plug had been found were given the nominal age 10½ days.

Dissection and fixation. Pregnant females were killed under ether anaesthesia; the uterus was removed, pinned out on cork, washed in Ringer's saline and allowed to cool for a few minutes so that muscular tone should diminish. Each horn was slit from end to end with round-nosed scissors, exposing the embryos in their membranes. The distribution of the embryos was noted and each was removed, with membranes and placenta, and transferred to an embryo cup containing saline at room temperature. The yolk sac was gently torn round near its junction with the placenta, using watchmakers' forceps; it was then pulled off the embryo, remaining attached to it by the vitelline vessels. After washing in saline the amnion was likewise removed. Embryos under examination remained alive in saline for up to 2 hr.; by then the heart beat was weak, the vitelline and umbilical vessels could be cut without excessive bleeding, the embryo was washed in saline and fixed by suffusion with Bouin's fluid. 720 embryos were dissected out, of which 140 were *CBA*; the distribution of the remainder, by stocks and ages, is given in Tables 1–3.

Microtomy. After fixation embryos were measured with a travelling microscope, transferred to fresh fixative, and outline drawings were made with the help of a camera lucida; after dehydration they were cleared in methyl benzoate (being stored at this stage if necessary) and embedded in paraffin wax (m.p. 54° C.). Orientation of the block was greatly helped by the outline drawings and the picric stain of the fixative, which made the embedded embryos easily visible. Sections were cut at 10 μ and usually stained with Delafield's haematoxylin and spirit-soluble eosin; various special staining techniques were used to detect the earliest signs of precartilage formation, notably Mallory's triple stain and Baker's modification of Masson's trichrome stain (vide Pantin, 1946). 379 embryos were sectioned in part or whole.

Reconstruction. Graphic reconstructions were plotted on to squared paper with the help of an eyepiece grid with 1 mm. squares. For projections on to a dorsal plane transverse sections were cut and the dorsal nerve tube used to give a median reference axis. Where sections were not accurately transverse, the angle of error was estimated by counting sections between the members of paired structures (e.g. dorsal intersegmental arteries) and the axis of plot offset by this angle.

For projections on to a parasagittal plane, transverse sections were cut and the outline of some easily recognizable feature, e.g. the top of the nerve tube, was plotted by reference to the edge of the ribbon; the latter was usually found by the use of slightly excessive amounts of albumen, which stained with eosin at the edge of the ribbon, or occasionally by direct observation of the dry slide before the wax was dissolved. The smooth curve so obtained gave a false origin to which all further reconstruction was referred, again using the nerve tube to define the median plane.

For projections of transverse sections on to a transverse plane, a false origin obtained as for the parasagittal projection was used, together with the median axis defined by the nerve tube, to enable outlines of sections to be correctly superimposed.

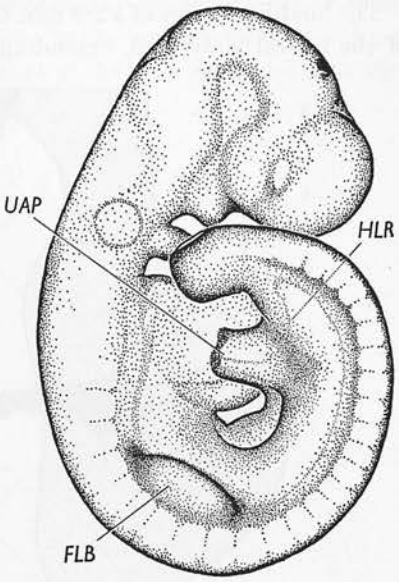
Graphical projections were made of parts of 87 embryos, mostly aged 10½ and 11½ days; those reproduced in this paper were chosen as typical of their classes.

Development of the hind limbs and associated structures in the normal mouse

10½-day stage. The 10½-day *CBA* embryo has a maximum dimension of about 2·9 mm. (range 1·9–3·9 mm.) (Text-fig. 1). The nerve tube is closed throughout, except at the posterior neuropore; the eye is represented by an optic cup, the ear by an otic vesicle. There are about twenty-six somites, but they do not yet extend into the tail bud; the first few somites are rather indistinct. Embryonic turning is complete and the embryo is curled up into a right-handed spiral, dorsum outwards, so that the tail bud lies between the heart and the right side of the forebrain. The heart is beating vigorously; posterior to it the embryo is open ventrally as far as the level of the posterior somites. At this level the umbilical vessels leave the embryo, passing to the right of it; the paired umbilical

arteries originate from the floor of the paired dorsal aortae and sweep somewhat anterad as they pass round the gut before joining and passing out of the embryo. The right artery is often slightly posterior to the left, and slightly smaller, but the disparity is never very great. The umbilical vein accompanies the artery from the placenta to the embryo proper; it then divides round the artery and the two branches pass anterad in the body walls to the liver and heart.

The fore limbs are represented by low buds which occupy a width of about five somites, somewhat posterior to the heart. The hind limbs are represented by slight thickenings of the lateral body walls, forming low ridges, at the level of the umbilical arteries. Owing to the heavy spiralization of the embryo, the hind-limb ridges and the fore-limb buds face one another, at opposite ends of a diameter across the embryo.



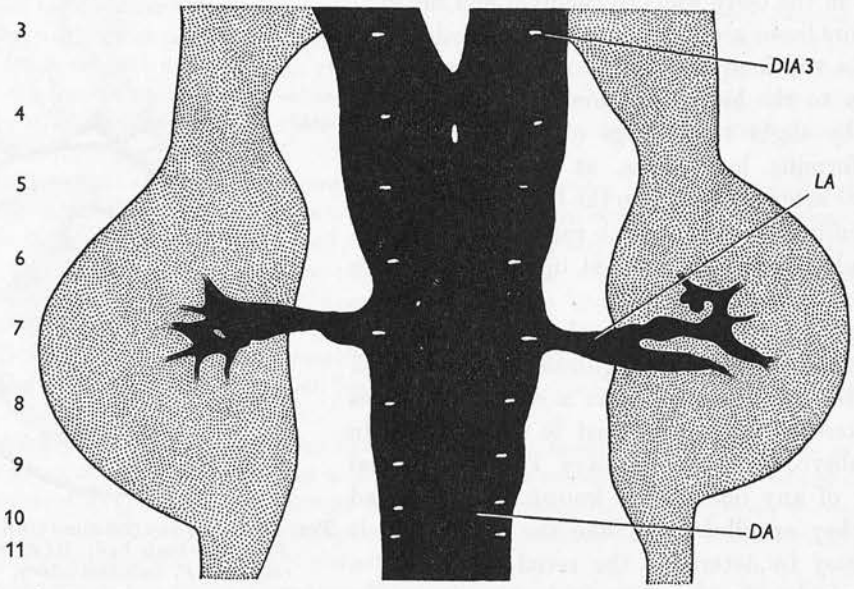
Text-fig. 1. 10½-day (2·9 mm.) *CBA* embryo. *FLB*, fore-limb bud; *HLR*, hind-limb ridge; *UAP*, umbilical artery.

Along the length of the dorsal aorta, arising from it, are small, paired, intersegmental arteries which supply the somites. They form a convenient series of markers which can be used to define levels in the embryo, provided always that the serial number of any one pair is known. In the head region they are ill-defined, like the somites, so it is not easy to determine the serial number of a given pair by direct reference to the first. Six pairs, however, bear a constant relationship to the fore-limb buds; two pairs lie, respectively, at the levels of the anterior angle and anterior margin of the bud, two pairs straddle the point of greatest bud width, and two pairs lie at the levels of the posterior margin and posterior angle (Text-fig. 2). In embryos of more than 3·0 mm. the third of these six pairs is associated with the root of the limb-bud artery; in what follows this pair has been arbitrarily numbered 7, because in the human embryo it is the seventh cervical pair of dorsal intersegmental arteries which is associated with the subclavian artery (vide Hamilton, Boyd & Mossman, 1945). In embryos of less than 3·0 mm. several pairs of dorsal arteries may be associated with small lateral arteries to the fore-limb buds; the serial numbers of these, however, become obvious in graphic reconstructions from their relationships to the buds. The identity of the seventh pair being thus established, the serial number of any other pair in a serially sectioned embryo can be determined by reference to the seventh.

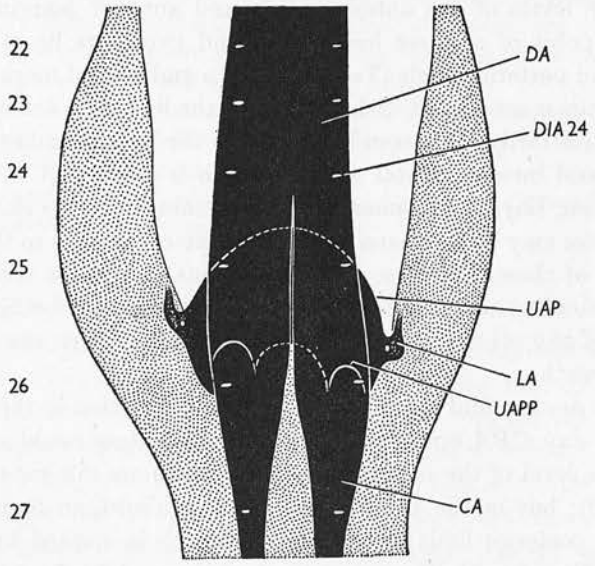
This method was used to find the normal amount of variation in the level of the hind-limb region in 10½-day *CBA* embryos. The hind-limb ridges could not themselves be used to indicate the level of the region, since their limits are not yet sufficiently clearly defined (Text-fig. 3); but in the 11½-day embryo the hind-limb buds bear a constant relationship to the posterior limit of the coelom, which is marked by the well-defined umbilical arteries (Text-fig. 7). The mean level of the umbilical arteries was therefore used in 10½-day embryos to indicate the level of the hind-limb region. Text-fig. 4 shows the data from twenty *CBA* embryos, the level of the artery being arbitrarily defined as

the level of the most posterior point on its anterior wall, which can easily be found from serial sections. In this sample it ranged over one and a half somites' width, from just anterior to the twenty-fifth intersegmental level to midway between the twenty-sixth and twenty-seventh; in only one embryo was it anterior to the twenty-fifth.

The hind-limb ridge of a 2·9 mm. *CBA* embryo consists of a rather ill-defined thickening of the lateral body wall, extending from about the twenty-third to about the twenty-

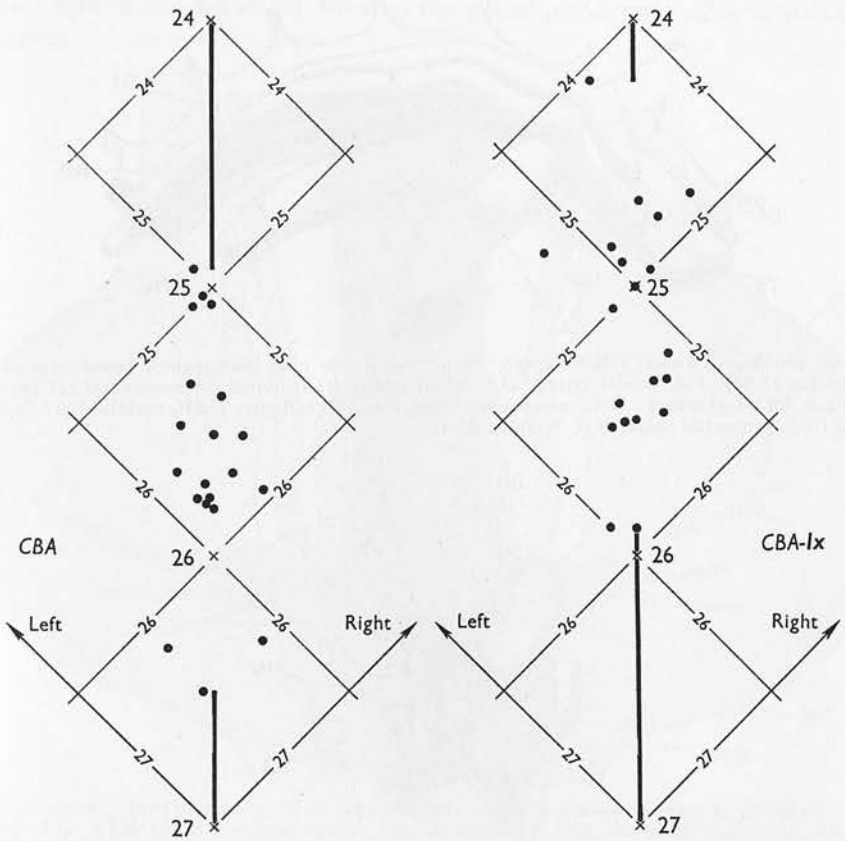


Text-fig. 2. 10½-day (3·1 mm.) *CBA* embryo. Structures in the fore-limb region projected on to a dorsal plane (×85). *DA*, dorsal aorta; *DIA*, dorsal intersegmental artery; *LA*, limb artery.



Text-fig. 3. 10½-day (2·9 mm.) *CBA* embryo. Structures in the hind-limb region projected into a dorsal plane (×85). *CA*, caudal artery; *DA*, dorsal aorta; *DIA*, dorsal intersegmental artery; *LA*, limb-bud artery; *UAP*, umbilical artery, primitive root; *UAPP*, most posterior level of the anterior wall of the umbilical root.

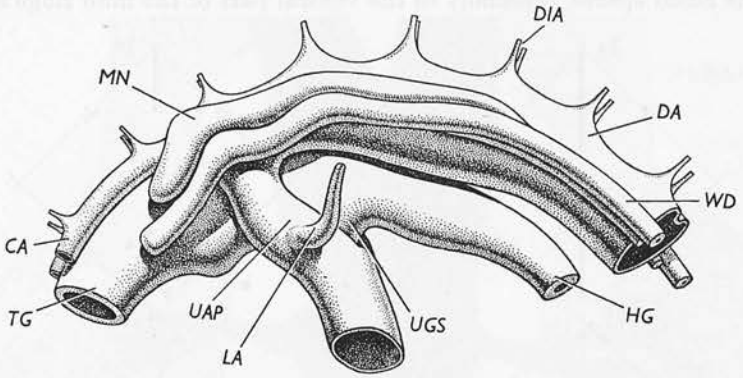
seventh intersegmental level (Text-fig. 3). It consists mainly of loose mesenchyme, lined by ectoderm externally and by mesothelium where it is adjacent to the coelom. The ectoderm consists of a single layer of cuboidal cells covered by a very thin pavement epithelium. In the ventrolateral part of the limb ridge the ectoderm is rather thicker than elsewhere; this is the region in which the *apical ectodermal ridge* will appear, but the cuboidal cells do not yet form a clearly delimited ridge. In the limb mesenchyme there are numerous blood spaces, especially in the ventral part of the limb ridge and near the



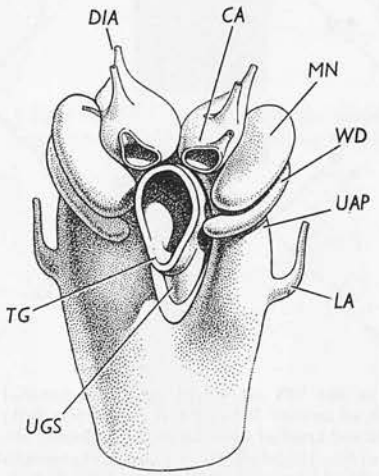
Text-fig. 4. Correlation diagrams of the left and right umbilical arterial levels, measured in terms of the neighbouring intersomite levels, of twenty 10½-day *CBA* embryos (left) and twenty *CBA*-**Ix** embryos. The levels of the left and right umbilical arterial roots have been plotted along the left and right diagonal axes; distance along the thick vertical line therefore gives a direct representation of the mean umbilical level of each embryo. The gap in the vertical line marks the range of the distribution. It is apparent that the mean of the *CBA*-**Ix** distribution is anterior, by more than half a somite, to that of the *CBA* distribution; the cranial quarter of the *CBA*-**Ix** distribution lies entirely anterior to that of the *CBA* distribution.

ectodermal and mesothelial surfaces, but they form a loose network rather than a well-defined vascular system. This network is drained by the posterior cardinal veins. It is fed by two routes. One is a small arterial ramus which leaves the lateral side of the umbilical artery ventral to the gut and runs dorsally and cranially round the end of the coelom into the limb ridge (Text-figs. 3, 5 and 6). The main blood supply, however, comes from the umbilical vein, which forms an annulus round the artery at the point where it joins the embryo; this annulus acts sphygmomanometerwise, pumping blood from the umbilical vein into the limb ridge at each arterial pulsation.

The Wolffian duct of a 2·9 mm. *CBA* embryo extends beyond the level of the corresponding umbilical artery (Text-fig. 5). Anterior to the artery it lies at mid-aortic level, but immediately posterior to the umbilical root it turns ventrally and somewhat mesiad, so that its blind posterior end lies close to the gut (Text-fig. 6). The nephrogenic cord is rather ill-defined; posterior to the twenty-fourth intersegmental level it widens into the metanephric blastema, also rather ill-defined; this extends beyond the umbilical root by



Text-fig. 5. 10½-day (3·2 mm.) *CBA* embryo. Structures in the hind-limb region; based on a parasagittal projection (×85). *CA*, caudal artery; *DA*, dorsal aorta; *DIA*, dorsal intersegmental artery; *HG*, hind gut; *LA*, limb-bud artery; *MN*, metanephric blastema; *TG*, tail gut; *UAP*, umbilical artery, primitive root; *UGS*, urogenital sinus; *WD*, Wolffian duct.

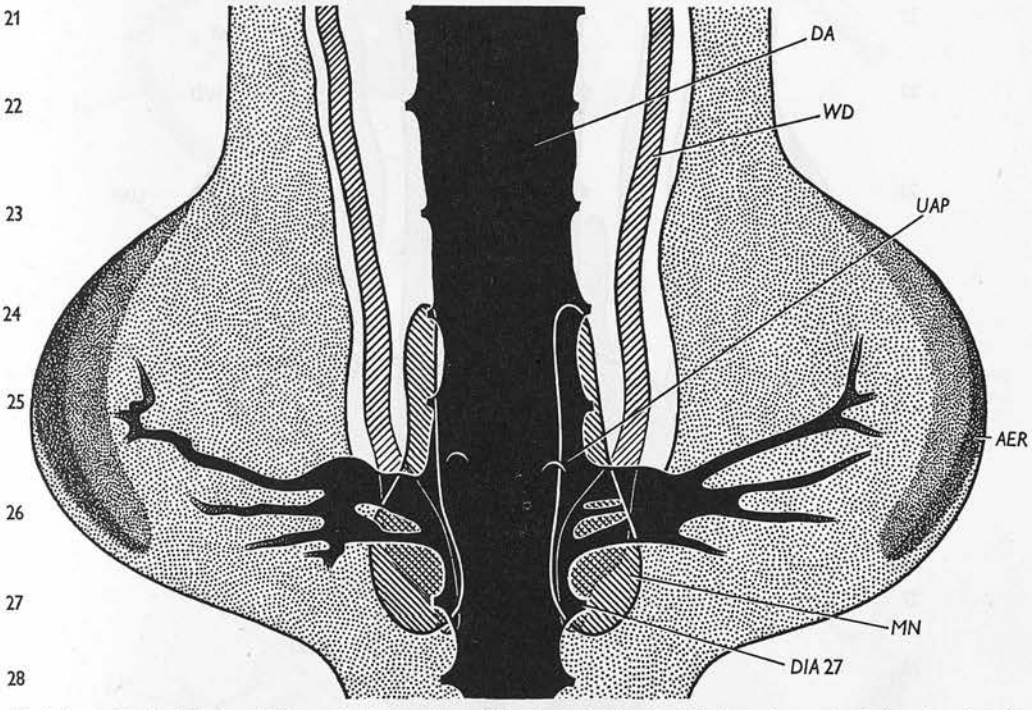


Text-fig. 6. 10½-day (3·2 mm.) *CBA* embryo, the same as in Text-fig. 5. Structures in the hind-limb region; based on a transverse projection (×85). For key see Text-fig. 5.

about one segment's width. The umbilical root passes *medial* to the Wolffian duct and metanephric blastema (Text-fig. 6). The hind gut is small and circular in section. Where it passes between the umbilical arteries the gut widens, forming from its floor a wide, shallow pouch, the future urogenital sinus. Posterior to the umbilical arteries the gut is in contact with the ventral ectoderm, but it is not open to the outside; it continues farther posterior as the tail-gut, which is very large at this stage.

The 2·9 mm. *CBA* embryo is at a stage of great morphogenetic activity, especially of the hind-limb region. It is also growing rapidly, its maximum dimension increasing by

about 0·1 mm., or 3%, per hour. Considerable morphogenetic changes are seen in the hind-limb region in larger embryos in the 10½-day range; and there is a close correlation between the state of development and the maximum dimension of embryos which have received similar handling and fixation. In 3·2 mm. embryos somites are visible in the tail bud. At 3·4 mm. the posterior neuropore is closed and the hind limbs are buds rather than ridges. At 3·6 mm. the apical ectodermal ridge is recognizable in sections; and vascularization of the posterodorsal part of the bud occurs, by the outgrowth of small arteries from the lateral wall of the aorta. At 3·8 mm. the apical ectodermal ridge is visible in the live embryo.



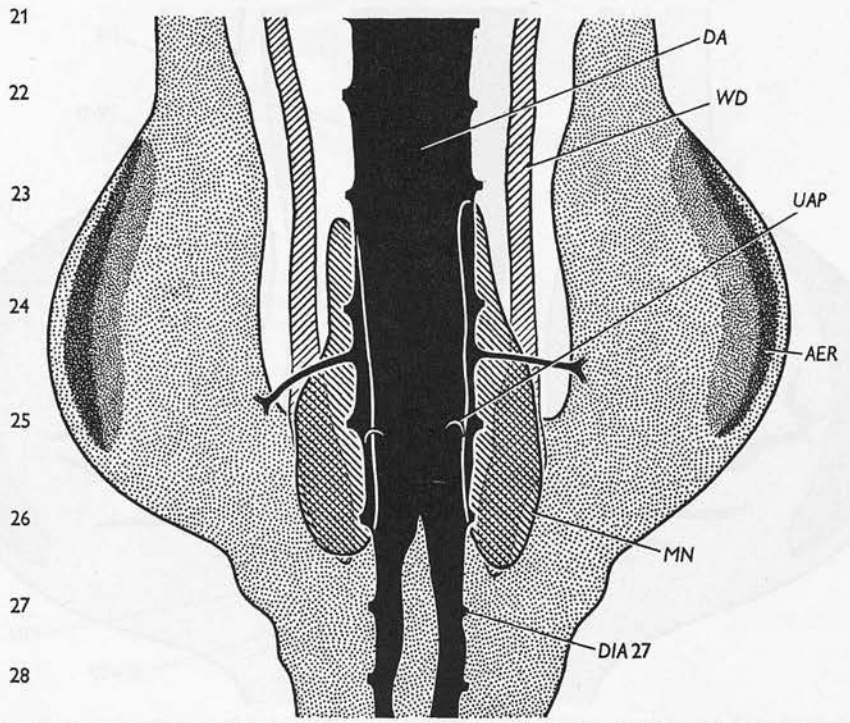
Text-fig. 7. Early 11½-day (4·2 mm.) *CBA* embryo. Structures in the hind-limb region projected on to a dorsal plane (×85). *AER*, apical ectodermal ridge; *DA*, dorsal aorta; *DIA*, dorsal intersegmental artery; *MN*, metanephros; *UAP*, umbilical artery, primitive root; *WD*, Wolffian duct.

11½-day stage. The 11½-day *CBA* embryo has a maximum dimension of about 5·0 mm. (range 4·0–5·9 mm.). The hind-limb buds have a width of about four somites. At 4·0 mm. the bud is still not as long as it is wide (Text-fig. 7); it is semicircular in embryos of about 4·6 mm.; at 5·4 mm. it has developed a marked anteroposterior asymmetry, being drawn over towards the posterior margin. There is not yet any footplate, but the apical ectodermal ridge is clearly visible.

Correlated with these changes in the limb bud there are extensive changes in the limb and umbilical arteries. Outgrowth of lateral branches of the aorta continues and now involves the anterodorsal as well as the posterodorsal part of the bud. The lateral aortic limb arteries give off ventral twigs which anastomose with the original limb arterial supply, coming up from a point on the umbilical artery ventral to the gut (Text-fig. 6). New arterial routes are thereby set up, in parallel with the paired umbilical arteries but,

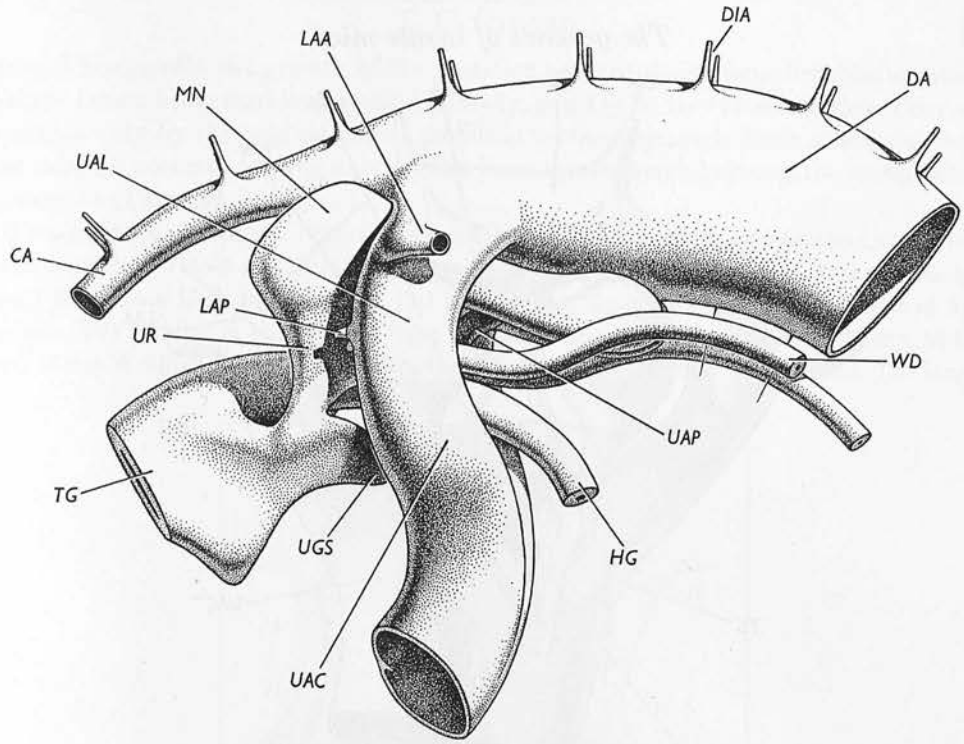
unlike them, passing dorsal and lateral to the Wolffian ducts and metanephric blastemata. During the next few hours these lateral arterial courses develop enormously, taking over almost the whole umbilical blood flow, while the original paired (medial) umbilical arteries regress to a small fraction of their erstwhile size (Text-figs. 9, 11). The process is complete in the 4·8 mm. embryo. The lateral umbilical arteries are always equal, or nearly equal, in cross-section (Pl. 2); the right artery is often a little posterior to the left, reflecting the fact that the common umbilical artery passes to the right of the embryo.

The Wolffian ducts and metanephric blastemata are now encircled by an arterial girdle (Text-fig. 11). At the same time each Wolffian duct becomes flattened and closely applied

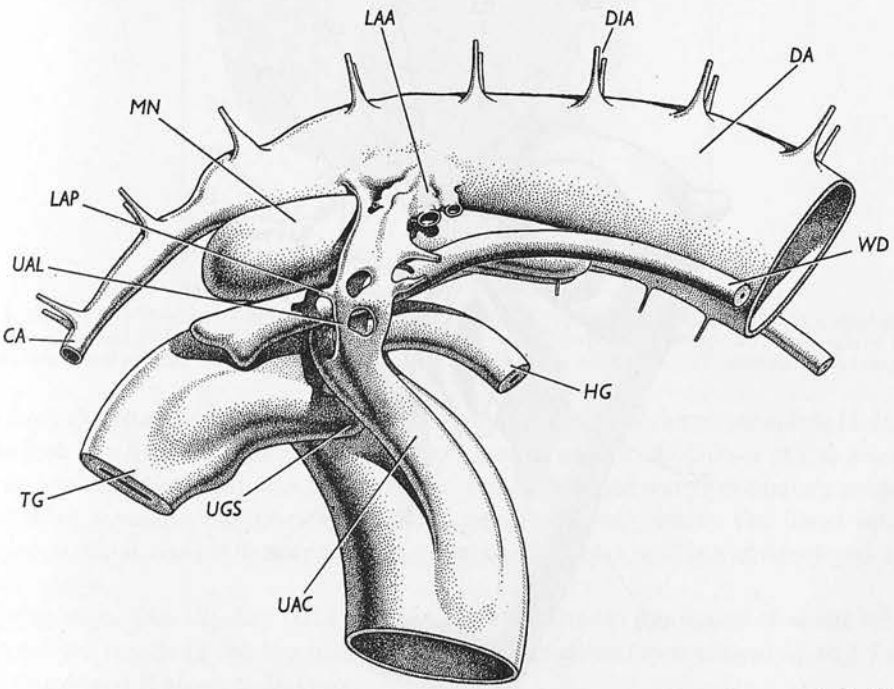


Text-fig. 8. For comparison with Text-fig. 7. Late 10½-day (4·2 mm.) *CBA*-**lx** embryo, probably **lxlx**. Structures in the hind-limb region projected on to a dorsal plane (× 85). For key see Text-fig. 7.

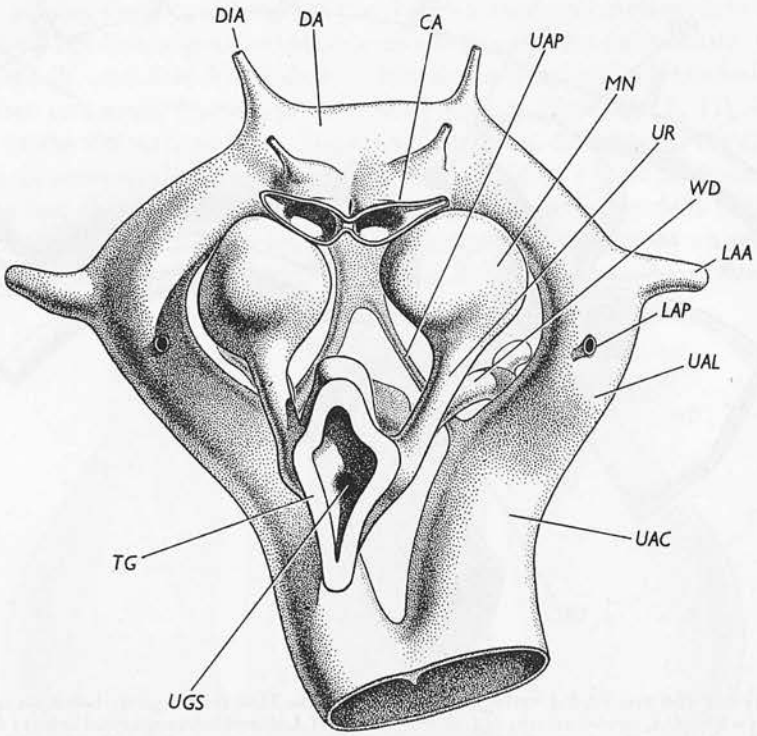
to the blastema where they pass through the girdle; it spirals laterally round the blastema, closely following its form, and thereby changes from a dorsolateral to a medioventral relationship to the blastema. In embryos of about 4·0 mm. a ridge forms on the lateral wall of the gut, immediately posterior to the urogenital sinus; simultaneously a duct buds ventrally from the Wolffian duct, near its posterior end, grows down towards and fuses with the ridge. Posterior to this duct the Wolffian duct becomes pycnotic and soon disappears. Slightly anterior to it a second duct, the ureter, buds off dorsally from the Wolffian duct and grows into the metanephric blastema. The ureter grows rapidly, both inside the blastema and between it and the Wolffian duct so that the posterior end of the blastema becomes enlarged and separated from the duct (Text-fig. 9). Inside the blastema the direction of ureter growth is anterad, so that it tends to pass through the umbilical girdle; it has not yet branched. An effect of the simultaneous development of



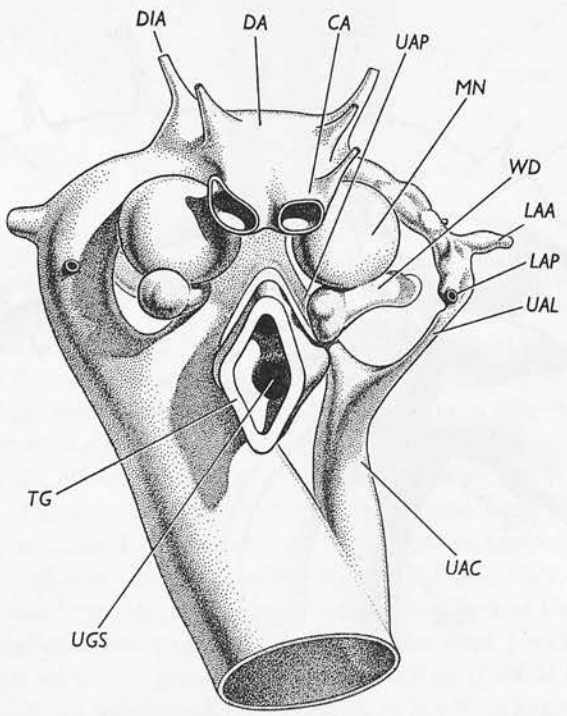
Text-fig. 9. 11½-day (5·4 mm.) *CBA* embryo. Structures in the hind-limb region; based on a parasagittal projection (× 85). *CA*, caudal artery; *DA*, dorsal aorta; *DIA*, dorsal intersegmental artery; *HG*, hind gut; *LAA*, *LAP*, anterior and posterior limb-bud arteries; *MN*, metanephros; *TG*, tail gut; *UAC*, *UAL*, *UAP*, the common umbilical artery and its lateral and primitive roots; *UGS*, urogenital sinus; *UR*, ureter; *WD*, Wolffian duct.



Text-fig. 10. For comparison with Text-fig. 9. 11½-day (5·2 mm.) *CBA*-**1x** embryo, probably **1x1x.** Structures in the hind-limb region; based on a parasagittal projection (× 85). For key see Text-fig. 9.



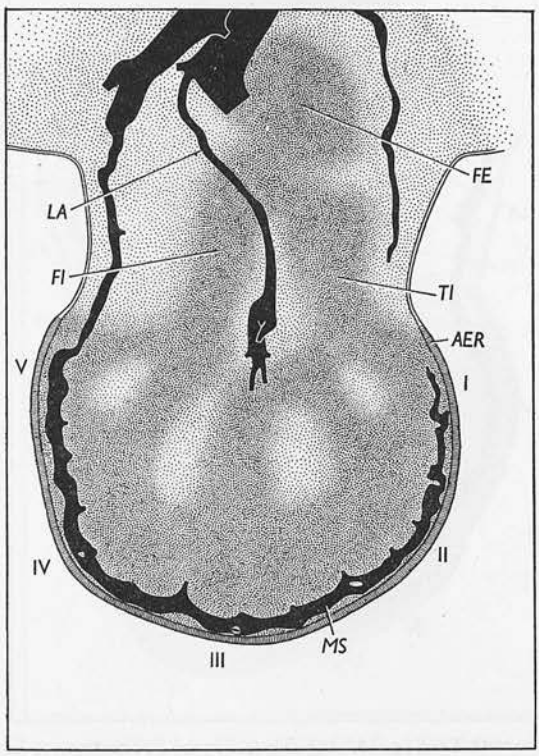
Text-fig. 11. 11½-day (5·4 mm.) *CBA* embryo, the same as in Text-fig. 9. Structures in the hind-limb region; based on a transverse projection (× 85). For key see Text-fig. 9.



Text-fig. 12. For comparison with Text-fig. 11. 11½-day (5·2 mm.) *CBA*-1x embryo, the same as in Text-fig. 10. Structures in the hind-limb region; based on a transverse projection (× 85). For key see Text-fig. 9.

the umbilical girdle and growth of the posterior parts of the metanephric blastemata is that the latter, being unable to expand laterally, come to lie very close together. They are separated only by the original paired umbilical roots, very much diminished; and these now migrate anterad, leaving only a little loose mesenchyme between the metanephric blastemata (Text-figs. 9, 11).

Though the lateral aortic branches originally developed as limb-bud arteries, only their distal parts now retain the simple limb supply function. In embryos of 4·0 mm. there are about four main limb-bud arteries, but limb supply later becomes concentrated in two arteries. The smaller of these arises from the posterior side of the umbilical artery, at the level of the Wolffian duct, and supplies the proximal postaxial part of the bud. The larger

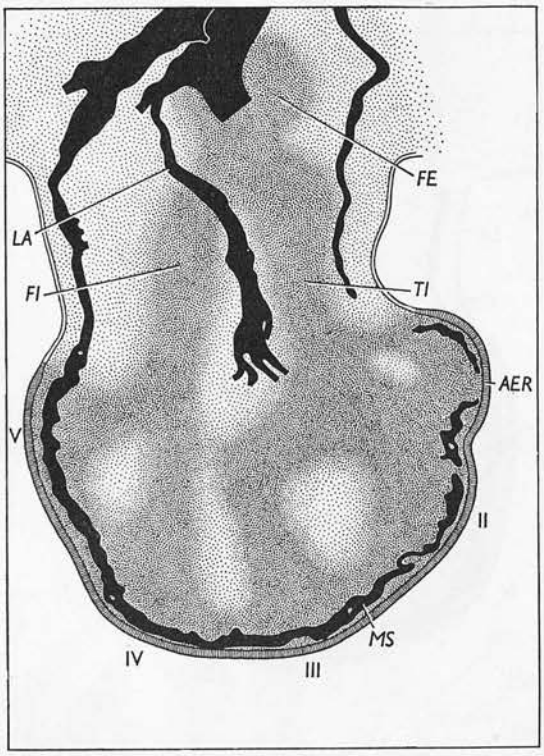


Text-fig. 13. 12½-day (8·0 mm.) *CBA* embryo. Structures in the right hind limb projected on to a plane parallel to the footplate (×85). *AER*, apical ectodermal ridge; *FE*, blastema of femur; *FI*, blastema of fibula; *LA*, limb axial artery; *MS*, marginal blood sinus; *TI*, blastema of tibia; I...V, position of future digits.

arises from the lateral side of the umbilical artery at the level of the metanephric blastema; it runs into the limb bud, along its axis, and breaks up into ill-defined radial arterioles running towards the apical ectodermal ridge. In the mesenchyme immediately subjacent to the ridge a venous marginal blood sinus now forms and drains the blood into the posterior cardinal vein; it first appears in embryos of 5·2 mm. and is well developed by the 5·6 mm. stage.

12½-day stage. The 12½-day *CBA* embryo has a maximum dimension of about 7·4 mm. (range 6·3–8·5 mm.): in the less inbred *CK* stock normal embryos ranged up to 8·7 mm.; in the cross-bred *Z* stock to 9·0 mm.

The first signs of footplate development are seen in embryos of 6·6 mm.; at 7·6 mm. they are almost circular in outline and at 8·0 mm. polygonal, the angles marking the positions of the future digits (Text-fig. 13). The apical ectodermal ridge is very well marked, extending all round the footplate. In the mesenchyme below, except at its limits, is the marginal venous sinus, at places in contact with the ridge, especially near the sites of the future digits, and elsewhere separated by a few mesenchyme cells. The sinus is narrow near the future digit I and progressively widens as it receives blood from the radial capillaries, being widest at digit V; thereafter it dives away from the ectoderm into the mesenchyme of the postaxial side of the leg and drains into the posterior cardinal

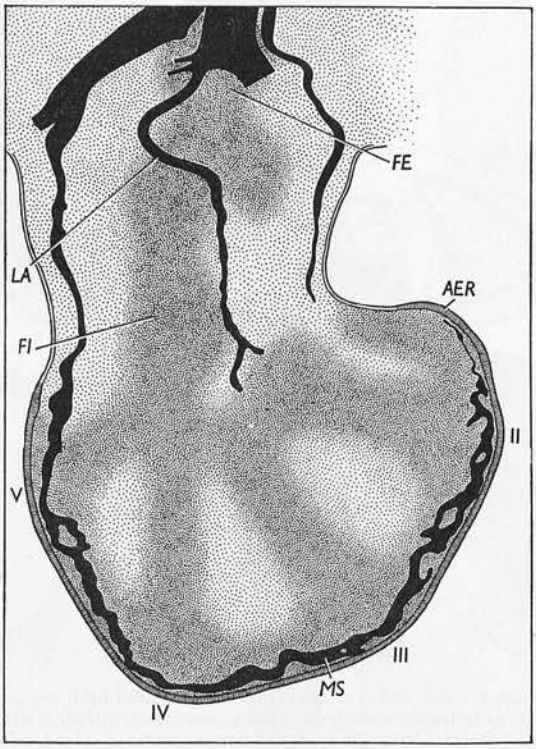


Text-fig. 14. For comparison with Text-fig. 13. 12½-day (8·0 mm.) *CK* embryo, **+1x** heterozygote. Structures in the right limb projected on to a plane parallel to the footplate (×85). Note excessive growth at the preaxial margin of the footplate. For key see Text-fig. 13.

vein; near the junction of the leg and body it receives tributaries from the dorsal surface of the leg. In live embryos a sluggish blood flow is visible near digit I, building up to a fast stream opposite digit V. There is a smaller vein, not continuous with the footplate marginal sinus, along the preaxial margin of the leg. The main blood supply to the limb is still by an axial artery, but this is now fed by the posterior (originally the smaller) of the two limb arteries arising from the umbilical artery; it crosses anterad across the base of the limb to reach the limb axis (Text-fig. 13). Condensation of mesenchyme to form the blastemal cords of the limb skeleton is first seen in embryos of about 7·2 mm.; it appears on the postaxial side of the limb, in the fibula and metatarsals III and IV, slightly earlier than on the preaxial side. The blastemata of the major elements of the limb skeleton are

visible in 8·0 mm. embryos (Text-fig. 13). At this stage outgrowths from the neural tube reach, but do not yet enter, the footplate.

Meanwhile growth of the ureters has continued. In the 6·3 mm. embryo the ureter tip has reached the level of the umbilical girdle and a bud appears on the posterior side of the ureter; this bud and the original tip form, respectively, the posterior and anterior pole tubules. As the ureter originally entered the metanephric blastema close to its posterior end, the posterior pole tubule soon approaches the end of the blastema; the anterior pole tubule, contrariwise, would have far to grow before it could reach the anterior end of the blastema. Simultaneous growth of the two tubules therefore tends to carry anterad the

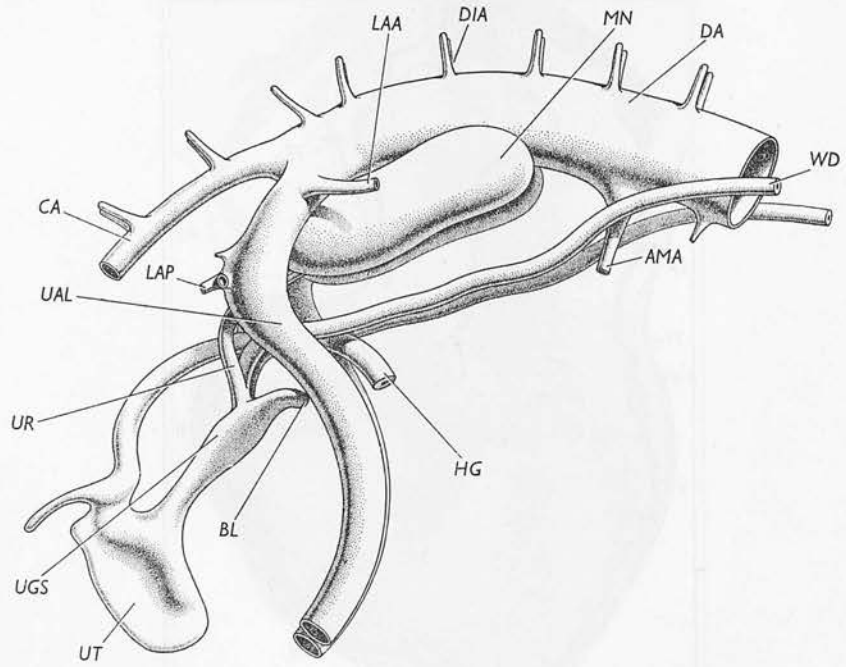


Text-fig. 15. For comparison with Text-figs. 13 and 14. 12½-day (7·7 mm.) *CK* embryo, **lxlx** homozygote. Structures in the right limb projected on to a plane parallel to the footplate (× 85). Note distortion of the footplate pattern and almost complete absence of a tibial blastema. For key see Text-fig. 13.

point at which the ureter enters the blastema, without any movement of the blastema as a whole; as a result of this process, at the 6·7 mm. stage the ureter enters the kidney at a level slightly anterior to the umbilical girdle. The pole tubules divide at about the 6·9 mm. stage, and again at the 7·1 mm. stage; this is accompanied by rapid growth of the preumbilical part of the blastema and by rapid migration of the whole kidney in an anterior, lateral and somewhat dorsal direction, with rotation about its long axis, so that the ureter enters its medial instead of its ventral surface (Text-fig. 16). This phase of migration appears to be due at least in part to mechanical pressure of the growing anterior half of the kidney on the umbilical girdle. Further migration, in a dorsal and lateral direction, takes the kidney into the hollow pocket on the dorsal wall of the coelom,

between the aorta and Wolffian ridge; this phase appears to be due to elastic tension of the peritoneum. In the course of migration the kidneys have had to pass each side of the primitive paired umbilical arterial roots. These are now very small and fused together at their origin; the left is larger than the right. They lose their ventral connexions with the lateral umbilical roots, and in older embryos the common median root remains as the posterior mesenteric artery (Text-fig. 17).

At the same time there are extensive morphogenetic changes in the ventral abdominal region (Text-fig. 16). The tail gut regresses rapidly. The cloaca is still closed from the outside by a membrane. The dorsal wall of the urogenital sinus grows caudally, almost

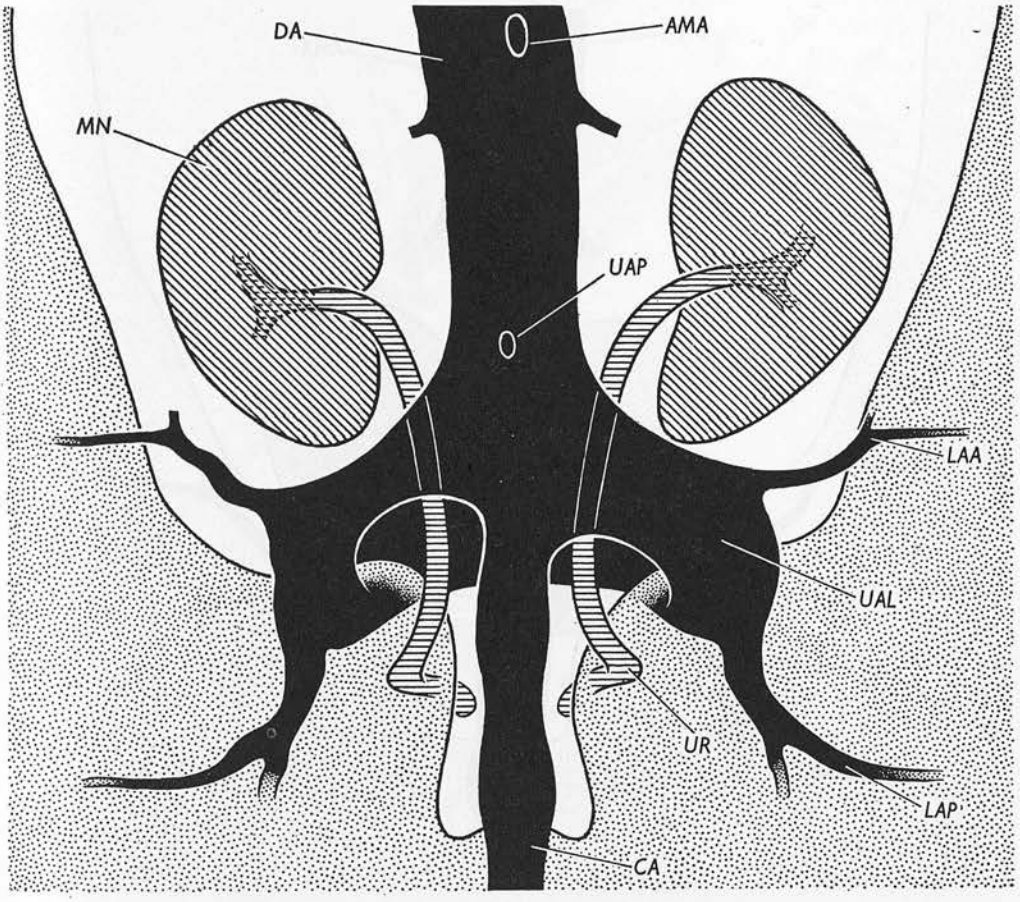


Text-fig. 16. 12½-day (7·1 mm.) *CBA* embryo. Structures in the hind-limb region; based on a parasagittal projection (×55). *AMA*, anterior mesenteric (omphalo-mesenteric, vitelline) artery; *BL*, urinary bladder; *CA*, caudal artery; *DA*, dorsal aorta; *DIA*, dorsal intersegmental artery; *HG*, hind gut; *LAA*, *LAP*, anterior and posterior limb arteries; *MN*, metanephros; *UAL*, umbilical artery, lateral root; *UGS*, urogenital sinus; *UR*, ureter; *UT*, urethra; *WD*, Wolffian duct.

separating the hind gut from the urogenital sinus and greatly increasing the length of the latter. The ventral wall of the urogenital sinus grows out to form two flat epithelial sheets, the urethral plate, which marks the centre of the newly forming urogenital papilla. At the proximal end of the urogenital sinus the urinary bladder appears as a small pouch, lying between the umbilical arteries; these are now greatly extended along the ventral abdominal wall. With the migration of the kidneys, the ureters change their relationships to the Wolffian ducts; each ureter now crosses dorsally over the corresponding Wolffian duct and lies lateral to it at their point of junction, a short distance from their opening into the urogenital sinus (Text-fig. 16).

13½-day stage. The 13½-day *CBA* embryo has a maximum dimension of about 9·0 mm. (range 8·2–10·1 mm.).

The hind footplate of an 8·2 mm. embryo is polygonal; the apical ectodermal ridge is still present, but not so well marked as at 12½ days. In embryos of 8·5–10·0 mm. there is rapid growth of the digits, so that the outline of the footplate becomes indented. At this stage the marginal sinus reaches its maximum development and carries a fast blood stream near digit V. Near digit I the marginal sinus is very small, but now continuous with the preaxial leg vein; the dividing point in the blood flow occurs at digit I, blood

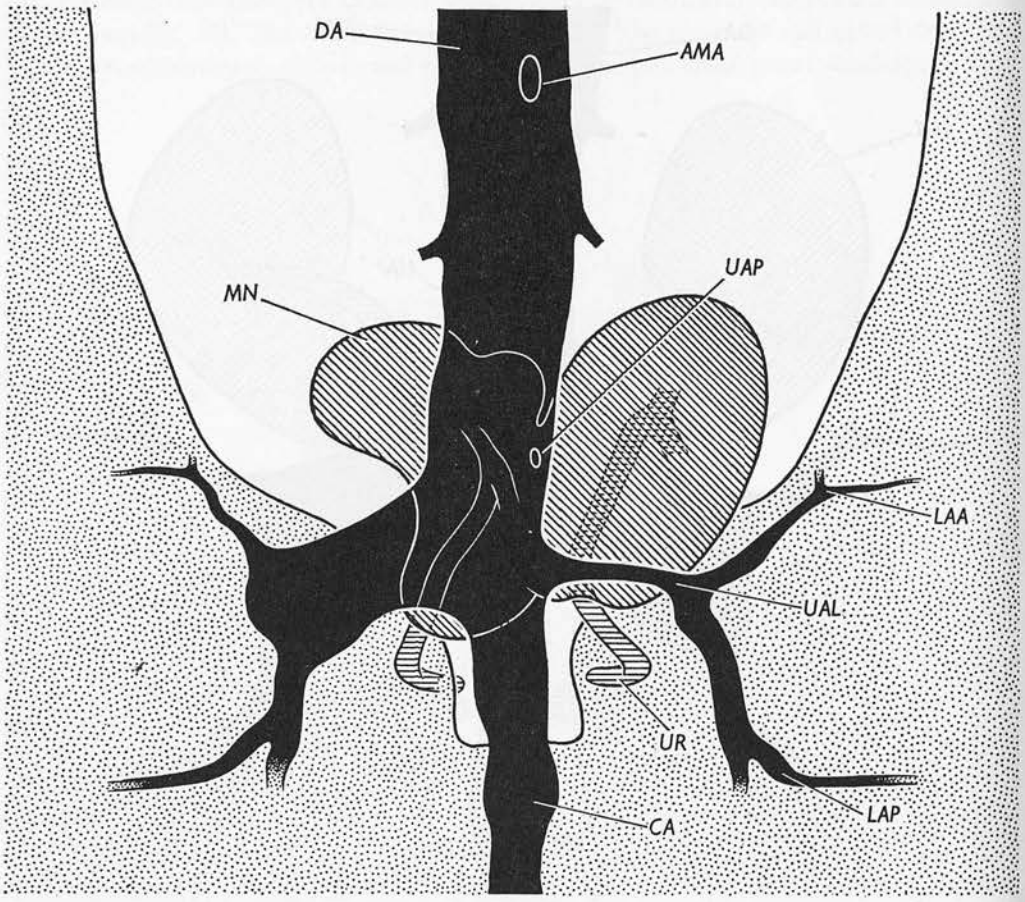


Text-fig. 17. 12½-day (8·5 mm.) *CK* embryo, probably $++^{lx}$. Structures in the hind-limb region projected on to a dorsal plane (×85). *AMA*, anterior mesenteric artery; *CA*, caudal artery; *DA*, dorsal aorta; *LAA*, *LAP*, anterior and posterior limb arteries; *MN*, metanephros; *UAL*, *UAP*, lateral and primitive (posterior mesenteric) roots of the umbilical artery; *UR*, ureter.

flowing away from this digit both pre- and post-axially. The main arterial supply to the limb is still through an axial artery arising from the umbilical artery at its most posterior point. A stainable matrix can be detected between the skeleton blastema cells of the leg and metatarsus from the 8·2 mm. stage, or even slightly earlier. This subsequently increases regularly, giving rise to precartilage. Nerves reach the metatarsus in embryos of about 9·0 mm.

The kidneys of a 9·0 mm. *CBA* embryo are clear of the umbilical girdle, lying lateral and slightly dorsal to the aorta (Text-fig. 19). The ureters lie very close to the umbilical

arteries in the posterior coelomic wall; each ureter now opens directly into the urogenital sinus rather than into the Wolffian duct, the point of entry of the ureter being now anterior, i.e. nearer to the bladder, than that of the Wolffian duct. The bladder has elongated greatly, growing along the ventral abdominal wall between the umbilical arteries; the urogenital sinus and urethral plate have also elongated, but are not yet open to the outside, and the sinus is almost closed off from the rectum. The anus is still closed by



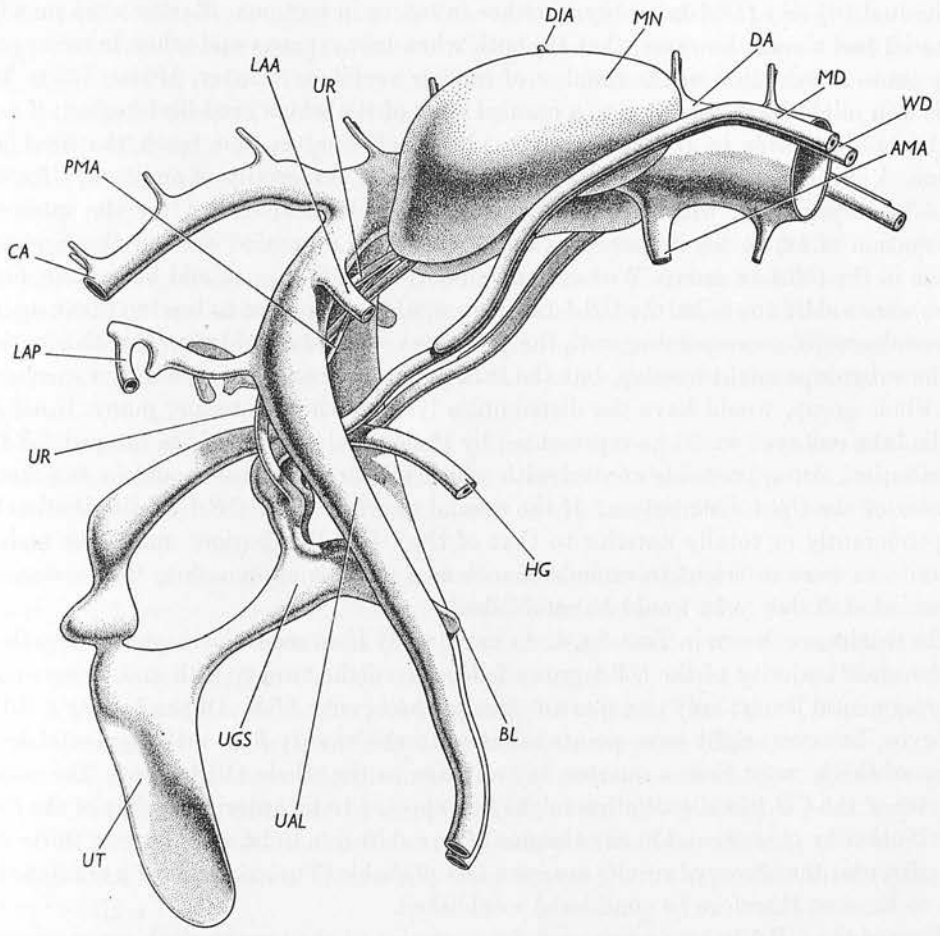
Text-fig. 18. 12½-day (8·3 mm.) **lxlx** *CK* embryo, sib of that shown in Text-fig 17. Structures in the hind-limb region projected on to a dorsal plane (×85). The kidneys are still within the umbilical girdle and fused together, the left element being badly impacted. The posterior mesenteric artery cuts deeply into the kidney. The right umbilical artery is very much reduced. For key see Text-fig. 17.

a membrane. The tail gut has disappeared. A Müllerian duct has appeared along the lateral side of each Wolffian duct, but it does not yet reach the urogenital sinus.

14½-day and later stages. In embryos of 14½ days and more, limb development consists largely of histodifferentiation, the main pattern being already apparent except in the tarsus and toes. The apical ectodermal ridge and marginal sinus disappear as toe development proceeds, the last traces of them being seen in 14½-day embryos. Chondrification occurs in the leg skeleton in late 14½-day embryos and in the foot, except the distal phalanges, at 15½ days. Ossification starts in the leg at 16½ days. Leg-muscle formation,

the first stages of which are detectable in some 13½-day embryos, becomes well defined at 14½ days; the limbs can be stimulated to active movement in some 14½-day and all 15½-day embryos.

Kidney migration is nearly complete by 14½ days, the kidneys now being in contact with the adrenal bodies. Glomeruli first appear at 15½ days. The bladder, lying between



Text-fig. 19. 13½-day (9·0 mm.) *CBA* embryo. Structures in the hind-limb region; based on a parasagittal projection (×55). *AMA*, anterior mesenteric (omphalo-mesenteric, vitelline) artery; *BL*, bladder: *CA*, caudal artery; *DA*, dorsal aorta; *DIA*, dorsal intersegmental artery; *HG*, hind gut; *LAA*, *LAP*, anterior and posterior limb arteries; *MD*, Müllerian duct; *MN*, metanephros; *PMA*, posterior mesenteric (primitive umbilical) artery; *UAL*, umbilical artery; *UGS*, urogenital sinus; *UR*, ureter; *UT*, urethra; *WD*, Wolffian duct.

the umbilical arteries, grows rapidly in girth, separating the arteries, which come to lie in its lateral walls. The ureters, through further anterad migration of their distal ends, by 14½ days open directly into the bladder instead of the urogenital sinus; this migration carries them close between the umbilical arteries and the urogenital sinus at the base of the bladder. Asymmetry of the umbilical arteries, the right becoming larger than the left, develops in most embryos at about 15½ days and is sufficiently well marked at birth to be visible through the abdominal wall; it is the origin of the asymmetry seen in the superior

vesical arteries of the adult, which represent the umbilical arteries of the embryo. The Müllerian ducts reach the level of the urogenital sinus at 14½ days and sex differentiation is apparent shortly thereafter; in the male they show regression at 15½ days.

DEVELOPMENT OF THE HIND LIMBS AND ASSOCIATED STRUCTURES IN MICE CARRYING **lx**

10½-day stage. No obvious morphological abnormalities were found on examination of individual 10½-day *CBA*-**lx** embryos, either *in toto* or in sections. Earlier work on adult material had shown, however, that **lx**, both when homozygous and when heterozygous, may cause a reduction in the number of lumbar vertebrae (Carter, 1951*a*, 1953). This reduction might have been due to a craniad shift of the whole hind-limb region; if so, it might be detectable in 10½-day embryos, in which somites first reach the hind-limb region. A statistical comparison was therefore made of two groups of embryos, *CBA* and *CBA*-**lx** respectively, which should be genetically identical except for the intercross segregation of **lx**, to see if there was any evidence of a craniad shift of the hind-limb region in the *CBA*-**lx** group. Without any shift the two groups should be similar; but if there were a shift due to **lx**, the *CBA*-**lx** group would be expected to tend to break up into three subgroups, corresponding with the genotypes **++**, **+lx** and **lxlx**. The distributions of the subgroups might overlap, but the **lxlx** subgroup, constituting about a quarter of the whole group, would have the distribution lying farthest anterior; many, if not all, of the **lxlx** embryos would be represented by the cranial quarter of the lumped *CBA*-**lx** distribution. An appropriate control with which to compare these would be the cranial quarter of the *CBA* distribution. If the cranial quarter of the *CBA*-**lx** distribution lay preponderantly or totally anterior to that of the *CBA* distribution, and if the scale of experiment were sufficient to exclude chance as a possible explanation, the existence of a craniad shift due to **lx** would be established.

The results are shown in Text-fig. 4. As has already been seen, the mean umbilical level of the great majority of the *CBA* group fell between the twenty-fifth and twenty-sixth intersegmental levels; only one was anterior to the twenty-fifth. Of the twenty *CBA*-**lx** embryos, however, eight gave points anterior to the twenty-fifth intersegmental level; seven of them, more than a quarter, lay anterior to the whole *CBA* group. The cranial quarter of the *CBA*-**lx** distribution might be expected to lie anterior to that of the *CBA* distribution by chance, and in the absence of any shift due to **lx**, only once in thirty-two experiments; the observed results are even less probable. The existence of a craniad shift due to **lx** must therefore be considered established.

None of the *CBA*-**lx** group gave a point posterior to the twenty-sixth intersegmental level, though a quarter of all *CBA*-**lx** embryos are expected to be $++^{lx}$ homozygotes, i.e. normal *CBA* embryos. This was probably a sampling effect. Only five of the *CBA*-**lx** group are expected to be $++^{lx}$ homozygotes, against twenty in the *CBA* group; the latter included only three giving points beyond the twenty-sixth level, so a group of five might easily by chance fail to include any.

When the *CBA*-**lx** distribution is truncated by the removal of its cranial quarter, putatively representing mainly the **lxlx** embryos, the mean level of the remainder is still anterior, by about a third of a somite, to that of the *CBA* distribution. This is presumably due to a craniad shift, of about half a somite, in the **+lx** heterozygotes.

Embryos from the cranial quarter of the *CBA*-**lx** distribution, presumed to be preponderantly **lxlx**, were compared with like-sized *CBA* embryos. No abnormalities were

found in the smaller ones; but the largest (4·2 mm., just outside the normal 10½-day range) showed marked differences from a like-sized (early 11½-day) *CBA* embryo. The hind-limb buds were narrower, three and a half somites wide, compared with four in the *CBA*; the difference was due largely to a craniad shift of the posterior margin of the bud, the anterior margin being only slightly displaced (Text-figs. 7, 8). The apical ectodermal ridge of the *CBA*-**lx** embryo was shorter. Posterior to the limb bud there was a marked step in the outline of the embryo, at the base of the tail; it was not present in the *CBA* embryo. (A step is sometimes seen in normal 11½-day embryos from other stocks, but not as strongly marked as in **lxlx** embryos.) The metanephric blastemata extended slightly beyond the limb bud in the *CBA*-**lx** embryo, to the level of the step, but not in the *CBA*. Outgrowth of lateral arteries from the aorta to the limb bud had scarcely started in the *CBA*-**lx** embryo, though it was well advanced in the *CBA*.

11½-day stage. Putatively homozygous **lxlx** embryos of the *CBA*-**lx** stock can be recognized from the 4·8 mm. stage, while still alive, by the abnormalities of their hind-limb buds, both in shape and in blood distribution. The bud is narrow and short; its apical ectodermal ridge is visible but short. From the dorsal view blood is visible in the base of the left hind-limb bud but not in the right. Anteroposterior skewness of the bud, which is well marked in a normal 5·4 mm. embryo, fails to develop to the same extent in **lxlx** embryos.

In sections it is found that the umbilical artery has developed its lateral course on the left side, but not on the right. Here there is a failure of the lateral aortic branches to establish proper connexion with the ventral limb arterial supply; nothing more than a flat network is formed (Text-figs. 10, 12; Pl. 2).

Development of the urogenital system also is slightly retarded. In a normal *CBA* embryo of 5·2 mm. a connexion is already established between both Wolffian ducts and the urogenital sinus, and the ureters have grown into the metanephric blastemata; in like-sized putatively homozygous *CBA*-**lx** embryos no connexion has yet been established with the sinus and the ureters are only just beginning to appear (Text-figs. 9, 10).

Identification of the abnormal embryos as **lxlx** homozygotes rests upon both morphological and genetic evidence. Morphologically they show the association of narrow hind limbs and defect of the right lateral umbilical artery seen also in those 12½-day and older embryos which are positively identifiable, *per continuitatem* from the shape of their footplates, as **lxlx** homozygotes. Genetically they appear in the expected, Mendelian proportions (Tables 1–3).

12½-day stage. Up to and including the 11½-day stage only the homozygous **lxlx** embryos could be identified as abnormal. By the 12½-day stage abnormalities are recognizable also in some heterozygotes.

Footplates first appear in normal embryos of about 6·6 mm. In some **lx**-carrying embryos of about 7·0 mm. excessive growth of the footplate is visible near the preaxial end of the apical ectodermal ridge. The marginal sinus is rather ill-defined in this region, without a clear blood-flow, and in places broken up into a network. The limb is drawn over slightly towards the preaxial side. These embryos are putatively the polydactylous **+lx** heterozygotes of the *CBA*-**lx** and *CK* stocks; they probably include some homozygotes in the weakly expressing *Z* stock. Blastemal condensation starts shortly afterwards, at the same stage as in normal embryos. Excessive condensation occurs in the preaxial part of the footplate, forming the blastemata of supernumerary digital elements (Text-fig. 14).

Putative homozygotes are recognizable without difficulty at the 12½-day and later stages by the hind legs, which are abnormally narrow, and the footplates, which are grossly distorted and pulled over preaxially. Preaxial to digit II the marginal sinus

Table 1. *Segregation of* **lx** *in CBA-***lx** *stock embryos*

	Phenotypes and numbers of embryos						
Age of embryo	Normal*	Polydactyl*	Total normal and polydactyl	Luxate*	Unclassified	Dead	Total
10½	?	?	13†	7†	25	6	51
11½	?	?	46‡	18‡	6	8	78
12½	6	11	17	3	1	3	24
13½	6	2	8	1	—	1	10
14½	4	3	7	2	—	3	12
15½	6	6	12	3	—	5	20
16½	—	—	—	—	—	—	—
17½	6	5	11	3	—	2	16
18½	1	2	3	3	—	—	6
Total observed	?+29	?+29	117	40	32	28	217
Total expected	—	—	*117·75*	*39·25*	—	—	—

* Classified by shape of footplate and leg, *per continuitatem*, unless specified otherwise. 'Polydactyl' means non-hemimelic polydactyl, **+lx** heterozygote. 'Luxate' means hemimelic **lxlx** homozygote.
† Classified by lumbar shift from reconstructed serial sections.
‡ Classified by width of limb bud and umbilical artery asymmetry.

Table 2. *Segregation of* **lx** *in CK stock embryos*

	Phenotypes and numbers of embryos						
Age of embryo	Normal*	Polydactyl*	Total, normal and polydactyl	Luxate*	Unclassified	Dead	Total
10½	—	—	—	—	8	—	8
11½	?	?	13‡	5‡	—	3	21
12½	19	26	45	13	—	8	66
13½	4	8	12	4	—	5	21
14½	4	2	6	4	—	2	12
15½	11	5	16	4	—	—	20
16½	4	3	7	—	—	—	7
17½	3	1	4	1	—	1	6
18½	2	—	2	2	—	—	4
Total observed	?+47	?+45	105	33	8	19	165
Total expected	—	—	*103·50*	*34·50*	—	—	—

*‡ See notes under Table 1.

Table 3. *Segregation of* **lx** *in Z-stock embryos*

	Intercross matings				Backcross matings			
Age of embryo	Normal*	Polydactyl*	Luxate*	Total†	Normal*	Polydactyl*	Luxate*	Total†
12½	11	10	6	27	12	9	20	41
13½	12	5	3	20	4	9	10	23
14½	3	3	2	8	10	12	16	38
15½ and older	8	8	4	20	10	2	9	21
Total observed	34	26	15	75	36	32	55	123
Total expected	*56·25*		*18·75*		*62·50*		*62·50*	

* See note under Table 1.
† The number of dead or otherwise unclassifiable embryos was not recorded for the *Z* stock.

is ill-defined or broken up into a network (Text-fig. 15). Blastemal condensation occurs at the normal time, but it is deficient in the preaxial half of the leg; it may be either deficient or excessive in the preaxial half of the footplate. Deficient condensation in the

footplate is associated with total or near absence of the tibial blastema; excessive condensation in the footplate is associated with a tibial blastema which is present but abnormally narrow.

Asymmetry of the umbilical arteries, the right being always the smaller, is strongly marked in 12½-day **lxlx** embryos; it is very strong in those of the *CBA*-**lx** strain. Some asymmetry is seen in many heterozygotes. This umbilical asymmetry has two effects on the urogenital system. First, as a result of the reduction of the right umbilical artery, the arterial hoop surrounding the right metanephric blastema and Wolffian duct, composed of the dorsal aorta and the right medial and lateral umbilical roots, is wider than normal. Secondly, the corresponding arterial hoop on the left side has to carry almost the whole umbilical circulation; the left lateral umbilical root becomes hyperplastic, regression of the left medial root is delayed, and the space through the hoop is consequently abnormally small (Text-fig. 12). In each case kidney migration may be delayed. The cause of delay on the left side appears to be simple mechanical drag, following impaction of the kidney in the hoop. On the right it may be that the anterior kidney pole, not being closely invested in an arterial hoop, is able to expand *in situ*, without migration of the kidney. Normal migration is laterad as well as craniad, and consequently leads to separation of the kidneys; growth without migration leads to contact and fusion of the kidneys, especially at their posterior poles, where lateral growth is limited by the lateral umbilical roots. The result is a horseshoe kidney (Text-figs. 17, 18). Asymmetry of the horseshoe follows directly from the hyperplasia of the left lateral umbilical artery; the left element of the kidney is displaced to the right, carrying with it the left ureter; the right element, with a migration route free of obstacles, eventually reaches almost its normal definitive position.

Rotation of the kidneys about their long axes, so that the ureter enters the kidney from the definitive medial instead of the primitive ventral aspect, occurs in a normal embryo by the time the kidneys clear the umbilical girdle. Fusion while they are still inside the girdle mechanically prevents this rotation; the ureters therefore always enter a horseshoe kidney on its ventral surface (Text-fig. 18; Pl. 2).

The posterior mesenteric artery is all that remains in the adult of the primitive medial umbilical arteries. These lie between the anterior poles of the kidneys at the time of fusion, the point of fusion being posterior to the arteries. In the course of migration, therefore, the posterior mesenteric artery is caught on the kidney isthmus and carried forward with it, giving rise to the prerenal origin of the artery invariably seen in the adult when the kidneys are fused (Text-fig. 18).

13½-*day stage*. Polydactylous **+lx** heterozygotes and homozygotes are recognizable at 13½ days by the external appearance of their hind legs and footplates.

Polydactylous heterozygotes show excessive growth of the footplate near the preaxial end of the apical ectodermal ridge, similar to that described by Chang (1939) as occurring in Fortuyn's (1939) polydactylous strain of mice. The marginal sinus in this region is represented by a network, partly following the apical ectodermal ridge at the periphery of the footplate and partly short-circuiting across the dorsal surface of the digit, below the ectoderm; the blood flow here is towards the preaxial marginal leg vein, the dividing point occurring in the interdigital space between digits I and II.

In hemimelic homozygotes the leg is narrow and the footplate heavily distorted, deviating towards the preaxial side. Apical ectodermal ridge is visible all round the foot-

plate. Preaxial to digit II the marginal sinus is very small, petering out altogether near digit I; no connexion is established with the preaxial marginal leg vein.

Deposition of matrix between the cells of the limb skeleton blastemata occurs at the same time as in normal embryos; as in them, there is a slight postaxial-preaxial gradient, matrix appearing in the fibula and fibular digits slightly before the tibia (where present) and tibial digits. Nerves grow into the foot at the normal rate.

Kidney migration is further delayed in horseshoe kidney cases.

14½-day and later stages. Histodifferentiation follows its normal course, and with the normal time relationships, in 14½-day and older embryos carrying **lx**. Limb development is generally similar to that seen in normal embryos, save that where a supernumerary blastema has been laid down, a supernumerary element develops; conversely, where a blastema is absent, the corresponding element is absent. When a blastema is present but abnormally small, the reduction of parts of the corresponding element may be exaggerated in later development. This is seen in embryos in which the tibial blastema is present but reduced. The proximal part of the blastema, still quite thick, gives rise to perichondrium and cartilage, apparently quite normal save that the cartilage is not as thick as in a normal embryo; the narrower distal part of the blastema gives rise to perichondrium-like tissue of normal thickness, but this occupies the whole width of the blastema; there is no central core left, such as usually forms the cartilage. This, presumably, is the manner of formation of the tibia in those adult **lxlx** homozygotes in which the tibia is ossified proximally but represented distally by a ligament; the ligament presumably represents the cartilage-less perichondrium of the embryo. When the blastema is further reduced, not even the perichondrium is formed and the distal part of the tibia is absent (Pl. 1). The converse effect is seen in the foot, where a preaxial digit may be well formed at the distal phalangeal level, but reduced or absent at the metatarsal and proximal phalangeal level. A similar effect is seen in some soft parts, e.g. the tendon of *m. flexor fibularis*, which may be thread-like at the metatarsal level but quite thick at the distal phalanx.

Dominance of the left over the right umbilical artery in many embryos becomes very marked in the later stages of gestation; in the *CK* stock (selected for horseshoe kidney) it occurs in a number of **+lx** heterozygotes as well as homozygotes. The dominant artery can be seen through the abdominal wall in many live newborn mice.

Studies with newborn and adult material (Carter, 1951*a*, 1953) have shown that hydronephrosis may be well established in the newborn, and that it shows strong statistical associations with hemimelia and with horseshoe kidney; it was attributed to partial occlusion of the ureter by kinks close to its distal end, where it passes through the muscular wall of the bladder. Hydronephrosis has not been seen in the *CBA*-**lx** stock. A search was therefore made of **lxlx** embryos from the *CK* and *Z* stocks, and brought to light two 15½-day embryos with considerable distension of one ureter (Pl. 2); both showed also horseshoe kidney and strong asymmetry of the umbilical arteries, and of the whole region near the base of the bladder. The distended ureter was abnormally contorted near the base of the bladder, where it passed close between the left umbilical artery and the urethra. (In one embryo the distended ureter had a diverticulum above this level, which ended blindly alongside the rectum; a diverticulum of this type was not found in any other embryo.) Glomeruli were present in the kidney. It seems probable that these embryos represent an early stage of hydronephrosis. Similar contortion of a ureter, but unaccompanied by distension, was seen in some 14½-day embryos.

DISCUSSION

(i) *Developmental analysis of the luxate syndrome*

In the *luxate* syndrome we see the action of a single mutant gene on three systems, namely, the axial skeleton, the hind limbs and the urogenital system.

The only sizeable effect on the axial skeleton is change of the twenty-sixth vertebra from the lumbar to the sacral form. This change, affecting especially the lateral processes of the vertebra, occurs sporadically in many mammals (Winckler, 1949). The relationship between the ilium and the vertebra in unilateral cases strongly supports the suggestion that sacralization of a vertebra is the result of an inductive action of the ilium. The effect of **lx** on the axial skeleton would thus be seen as secondary, and due to the anterior end of the ilium lying anterior to its normal level. This implies either that the ilium must be longer than usual, or that the whole pelvic girdle must be displaced craniad. There is no evidence to support the former suggestion; on the contrary, the innominate bone tends to be abnormally short in **lxlx** homozygotes (Carter, 1951*a*). This evidence, together with that from $10\frac{1}{2}$-day embryos, must be considered to establish that a craniad displacement of the pelvic girdle is a fundamental effect of **lx**.

The effects of **lx** on the hind limb and girdle involve primarily its preaxial side, effects on the postaxial side being apparently secondary (Carter, 1951*a*). That the preaxial end of the vertebrate hind-limb field is peculiarly sensitive to developmental disturbance is evident from the frequency with which preaxial polydactyly is found in many species. It is one of the commonest hind-limb anomalies in man (Gates, 1946), and is well known in poultry, pigeons, cats, dogs, mice and many other species (see Hollander & Levi (1942) for pigeons; Danforth (1947) for cats; Guerreiro (1947) for dogs; Murray (1932), Fortuyn (1939), Holt (1945) and Chase (1951) for mice). In the mouse it occurs sporadically in many cross-bred and some inbred stocks, including the *CBA* strain (Carter & R. J. S. Phillips, unpublished data). It frequently appears in chick embryos following operative procedures on the hind-limb region (Rudnick, 1945; Wolff & Kahn, 1947). It first becomes visible at the time of footplate morphogenesis; but L. B. Russell (1950), who induced it in mouse embryos by X-irradiation of the pregnant female, made the significant observation that it was most easily induced when the embryo was irradiated at the $8\frac{1}{2}$- and $9\frac{1}{2}$-day stages, before hind-limb bud formation. This strongly suggests that its cause may be more than a mere local disturbance of footplate development. This is emphasized by her observation that irradiation at the $11\frac{1}{2}$- and $12\frac{1}{2}$-day stages, when footplate morphogenesis is occurring, has the direct effect on footplate development that might have been expected, namely, damage and loss of digits.

The sensitivity of the preaxial part of the hind-limb field is shown also by the ease with which polydactyly of genetic origin can be modified or suppressed by environmental factors. Sturkie (1943) and Warren (1944) were able to suppress it in the chick by applying temperature shocks to the embryo; Gabriel (1946) and Bretscher (1950) by colchicine; Landauer (1948) by insulin. Holt (1948) found that in her mice it was affected by an intra-uterine environmental factor, maternal age.

The sensitivity of the preaxial part of the hind limb to developmental disturbance is shown also by the many examples of loss of the anterior part of the field. Besides numerous genetic examples, environmental effects have been reported here also. Tibial hemimelia appeared in rat embryos when the mother was kept on a deficient diet (Warkany & Nelson,

1941), or injected with trypan blue (Gillman, Gilbert & Gillman, 1948); in mouse embryos it was induced by X-radiation at the 9½- and 10½-day stages (L. B. Russell, 1950). That there is some close developmental relationship between excessive digital and deficient limb induction is clear from the many gene-controlled conditions in which the low-grade expression is preaxial polydactyly while the high-grade expression involves loss or reduction of the tibia or radius. This relationship is seen in guinea-pigs carrying the gene **Px** (Wright, 1935), in polydactylous poultry (Landauer, 1948) and in many conditions in man (O'Rahilly, 1951).

The suggestion was made above that the ligament replacing the distal part of the tibia in weakly manifesting **lxlx** homozygotes arises from a qualitatively normal but quantitatively reduced blastema, the outer shell of which gives rise preferentially to perichondrium and the remaining inner core, if any, to cartilage. This explanation implies that there is, for each cartilaginous skeletal element or part of an element, a critical blastemal size below which chondrification will not occur. Studies of mice carrying the *undulated* (**unun**) and *Danforth's short-tail* (**Sd+**) mutants led Grüneberg (1950*a*, *b*, 1953) to put forward the same suggestion to explain the abnormalities seen in them.

Those parts of the *luxate* syndrome which may show asymmetrical expression fall into two groups. In the first group, which comprises sacralization of the twenty-sixth vertebra and preaxial polydactyly in heterozygotes, expression is often unilateral, and in about three-quarters of these cases it is the right side which is affected. Landauer (1948) concluded that unilateral expression of polydactyly in poultry is mediated by naturally occurring lateral asymmetries of, or local differentials in, the growth intensities of the regions concerned. That such asymmetries must exist is apparent from the fact that the chick embryo develops a left-handed twist; the mouse embryo, conversely, develops a right-handed twist, its tail passing to the right of the head; it is significant that unilateral polydactyly is predominantly left-sided in the chick and right-sided in the mouse.

The second group of asymmetrical parts of the syndrome comprises horseshoe kidney, gross reduction of an umbilical artery and hemimelia; here the expression is invariably stronger on one particular side, namely, the left in horseshoe kidney, the right in umbilical reduction and the right (with a few possible exceptions among thousands of individuals) in tibial hemimelia. This regularity points to a close causal dependence of these three parts of the syndrome on the normal right-handed spiralization of the embryo. Perhaps the physiological necessity of an umbilical circulation militates against a tendency, inherently bilateral, to umbilical arterial defect; and the natural growth differentials, initially slight, determine which artery shall carry the umbilical circulation. The greater abnormality of the right hind limb would then be seen as a result partly of the greater reduction of its blood supply; the greater displacement of the left kidney as a result of the interference with its migration by the hypertrophied left umbilical artery.

Some inductive relationship between the limbs and the nephric system in vertebrates has often been suspected, but clear evidence is lacking. There are numerous instances of syndromes in which renal agenesis accompanies limb defects, of which only the *wingless syndrome* (Zwilling, 1949) in chicks and symmelic monsters in mouse and man need be cited. On the other hand, normal kidneys and ureters can develop in a mouse totally devoid of hind limbs, and in which the pelvic girdle is represented only by small ilia and ischia (Carter, unpublished observation): conversely, normal limbs develop in mice carrying the *Danforth's short-tail* mutant, which may entirely lack ureters and kidneys

(Gluecksohn-Schoenheimer, 1945). Any inductive relationship involving limbs and metanephroi cannot, therefore, be a direct one.

The main object of the present work was to search for causal connexions between the many parts of the *luxate* syndrome. While the existence of a causal connexion cannot be proved by a purely descriptive investigation, many possible hypotheses can be excluded. There are two main arguments. (i) *Sine hoc, ergo non propter hoc.* Incomplete manifestation of a syndrome, either in the adult or in the embryo, establishes that development of those defects which are present is not causally dependent upon those which are absent. With this in view, an account has already been given of the association of the limb and urogenital defects with lumbar reduction in adult mice carrying **lx** (Carter, 1953). (ii) *Ante hoc, ergo non propter hoc.* Development of one part of a syndrome cannot be causally dependent on the existence of another part which develops later. The order in which the various parts of the *luxate* syndrome could be detected in the embryo was found to be: craniad shift of the hind-limb region, narrowness of the hind-limb bud and shortness of its apical ectodermal ridge, retardation of hind-limb bud vascularization, defect of the right umbilical artery and hyperplasia of the left, abnormal preaxial limb blastemal condensation, retarded kidney migration, horseshoe kidney and prerenal posterior mesenteric artery, ureteric kinks, hydronephrosis.

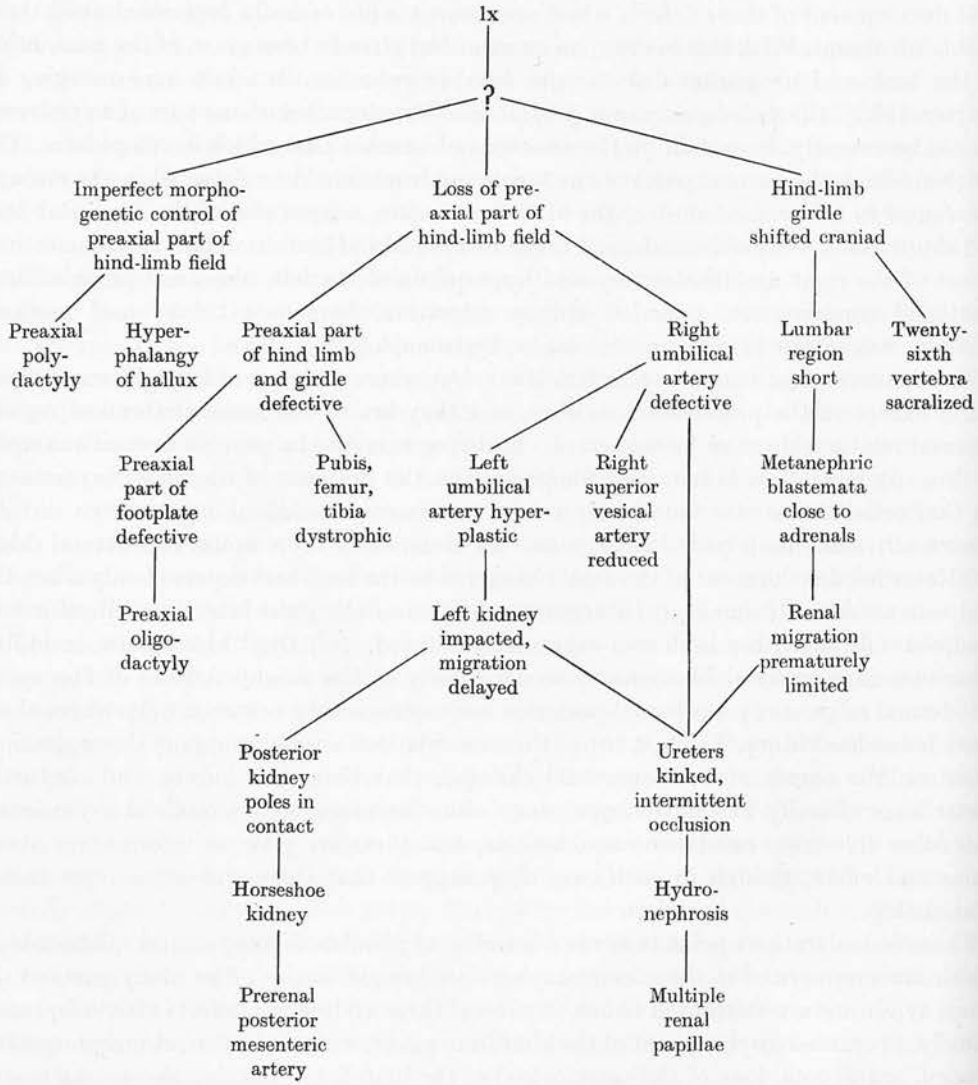
The converse arguments may be fallacious; but where one part of a syndrome is never found except in the presence of another, and they are in the same anatomical region, a causal relationship may be suspected. Embryos carrying **lx** provide several examples of this. (i) Horseshoe kidney was found only in the presence of umbilical asymmetry. (ii) Contorted ureter was found only in the presence of umbilical asymmetry. (iii) An abnormally small limb bud always carried an abnormally short apical ectodermal ridge. (iv) Retarded development of the aortic branches to the limb bud occurred only when the bud was abnormally small. (v) Extreme reduction of the right lateral umbilical artery occurred only when the limb was very much reduced. (vi) Digit blastemata, including supernumerary preaxial blastemata, occurred only in the neighbourhood of the apical ectodermal ridge. (vii) A prerenal posterior mesenteric artery occurred only where there was a horseshoe kidney. The first two of these associations strongly support the suggestion, based on the course of developmental changes, that horseshoe kidney and contorted ureter were causally dependent upon, *inter alia*, the presence of umbilical asymmetry. The other five were one-to-one associations, and therefore give no information about cause and effect, though in each case they suggest that there was some close causal relationship.

These considerations point towards a number of possible developmental relationships, which are summarized in the accompanying 'pedigree of causes'. The many parts of the *luxate* syndrome are attributed to one or more of three underlying defects of development, namely, (i) craniad displacement of the hind-limb girdle, with (ii) imperfect morphogenetic control, or (iii) total loss, of the anterior end of the limb field. Lumbar shortening is seen as a result of the craniad shift of the limb girdle. Preaxial hyperphalangy and polydactyly are seen as the effects of imperfect control of the anterior end of the limb field. Hemimelia and right umbilical defect are seen as the effects of loss of its anterior end. The kidney defects are seen as the secondary effects of right umbilical defect and of shortening of the lumbar region.

The interpretation that the urogenital defects may be due to mechanical interference

with the developing urogenital system by the abnormal umbilical arteries agrees with all the observations except, perhaps, for two rather puzzling features, namely, (*a*) the slightly retarded development of the ureter bud and of the connexion between the Wolffian duct and the urogenital sinus in **lxlx** embryos; (*b*) the absence of horseshoe kidney and hydronephrosis from the *CBA*-**lx** strain, in which umbilical asymmetry is very strong.

Pedigree of causes of the *luxate* syndrome



The slightly retarded development of the ureter bud and of the connexion between the Wolffian duct and urogenital sinus may merely reflect the retarded growth of the hind-limb region; or possibly some inductive relationship may be involved. Two possible inductive systems involving the umbilical arteries are suggested by their regular relationships with parts of the urogenital system. (*a*) The urogenital sinus always develops from the gut where it passes between the primitive umbilical roots, even though these may

vary in level by up to two somites' width. (*b*) The bladder grows out from the urogenital sinus between the lateral umbilical arteries and enters into a close relationship with them. In symmelic monsters, which have a single, median umbilical artery (representing the primitive roots (?)), the bladder is absent, even though the rest of the urogenital system may be present (Shryock, Janzen & Barnard, 1942; Asang, 1952). More commonly, however, the urinary system is absent (Hung, 1950, for man) or the complete urogenital system (Gluecksohn-Schoenheimer & Dunn, 1945, for the mouse). The present investigations provide no means of determining whether gross asymmetry of the region of the base of the bladder in mice carrying **lx** is merely a mechanical result of umbilical asymmetry, or whether inductive agencies are also at work.

The absence of horseshoe kidney and hydronephrosis from the *CBA*-**lx** strain might be explicable as an effect of the genetic milieu on growth rates in the hind-limb region; the metanephros fits into its arterial hoop very closely, and slight differences of growth rate might make all the difference between impaction and free migration. Hydronephrosis, in the *CK* stock, is closely correlated with horseshoe kidney (Carter, 1953); its absence from the *CBA*-**lx** strain may follow on the absence of horseshoe kidney. On the other hand, hydronephrosis and gross umbilical asymmetry are both found in mouse embryos showing the *myelencephalic blebs* syndrome (Brown, 1931), but horseshoe kidney has not been found in them.

The *luxate* syndrome appears to be the first instance of a syndrome including horseshoe kidney which has been investigated developmentally. Horseshoe kidney occasionally appears in mice carrying the *tailless* and *uro-recto-caudal syndrome* mutants, **Tt^0 urur** (Dunn & Gluecksohn-Schoenheimer, 1947); but all other instances appear to have been sporadic. The findings with **lx** fully confirm the inspired guess made by Lewis & Papez (1915) that horseshoe kidney arises by the kidneys growing together while they still lie between the umbilical arteries; they also confirm the suggestion (Jazuta, 1924), which has not found universal favour, that it may be associated with instability and craniad shift of the sacral region. Sections of 12½-day mouse with horseshoe kidney (Pl. 2) are strikingly similar to the illustration reproduced by Boyden (1931) of horseshoe kidney in a human embryo figured by Keibel (1896). The results with mice emphasize, however, that the origin of horseshoe kidney lies in a delay of renal migration; and the existence of this delay suggests that the umbilical arteries may play an important, albeit passive, role in at least one phase of normal kidney migration, though there is reason to believe that other factors affect its other phases (Gruenwald, 1943).

The occurrence of a hydropic ureter in embryos of 15½ days indicates that glomerular function has started by this stage; the negative observation that hydronephrosis was not seen in 14½-day embryos is less significant, but together they show that kidney function must start in the mouse at about the time at which glomeruli become histologically recognizable. This is in line with the experimental results of Gersh (1937) on rats and other species.

A question which can never be answered with certainty is whether the now extinct hemimelic mice, *souris luxées*, described by Rabaud (1914), carried **lx**, or whether some other gene was involved. A comparison of the adult morphology of **lxlx** homozygotes with that of the *souris luxées*, as described by Hovelacque (1920), led to the conclusion that 'there are no phenotypic differences... which cannot be attributed to differences of residual genotype or observational technique' (Carter, 1951*a*). The development of the

hind legs of the *souris luxées* was investigated by Hovelacque & Noël (1923). The first sign of abnormal development which they found, in 7·5 mm. embryos, consisted of failure of the tibial blastema to lay down an intercellular matrix; this was followed, at the 8·5 mm. stage, by lack of chondrification in the distal part of the tibia. This description agrees with the appearance of **lxlx** embryos in the weakly manifesting *Z* stock, which provides the best comparison with the *souris luxées*, since these were maintained by *inter se* matings of abnormals. It therefore remains quite possible that the *souris luxées* represent an earlier occurrence of **lx.**

(ii) *A unitary hypothesis for the luxate syndrome*

It seems unlikely that purely descriptive embryological investigations, with the techniques at present available, will enable analysis of the development of the *luxate* syndrome to be taken very much further back into embryogeny; and the techniques of experimental embryological investigation of mammals are not yet sufficiently developed to hold much promise of an early experimental attack on the problem. Identification of a hypothetical common antecedent of the three developmental upsets must therefore at present be conjectural, resting largely on the findings of experimental investigations of avian and amphibian material.

Several theories have been put forward to explain the origin of preaxial polydactyly. Scott (1937) attributed it, in guinea-pigs carrying **Px**, to a generalized acceleration of growth at a particular stage of development, leading to abnormal morphological relationships. Gabriel (1946) adopted the view that it arises by partial duplication of the limb field, an incomplete, mirror-image field occurring anterior to the normal field. This theory receives strong support from some rare abnormalities of the arm in man, e.g. diantebrachia, where there may be three bones in the forearm (ulna, radius, ulna) and up to nine digits (V, IV, III, II, I, II, III, IV, V) (vide O'Rahilly, 1951). Against it, such complete duplication does not seem to occur in the hind limbs (except where there is duplication of the body axis). Danforth (1947) concluded that the main, perhaps only, effect of the polydactyly gene in cats is to induce an early excess in the number of cells on the preaxial border of the limb, and that all other changes are secondary to this increase. Landauer (1948) held that the polydactylism gene in poultry is responsible for a disturbance of the proper organization of the limb field, the exact nature of the disturbance being unknown; of the abortive limb field duplication theory he concluded that 'such explanations are too narrow in view of the evidence from ectrodactylism and abnormalities of the radius'; these he considered 'a rare but significant feature of polydactylous stocks'.

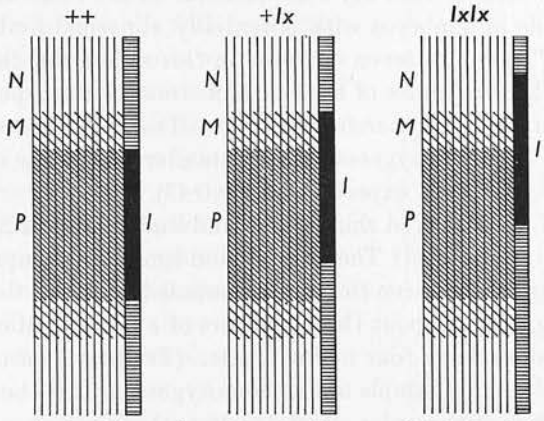
The appearance of polydactyly in **+lx** heterozygotes supports the abortive duplicate limb field theory; but this theory leaves unexplained some of the essential features of the expression of **lx.** In particular, (i) why is the duplicate field truncated, so that it is never represented by more than three digits? (ii) what is the connexion between limb field duplication, as seen at the phalangeal level in low grades of expression, and partial suppression of the normal field, as shown by loss of the long bones and of the preaxial digits in the higher grades of expression? (iii) why is the normal limb field shifted craniad?

An important advance in the understanding of limb developmental mechanics was made by Saunders (1948), who showed that the morphogenetic pattern of the chick's wing is laid down in a proximo-distal sequence and that the apical ectodermal ridge plays an important role in the ordering of this pattern. When part of the ridge was removed

from an early limb bud, the limb which developed was deficient of the corresponding sector. When extirpation was performed at an early stage, a large part of the limb was deficient, proximal as well as distal; late operation led to a limb lacking only distal parts. Zwilling (1949) subsequently showed that the apical ectodermal ridge is partly or completely absent from chick embryos showing the *wingless* syndrome. These results lend emphasis to the finding that in the earliest identified **lxlx** homozygotes the apical ectodermal ridge was abnormally short.

Saunders's findings suggest the following unitary hypothesis to account for the three underlying defects to which the parts of the *luxate* syndrome have been traced:

(i) *In the mouse embryo the hind limb is formed through an interaction between limb-potent tissue, restricted to a region in the neighbourhood of a few specified somites, and a limb inductor, extending along most (but not all) of the limb-potent tissue.*



Text-fig. 20. Unitary hypothesis of the action of **lx**. In the normal individual (left) a limb inductor (I) lies adjacent to a region of limb-potent tissue (*P*). In a **+lx** heterozygote (centre) the inductor is displaced craniad, so that its anterior end lies adjacent to a marginal region of limb-potent tissue (*M*), supposedly leading to poorly controlled limb-forming response, whence preaxial polydactyly. In a **lxlx** homozygote (right) there is a further craniad displacement of the inductor: its anterior end now lies adjacent to tissue lacking limb potency (*N*), whence tibial hemimelia.

(ii) *Any slight craniad displacement of the limb inductor relative to the limb-potent tissue may result in the induction of supernumerary digits from the normally inactive limb-potent tissue immediately anterior to the limb inductor.*

(iii) *The action of* **lx** *is to shift the limb inductor craniad, slightly when* **lx** *is heterozygous, heavily when homozygous.*

Thus the slight displacement of the limb inductor caused by **lx** when heterozygous would tend to result in preaxial polydactyly; and the greater shift when **lx** is homozygous would move the anterior part of the limb inductor beyond the region of limb potency, leading to preaxial hemimelia (Text-fig. 20).

What are the limb inductor and the limb-potent tissue? Saunders's results suggest that they may be the precursors of, respectively, the apical ectodermal ridge and the lateral mesenchyme at hind-limb level. If this is correct, then it becomes an essential feature of the hypothesis to suppose that the precursor of the lateral mesenchyme slightly anterior to the hind-limb bud lacks limb potency. Is this in agreement with the known facts of limb induction in the chick? The crucial experiment, transplantation of limb-bud apical ectoderm into this region, does not appear to have been

reported. Harrison (1931) was unable to induce limb formation when he grafted limb ectoderm to other sites in the flank of *Amblystoma*; likewise Balinsky (1933) was unable to induce supernumerary limbs in a region of two somites' width anterior to the hind-limb buds (though he succeeded in inducing them almost everywhere else in the lateral mesoderm). However, it cannot be assumed that what is true of an amphibian is necessarily true also of amniotes, since it is known that the ectoderm plays a relatively less important role in limb formation in amphibians (Harrison, 1931). Wilde (1950) has pointed out that an apical ectodermal ridge is not even a constant feature of the fore-limb bud of *Amblystoma*.

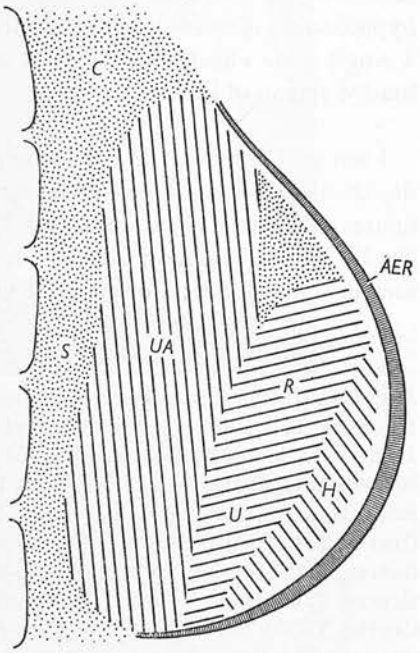
To be acceptable, a hypothesis must agree with the observed results not only qualitatively but also quantitatively. Since polydactyly is rare in the *CBA* strain, it must be assumed to be absent from a sample of twenty *CBA* embryos; hence that part of the *CBA*-**lx** distribution which in Text-fig. 4 lies anterior to the *CBA* distribution must, on this hypothesis, include all embryos with potentially abnormal limbs, heterozygotes as well as homozygotes. There are seven embryos in this tail of the distribution; are they enough? In the **+lx** heterozygotes of the *CBA*-**lx** strain **lx** has a penetrance of 36·0%; twenty intercross embryos would therefore be expected to include 8·6 with abnormal limbs (5·0 homozygotes and 3·6 heterozygotes), with a standard deviation of 2·2 embryos. The observed number, 7, agrees with expectation ($P = 0{\cdot}47$).

Is the magnitude of the observed shift of the hind-limb region sufficient to account for the extent of the loss of limb field? The normal hind-limb bud occupies a width of about four somites; the difference between the farthest craniad points of the *CBA* and *CBA*-**lx** distributions (Text-fig. 4) was about three-quarters of a somite. Most homozygous **lxlx** mice of the *CBA*-**lx** stock have four or five digits. (Tridactyls occur but are rare; one would hardly be expected in a sample of five homozygotes.) It is therefore clear that the magnitude of the shift is of the order which the hypothesis requires.

A hypothesis about the developmental mechanics of **lx** must also provide an explanation of the order, in some respects rather irregular, in which the parts of the limb appear and disappear with increasing gene expression. It has been recognized from a study of the adult condition (Carter, 1951*a*) that the spectrum of expression of **lx** consists of two phases: (i) An addition phase, in which extra digital elements are added to the foot, until there are seven triphalangeal digits. (ii) A loss phase, in which there is progressive loss of digits, metatarsals, tarsals, tibia, femur and pubis, in a sequence that is mainly preaxial-postaxial but shows some ordering along the limb axis; thus loss in the foot and leg spreads both distally and proximally from the metatarsus, so that the head of the tibia and distal phalanx of a digit may remain after the intermediate parts have disappeared; likewise loss of the pubis and proximal part of the femur may occur, but not of the distal part of the femur. These peculiarities of order in the loss phase are predicted by the present hypothesis if it is assumed that the presumptive map of the mouse's hind-limb bud is zigzag, like that of the chick's wing. There the main elements lie obliquely, the presumptive humerus pointing towards the postaxial margin, radius and ulna preaxial, metacarpals and digits again postaxial (Saunders, 1948; Warren, 1934). If the mouse's hind-limb map were similar, failure of successive levels of the field, starting with its preaxial end and progressing postaxially, would be expected to manifest itself by early loss of the more preaxial parts of the map; femur, metatarsals and digits would therefore disappear proximodistally, tibia distoproximally, as they in fact do.

Does the hypothesis account for the stronger limb defect expression of **lx** in the *CBA*-**lx** than in the *CK* genetic milieu? About 80% of adult *CBA* mice have only five lumbar vertebrae; **+ +** homozygotes in the *CK* stock have the usual six; both stocks have thirteen thoracic vertebrae. The *CBA* genetic milieu is therefore one in which the pelvic girdle, and presumably also the hind-limb inductor, is displaced somewhat craniad, even in the absence of **lx**; in the presence of **lx** displacement should therefore be very strong, with very strong effects on the limb field. Why, then, are there no **lxlx** homozygotes in this stock with only four lumbar vertebrae? Presumably because the mechanism which sets an anterior limit to the limb field limits also the pelvic girdle; a homozygote with extreme expression of **lxlx** has a shortened ilium as well as defect of the pubis (Carter, 1951*a*, pl. 1*c*).

The hypothesis suggests that genetic milieux in which **lx** expresses very strongly may generally be those in which the hind-limb inductor tends to be displaced craniad; such a genotype might, in the absence of **lx**, manifest itself by polydactyly and lumbar reduction. The *fidget* mutant is one with which **lx** interacts strongly, most double heterozygotes being hemimelic (Carter & Grüneberg, 1950); polydactyly is an irregular expression of **fi**, but it is not known whether **fi** has any action on the number of lumbar vertebrae.



Text-fig. 21. Presumptive map of a chick wing bud, modified after Saunders (1948). The direction of the cross-hatching indicates the long axes of the main bones. *AER*, apical ectodermal ridge; *C*, coracoid; *H*, hand; *R*, radial region of forearm; *S*, scapula; *U*, ulnar region of forearm; *UA*, upper arm.

(iii) *Conclusions*

The results of this investigation make it clear that the development of the *luxate* syndrome is compatible with a unitary hypothesis of the action of the **lx** gene, provided that certain assumptions are made about developmental mechanisms of the mouse, all of which are in accord with present knowledge of mechanisms in the chick. Some aspects of the problem are incompletely elucidated; for example, why is the establishment of a connexion between the Wolffian duct and the urogenital sinus delayed in **lxlx** embryos? More important, why does the mouse hind-limb bud so often react to developmental disturbance, genetic or other, by the production of preaxial polydactyly? These and other problems must for the present remain unanswered. Meanwhile two questions, which should be answerable, must be posed. Are ectrodactyly and hemimelia in other material associated with a displacement of the limb buds along the body axis? Does the lateral mesoderm immediately adjacent to a limb bud lack limb potency?

SUMMARY

This paper describes the development of the *luxate* syndrome in mice. The manifold defects of limbs, urogenital system and lumbar vertebrae are traced back to three underlying causes, namely, (i) a craniad shift of the hind-limb girdle, with (ii) imperfect

morphogenetic control, or (iii) loss, of the anterior end of the limb field. Craniad shift of the hind-limb region is demonstrable in 10½-day embryos. The limb defects give rise to defects of the umbilical arteries; these lead to the urogenital defects. A possible unitary hypothesis is discussed, whereby all the effects of the *luxate* gene could be attributed to a single gene effect, craniad shift of the hind-limb inductor relative to a supposedly limited region of limb potency.

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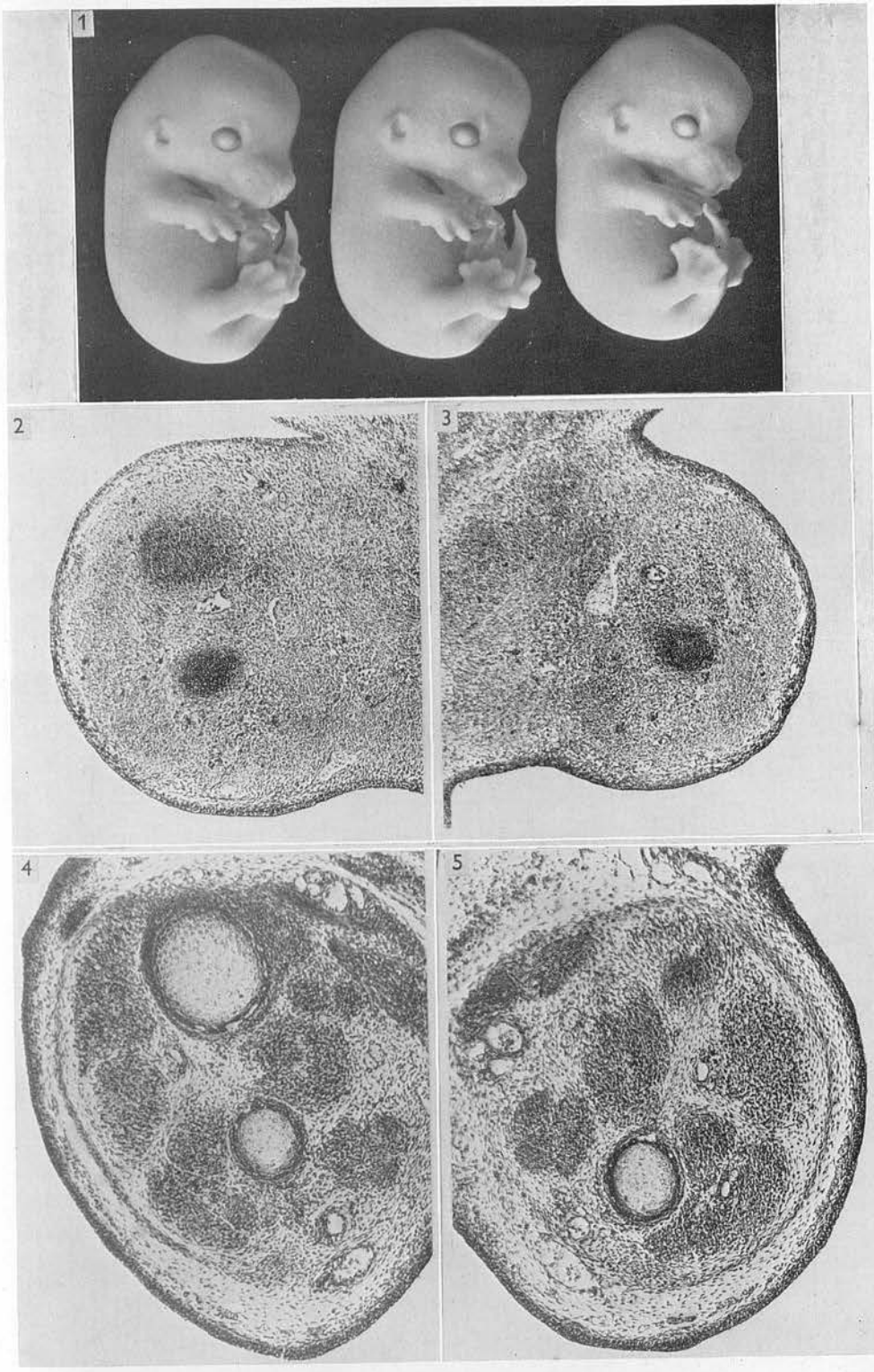
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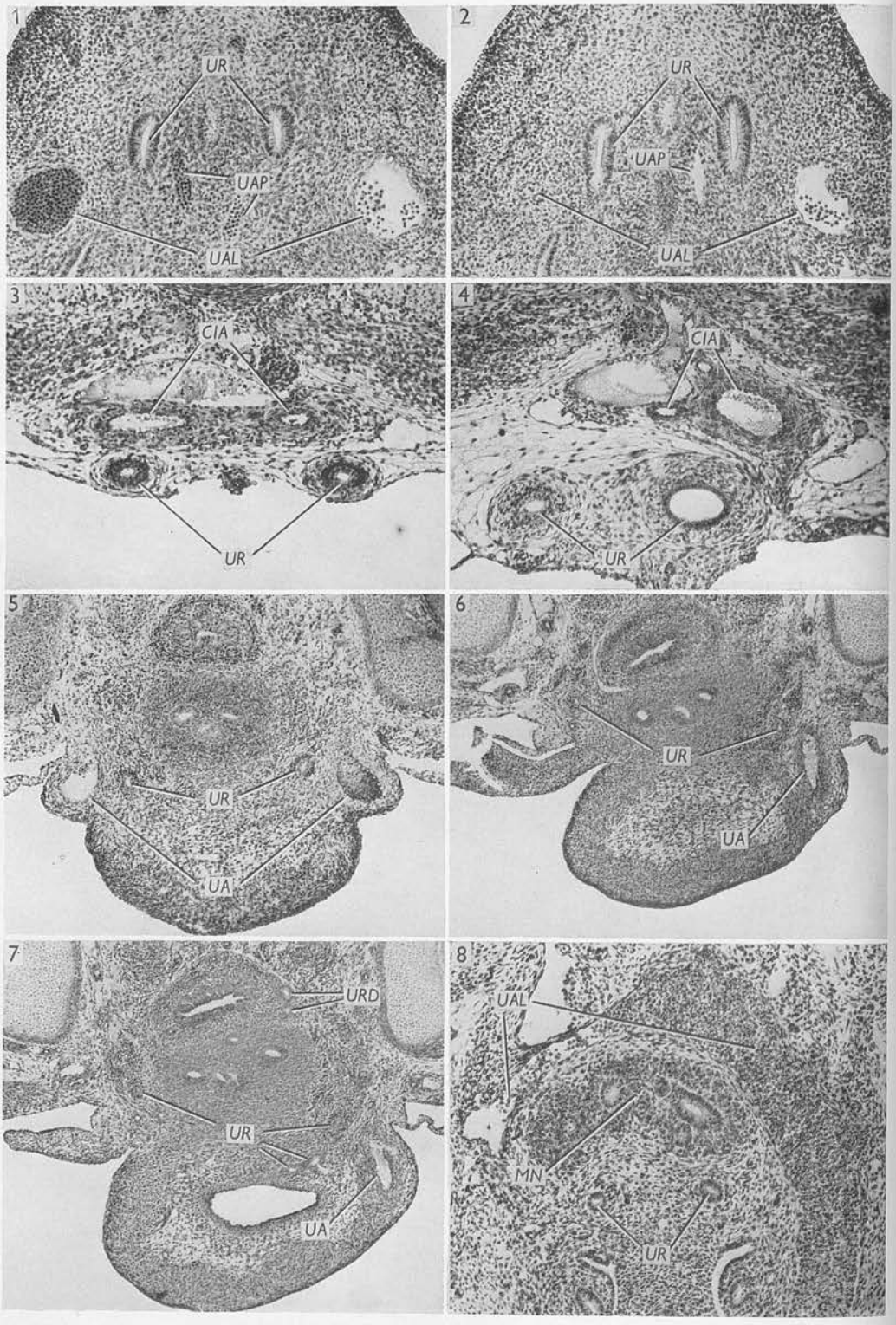
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EXPLANATION OF PLATES

PLATE 1

Fig. 1. 14½-day *CBA*-**lx** embryos; normal (left), polydactylous **+lx** heterozygote and hemimelic **lxlx** homozygote.

Figs. 2–5. Late 12½-day and 15½-day embryos sectioned transversely to the leg; normal left legs and **lxlx** right legs; preaxial at the top of the photograph. Note the absence of a tibia from the abnormal legs.

PLATE 2

All sections in this plate are seen as though looking towards the tail with the dorsum at the top; the right side of the embryo therefore appears at the left of the photograph.

Figs. 1, 2. 11½-day *CBA* and *CBA*-**lx** embryos sectioned through the base of the tail; owing to the curvature of the embryo, the primitive and lateral roots of the umbilical artery (*UAP*, *UAL*) and the ureter buds (*UR*) are cut transversely. Note the defective right umbilical artery of the *CBA*-**lx** embryo. (×90.)

Figs. 3, 4. 15½-day *CK* embryos, normal and **lxlx**, sectioned transversely slightly posterior to the bifurcation of the aorta, showing the ureters (*UR*) and common iliac (lateral umbilical) arteries (*CIA*). Note the hydropic left ureter of the **lxlx** embryo. (×90.)

Figs. 5–7. 15½-day *CK* embryos; 5 normal, 6 and 7 the same embryo as 4. 5 and 6 sectioned transversely through the base of the bladder, showing the asymmetry of this region in the **lxlx** embryo; 7 showing contortion of the left ureter (*UR*) where it passes near the umbilical artery (*UA*), and a diverticulum (*URD*) of the left ureter lying near the rectum. (×55.)

Fig. 8. 12½-day *CK* embryo, **lxlx**, sectioned transversely through the lateral umbilical arterial roots (*UAL*). (The same embryo is shown in Text-fig. 18.) Note the fused kidneys (*MN*) with the ureters (*UR*) lying ventral to them. (×90.)

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ICHTHYOSIS, A NEW RECESSIVE MUTANT

In the House Mouse

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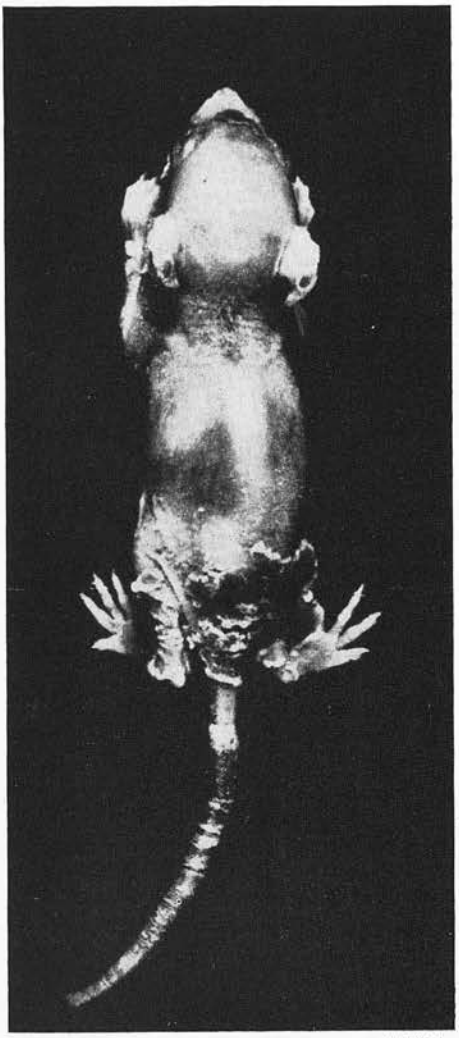
A NUMBER of mutants are known in *Mus musculus* which affect the coat or skin or both. Most of them fall into one of two main groups, the hair-waving and the hypotrichosis mutants respectively. Grüneberg[3] has summarized previous work on them. Descriptions of three more have subsequently become available, namely *wellhaarig*[4] a recessive hair-waving gene; *fuzzy*[5] a recessive gene which causes the coat to be sparse and curly; and *crinkled*[2] a recessive gene with manifold effects on the hair follicles and skin.

During 1948 an inherited ichthyotic condition was found in a sib-mated stock* maintained at Edinburgh; it occurred in two of several parallel sublines, all derived from three foundation animals. It presumably arose by spontaneous mutation in a common ancestor.

Description

The vibrissae of a two-day-old ichthyotic mouse are straight but they appear to be somewhat shorter than those of its normal sibs. At three days the vibrissae begin to lose their regular arrangement; there is a certain amount of criss-crossing, especially in the region nearest the tip of the nose, and some of them are also curved. At four days most (but not all) of the vibrissae are seen to curve upwards and inwards towards the tips of the nose; dorsal guard-hairs are scarcely visible, though they are clearly visible in the normal mouse of the same age. At six days the dorsal guard-hairs of an ichthyotic mouse are still scarcely visible, being represented only by a little fluffy down on the head and neck; its skin looks rough and papillate, and a number of creases, like those of the palm of a hand, are clearly visible in the skin of its sides, back and tail.

During the second week, when a normal mouse grows a full coat, an ichthyotic rarely grows more than a thin, short coat, in which the density of hairs appears to be low and the hairs themselves curly; the vibrissae are



YOUNG ICHTHYOTIC
Figure 5

During the third and fourth weeks the skin of the ichthyotic mouse becomes hard and scaly and in a few days cracks and is finally sloughed. This mouse has scaly plates of old skin on the nose, ears, legs and tail.

*The authors wish to thank Miss Catherine Scott, who found the first ichthyotic mice, and Miss Esme Mavor for their technical assistance and care in maintaining the stock.

short and heavily curled. The ichthyotic grows more slowly than its normal sibs and is less viable.

During the third and fourth weeks an ichthyotic mouse usually falls far behind its sibs and undergoes a developmental crisis. The skin dries, hardens and becomes scaly; a few days later it cracks and is finally sloughed. There is considerable variation between individuals in the severity of this process. In mild cases the scales are small, confined chiefly to the back, legs and tail and are shed without apparent difficulty. In severe cases the scales form rigid plates, covering the whole body, which appear to make respiration and movement of the limbs difficult. On the tail the dry skin often forms a number of rings; these presumably interfere with the circulation to more distal parts, since necrosis of the tip of the tail is an almost invariable feature of the condition. The sloughing does not occur all over the body simultaneously, but proceeds in an anteroposterior and dorsoventral direction. However, odd scales may remain attached to the nose, ears and legs after the process is completed in neighboring regions. Viability is low during the sloughing process.

An older ichthyotic mouse often grows a thin, curly coat, which never becomes as thick as in a normal mouse; at the most it resembles the coat of a *fuzzy* mouse[6]; at the least the ichthyotic is competely bare except for a few short, heavily curled vibrissae. Ichthyotics usually remain small and are often subject to respiratory disorders. Some are mute, but it is not known whether this is true of most or all of them. The eyelids may be thickened, the lower lip retracted and the claws may be long and spiralled, but these are not regular features.

Most ichthyotics are sterile. An external vaginal opening appears in the female, but it is usually displaced forwards, over the pubic symphysis. The skin between the vagina and anus is then stretched tightly over the posterior edge of the pelvic girdle; the vaginal canal, pinched between the skin and symphysis, is thereby effectively closed. A few females are fertile and they can suckle their litters, (though poorly) but their breeding lives are short and none has yet borne more than two litters. Most male ichthyotics fail to develop a scrotum and the penis is usually deflected forwards; cryptorchid males are invariably sterile, but males with one or both testes descended are usually fertile.

There is some variability in the severity of the ichthyotic condition, but the range of expression does not approach the normal condition. There is never any doubt whether a mouse should be classified as ichthyotic or not.

Inheritance of Ichthyosis

No ichthyotics have yet bred when mated *inter se,* even when both mates had previously been proved fertile.

Matings between ichthyotics and unrelated mice of either sex produced only non-ichthyotic young, thereby establishing that the condition is not inherited as an autosomal dominant, nor is it due to a mutant gene in the differential segment of the sex-chromosome.

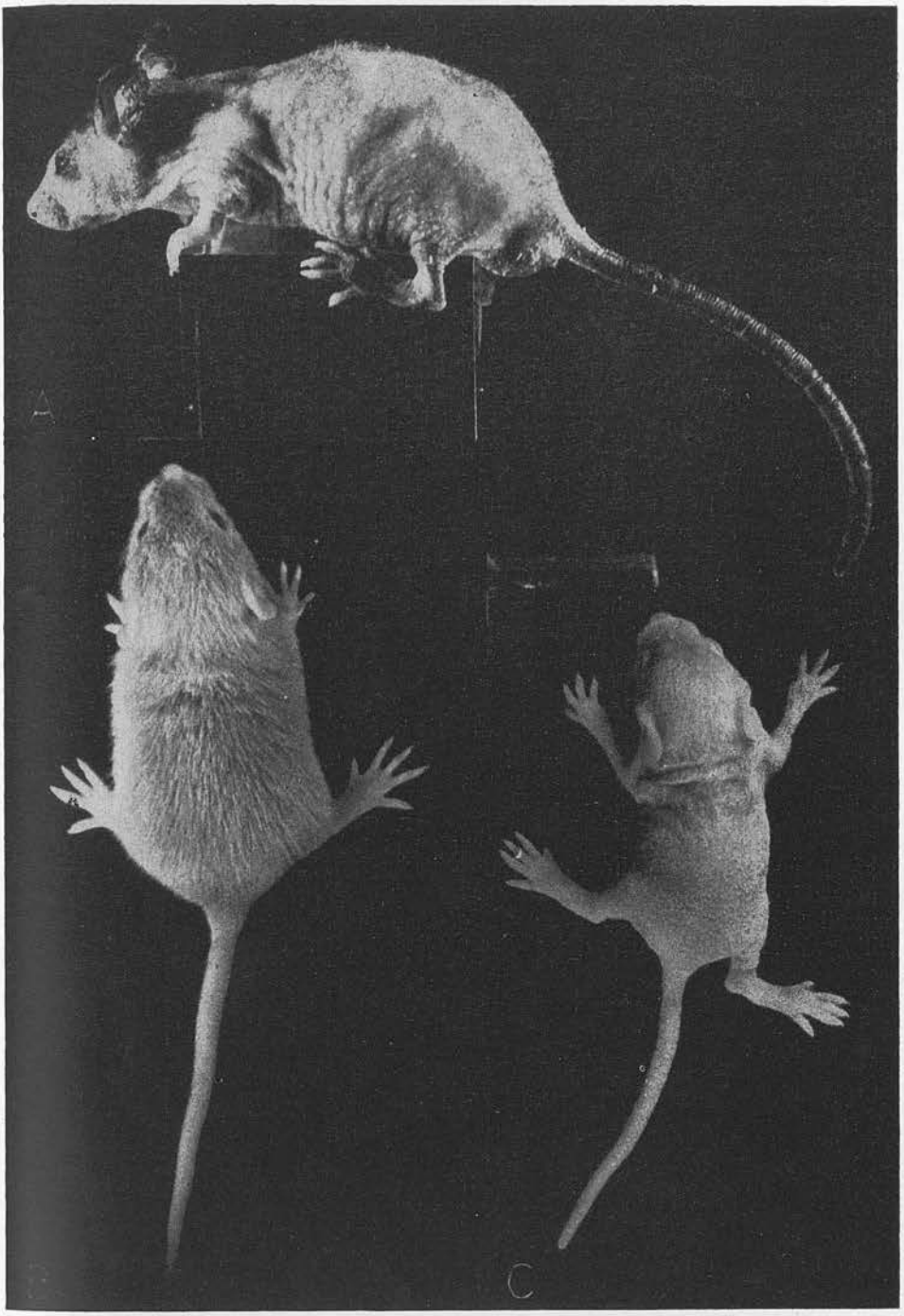
Ichthyotics were recovered in the F_2 and backcross generations, but in numbers which were significantly lower than expectation on the basis of a single Mendelian recessive. The data, nevertheless, are held to support a one-gene hypothesis and the symbol *ic* is proposed for the mutant gene. The deficiency of ichthyotics is attributed to high early

TABLE I.—Segretation of *ic*.

PARENTS	PROGENY									SEGREGATION	
	Classified						Unclassified				
Type of mating	Lived			Died			Died			Ichthyotics Calculated	born Expected
	T	N	I	T	N	I	T	N*	I*		
Intercrosses:—											
F_1 ♂ × F_1 ♀ (*Ca* not present)	66	50	16	—	—	—	2	?	?	16.00	16.50
F_1 ♂ × F_1 ♀ (*Ca* present)	415	363	52	55	11	44	32	6.4	25.6	121.60	125.50
Normal ♂ × normal related ♀ (*Ca* present)	147	116	31	16	12	4	14	10.5	3.5	38.50	44.25
Backcrosses:—											
Ichthyotic ♂ × F_1 ♀ (*Ca* present)	12	9	3	—	—	—	—	—	—	3.00	6.00
Outcrosses:—											
Ichthyotic ♂ × normal ♀	43	43	—	—	—	—	—	—	—	—	—
Normal ♂ × ichthyotic ♀	11	11	—	—	—	—	—	—	—	—	—
Hairless ♂ × ichthyotic ♀	5	5	—	—	—	—	1	—	—	—	—
Ichthyotic ♂ × hairless ♀	12	12	—	1	1	—	—	—	—	—	—
Ichthyotic ♂ × fuzzy ♀	6	6	—	—	—	—	—	—	—	—	—

T = total; N = normal; I = ichthyotic.

*The figures in the columns marked with an asterisk were obtained by dividing the number in the 'unclassified, died, total' column in the ratio of the numbers in the 'classified, died, normal' and 'classified, died, ichthyotic' columns. Ten complete litters, totalling 28 young, all of which were unclassified and disappeared within five days of birth, have been excluded from the data.



DEVELOPMENT OF ICHTHYOTIC MICE

Figure 6

A shows an adult male with typical very short thin coat and tail shortened by necrosis of the tip. *B* shows a normal mouse fifteen days old and *C* shows an ichthyotic littermate. The coat of the latter is shorter, but skin lesions have not yet developed.

postnatal mortality of homozygotes, for the following reasons. The strain in which the first ichthyotics occurred was homozygous for the dominant hair-waving mutant *Caracul*[1]; consequently classification for ichthyosis of the original F_2 generation, which provided the greater part of the data, could not be based on the appearance of the vibrissae at three days, but had to be delayed until seven to ten days, when the coat abnormalities are clearly visible. A number of young died before the age of classification; more died between the ages of classification and weaning, and among these the number of ichthyotics was disproportionately high. If two plausible assumptions are made, namely (1) that when a complete litter died before classification, death was due to poor maternal care and did not depend on the genotype of the young, and (2) that the proportion of ichthyotics among the remaining pre-classification deaths was the same as that among the post-classification deaths, then an estimate may be made of the number of ichthyotic young born. This is found to be in good agreement with Mendelian expectation (Table I).

The *ic* gene has been proved to be non-allelomorphic with three other mutants which affect the coat, namely *fz*, *hr* and *Ca*. Non-allelomorphism of *ic* with *fz* and *hr* was proved by direct test; both crosses gave an all-normal F_1 generation. Non-allelomorphism of *ic* with *Ca* is established by the fact that segregation of *ic* and *Ca* occurs in F_1 mice who received both mutants from the same parent.

Summary

Ichthyosis, *ic*, is a recessive mutant in *Mus musculus* which affects the skin and its derivatives. It was found in a laboratory stock at Edinburgh in 1948, having presumably arisen by spontaneous mutation. An *icic* homozygote is distinguishable at three to four days by its curly vibrissae; the first coat is very short and sparse and the individual hairs are curly. During the third and fourth weeks the skin may harden, dry and be sloughed and the distal part of the tail becomes necrotic; the adult may grow a thin, curly coat. Other effects of the mutant may include thickening of the eyelids, retraction of the lower lip, mutism, overgrowth of the claws and cryptorchism. Cryptorchid males and most females are sterile. Viability is impaired. *ic* is not allelomorphic with *fz*, *hr* or *Ca*.

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Ragged, a semidominant coat texture mutant in the house mouse.

by

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INTRODUCTION

There are three main classes of mutant gene in *Mus musculus* with an effect on coat texture: those which wave the coat (e.g. *Rex*), those which remove it (e.g. *Naked*) and those which change the relative numbers of the hair types (e.g. *crinkled*).

This paper describes a new semidominant mutant which belongs to the last class; its locus is at one end of Linkage Group V.

ORIGIN

The first *Ragged* mouse, a female, was one of a litter of ten sired in a test of her father's fertility; he was from a cross-bred stock in which a translocation had been induced five generations earlier by X-rays. The *Ragged* phenotype was subsequently proved to be due to a gene which is semidominant; it must, therefore, have arisen spontaneously, either in one of the gametes which gave rise to the mutant animal, or as a sectorial mutation in one of her parents' gonads. Her father, having been found not to carry the translocation, was killed; it was thus impossible to test him, after discovery of the *Ragged* mouse, for a mosaic gonad. Her mother was test-mated to an unrelated wild-type male, but bore only eight further young; all were normal. The *Ragged* female was mated to the same male and bore forty-seven young, of which twenty-four were *Ragged*.

RAGGED HETEROZYGOTES

Heterozygotes can often be recognised at birth, in segregating litters, by their vibrissae, which are slightly shorter than normal; but this classification

is not always reliable. Between two and four days, when the pigmentation of normal mice first becomes visible, heterozygotes colour abnormally slowly; this can form the basis of a reliable classification, provided that colour mutants are not also segregating. Between three and six days down can be seen along the dorsum of a normal mouse when it is held silhouetted against a light; in heterozygotes the down is shorter, sparser, and develops about a day later. By nine days the coat of a heterozygote appears macroscopically different from that of a normal sib, being obviously thinner; this difference remains throughout life.

The coat of the adult heterozygote (Fig.) looks sparse and lacking cohesion; its appearance suggests that many of the hairs are absent. There is no waving. Many guard-hairs are present, and they seem to stand out from the coat more than in the normal mouse; this was the feature which gave rise to the name 'Ragged'. Pinches of hair from the mid-dorsal region include many guard-hairs, awls and auchenes, but the proportion of zigzags is much lower than in normal mice. Vibrissae are present and grossly normal. The distribution of the yellow agouti pattern is abnormal, the whole coat being unusually dark; this effect is strongest in the region of the dorsal midline, which may be completely black; the cheeks are affected least, and when colour mutants are segregating classification for agouti is most easily based on their appearance. Tail rings are present. The claws and eyelids appear normal.

The distinction between adult heterozygotes and normal mice is always quite clear; there is no reason to suppose that normal overlapping may occur.

Heterozygotes may develop a little more slowly than their normal sibs, especially in large litters; but viability does not appear to be appreciably

affected. Breeding performance is normal; segregation data are given in Table 1.

RAGGED HOMOZYGOTES

When Ragged heterozygotes were mated together, they produced Ragged and normal young in a 2-to-1 ratio (Table 1), suggesting that there are no homozygotes in the Ragged class. In addition these matings gave some abnormal young which formed a homogeneous group, characterised at birth by the non-eruption of all but a few sinus hairs; the few which had erupted were the most posterior of the moustache hairs, and they were very short. Many of the abnormal young had also a grotesquely blown-up appearance, due to generalised oedema; most of these died shortly after birth. Survivors grew slowly, but some became adult and were proven by breeding test to be RaRa homozygotes. Adult homozygotes (Fig.) are naked, their few isolated pelage hairs being confined to the posterior and ventral part of the body. Sinus hairs are few and may be short; the moustache is represented only by the two or three posterior hairs in each row; the postorbital sinus hair is present, but the postorals are absent and the supraorbitals reduced from two to one each side. The ears and tail are pigmented. Tail rings are present and there are no kinks in the tail. The claws appear normal.

There is nothing to suggest any overlap of the homozygous and heterozygous phenotypes; Ra is thus incompletely dominant.

The intercross segregation data of Table 1 shew that the abnormal group could only account for about half of the expected homozygotes. However, there were also some stillborn young, many of which were partly eaten and unclassifiable; some may have been homozygotes. Other homozygotes could have died and

been eaten before examination of the litters. Thus there is no conclusive evidence of prenatal mortality of homozygotes.

LINKAGE TESTS

Ragged mice were outcrossed to standard linkage-testing stocks (Carter & Falconer, 1951) and multiple heterozygotes from the F1 generation were back-crossed. This provided segregation data testing Ra for linkage with markers in all the known linkage groups except Group IV. Statistically significant evidence was found of linkage with the closely-linked marker genes short-ear, se, and blue dilution, d, in Group II and with nonagouti, a, and Dunforth's short-tail, Sd, in Group V. (See Table 2).

The evidence of linkage with se and d came entirely from coupling data, where it was largely due to a shortage of the triply-mutant phenotype; repulsion data failed to support the suggestion of linkage. It is probably spurious.

The evidence of linkage with a was highly significant ($25.7 \pm \frac{3.2}{}$%), with Sd less so (35.4 ± 5.3%). To establish the position of Ra in Group V, three-point tests were made with pallid, pa, which lies between a and Sd (Borger, 1950) and shews about 17% recombination with a; thus Ra would be expected to shew close linkage with pa if the order were a, pa, Ra, but loose linkage if it were Ra, a, pa. The tests established without question that the order is Ra, a, pa (see Table 3). Three-point tests with Sd indicated the order Ra, a, Sd, (Table 4), confirming that Ra lies beyond an end marker of its group.

Joint estimates of the recombination percentages of Ra with a, pa and Sd, based on all available data, are given in Table 5.

NON-ALLELISM OF *RAGGED* WITH OTHER COAT TEXTURE MUTANTS

The location of *Ra* at one end of Linkage Group V establishes that it is not allelic with any of the known coat texture mutants. Only two of them belong to Group V, *we* and *ro*, and both are located close to *pa* (Fisher, (1949) for *we*; Falconer (1954) for *ro*).

DISCUSSION

Ragged promises to be an interesting and useful mutant from two viewpoints, those of formal and developmental genetics.

Its formal interest lies in the fact that it extends, by more than 20 units, a linkage group map that is already long. It is not surprising that Group V should prove to be long, since Slizynski (1952) has shown, through cytological study of a tagged translocation induced and analysed by Snell (1946), that this group is carried in one of the longest autosomes. Its map is now of such a length that it is no longer sufficient to use only the two end markers, *Ra* and *Sd*, when testing it for linkage with a new mutant.

The developmental interest of *Ragged* lies chiefly in the opportunity which it affords for testing concepts about the development of the coat, and especially the hypothesis that each type of hair fibre arises from its characteristic type of follicle (Dry, 1926) and that the various types of follicle appear in a time sequence (Falconer, Fraser & King, 1951). Comparative studies of *crinkled* and normal mice by the latter authors shewed that follicle initiation occurs in four main phases: (i) in embryos of 12½-13½ days, (ii) in those of 14½-17½ days, (iii) from 17½ days gestation to birth, (iv) in the first few days after birth. Follicles of the first phase give

rise to sinus hairs, those of the third phase to awls and anchenes. Ragged resembles crinkled in having few zigzags, but differs in having plenty of guard hairs; it therefore constitutes ideal material for testing the remainder of the hypothesis, namely that the second phase of follicles gives rise to guard hairs and the fourth to zigzags.

ACKNOWLEDGMENT

We are grateful to Miss M. M. Manson, who found the first Ragged mouse, and to Miss G. I. E. Mavor for their technical assistance; and to Mr D. W. M. Pinkney who took the photographs.

SUMMARY

Ragged, Ra, is a new semidominant coat-texture mutant in the house mouse. Heterozygotes have a sparse coat in which the proportion of zigzag fibres is low; in agouti mice the coat is abnormally dark, especially on the dorsum. Most homozygotes shew a generalised oedema and die soon after birth; the few survivors are naked. Ra is located at one end of Linkage Group V; it shews 24% recombination with nonagouti and 39% with pallid.

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Revised version

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TABLE 1. Segregation of *Ra*.

Genotypes of parents	Phenotypes of progeny		
	RaRa	*Ra*	+
Ra+ x ++	0	388	374
Ra+ x *Ra*+	21*	81	44
RaRa x ++	0	31	0
RaRa x *Ra*+	19	26	0

*Some of fourteen unclassified stillborn may have been *RaRa* homozygotes.

TABLE 2. Independence tests with Ra.

Linkage group	Marker gene, M or m	Mating type, phase*	Phenotypes of progeny: Ra M	Ra m	+ M	+ m	Recombination percentage †
I	c^e, c^{ch}	BC	52	69	57	84	48.1 ± 3.1
"	p	BC	9	8	6	7	46.7 ± 9.1
II	d, se	BC	81	41	50	61	
"	" "	BR	15	10	17	16	41.9 ± 2.9
III	s	BC	63	60	67	45	54.0 ± 3.3
"	W^v	BC	81	93	81	89	50.6 ± 2.7
V	a	BC	32	8	16	35	
"	"	BR	17	35	40	8	25.7 ± 3.2
"	Sd	BR	14	28	25	15	35.4 ± 5.3
VI	Ca	BC	38	24	40	28	
"	"	BR	12	13	8	6	48.5 ± 3.8
"	bt	BC	8	4	7	1	55 (>32)
VII	Re	BC	9	7	8	10	
"	"	BR	28	21	15	29	56.7 ± 4.4
"	wa-2	BC	6	5	7	4	55 (>33)
VIII	b	BC	70	56	68	59	49.0 ± 3.1
IX	T	BC	1	1	1	=	
"	"	BR	60	35	47	37	54.4 ± 3.7
X	v	BC	3	2	1	4	30 (>7)
XI	Mi^{wh}	BR	16	9	9	5	53.8 ± 8.0
XII	ru	BC	2	1	8	10	43 (>22)
"	je	BC	2	1	9	8	50 (>28)
XIII	fz	BC	5	-	4	1	40 (>13)
"	ly/n	BC	5	6	6	4	57 (>35)
XIV	f	BC	1	2	12	3	78 (>47)
-	Va	BR	-	1	2	2	60 (>6)
XX	d	BC	21	25	26	18	
"	"	BR	59	80	70	82	50.4 ± 2.5

B = backcross, C = coupling, R = repulsion. d treated as a dominant.
† Where the total number of progeny classified exceeds 25, recombination percentage and its standard error are quoted; otherwise the recombination percentage and its lower (2½%) fiducial limit.

Revised Table

TABLE 3. Three-point tests of Ra with a^t and pa.

Genotype of parents	Phenotypes of progeny								
	Ra++	Ra+pa	+++	++pa	Raa^t+	Raa^{t}pa	+a^t+	+a^{t}pa	Total
+a^{t}pa/+a^{t}pa♂ x Ra++/+a^{t}pa♀	27	2	11	0	0	1	9	7	57
+a^{t}pa/+a^{t}pa♂ x Raa^{t}pa/+++♀	2	0	6	1	1	3	0	1	14

TABLE 4. Three-point tests of Ra with a and Sd.

Genotype of parents	Phenotypes of progeny								
	Ra+Sd	Ra++	++Sd	+++	RaaSd	Raa+	+aSd	+a+	Total
+a+/+a+♂ x Raa+/++Sd♀	1	0	1	0	3	2	3	1	11
Ra+Sd/+a+♂ x +a+/+a+♀	19	18	10	8	6	3	24	35	123

TABLE 5. Recombination of Ra with a or a^t, pa and Sd, from the data of Tables 2, 3 and 4.

Locus	Recombination with Ra and standard error (%)
a	24.2 ± 2.5
pa	39.4 ± 5.9
Sd	41.2 ± 5.3

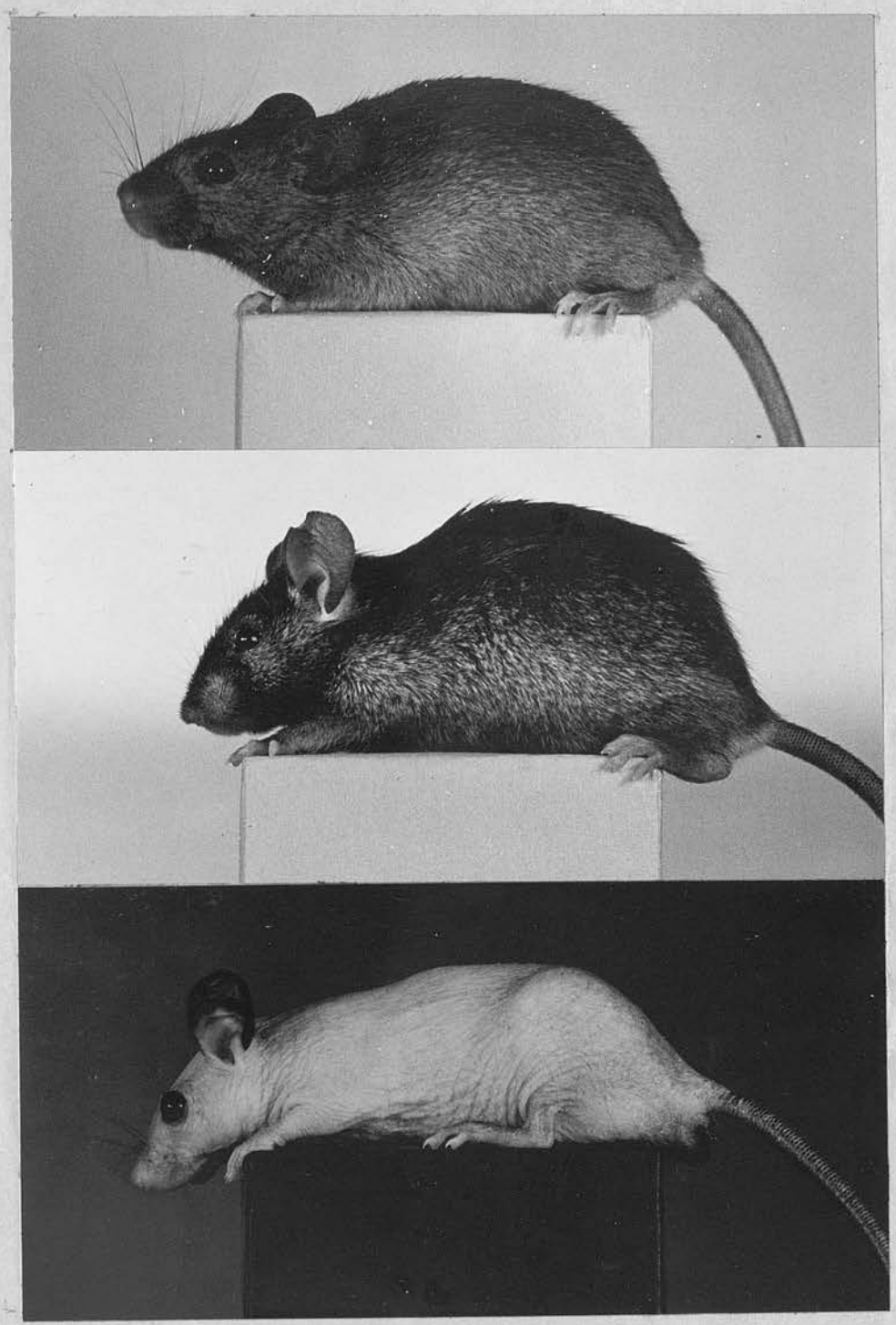

Fig. 1. Top, wild type mouse; middle, Ragged heterozygote; bottom, Ragged homozygote.

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Malformations in Mouse Embryos induced by Trypan Blue

Gillman, Gilbert, Gillman and Spence in a recent paper[1] reported a series of malformations obtained in newborn rats as a result of injection of the dye trypan blue into female rats during varying stages of pregnancy. One of the interesting features of these malformations in rats is their remarkable resemblance to abnormalities known to occur in mice as effects of mutations[2,3].

A series of experiments was therefore undertaken to determine whether the trypan blue treatment might have a similar effect in the house mouse, and if so, whether developmental disturbances, like those found to be responsible for the genetically determined mouse abnormalities, were also involved in the malformations induced by the trypan blue dye.

Females of Bagg Albino mice, 80–88 generations inbred (kindly supplied by Dr. E. C. MacDowell), were injected twice with $\frac{1}{4}$–$\frac{1}{2}$ c.c. of a 0·5 per cent trypan blue solution, first at about eight days prior to fertilization, and then at seven to eight days after fertilization.

From twenty females injected, sixty-seven newborn mice were recovered, of which sixteen showed different types and degrees of tail abnormalities, resembling the well-known tail mutations of the mouse such as kink, brachy and fused[4]. Of the sixteen newborn mice with tail abnormalities, twelve were males and only four were females. The possibility that the treatment may affect the sex ratio is at present being investigated.

After it had thus been established that the trypan blue treatment was actually effective in inducing abnormalities in newborn mice, too, preliminary embryological examinations were performed. Twenty-seven Bagg Albino females were injected in the manner described above, and then killed 10, 11, 12, 13 and 14 days after fertilization, and their litters removed for gross examination and subsequent sectioning.

Eighteen of these mice yielded a total of 118 embryos, and observations on these embryos varying from 10–14 days in copulation age were as follows.

(1) Sixty-eight of the 118 embryos were abnormal.

(2) If the dye was injected between seven and eight days after fertilization, the majority of embryos showed two types of abnormalities : (*a*) abnormalities of the posterior axial region, such as kinky, short or curled tails ; in the 12-day embryos a constriction

at various levels of the tail is visible, a condition resembling that leading to the shortening of the tail in the brachy mice[2]; (*b*) abnormalities of the anterior axial region, for example, failure of the head folds to close, or an everted brain, sitting like a cap on top of the developing skull, resembling the mutation pseudencephaly[5]. The abnormalities of some embryos were so severe that they probably could not have survived to term. This would account, for example, for the absence of the pseudencephalic condition among the newborn mice and also for the fact that litter size was smaller at birth than at 10–14 days after fertilization. Furthermore, it would explain the higher incidence of abnormals among embryos (68 out of 118) than among the newborn (16 of 67).

(3) Both anterior and posterior types of abnormalities were visible at gross inspection as early as twelve days after fertilization, when growth and differentiation are most active.

The gross examination of embryos of female mice treated with trypan blue definitely shows that there exist similarities between the morphology of embryonic stages of malformations produced by trypan blue and of those genetically determined. A detailed histological examination of developmental systems, such as notochord, nervous system, etc., of the abnormal embryos will show how these are affected by the trypan blue treatment, and how their abnormalities compare with those described as effects of mutations in mice.

This study was begun at the suggestion of Dr. S. Gluecksohn-Waelsch, under whose guidance it is being carried out. It is being supported by a fellowship grant from the U.S. Public Health Service, Bethesda, Md.

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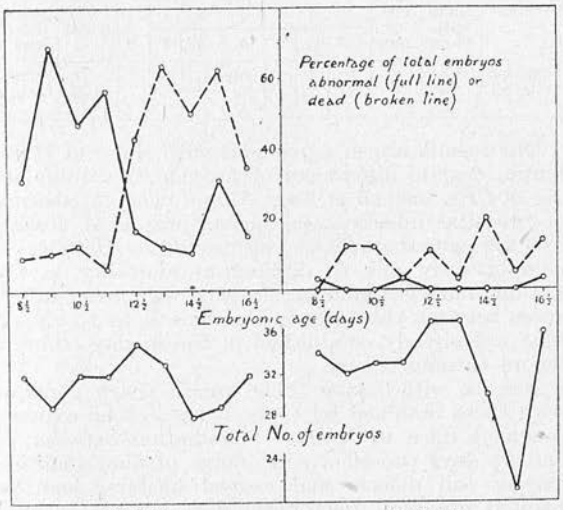
New York 27, N.Y. Nov. 1.

[1] Gillman, J., Gilbert, Ch., Gillman, Th., and Spence, I., *S. Afr. J Med. Sci.*, **13**, 47 (1948).
[2] Dunn, L. C., Harvey Lectures, **35**, 135 (1940).
[3] Gluecksohn-Schoenheimer, S., *Genetics*, **23**, 573 (1938).
[4] Gluecksohn-Waelsch, S., "Advances in Genetics", **4**, 2 (1951).
[5] Bonnevie, K., *Skrif. Norske Vid. Akad.*, 2, 1 (1936).

EXPERIMENTS in this laboratory, using inbred mice of the *CBA* strain, have given results similar to those described above by Hamburgh on the Bagg Albino strain. After some preliminary experiments, we used the following procedure. Males were placed with females overnight and the females were examined next morning for copulation plugs. Seven days after

the plug, each experimental female was given a single subcutaneous injection of ½ c.c. of 1 per cent aqueous trypan blue; an equal number of females were kept as untreated controls. Embryos were dissected out 1, 2 . . . 9 days later, that is, at 8½, 9½ . . . 16½ days embryonic age. Four females were used at each stage in each series; females without implantations were discounted and replaced.

The accompanying graph shows the results. By twenty-four hours after injection there is already a raised incidence of abnormal live embryos; this falls sharply at 12½ days, when there is a compensating rise in the incidence of dead embryos. The abnormal live embryos are of a uniform type at each stage. At 8½ days (presomite or early somite stage) the equatorial part of the egg cylinder (presumptive yolk-sac) is stained blue, in sharp contrast with the embryo proper; the latter is often retarded and shows sub-ectodermal blebs near the somites. At 9½ days, when embryonic turning normally occurs, the blebs are still present dorsally; the pericardium is also abnormally large and this apparently interferes with the turning process, so that the posterior part of the body fails to slip round the allantois and remains deflected along the right side of the anterior part. At 10½ days these defects are still present: in addition the neural tube, which should by then have closed anteriorly, may still be open; if closed, it is often convoluted in the thorax and the head is abnormally broad. By 11½ days the deflected post-umbilical



TREATED
Total: 282 embryos

CONTROL
Total: 293 embryos

body is very small and retarded, the pericardium relatively enormous. By 12½ days death intervenes. Between 12½ and 16½ days those embryos which successfully completed the turning process may develop hæmatomata on the head or, more often, the tail, or both. The commonest sites on the head are at the base of the nose and below the ear ; on the tail, near the tip. A tail hæmatoma apparently interferes with development of the distal part of the tail ; of the 58 live treated embryos aged 13½ days or more, seventeen had short tails (with or without head hæmatomata), three had head hæmatomata alone and one was otocephalic. Of 293 control embryos of all ages, 264 were normal, 26 dead, two moribund and one was unilaterally microphthalmic.

In a second series of experiments litters were carried to term. Experimental litters were small (and often cannibalized) ; they included many young with tail defects, some with head hæmatomata and a few which developed corneal opacity. The last, however, has been seen in untreated related stocks. A striking feature was a great deficiency of females in the experimental litters, among both normal and abnormal young (see table). Of 186 classified control young, 183 were normal, two had head hæmatomata and one clubfoot.

LITTERS CARRIED TO TERM
(*A* = abnormal, *N* = normal, ? = partly cannibalized)

Series	Females with plugs	Litters of zero	Progeny examined							Total	Mean litter size
			♀			♂			?		
			N	*A*	?	*N*	*A*	?			
Treated	31	8	10	4	1	26	13	10	7	71	2·29
Control	43	9	84	1	1	99	2	1	7	195	4·53

Our results are in agreement with those of Hamburgh, despite differences of procedure, notably (*a*) use of *CBA* instead of Bagg Albino mice, (*b*) absence of precoital injection, (*c*) higher postcoital dosage. The sex ratio disturbance, also noted by Hamburgh, is apparently due to differential mortality in the 11½-day embryos, and implies that significant differences between the sexes in their reactions to trypan blue are already established in the 9½-day embryo, before turning occurs.

Results with trypan blue are in sharp contrast with those obtained by L. B. Russell[1], who exposed pregnant mice to X-rays. Irradiation between 7½ and 9½ days caused a wide range of abnormalities, besides tail defects and cranial blisters, such as vaulted cranium, microphthalmia, coloboma, open eyelids, narrow snout, imperforate anus, hypospadias

and other urogenital anomalies, overgrowth of the limbs and feet. None of these defects was seen in our treated mice (the sole case of microphthalmia being in a control).

We are indebted to Messrs. Imperial Chemical Industries (Dyestuffs Division), Ltd., for a sample of purified trypan blue; the work was carried out under a research grant from the Medical Research Council.

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[1] Russell, L. B., *J. Exp. Zool.*, **114**, 545 (1950).

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A Note on Abnormalities induced in Mouse Embryos by Trypan Blue

by C. H. WADDINGTON *and* T. C. CARTER[1]

From the Institute of Animal Genetics, Edinburgh

INTRODUCTION

In 1948 Gillman, Gilbert, Gillman & Spence reported the results of experiments in which rats which had been injected at fortnightly intervals with 1 c.c. of 1 per cent. trypan blue were allowed to produce offspring. A most remarkable variety of anatomical abnormalities, often of a very severe character, was found in the young. The malformations included hydrocephalus, spina bifida, eye and tail defects, and alterations in internal organs. Some of the defects were similar to abnormalities which can be produced by mutant genes, either in the rat or in other mammals, and could therefore be regarded as belonging to the ill-defined class of 'phenocopies'. Since the mammal embryo is notoriously difficult of access, and comparatively little is known of its developmental physiology, the phenomena discovered by the South African authors seemed worthy of further investigation. Moreover, there is a considerable intrinsic interest in the possibility of the induction of pathological development by influences emanating from the maternal body, particularly in connexion with the fact that certain virus infections of the mother may be followed by effects of this kind (Gregg, quoted by Gillman, Gilbert, Gillman & Spence, 1948). We therefore decided to make an exploratory study of the effects of trypan blue in the mouse embryo. Since this did not reveal such striking modifications of development as were found in the rat, it has not seemed worth while to carry the investigations into all the possible ramifications of time and dosage, and the results obtained so far are here put on record. A preliminary note on the work has appeared elsewhere (Waddington & Carter, 1952).

MATERIAL AND METHODS

Since some of the abnormalities which may be produced by treatment with trypan blue may be expected to simulate genetic effects, it is desirable to carry out the experiments with strains of animals in which there is some reason to believe that mutant genes of the relevant kinds are absent. For this reason, and also because they were more easily available, we have worked with mice instead

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of the rats used by Gillman *et al.* The strain employed was the *CBA* inbred strain, in which gross abnormalities (such as hydrocephalus, tail abnormalities, &c.) do not occur with any appreciable frequency.

Gillman and co-workers injected their rats at fortnightly intervals, the times of injection not being related in any specific way to the time of conception of the offspring. In the analysis of their results they found that the greatest effect was produced in those animals which happened to have been injected on the seventh or eighth days of pregnancy; and the effect was greater still if there had been an earlier injection a fortnight previously. We decided to concentrate attention on the effects of injections given at the time of maximum efficiency. After some preliminary trials the following procedure was adopted. Females were left with males overnight, and examined next morning for copulation plugs. Seven days after the plug was found a single subcutaneous injection of 0·5 c.c. of a 1 per cent. aqueous solution of purified trypan blue was given.

In the first series of experiments four of the injected females were killed each day and the embryos dissected out, starting one day after the injection; that is to say, embryos were obtained at daily intervals between $8\frac{1}{2}$ and $16\frac{1}{2}$ days after conception. If a female contained no implanted embryos it was discounted and a new one prepared in place of it. A similar number of non-injected females were dissected in the same way to serve as controls. Embryos were fixed in Bouin's fluid, stained in Delafield's haematoxylin or borax carmine, and cleared and examined in methyl benzoate. A certain number of representative specimens were sectioned for more detailed study.

In a second series of experiments the same procedures were followed, but the females were allowed to come to term and to bear their litters, which were examined as soon after birth as possible.

RESULTS

A. *First Series*

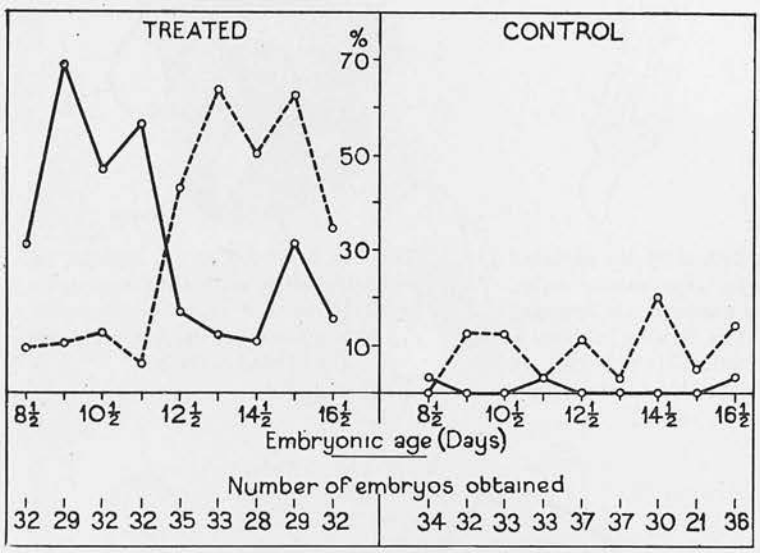
The general results of the experiments are shown in Text-fig. 1. It will be seen that, as was to be expected, the controls showed comparatively few abnormal or dead embryos, the number of prenatal deaths being well within the normal range for the mouse. The only marked anatomical abnormality was in one embryo which was unilaterally microphthalmic.

In the treated series, on the other hand, a very considerable number of abnormal or dead embryos were found. Already in the $8\frac{1}{2}$-day embryos (1 day after injection) the percentage of abnormals was considerably higher than in the controls. In the next few days the percentage of abnormals rose considerably, to fall again sharply in the $12\frac{1}{2}$-day and later samples, by which time the frequency of dead embryos had greatly increased. Clearly many of the embryos which show abnormalities in the earlier period die before the end of pregnancy.

The abnormal embryos exhibited a considerable variety of conditions. It will perhaps be best to describe the appearances characteristic of each successive day

of development before attempting to arrange the abnormalities into connected causal sequences.

8½-day embryos. In the normal 8½-day embryo the main axis of the body has only recently appeared, and comparatively few somites are formed, so that there is not yet much scope for morphological irregularities. In the treated embryos there is often a suggestion that development has been somewhat retarded, but the normal variation is fairly wide and it is difficult to be certain that the inhibited embryos are outside the normal range. The most striking phenomenon in the



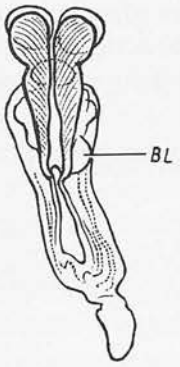
TEXT-FIG. 1. Numbers of embryos, including dead implantation sites, obtained at each stage in the experimental (left) and control series; and percentages of the total which were abnormal (full line) or dead (broken line).

embryos dissected out of the uterus is the concentration of blue dye in the central region of the egg cylinder, the future yolk-sac, which may be very deeply stained. Both the embryo proper and the amnion remain of their normal colour and do not take up the dye to any noticeable extent.

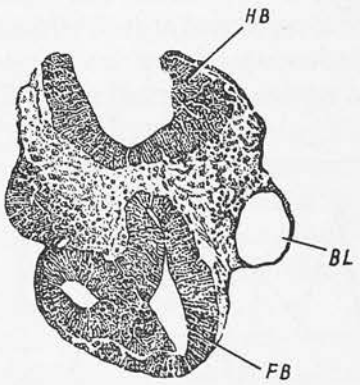
The dead embryos which are found at this stage are usually already disintegrated and appear as small more or less featureless lumps of necrotic tissue. They probably represent the amount of mortality which is normal in this strain.

9½-day embryos. The blue staining of the yolk-sac is still very noticeable. It is also quite clear that in many embryos the general rate of development has been slowed down, the stage of closure of the neural folds and the number of somites formed being definitely less than would be expected in a normal 9½-day embryo. Morphological abnormalities are also obvious in many cases. The neural tube often closes abnormally, remaining open in the brain region at a time when its condition elsewhere would lead one to expect it to be closed. It may also not be

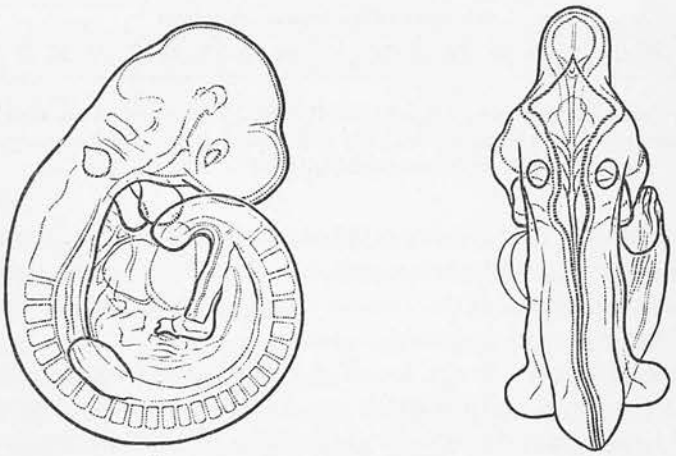
stretched out straight, but show kinks and bends. Sub-epidermal blisters, normally fairly small, appear in some embryos, particularly on the sides of the hind-brain or in the thoracic region, above or lateral to the first few pairs of somites (Text-figs. 2 and 3).



TEXT-FIG. 2. 9½-day unturned embryo, experimental series, seven-somite stage, dissected out of its amnion; it shows a subectodermal bleb (BL) on the right side in the somite region.



TEXT-FIG. 3. 9½-day turned embryo, experimental series, sectioned almost transversely in the head, showing subectodermal bleb (BL) between the fore-brain (FB) and hind-brain (HB).

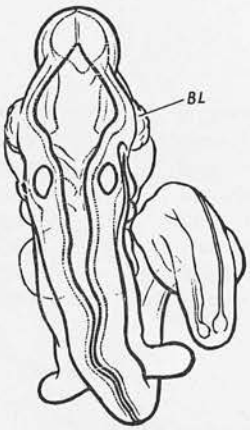


TEXT-FIG. 4. 10½-day normal embryo, control series. *A*, seen from the right side; *B*, the dorsal aspect of the myelencephalon.

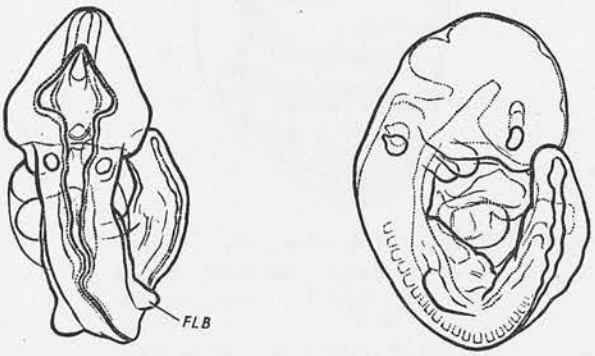
In the normal 9½-day *CBA* embryo the turning process occurs, whereby the embryo comes to be rolled up into a complete gyre of a right-handed spiral, so that the dorsum of the lumbar region faces in the opposite direction to that of the myelencephalon. The result of this rolling up is shown in Text-fig. 4. In many

abnormal embryos the posterior part of the body, from about fore-limb level, is deflected to the right; the dorsum of the lumbar region then faces to the right of the embryo, or even in the same direction as that of the myelencephalon (Text-figs. 5 to 8).

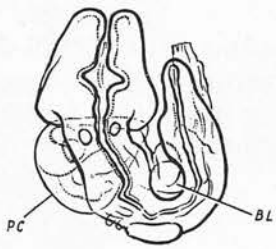
10½- and 11½-day embryos. The blue staining of the yolk-sac is still visible, but is becoming less intense except in the region near the placenta. There are still some very strongly retarded embryos in which the neural folds are not yet completely closed. Sub-epidermal blebs may also still be found.



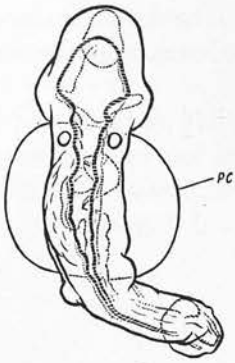
TEXT-FIG. 5. 10½-day embryo, experimental series, showing bleb (BL) on each side of the head near the maxillary process, dilated mid- and hind-brain, and slight convolution of the neural tube at the level of the heart.



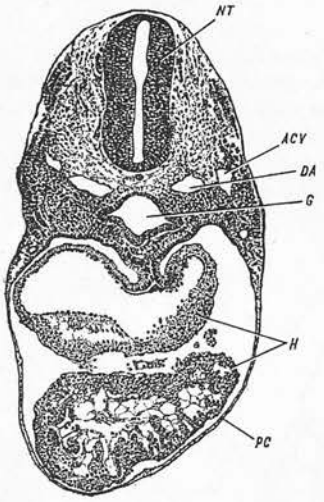
TEXT-FIG. 6. 10½-day embryo, experimental series, showing enlarged pericardium, body posterior to the fore-limb buds (FLB) deflected to the right, head oedematous, hind-brain dilated, and neural tube somewhat convoluted.



TEXT-FIG. 7. 10½-day embryo, experimental series, showing retarded development, enlarged pericardium (PC), subectodermal bleb (BL) dorsal to right fore-limb bud, failure of turning, oedematous head, failure of neural folds to close in the head, and convolution of the neural tube at the level of the heart.

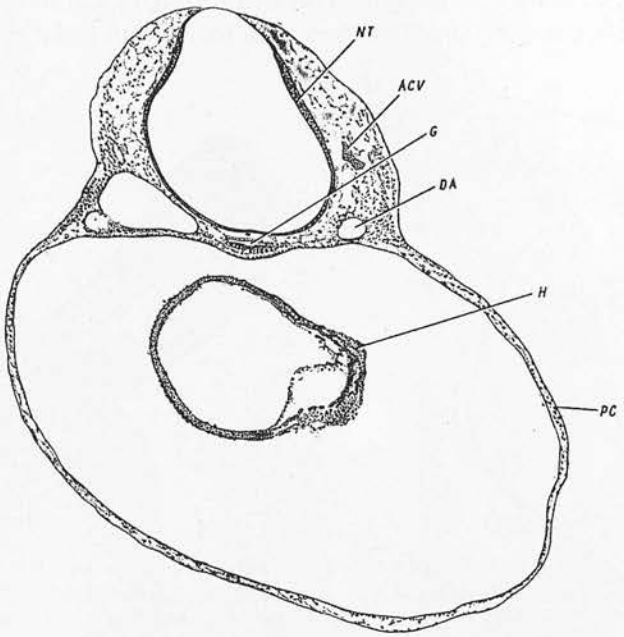


TEXT-FIG. 8. 11½-day embryo, experimental series, showing retarded development, greatly enlarged pericardium (PC), posterior part of the body deflected to the right, head oedematous, mid- and hind-brain dilated, and neural tube convoluted.



TEXT-FIG. 9. 10½-day embryo, control series, sectioned transversely at the level of the heart. For comparison with Text-fig. 10. ACV, anterior cardinal vein; DA, dorsal aorta; G, foregut; H, heart; NT, neural tube; PC, pericardium. The rest of the section, which shows also the hind-limb region and tail-bud, has not been drawn.

The most characteristic features of the abnormal embryos of this age, however, are four effects which it seems probable are closely connected with one another. These are (i) an enormous dilation of the pericardium, sometimes but not always accompanied by a corresponding increase in the size of the heart; (ii) a swelling of the embryonic blood-vessels, and general oedema of the tissues, affecting particularly the head; (iii) a deflexion of the posterior part of the body



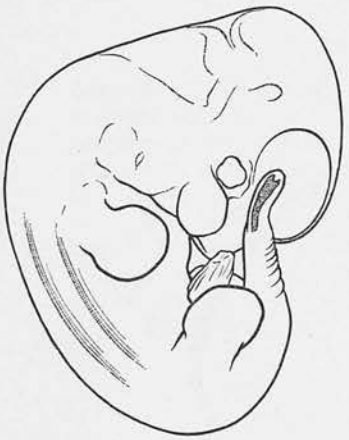
TEXT-FIG. 10. 11½-day embryo, experimental series, sectioned as in Text-fig. 9. Note the enormous pericardium, heart cut only once, dilated left dorsal aorta, small gut, and general sparseness of the tissues, especially the dilated neural tube. Abbreviations as in Text-fig. 9.

to the right side; and (iv) dilation of the neural tube, which is often kinked. Illustrations of these effects are shown in Text-figs. 5 to 8, from which their character will be sufficiently obvious without further description in words. The enlarged pericardium may reach extraordinary dimensions, so that the embryo looks almost like that of a teleost, with the pericardium taking the place of the teleost yolk-sac. This may be seen in Text-figs. 9 and 10, in which an abnormal and retarded embryo is compared with a normal one of about the same stage of development.

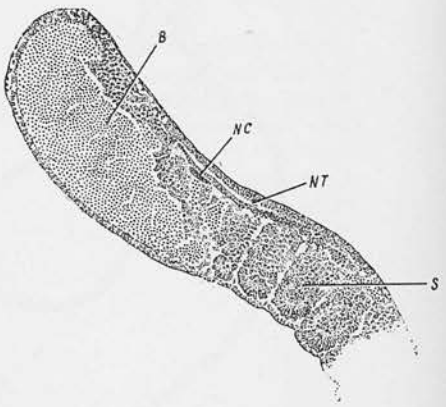
12½-day embryos. In the later 11½-day embryos, in which the posterior part of the body has been deflected to the side, it is usually small and retarded in development. By 12½ days this region has become very abnormal and is usually

necrotic. In these cases death of the whole embryo seems to occur towards the end of this period.

In those embryos in which the turning is more satisfactorily completed, morphological abnormalities are usually comparatively slight. They are more or less confined to the appearance of haematomata, particularly on the tail and in the head, although the tail may also show phenomena suggestive of degeneration without any marked escape of blood into the tissues. An example is shown in Text-fig. 11; Text-fig. 12 illustrates a section through a tail haematoma; Text-fig. 13 shows a section through a tail in which the tail-gut is deflected.



TEXT-FIG. 11. 12½-day embryo, experimental series, showing extravasation in the tip of the tail.



TEXT-FIG. 12. 12½-day embryo, experimental series; longitudinal section through the tip of the tail, showing large conglomeration of blood (B). NC, notochord; NT, neural tube; S, somite.

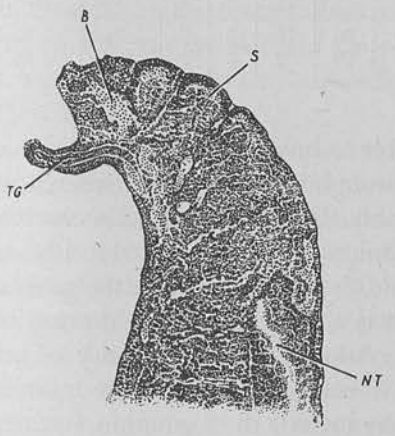
Later embryos. In embryos which survive till after the thirteenth day, the only abnormalities which have been found are shortening and other defects of the tail, with or without the extravasation of blood, and the occasional occurrence of haematomata on other sites. Among 58 embryos which survived till 13½ days or more, 17 had shortened tails, with or without haematomata on the head in addition, and 3 had head haematomata with normal tails. Sections of shortened tails show regions of necrosis near the tip and sometimes abnormal relationships of the tail-gut or notochord (Text-figs. 13 and 14).

The only other abnormality was a single case of otocephaly.

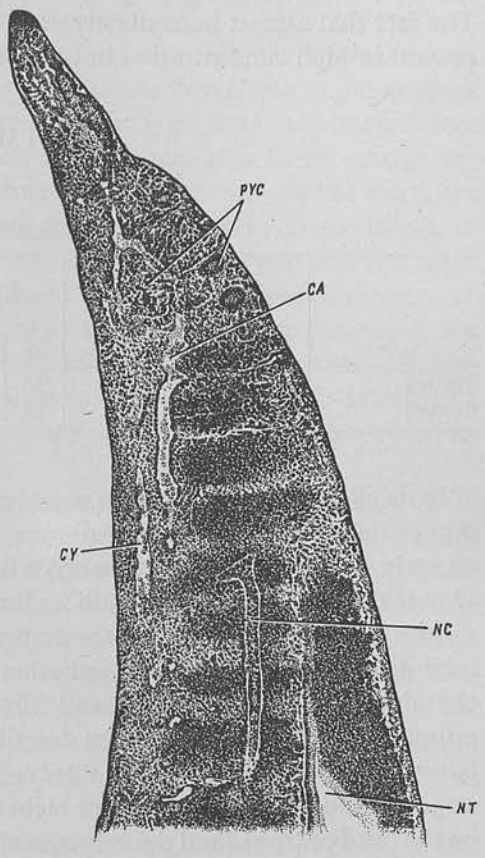
B. *Second Series*

The litters produced by those animals which were allowed to bring their young to term were usually small; and many of the new-born mice were eaten by their mothers in whole or in part. The numbers of animals examined are shown in the Table. The abnormals among the treated litters were mostly animals with tail

defects (shortening or bending). Some also exhibited head haematomata. A few developed corneal opacity, but since this condition has been seen in untreated related stocks, the occurrence of the defect here cannot be certainly attributed



TEXT-FIG. 13. 12½-day embryo, experimental series; longitudinal section through the tip of the tail, showing conglomeration of blood (B) and, immediately proximal to it, ventral branch of the tail containing tail-gut (TG). NT, neural tube; S, somite.



TEXT-FIG. 14. 13½-day embryo, experimental series; longitudinal section through the tail, showing deflected notochord (NC) and pycnotic tissue (PYC) near the tip. CA, caudal artery; CV, caudal vein; NT, neural tube.

to the trypan blue injections. The three abnormals among the controls consisted of two cases of head haematomata and one with club foot.

The data recorded in the Table show a very marked deficiency of females among both the normals and abnormals in the treated litters. Whereas of the classifiable young in the control litters there were 86 ♀ to 102 ♂, in the treated litters there were only 15 ♀ to 49 ♂. It is most plausible to attribute this disproportion to a greater relative frequency of death and resorption of female embryos.

DISCUSSION

The malformations of the embryos in the first series exhibit a rather considerable variety, and it is of interest to consider whether all the different abnormalities can be regarded as consequences of one original effect of the dye. The fact that almost immediately after the injection the dye can be seen to be present in high concentration in the yolk-sac gives us some clue as to the kind

TABLE

Litters carried to term

(A = abnormal, N = normal, ? = partly cannibalized)

Series	*Females with plugs*	*Litters of zero*	*Progeny examined*								*Mean litter size*
			♀			♂					
			N	*A*	?	*N*	*A*	?	?	*Total*	
Treated .	31	8	10	4	1	26	13	10	7	71	2·29
Control .	43	9	84	1	1	99	2	1	7	195	4·53

of basic effect which it would be most reasonable to invoke. The yolk-sac comes into contact with Reichert's membrane, and through it the maternal decidua, at an early stage; and in rodent embryos it probably then forms the main channel of nutrition. An interference with its function might well be expected to lead to disturbance of the nutrition of the embryo, and this may account for the general inhibition of growth and differentiation which is a marked feature of many of the abnormal embryos of $9\frac{1}{2}$ and $10\frac{1}{2}$ days. Another major category of abnormalities can, very broadly, be described as affecting the body fluids or circulatory system of the embryo. In this type we may include the formation, in early stages, of sub-epidermal blisters or blebs filled with clear fluid and located usually on the head or trunk, and the subsequent dilation of the neural tube. The haematomata which appear in rather later embryos are also local accumulations of body fluid, in this case of blood. They, like the earlier blisters, are formed more or less superficially, for instance under the epidermis of the head. When they form, as they often do, in the tail, they are in a small, rapidly growing region of the body, and it is only to be expected that for merely mechanical reasons their presence would cause considerable disturbance of the organization of the tail, e.g. in the direction of growth of the notochord or tail-gut, which might easily account for the kinks, bends, and shortening seen in the newborn.[1]

[1] Experiments on the effect of trypan blue on amphibian embryos, conducted while this paper was in the press, have shown that in those forms the dye suppresses the differentiation of neural structures and of the notochord. One must therefore keep in mind the possibility that the comparatively slight abnormalities of those organs seen in the mouse embryos may not be only secondary consequences of mechanical disturbance but may in part be produced by a direct action of the dye.

An even more drastic effect on the embryonic body-fluid system is revealed by the enlargement of the pericardium, heart, and embryonic vessels particularly in the head region. The oedema of the head is presumably also a part of this syndrome. It is the enlargement of the pericardium which is carried to the most extravagant degree. This structure is often hypertrophied out of all proportion to the heart, even when the latter is somewhat enlarged.

There would seem to be two alternative ways of regarding the whole complex of effects on the embryonic body fluids. On the one hand, one might suppose that the primary effect was on the nature or quantity of the fluids (for instance by a decreasing of the passage of maternal substances into them through the yolk-sac) and that the formations of blisters, the hypertrophy of the heart, the swelling of the blood-vessels, and the oedema were secondary consequences; or one might suppose that the primary effect was a direct morphological alteration in the heart region, leading to hypertrophy of the heart itself and of the pericardium, and that the circulatory disturbances were secondary consequences of this. In many ways the former alternative would seem the more attractive, although the enormous expansion of the pericardium is not quite easy to fit into such a scheme. But the matter can hardly be settled by the kind of evidence yielded by these experiments.

The enlargement of the pericardium, whether as a primary or secondary effect, would seem to provide the explanation for most of the other abnormalities which remain to be discussed. It is clear that the failure of the normal turning movement is closely connected with the presence of the enlarged organ in the very region towards which the tail bud usually moves. The occurrence of thoracic blisters may also sometimes impede the movement. It is to the failure of the turning, and to the consequent abnormal bending of the axis and impeding of the circulation to which this leads, that one can attribute the later degeneration of the posterior end of the body and the eventual death of many embryos at about $12\frac{1}{2}$ days.

The only types of abnormality which have not so far been fitted in to this scheme are the delay of closure of the neural folds in the head region and the appearance of kinks or convolutions in the neural tube. The first of these is most probably a relatively unspecific effect of general inhibition. A similar failure of closure is of common occurrence in chick embryos grown under abnormal conditions, as for instance *in vitro* (Waddington, unpublished observations). Kinks of the neural tube are also often seen in such embryos and are probably to be attributed to disproportionate growth, such as may result when a general inhibition affects the surrounding tissues more severely than it does the neural tube itself. Thus both the neural effects can probably be considered as consequences of general retardation, and do not call for the postulation of a specific and particular cause.

It therefore appears probable that the whole range of abnormalities can be accounted for in terms of a general inhibition of development acting at about the

time that the circulation appears and a more specific effect on the body fluids and the associated circulatory organs.

It is to be noted that these effects begin to come into operation very soon after the dye is injected. We have seen that the second series of experiments gives evidence of a greater sensitivity in female than in male embryos; it is presumably operative before $12\frac{1}{2}$ days, at which time the greatest uterine mortality takes place. In fact it would seem likely that the difference in sensitivity is exhibited in the $8\frac{1}{2}$-day and $9\frac{1}{2}$-day embryos in which the abnormalities are initiated which may eventually lead to death *in utero*. At such a time the sex differentiation of the embryos has not yet made its appearance, and the greater sensitivity of females than of males must be one of the earliest sex differences yet discovered.

Simultaneously with the work reported in this paper, a rather similar investigation has been carried out by Hamburgh and has been briefly reported (1952). He used an inbred Bagg Albino strain of mice, and gave two injections, each of $\frac{1}{4}$ to $\frac{1}{2}$ c.c. of 0·5 per cent. trypan blue, the first some 8 days before fertilization and the second 7 to 8 days after it. He also found that the main abnormalities in the young born after such treatment affected the tail, and that in the 10- to 14-day embryos abnormalities of the anterior part of the axis also occurred. He appears, in fact, to have encountered cases of pseudencephaly, which denote the survival, to a much later stage than in our experiments, of embryos with a profound disturbance of the anterior part of the neural system. He does not, in the preliminary communication, draw attention to any hypertrophy of the pericardium, or of the heart and embryonic blood-vessels, and he does not mention any failure in the turning of the posterior end of the body. The publication of a full account of his experiments will be awaited with interest; it may prove that his stock of mice reacts differently to ours, either because of genetic differences, or as a consequence of the difference in the timing and concentration of the injections.

The effects reported here, as well as those of Hamburgh, differ from those of Gillman, Gilbert, Gillman & Spence in the fact that the doses used on mice have had more profound effects and led to the death *in utero* of all the more extreme abnormalities, so that only minor tail defects remain alive till birth. It is difficult to tell whether other dosages, which allowed more abnormal embryos to struggle through to term, would have produced embryos showing the same types of defect as those authors described in their rats. It may perhaps be doubted whether this would have been the case, at least with the *CBA* mice. Gillman *et al.* found many cases of pseudencephaly and spina bifida, whereas the data from our series suggest that the disturbance of the turning movement would always prove lethal before the neural tube was affected as drastically as that. It may be that this is a result of the particular time at which our injections were given, but it must be remembered that the time of closure of the neural folds and the time of embryonic turning are not very different, and it is perhaps more plausible to suggest that there is a real difference in the pattern of sensitivity between rats and *CBA* mice. The fact that Hamburgh observed pseudencephaly among his

embryos may indicate that Bagg Albino mice are more similar to rats in this respect.

Ancel (1947) has described experiments in which trypan blue was injected into chick eggs. A certain similarity with our results is shown in the fact that the dye had a primary effect on the circulation, causing haemorrhages, which in the chick embryos often led to the local destruction of the body wall and the appearances of hernias of the type known as coelosomy.

SUMMARY

1. Females of an inbred *CBA* strain of mice, kept in the presence of males, were injected with $\frac{1}{2}$ c.c. of 1 per cent. trypan blue solution 7 days after the detection of a copulation plug. In one series of experiments the animals were killed and the embryos recovered 1, 2, 3, &c., days after the injection; in another series the females were allowed to bring their embryos to term.

2. The injected dye soon becomes accumulated in the yolk-sac, and a little later is visible in the region of the placenta.

3. In many $9\frac{1}{2}$-day embryos the rate of development has been slowed down; the closure of the neural tube is abnormal, and the tube itself may show bends and kinks. Sub-epidermal blebs may be found in various regions.

4. The rolling up of the embryo into a right-handed spiral, which normally occurs at about $9\frac{1}{2}$ days, is very often affected, the posterior part of the body being deflected to the right. At the same time, the pericardium begins to be very markedly inflated; and it seems probable that the abnormal turning of the embryo is caused by the mechanical impediment presented by the hypertrophied pericardium.

5. There is often a considerable dilation of the embryonic blood-vessels, particularly in the head; and fluids tend to escape from the vessels. There are often haematomata in the tail region.

6. There is a high death-rate of embryos at 12–13 days. Those which survive to term usually show few abnormalities other than shortening or kinking of the tail.

7. There is a marked shortage of females in the surviving young. This is presumably due to a differential death-rate, and since many deaths occur by 12 days, the females must be more sensitive than the males at a still earlier period, well before the time at which sexual differentiation takes place.

8. It is suggested that the main effects of the dye are firstly a general inhibition of development and secondly a more specific effect on the body fluids and circulatory organs.

We should like to express our gratitude to Miss M. M. Manson and Miss E. Paton for technical assistance; to Mr. E. D. Roberts, who drew the figures; to Messrs. Imperial Chemical Industries (Dyestuffs Division) Ltd. for a sample of

purified trypan blue, and to the Agricultural Research Council for financial support of the general work of the laboratory.

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AN EXPERIMENTAL ATTEMPT TO INVESTIGATE THE INDUCTION OF VISIBLE MUTATIONS IN MICE BY CHRONIC GAMMA IRRADIATION

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INTRODUCTION

A CONSEQUENCE of the increasing use of nuclear energy is the exposure of sections of human populations to long-continued dosage, at very low rates, with gamma radiation. It cannot be doubted that this exposure is having genetic effects on the populations concerned; and it is therefore important to try to evaluate, as soon as possible, the extent of these effects.

For any attempt to make such an evaluation, information is needed on many different characteristics of the population. Some of these, such as the degree of inbreeding and the proportion of the population exposed to radiation, can only be dealt with by a study of the population itself. However, three questions, all of them fundamental to the problem, can be dealt with in part by an experimental approach. They are the following: (i) is the genetic effect independent of the dosage rate when the latter is very low? (ii) is the genetic effect proportional to the accumulated dose when this is very low? (iii) if genetic effect is proportional to the accumulated dose, what is the constant of proportionality? What is the dose which makes the total mutation rate twice the spontaneous rate?

These questions have been extensively studied, using *Drosophila melanogaster* as experimental material. The work of Caspari and Stern (1948) using gamma radiation, of Spencer and Stern (1948) using röntgen rays, and of Uphoff and Stern (1949) indicates that the proportionality of dose and effect holds good down to doses of the order of 25 r; the effect measured was the induction of sex-linked lethals and a dose of the order of 30 r to 50 r was required to double the spontaneous mutation rate. On the other hand, Bonnier and Lüning (1949), who studied mutation at the *white eye* and *forked* loci, detected apparent departures from a strictly linear law when they used röntgen ray doses of the order of 16 r or less.

Biological Hazards of Atomic Energy, pp. 73–81.

This finding may be particularly important. If it is generally true, and not merely a peculiarity of the loci studied by Bonnier and Lüning, it suggests that the slope of the effect/dose curve may be much higher, in the important part of the range, than the average over the whole range; the rate-doubling dose may then be much smaller than that expected on the basis of a strictly linear law (Fig. 1).

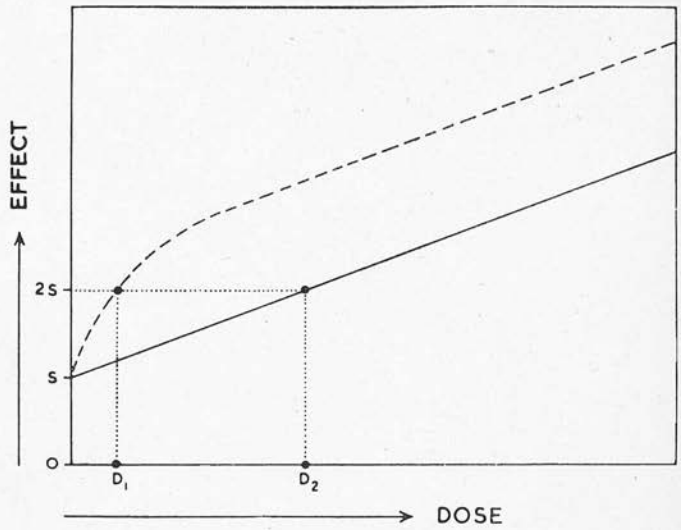

FIG. 1. Dose/effect relationship, (*a*) on a strictly linear law (full line), (*b*) on a law of the type suggested by Bonnier and Lüning (broken line). S, spontaneous rate; D, rate-doubling dose.

However, it is a long way from *Drosophila* to man; and there is a clear need for studies on some species more closely related to man. The need to raise enormous numbers of individuals, which is inherent in mutation-rate studies, virtually prohibits the use of a mammal to study the first two questions; but it is not beyond practical bounds to use the mouse, for example, to obtain an answer to the third question. It must be emphasized that a measurement made on mice cannot supply all the information needed. There is no *a priori* reason for supposing that the mutation-rate-doubling dose applicable to the mouse is also applicable to man; but if this dose is experimentally found to be the same for a number of species, including forms as diverse as dipterans and mammals, then one would have more confidence in assuming that the same figure is also applicable to man.

Experimental work with mice has therefore been started at Edinburgh in

an attempt to measure the spontaneous mutation rate and the rate-doubling dose of gamma radiation. Recessive visibles were chosen as the class of mutation to be studied, partly because in the mouse they are more easily dealt with than the sex-linked lethals commonly studied in *Drosophila melanogaster*, and partly because they may constitute an important fraction

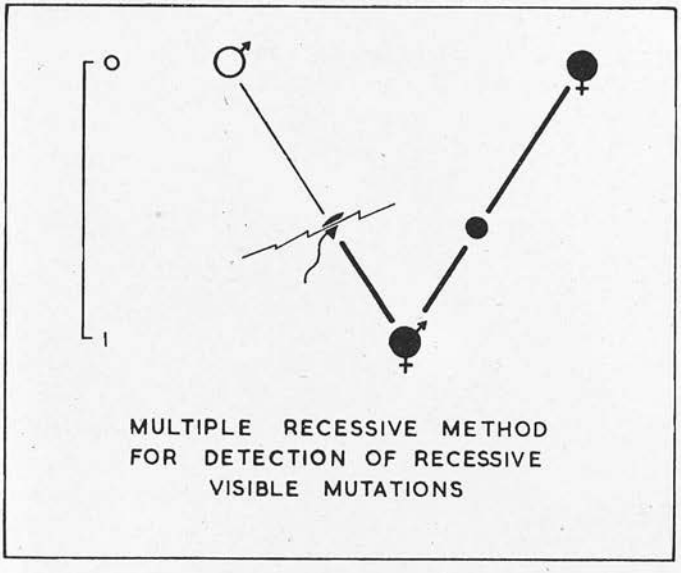

FIG. 2. Multiple recessive method for the detection of recessive visible mutations at specified low. Top left, irradiated male; top right, multiply recessive female; a sperm has mutated under the influence of the radiation and the F_1 mouse is consequently homozygous for the mutant.

of the genetic changes induced in human populations. Information about the mutation of dominant visibles accrues at the same time. The work is on a comparatively small scale, since it is at present merely a pilot experiment, intended to provide information about the best methods of attacking the problem.

Methods and materials

The two main methods available for measuring a recessive visible mutation rate have both been used.

In the first method attention is confined to a small number of gene loci. A wild-type mouse carrying the gamete under test is mated to one which is homozygous for the recessive allele at the specified loci; the ensuing F_1 animal is multiply heterozygous, and therefore wild-type, unless the gamete

being analysed has mutated to the recessive allele at any of the specified loci (see Fig. 2). This method has the advantage that only one generation is required for the analysis and therefore large numbers of gametes can be analysed; and the subjective element can be minimized by the choice of easily recognized recessives. It has the disadvantage that the number of loci analysed is limited to the number for which a stock of mice can be made homozygous without impairing fertility. This number is probably not much above eight: the stock at present in use carries seven (*a*, *b*, c^{ch}, *d*, *p*, *s*, and *se*). The results obtained by this method are not described in the following sections.

The second main method is to analyse the gamete for recessive mutation at all possible loci. It calls for at least three generations of breeding and therefore only a comparatively small number of gametes can be analysed when cage-space is limited. There is also a much greater subjective element in the detection of mutations, since past experience is no guide to the recognition of a new mutant. Two different forms of this method have been used, namely, the backcross method as used by P. Hertwig (1948) and the sib-mating method.

All the mice irradiated are of the Strong CBA inbred strain descended from a single pair obtained in 1947. Gamma radiation is obtained from a 400-millicurie radium source and variation of dosage-rate is obtained by varying the distance from the source.

Gamete analysis by the backcross method

CBA males for irradiation are housed in small, aluminium cages; these are carried on three racks, at different distances from the radium source, so arranged that the mice receive 6·0 r, 1·2 r, or 0·24 r in 16 hours (Table I). Control mice are kept in similar cages and in the same room, but shielded from the source. The source is exposed for 16 hours on each of 5 nights per week. Mice enter the field when 8 weeks old and irradiation continues for 25 weeks; they are placed with CBA females for 2 nights after 5 weeks' exposure and again for a week after completion of 25 weeks' irradiation. However, it was found that all mice irradiated for 25 weeks at the highest dosage-rate were sterile, and exposure at this level was reduced to 10 to 15 weeks.

Each F_1 mouse represents one irradiated or control spermatozoon; if this spermatozoon carried a recessive mutation, the F_1 animal must be heterozygous for it. Males of this generation are therefore tested by a variant of the technique used by P. Hertwig (1942) (Fig. 4); they are crossed with their sisters, and their daughters are then backcrossed to them. If the F_1

male is heterozygous for a recessive mutant, half of his daughters will be expected to be heterozygous; and when a heterozygous daughter is back-crossed to him, one-quarter of her young will be expected to be homozygous and therefore to show the recessive phenotype.

The method has several disadvantages. First, it discloses any recessive mutant carried by the F_1 male, irrespective of origin. Such a recessive need

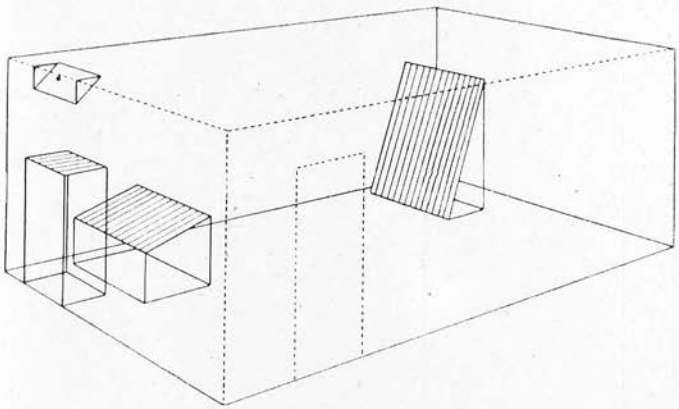

FIG. 3. Gamma-irradiation room. The radium source, surrounded by a lead control-mechanism, is at ceiling level, top left; the irradiated mice are in cages on three racks, at different distances from the source. A fourth, shielded rack, carrying control mice, is not shown.

not have been radiation-induced; it could have mutated spontaneously in the treated spermatozoon; it could have been present heterozygously in the irradiated P_1 mouse, having mutated spontaneously in an ancestor; or it could have mutated in the untreated P_1 female or in an ancestor of hers.

TABLE I

Accumulated doses of gamma radiation received by CBA males

Level	*Dose/16 hrs. (r)*	*Dose/week (r)*	*Maximum exposure (weeks)*	*Maximum accumulated dose (r)*
A	6	30	15	450
B	1·2	6	25	150
C	0·24	1·2	25	30
K	—*	—	25	—

* The mice in level K are shielded from the direct radiation: the dose due to scatter does not exceed 50 mr per 3 days.

It is sometimes possible to discover, from breeding tests, when the new mutant was carried heterozygously by either P_1 animal; but distinction between the other possibilities can only be made on a statistical basis.

A new mutation may remain undetected simply because all the daughters backcrossed to the F_1 male happened, by chance, not to be heterozygotes;

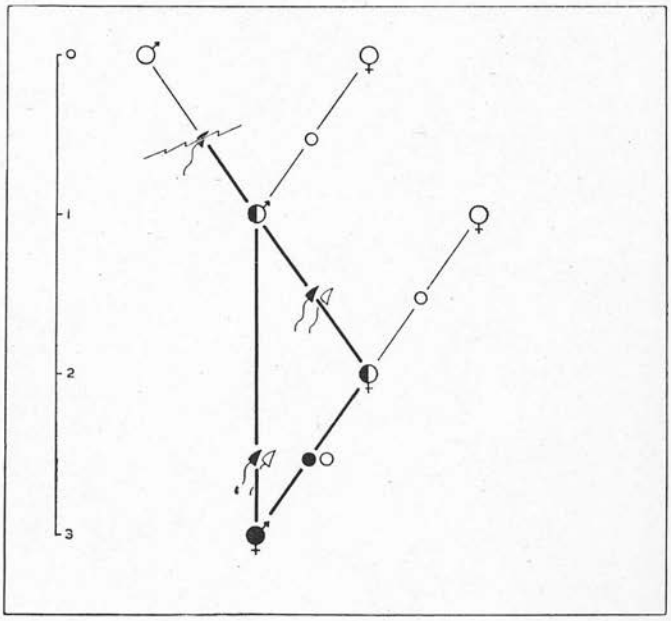

FIG. 4. Backcross method for the detection of recessive visible mutations at any locus. Top left, irradiated male. A sperm has mutated under the influence of the radiation and the F_1 male is consequently heterozygous; he is outcrossed, a daughter is backcrossed to him, and a homozygote has occurred in the third generation.

or because their progeny happened, by chance, not to include a homozygote. This introduces a statistical problem which has a considerable bearing on the design of the experiment; it has been discussed by Falconer (1949), who introduced the 'completeness-of-test' concept and pointed out that it may be more efficient to test many F_1 animals sketchily than to test a few completely.

Two newly mutated recessives have been found by this method. One was a recurrence of the well-known pink-eye mutant (Carter and Phillips, 1950); the other, previously unknown, causes a form of paralysis and has been called 'ataxia'. Both were found in the descendants of control mice; they were therefore not radiation-induced.

Gamete analysis by the sib-mating method

The sib-mating method of analysis of gametes for recessive mutation at all possible loci has recently been introduced into the experiment. By this

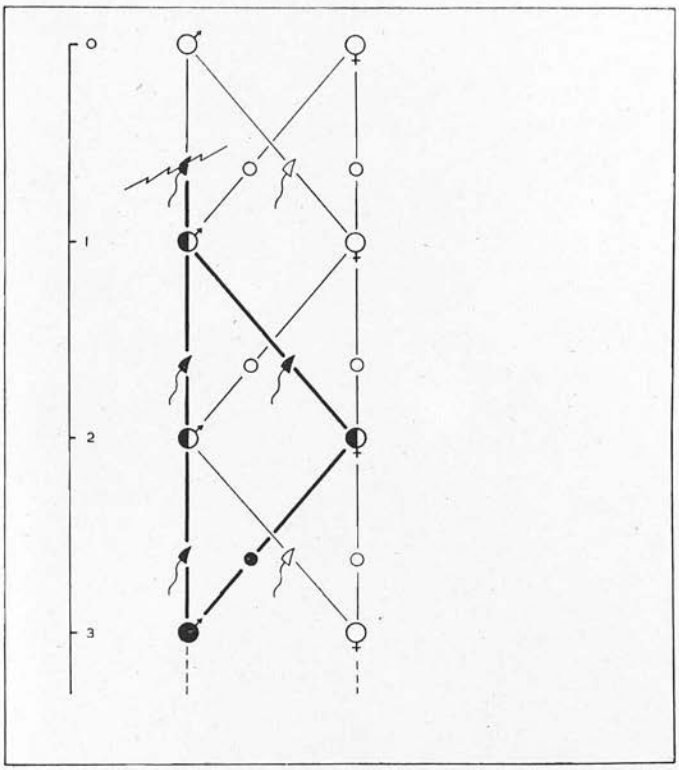

FIG. 5. Sib-mating method for the detection of recessive visible mutations. A sperm carried by an irradiated male (top left) has mutated under the influence of the radiation; one mate in generation 1 is consequently heterozygous and a homozygote has appeared in generation 3.

method parallel sib-mated sublines, all derived from a common ancestral pair, are maintained in the radiation field or as controls. Those maintained in the field are exposed to the radium source simultaneously with those treated by the multiple recessive method. If one of the mates in a subline carries a new recessive mutation, the two animals chosen as mates for the next generation may both receive it and homozygotes may appear in the second generation; or it may by chance remain in the subline in the heterozygous state, and fail to segregate out until the third generation or later; or it may by chance be lost altogether (Fig. 5).

The sib-mating method has the disadvantage that the statistical interpretation of the results is more complicated than with the backcross method. The probability that any mutation occurring will be detected is low, less than one-half; but this is compensated by the fact that both sexes contribute induced or control mutations. In this it differs fundamentally from the backcross method, in which only males are exposed to the radiation. The sib-mating method has the great advantage of technical simplicity; each mouse remains in the same cage, with the same mate, throughout its useful life.

One newly mutated recessive has been found by this method. It affects the tail, which is wound up; affected mice die of unknown causes at about 3 weeks. It also was found in a control line and therefore was not radiation-induced. It has been called 'gyre-tail'.

Results and conclusions

A summary of the results obtained by both methods is shown in Table II. The figures given for the number of gametes tested are the equivalent numbers of completely tested gametes (Falconer, 1949), not the actual number subjected to test.

TABLE II

Summary of data

Method of analysis	*Type of gamete*	*Completely analysed equivalent*	*Recessive visibles found*	*Mean accumulated dose (r)*
Backcross	Irradiated	72·3717	—	76·5
,,	Control	145·8265	2	—
Sib-mating	Untreated	193·7495	1	—

As none of the mutants found was radiation-induced, no estimate can yet be made of the mutation rate under irradiation; the data can be used, however, to set an upper fiducial limit, which has been obtained from the tables given by Stevens (1942) (Table III).

TABLE III

Mutation rate estimate and fiducial limits ($2\frac{1}{2}\%$)

Mutation rate	*Lower limit*	*Mean*	*Upper limit*
S	0·0018	0·0088	0·0258
S+½I	—	—	0·0510

S = spontaneous; I = induced. The factor ½ enters because only one of the two P_1 mice was irradiated.

The rough estimate obtained for the spontaneous mutation rate, 0·88 recessive visibles per 100 completely tested gametes, will surprise many who work with mice. It shows the need for extensive control experimentation in this type of work and emphasizes the danger of assuming that a new mutant was artificially induced, simply because it arose in a stock treated with a mutagenic agent.

The absence of mutants in the irradiated series, which received an average of over 75 r, suggests that the rate of induction cannot be very high; in particular, our data make it improbable that the true rate-doubling dose is of the order of 3 r, a figure which has been considered as being possibly applicable to human populations (Sewall Wright, 1950).

The work described does not, of course, constitute a completed experiment. This is a progress report on a pilot experiment intended to provide information on which to base a choice between alternative experimental techniques. As a result of the experience gained, the backcross method of gamete analysis has been discontinued in favour of the sib-mating and multiple-recessive methods; no positive conclusions can be drawn from the results obtained to date about the effect of chronic gamma irradiation on the mutation rate in mice.

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A mathematical treatment of genetical recombination using a four-strand model

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A mathematical treatment of genetical recombination is given, using a four-strand model.

Two distinct kinds of interference are recognized: *chiasma position interference* ('chiasma interference'), affecting the distribution of the chiasmata along the chromosome, and *chiasma type interference* ('chromatid interference') affecting the strands which take part in adjacent chiasmata. Their treatment is based on the methods of Owen and Weinstein respectively.

Type interference is of two kinds, according as adjacent chiasmata more frequently involve two or four strands.

When chiasma pairs involving two strands are much more frequent, the recombination fraction rises with map distance to about 25 % and thereafter creeps up slowly towards the limiting value, 50 %; with map distances of the length met in most material, the recombination fraction might scarcely rise above 25 %. When all chiasma pairs involve only two strands, the limiting recombination fraction is 25 %.

When four-strand chiasma pairs are more frequent than two-strand pairs, the recombination fraction rises with increasing map distance to a maximum above 50 % and thereafter performs damped oscillations round 50 %.

When all chiasma pairs involve four strands, the maximum recombination fraction, in the absence of position interference, is 53·34 %. When position interference is present, it enhances the effect of type interference and higher maxima are possible; 100 % recombination would occur between points separated by two localized chiasmata always involving four strands.

It is thought possible that 'second-order linkage' may be detectable in some material, i.e. linkage of less than 50 % between two loci, each of which shows linkage exceeding 50 % with an intermediate marker.

1. Introduction

The discovery of genetical linkage by Bateson and Punnett in 1906 marked the beginning of a period in which the new science of genetics was to be drawn into a close relationship with cytology. Development was rapid, and it was soon shown that genes were organized in linkage groups; that within each group they were arranged in a linear order; that each linkage group corresponded with a chromosome; and that the mechanism of crossing-over was chiasma formation. By 1913 Sturtevant was able to construct the first linkage-group maps.

It became apparent, however, that linkage-group maps were strictly accurate only when closely linked genes were involved; the linkage between two widely separated marker genes A and C was always found to be less than that predicted by summing the linkages of A and C with an intermediate marker B. It was recognized that this deficiency must be due to double crossing-over between A and C; and in 1919 Haldane gave a mathematical analysis of the problem, based on a model in which crossing-over took place by exchange of parts between flexible chromosomes. He used the concept of a strictly additive map-distance, of which the unit should be a morgan, and to which small linkage values should approximate closely; and he showed that with fully flexible chromosomes (and consequently with randomly distributed chiasmata) the recombination fraction, y, would be related to the map distance, x, by the equation

$$y = \tfrac{1}{2}(1 - e^{-2x}). \tag{1}$$

With this model, therefore, recombination fractions exceeding 50 % were impossible.

Haldane also examined the effect of imperfect flexibility of the chromosomes; this would reduce the probability of a chiasma forming in the near neighbourhood of an existing chiasma; and he showed that the effect would be to shift the recombination-fraction/map-distance curve so that it lay between the curve of (1) and the straight line $y = x$. Recombination exceeding 50 % becomes possible with this modified two-strand model.

Meanwhile it appeared from cytological and genetical studies that the formation of chiasmata during meiosis occurs after each chromosome has split longitudinally into two chromatids, and that only one chromatid of each pair takes part. Following earlier work by Bridges (1916), this was firmly established by Anderson (1925). It was followed by mathematical studies of the four-strand model, notably by Weinstein (1928, 1932, 1933, 1936, 1938). He was mainly concerned with analyzing the effects of the additional degree of freedom introduced by changing from a two-strand to a four-strand model. Suppose, for example, that the four strands are A and A', a and a' (figure 1), and that an existing chiasma involves A and a; then a new neighbouring chiasma may involve any of the pairs Aa, Aa', $A'a$ and $A'a'$. Furthermore, the probabilities may not be the same of forming the two-strand ('reciprocal', 'regressive') type Aa, the three-strand ('diagonal', 'progressive') types Aa' and $A'a$, and the four-strand ('complementary', 'digressive') type $A'a'$.

Two distinct sorts of interference may thus be recognized. First, the probability that a new chiasma will form in a given region may be influenced by the position of

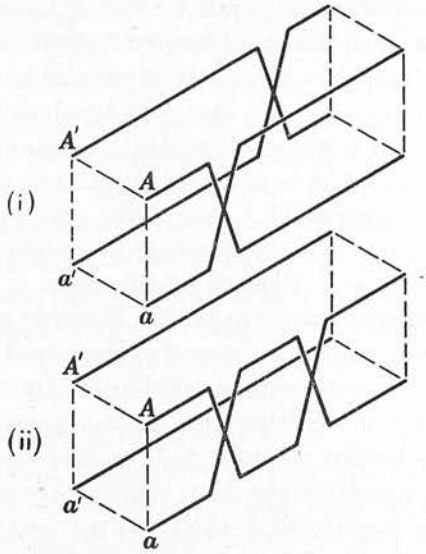

FIGURE 1. The four strands which constitute one arm of a bivalent. ***AA'***, ***aa'*** are pairs of sister chromatids. (i) shows a four-strand pair of chiasmata, (ii) a two-strand pair.

that region relative to an existing neighbouring chiasma; being the only sort of interference which could occur in the early, two-strand model, this was called simply 'chiasma interference'. Secondly, the probability that a given strand will take part in the new chiasma may depend on whether or not it took part in the neighbouring chiasma; this sort of interference, which can only exist in multi-strand systems, has been called 'chromatid interference'. These terms, however, are open to objection. They lack clarity, for all chiasmata are formed between chromatids, and interference of either sort must be exerted through chromatids. Worse, they fail to express the essential difference between the two sorts of interference, which is that they are concerned with, respectively, the position and the type (two-, three- or four-strand) of a new chiasma relative to an existing one. We have therefore found it necessary to adopt some alternative terms; and the best available seemed to be 'position interference' and 'type interference', the prefix 'chiasma' being understood in each case.

It follows from Weinstein's results that, with a four-strand model, recombination exceeding 50 % is possible in a chromosome segment of suitable length, but only when both of two conditions are satisfied, namely, (i) there must be type interference whether or not there is also position interference, and (ii) the type interference must be such that adjacent chiasmata involve four strands more often than two.

Further contributions to the problems of genetical recombination and chiasma formation were made by Jennings (1923), Winge (1936) and Mather (1933, 1936, 1937, 1938, 1940). It was suggested that chiasma formation may be a sequential process, starting from the centromere and proceeding distally, the distribution of the point of formation of each new chiasma being dependent only on the position of the immediately preceding chiasma. With some changes, this concept was adopted and developed into a mathematical theory of genetical recombination by

Owen (1949, 1950), following earlier papers by Fisher, Lyon & Owen (1947) and by Fisher (1948). Owen assumed a certain form of interference probability function and found that, when applied to his model, it gave recombination-fraction/map-distance curves of two kinds. In both, the recombination fraction tended to 50 % for very large map distances, but they differed in the nature of the curve for medium map distances. In one class the recombination fraction rises monotonically with increasing map distance, approaching (but never exceeding) the value 50 %; in the other class the recombination fraction rises to a maximum greater than 50 % and then executes damped oscillations round this value.

One fundamental feature of Owen's approach, however, seems open to objection; he confines attention to events on one strand of the tetrad, disregarding events on the other three strands. Some chiasmata are thus totally neglected. So, too, is the important fact that events on the other three strands are not independent of events on the strand to which attention is confined. A result of this one-strand approach is that the distinction between sister chromatids is lost; the equations appropriate to a two-strand model then become applicable, and are used by Owen; it is therefore impossible to incorporate an explicit treatment of type interference, which is essentially a multi-strand phenomenon. Owen (1950) recognizes that, with a four-strand model, type interference is necessary for the existence of recombination in excess of 50 %; but he holds that the effects of both type and position interference can be adequately treated when they are subsumed into a single interference parameter. This assertion seems to us unsupportable, since they are quite distinct phenomena, each of which can be visualized as existing independently of the other.

It therefore seemed desirable to re-examine the effects of type and position interference, frankly using a four-strand model. To do this we have used Weinstein's treatment of type interference and Owen's elegant mathematical methods to deal with position interference; these have been integrated with a type-position interference interaction to yield a new set of recombination-fraction/map-distance relationships.

Our model therefore has three parameters, I, g_0 and λ, referring to position interference, type interference and their interaction respectively. The position interference parameter I is essentially the same as Owen's interference parameter, but its application differs in that we take all chiasmata into account; we have, however, restricted ourselves to the simple case, dealt with in detail by Owen, when I has the value $\frac{1}{2}$. The type interference parameters g_0 and λ govern the excess probability that a newly formed chiasma and its immediate predecessor will involve two strands, rather than four; the excess is given by $g_0 e^{-\lambda u}$, where u is the distance between the chiasmata.

Fundamental four-strand model equations are derived, relating recombination fraction and map distance to chiasma frequency and type-interference strength. These are first applied to some simple cases in which position interference is absent but type interference present; the general case is considered next, including the special case in which position interference is present but type interference absent.

2. The dependence of recombination fraction and map distance on chiasma frequency

The fundamental recombination-fraction and map-distance equations obtained in this section are based on the following assumptions. (*a*) Chiasma formation involves two non-sister chromatids in a four-strand bivalent, of which only one arm is considered. (*b*) Type interference may exist and is measured by $(\alpha-\gamma)$, where α is the probability that a chiasma and its immediate predecessor will involve two strands only, and γ is the probability that they will involve four. (*c*) Position interference may exist, but the equations obtained are of general validity, whether it exists or not.

(i) *Recombination fraction*

The recombination fraction between two loci A and B is, by definition, that fraction of all gametes, and therefore of all emergent chromatids, which shows recombination between A and B. The total population of chromatids can be subdivided into classes according as they were derived from bivalents which carried $0, 1, 2, \ldots, n, \ldots$ chiasmata between A and B. Let the frequencies of these classes be $P_0, P_1, P_2, \ldots, P_n, \ldots$ respectively, so that $\sum_{n=0}^{\infty} P_n = 1$; and let q_n be the frequency of recombinant chromatids in the class derived from bivalents having n chiasmata between A and B. Then the recombination fraction is the weighted mean value of q, the weights being the class frequencies; so we have

$$y = P_0q_0 + P_1q_1 + P_2q_2 + \ldots + P_nq_n + \ldots. \tag{2}$$

For $n = 0$ none of the chromatids is a recombinant, whence $q_0 = 0$; for $n = 1$, two of the four chromatids are involved in the single chiasma, when $q_1 = \frac{1}{2}$. Weinstein (1938, 1948) has given the values of q for higher values of n. The results which he quotes are

$$q_3 = q_5 = \ldots = q_{2n+1} = \tfrac{1}{2}, \tag{3a}$$

$$q_2 = \tfrac{1}{2} - \tfrac{1}{2}(\alpha_2 - \gamma_2), \tag{3b}$$

where α_2 and γ_2 are the values of α and γ appropriate to the formation of the second chiasma. Similarly

$$q_{2n} = \tfrac{1}{2} - \tfrac{1}{2}(\alpha_2 - \gamma_2)(\alpha_4 - \gamma_4)\ldots(\alpha_{2n} - \gamma_{2n}). \tag{3c}$$

When equations (2) and (3) are combined, they give the fundamental equation relating recombination fraction to chiasma frequency and type interference:

$$\begin{aligned} y &= 0.P_0 + \tfrac{1}{2}P_1 + \tfrac{1}{2}\{1-(\alpha_2-\gamma_2)\}P_2 + \tfrac{1}{2}P_3 + \tfrac{1}{2}\{1-(\alpha_2-\gamma_2)(\alpha_4-\gamma_4)\}P_4 + \ldots \\ &= \frac{1}{2}\sum_{n=1}^{\infty} P_n - \frac{1}{2}\sum_{n=1}^{\infty} P_{2n}\prod_{i=1}^{n}(\alpha_{2i}-\gamma_{2i}) \\ &= \tfrac{1}{2}(1-P_0) - \frac{1}{2}\sum_{n=1}^{\infty} P_{2n}\prod_{i=1}^{n}(\alpha_{2i}-\gamma_{2i}). \end{aligned} \tag{4}$$

(ii) *Map distance*

The map distance between A and B is the sum of the recombination fractions of the indefinitely large number of indefinitely small subsegments between A and B.

Each of these is assumed to be so small that it can never contain more than one chiasma; therefore for each subsegment

$$\begin{aligned} y &= P_0 q_0 + P_1 q_1 \\ &= \tfrac{1}{2} P_1. \end{aligned}$$

This is half the mean number of chiasmata in the subsegment. The map length of the whole segment AB is therefore half the mean number of chiasmata in the segment, i.e.

$$\begin{aligned} x &= \tfrac{1}{2}(P_1 + 2P_2 + 3P_3 + \ldots + nP_n + \ldots) \\ &= \frac{1}{2} \sum_{n=1}^{\infty} nP_n. \end{aligned} \tag{5}$$

This is the fundamental equation relating map distance to chiasma frequency.

3. The effect of type interference alone

We shall first examine three special, simple cases in which there is no position interference and where type interference has only certain constant, limiting values. When there is no position interference, the relative frequencies of $0, 1, 2, \ldots, n, \ldots$ chiasmata in a marked segment AB are given by the Poisson terms $e^{-m} m^n/n!$ (Haldane 1919), where $m = 2x$ is the mean number of chiasmata in the segment.

(i) *Type interference absent*

When type interference is also absent, so that $\alpha - \gamma = 0$, equation (4) degenerates into

$$\begin{aligned} y &= \tfrac{1}{2}(1 - P_0) \\ &= \tfrac{1}{2}(1 - e^{-m} m^0/0!) \\ &= \tfrac{1}{2}(1 - e^{-2x}). \end{aligned} \tag{6}$$

This is Haldane's no-interference equation.

(ii) *All chiasma pairs two-strand*

When all chiasma pairs involve two strands, so that $\alpha - \gamma = 1$, equation (4) becomes

$$\begin{aligned} y &= \tfrac{1}{2}(1 - P_0) - \frac{1}{2} \sum_{n=1}^{\infty} P_{2n} \\ &= \frac{1}{2} - \frac{1}{2} \sum_{n=0}^{\infty} P_{2n} \\ &= \tfrac{1}{2} - \tfrac{1}{2} e^{-m} \left[1 + \frac{m^2}{2!} + \frac{m^4}{4!} + \ldots \right] \\ &= \tfrac{1}{2} - \tfrac{1}{2} e^{-m} [\tfrac{1}{2}(e^m + e^{-m})] \\ &= \tfrac{1}{4}(1 - e^{-2m}) \\ &= \tfrac{1}{4}(1 - e^{-4x}). \end{aligned} \tag{7}$$

y rises monotonically and is asymptotic to the line $y = \frac{1}{4}$, i.e. the limiting recombination fraction is 25 %.

(iii) *All adjacent chiasma pairs four-strand*

When all adjacent chiasma pairs involve four strands, so that $\alpha-\gamma=-1$, equation (4) becomes

$$\begin{aligned} y &= \tfrac{1}{2}(1-P_0)-\tfrac{1}{2}(-P_2+P_4-\ldots) \\ &= \tfrac{1}{2}-\tfrac{1}{2}(P_0-P_2+P_4-\ldots) \\ &= \tfrac{1}{2}-\tfrac{1}{2}\mathrm{e}^{-m}\left[1-\frac{m^2}{2!}+\frac{m^4}{4!}-\ldots\right] \\ &= \tfrac{1}{2}-\tfrac{1}{2}\mathrm{e}^{-m}\cos m \\ &= \tfrac{1}{2}(1-\mathrm{e}^{-2x}\cos 2x). \end{aligned} \tag{8}$$

y has a maximum value of 53·34 % recombination for a segment about 120 cM long and thereafter executes damped oscillations round the 50 % value. This curve appears to coincide with that obtained by Owen for recombination in a segment infinitely distant from the centromere when $I=\frac{1}{2}$.

4. The effects of type and position interference acting together

(i) *Description of the model*

Our model has the following features:

(*a*) Each arm of the bivalent in which chiasmata are to be formed consists of two homologous chromosome arms, each of which has divided longitudinally into two chromatids. Each arm is indefinitely long.

(*b*) Chiasma formation consists of the occurrence of breaks at the same position in two non-sister chromatids, followed by non-restitutional joining. Chiasmata are formed sequentially, the first being nearest to the centromere.

(*c*) Distance along the bivalent arms from the centromere is measured in terms of the metric t used by Owen (1949).

(*d*) Position interference is present. If $f_r(t)\,\delta t$ is the probability of the rth chiasma being in a small region of length δt, then $f_r(t)$ is dependent only on the position of the $(r-1)$th chiasma. The centromere behaves as though it were a chiasma. The strength of the position interference is such that the probability is $u\,\mathrm{e}^{-u}\,\delta u$ that the next chiasma to be formed will lie in an element δu at a distance u from the last-formed (or, in the case of the first chiasma, from the centromere). This is equivalent to the statement that $I=\frac{1}{2}$ (Owen 1949).

(*e*) Type interference is present. The probability that a new chiasma and the last-formed will involve only two strands exceeds the probability that they will involve four by an amount g, which is a function of u, the distance between the chiasmata. As a convenient model we shall assume that $g=g_0\,\mathrm{e}^{-\lambda u}$; the magnitude of the excess probability therefore declines continuously as the distance between chiasmata increases.

(ii) *Map distance between two markers in the same arm of the bivalent*

From the definition of map distance, x, given in § 2 above, it follows that the map lengths of two adjacent segments are simply additive, i.e. if A, B, C are three marker genes lying in that order on a chromosome, then the map distance between A and C is the sum of the map distances between A and B and between B and C.

In Owen's (1949) discussion of interference he showed that, with position interference of the strength assumed here, the probability of there being n chiasmata between the centromere and a marker gene at a metric distance t from it is

$$P_n = \mathrm{e}^{-t}\left[\frac{t^{2n}}{(2n)!}+\frac{t^{2n+1}}{(2n+1)!}\right]. \tag{9}$$

The map distance between the centromere and the marker gene then follows from equation (5):

$$\begin{aligned} x(t,0) &= \frac{1}{2}\sum_{n=1}^{\infty}\mathrm{e}^{-t}n\left[\frac{t^{2n}}{(2n)!}+\frac{t^{2n+1}}{(2n+1)!}\right] \\ &= \tfrac{1}{4}\mathrm{e}^{-t}\sum_{n=1}^{\infty}\left[\left\{2n\frac{t^{2n}}{(2n)!}+(2n+1)\frac{t^{2n+1}}{(2n+1)!}\right\}-\frac{t^{2n+1}}{(2n+1)!}\right] \\ &= \tfrac{1}{4}\mathrm{e}^{t}\left[\left(t+\frac{2t^2}{2!}+\frac{3t^3}{3!}+\ldots\right)-\left(t+\frac{t^3}{3!}+\frac{t^5}{5!}+\ldots\right)\right] \\ &= \tfrac{1}{4}[t-\tfrac{1}{2}\mathrm{e}^{-t}(\mathrm{e}^{t}-\mathrm{e}^{-t})] \\ &= \tfrac{1}{4}[t-\tfrac{1}{2}+\tfrac{1}{2}\mathrm{e}^{-2t}]. \end{aligned} \tag{10}$$

Hence when t is large, $x(t, 0)$ become equal to $\frac{1}{4}(t-\frac{1}{2})$. Furthermore, it follows from the definition of x that

$$x(t_2, t_1) = x(t_2, 0)-x(t_1, 0).$$

Hence when t_1 is large, $x(t_2, t_1)$ becomes equal to $\frac{1}{4}(t_2-t_1)$.

(iii) *Recombination between two markers in the same arm of the bivalent*

When there are two markers, A and B, in an arm of a bivalent (see figure 2), it may be considered to consist of the following segments:

(*a*) A segment of length t_1, which may or may not contain chiasmata, extending from the centromere to the locus of A.

(*b*) A segment of length l_1, extending from A to the first chiasma distal to A.

(*c*) Segments of lengths $l_2, l_3, \ldots$, between the successive chiasmata lying between A and B. We are at present concerned only with the case when there are $2n$ chiasmata and therefore $(2n-1)$ of these segments.

(*d*) A segment extending from the $2n$th chiasma after A to the locus of B, which lies at t_2.

(*e*) The rest of the bivalent arm distal to B.

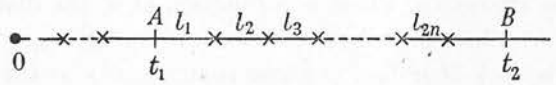

FIGURE 2. One arm of a bivalent. ● = centromere. × = chiasma. A, B, marker loci. 0, t_1, t_2, positions of centromere, A and B in terms of position interference metric.

It has been shown in § 2 that the recombination fraction between A and B is given by the fundamental equation

$$y = \tfrac{1}{2}(1-P_0)-\frac{1}{2}\sum_{n=1}^{\infty}P_{2n}\prod_{i=1}^{n}(\alpha_{2i}-\gamma_{2i}). \tag{4}$$

It is therefore necessary to find the values of P_0 and $P_{2n}\prod_{i=1}^{n}(\alpha_{2i}-\gamma_{2i})$ appropriate to the region AB under the given interference conditions.

Consider first the term $P_{2n}\prod_{i=1}^{n}(\alpha_{2i}-\gamma_{2i})$. There is some general distribution function $f(l_1, l_2, \ldots, l_{2n})$ for the positions of the $2n$ chiasmata between A and B: also a number of type interference functions $g(l_2)$, $g(l_3)$, ..., $g(l_{2n})$ appropriate to the formation of these chiasmata. Then

$$P_{2n}\prod_{i=1}^{n}(\alpha_{2i}-\gamma_{2i}) = \int\int\ldots\int f(l_1, l_2, \ldots, l_{2n})\, g(l_2)\, g(l_4)\ldots g(l_{2n})\, \mathrm{d}l_1 \mathrm{d}l_2 \ldots \mathrm{d}l_{2n}.$$

But the $(2n-1)$ segments between the various successive chiasmata are all on exactly the same footing. It is therefore irrelevant which suffix is attached to any particular g, provided that no two g's have the same suffix; hence

$$P_{2n}\prod_{i=1}^{n}(\alpha_{2i}-\gamma_{2i}) = \int\int\ldots\int f(l_1, l_2, \ldots, l_{2n})\, g(l_2)\, g(l_3)\ldots g(l_{n+1})\, \mathrm{d}l_1 \mathrm{d}l_2 \ldots \mathrm{d}l_{2n}.$$

The position-type interference interaction may now be introduced by substituting $g_0 \mathrm{e}^{-\lambda l_2}$ for $g(l_2)$, etc.,

$$P_{2n}\prod_{i=1}^{n}(\alpha_{2i}-\gamma_{2i}) = \int\int\ldots\int f(l_1, l_2, \ldots, l_{2n})\, g_0^n \exp[-\lambda(l_2+l_3+\ldots+l_{n+1})]\, \mathrm{d}l_1 \mathrm{d}l_2 \ldots \mathrm{d}l_{2n}.$$

But $l_2+l_3+\ldots+l_{n+1}$ $(=u)$ is merely the distance from the first to the $(n+1)$th of the chiasmata distal to A. The integral may therefore be split into two parts, namely, (*a*) the distribution of l_1; (*b*) the distribution of u, the distance between A and the $(n+1)$th chiasma distal to it, conditional on l_1, together with the type-interference term:

$$P_{2n}\prod_{i=1}^{n}(\alpha_{2i}-\gamma_{2i}) = \int_0^{t_2-t_1}\phi(l_1)\int_0^{t_2-t_1-l_1}\psi(u \mid l_1)\, g_0^n \mathrm{e}^{-\lambda u}\, \mathrm{d}u\, \mathrm{d}l_1. \tag{11}$$

It is therefore necessary to derive $\phi(l_1)$ and $\psi(u \mid l_1)$ from the postulated form of the position-interference function.

Consider first $\phi(l_1)$. If $f_r(z)$ is the distribution function of the rth chiasma, then the probability that the $(r+1)$th will lie in an element δv at a distance v from the centromere $(v \geqslant t_1)$ and that it shall also be the first chiasma after A, is

$$\left[\int_0^{t_1}(v-z)\, \mathrm{e}^{-(v-z)} f_r(z)\, \mathrm{d}z\right]\delta v.$$

Also the probability that the first chiasma from the centromere shall lie in δv is $v\mathrm{e}^{-v}\delta v$. Hence the distribution of the first chiasma distal to A is given by

$$\phi(v) = v\,\mathrm{e}^{-v} + \sum_{r=1}^{\infty}\int_0^{t_1}(v-z)\, \mathrm{e}^{-(v-z)} f_r(z)\, \mathrm{d}z. \tag{12}$$

But we know from Owen (1949) that

$$f_r(z) = \frac{z^{2r-1}}{(2r-1)!}\mathrm{e}^{-z}. \tag{13}$$

Hence
$$\phi(v) = v\,e^{-v} + \int_0^{t_1} (v-z)\,e^{-(v-z)}\left(z + \frac{z^3}{3!} + \ldots\right) e^{-z}\,dz$$
$$= v\,e^{-v} + e^{-v}\int_0^{t_1} (v-z)\sinh z\,dz$$
$$= v\,e^{-v} + e^{-v}\,[v\cosh z - z\cosh z + \sinh z]_0^{t_1}$$
$$= v\,e^{-v} + e^{-v}\,[v\cosh t_1 - t_1\cosh t_1 + \sinh t_1 - v].$$

Therefore
$$\phi(l_1) = \exp[-(l_1+t_1)]\,[l_1\cosh t_1 + \sinh t_1], \tag{14}$$
since $v = t_1 + l_1$.

Now consider $\psi(u \mid l_1)$, the distribution of the position of the $(n+1)$th of the $2n$ chiasmata between A and B, conditional on the position of the first. It can also be derived from Owen's (1949) results. We have

$$\psi(u \mid l_1) = \frac{u^{2n-1}}{(2n-1)!}\,e^{-u}\left[\frac{(t_2-u-l_1)^{2n-2}}{(2n-2)!} + \frac{(t_2-u-l_1)^{2n-1}}{(2n-1)!}\right]\exp[-(t_2-u-l_1)]. \tag{15}$$

Both parts of the right-hand side of equation (11) have now been found, and can be substituted in equation (4); to complete this equation it remains only to find P_0, the probability of there being no chiasma between A and B. This is the same as the probability that the first chiasma distal to A shall also be distal to B. Hence, from (14),

$$P_0 = \int_{t_2}^{\infty} e^{-v}[(v-t_1)\cosh t_1 + \sinh t_1]\,dv$$
$$= e^{-t_2}[(t_2-t_1)\cosh t_1 + e^{t_1}]. \tag{16}$$

Equation (4) can now be used to yield the recombination fraction y. No simple integrals exist, so numerical methods must be used.

(iv) *Recombination across the centromere*

We have so far been dealing only with a single arm of the chromosome. If we assume that there is no interference across the centromere, then the chiasmata formed in the two arms are independent of each other. The recombination fraction y_{12} between two loci in different arms whose recombination with the centromere is y_1, y_2, respectively, is then given by Trow's formula:

$$y_{12} = y_1 + y_2 - 2y_1y_2.$$

If y_1 and y_2 vary from zero to a maximum between 50 and 100 %, examination of y_{12} shows that such a function is at its maximum when either y_1 or y_2 is zero and the other is at its maximum. That is to say, of all regions containing the centromere, that with the maximum recombination value will have the centromere at one end. For the limited purpose of discussing the maximum, therefore, we can confine attention to a single arm.

(v) *The effect on recombination of proximity of the centromere to the marked sector*

It will be seen from the equations that the effect on recombination of the position of the centromere relative to the marked sector AB is expressed through $\phi(l_1)$, the distribution of the first chiasma beyond A. When A is remote from the centromere,

$\phi(l_1)$ takes a limiting form which is independent of t_1, i.e. of the distance from A to the centromere. A close approximation to this limiting form is maintained, as t_1 decreases, until A is as close as 5 cM to the centromere. Figure 3 shows the recombination-fraction/map-distance curves for the limiting cases when A coincides with the centromere ($t_1 = 0$) and when A is remote from it ($t_1 = \infty$): they are plotted for $\lambda = 0$ and for three values of g_0. The two curves of each pair resemble one another closely and the differences between them will not usually be important; in the following sections, therefore, only the more general case is considered ($t_1 = \infty$).

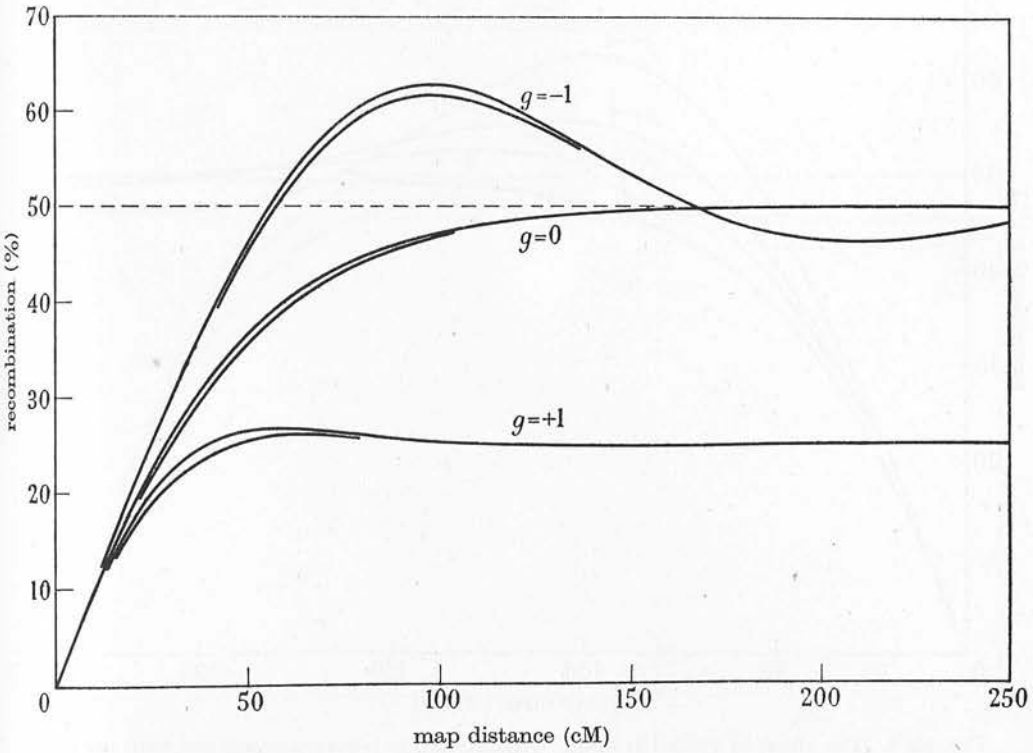

FIGURE 3. The effect of variation in t_1. Recombination percentage against map distance for $t_1 = 0$ (lower curve in each pair) and for $t_1 = \infty$; in each case $\lambda = 0$.

(vi) *The effect on recombination of type interference strength*

Figure 4 shows the recombination-fraction/map-distance curves obtained when the type interference parameter, g_0, takes various values. They are calculated for $\lambda = 0$, i.e. on the assumption that type interference does not decrease as interchiasma distance increases.

When all adjacent chiasma pairs involve four strands ($g_0 = -1$), there is recombination in excess of 50% for marker separations between 60 and 170 cM; it rises to a maximum of about 62 % for a marker separation of about 100 cM. Beyond 170 cM recombination is again less than 50 %; for a separation of about 210 cM it has a minimum below 47 %. For greater separations the recombination fraction oscillates round the 50 % value, but the excursions are small.

For values of g_0 between -1 and 0 interest lies chiefly in the positions of the first maximum and first minimum. These are shifted to the right as $|g_0|$ diminishes and deviate progressively less from 50 % recombination.

When $g_0 = 0$, so that there is no type interference, though position interference is still present, the oscillatory nature of the curve vanishes; it becomes a simple monotonic curve, asymptotic to $y = \frac{1}{2}$ and lying in the region bounded by Haldane's curve $y = \frac{1}{2}(1-e^{-2x})$ and the straight lines $y = x$ and $y = \frac{1}{2}$.

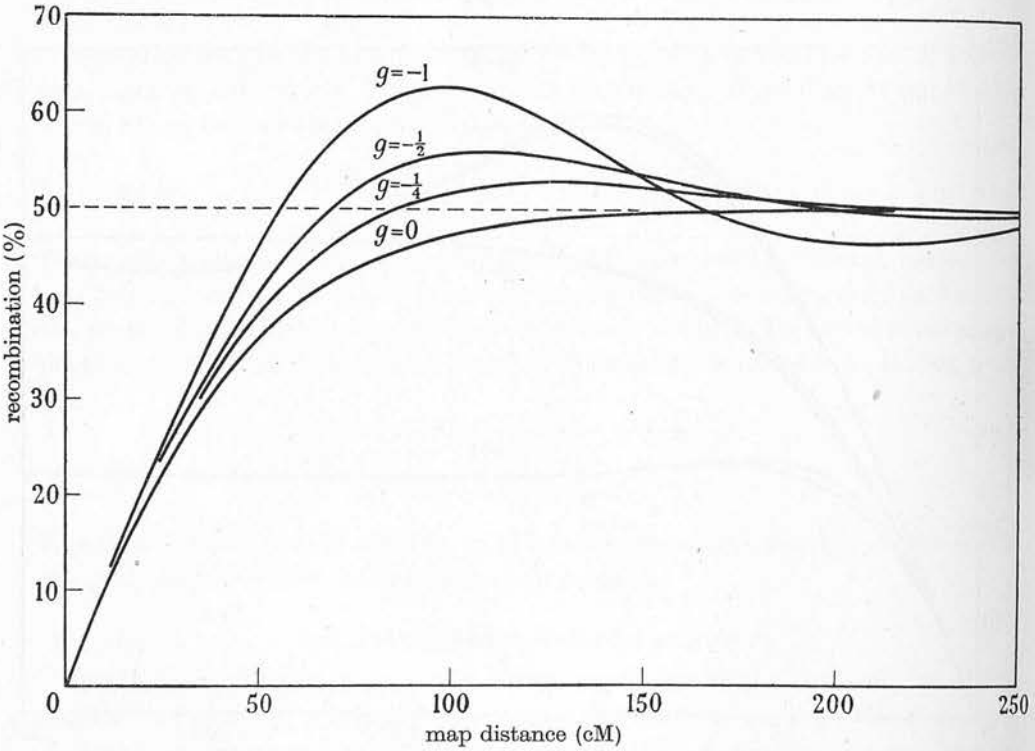

FIGURE 4. The effect of variation in g_0. Recombination percentage against map distance plotted for various values of g_0; in each case $t_1 = \infty$ and $\lambda = 0$.

For positive values of g_0 in the range $0 < g_0 < 1$, the recombination fraction initially rises in step with map distance, but the curve flattens out and lies below the curve for $g_0 = 0$; the limiting value of the recombination fraction is still 50 %. When g_0 is very nearly unity, the flattening of the curve occurs at about 25 % recombination; thereafter it creeps up very slowly towards the 50 % value; for all except the longest linkage groups, then, the maximum recombination may scarcely exceed 25 %. When $g_0 = 1$, the limiting value of the recombination fraction is 25 %. This result follows from the fact that all chiasma pairs involve the same two strands; two of the four chromatids, therefore, never take part in a chiasma and are always non-recombinant; the other two are recombinant or non-recombinant according as the number of chiasmata is odd or even. Thus on the average there are two recombinant chromatids in eight, i.e. 25 % recombination.

(vii) *The effect on recombination of type-position interference interaction*

For a given amount of type interference, g_0, an increase in the type-position interference interaction parameter, λ, leads to a decrease in the mean value of $|g|$. An increase in λ, therefore, affects the recombination-fraction/map-distance curve in much the same way as a decrease in $|g_0|$; thus in a curve showing more than 50 % recombination, the magnitude of the first maximum can be reduced either by a reduction in $|g_0|$ or by an increase in λ. In contrast to the effect of g_0, the variation in λ does not much influence the position of the first maximum (figure 5).

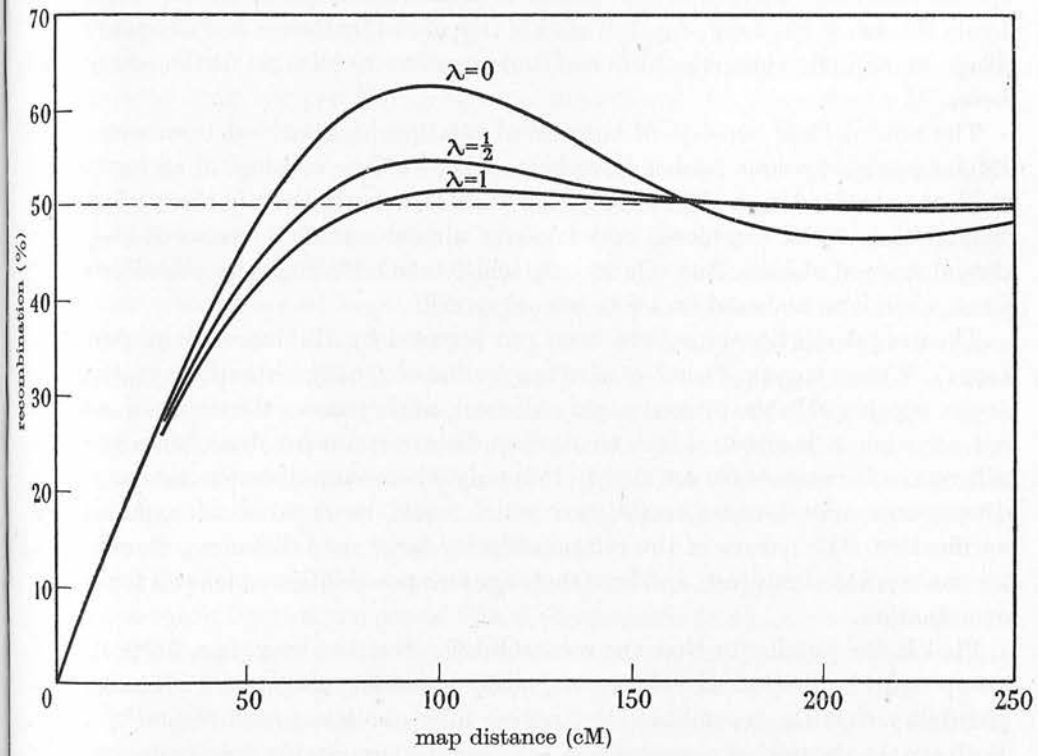

FIGURE 5. The effect of variation in λ. Recombination percentage against map distance plotted for three values of λ; in each case $t_1=\infty$ and $g_0=-1$.

(viii) *The effect on recombination of position interference strength*

The foregoing paragraphs in this section were based on the assumption that position interference was of the strength defined by the value $\frac{1}{2}$ for Owen's (1949) interference parameter I. The behaviour of the recombination fraction in the absence of position interference was examined in § 3. At the other extreme, when position interference is complete in the sense that the distributions of the chiasmata in a bivalent arm do not overlap, and when type interference is also complete ($g=-1$), recombination is given by $y=x$ $(0\leqslant x\leqslant 1)$ for points separated by not more than two chiasmata; under these conditions, therefore, 100 % recombination is possible. It is clear, without more extensive analysis, that the general effect of

an increase in position interference, acting in the presence of type interference such that g is negative, will be to raise the maxima in the recombination-fraction/map-distance curve, and depress the minima.

Discussion

The many formulae that have been proposed for the conversion of recombination fractions into map distances fall into two classes. The first consists of empirical relationships. A curve in this class usually meets a threefold specification (i) $y/x \to 1$ as $x \to 0$, (ii) $y \to \frac{1}{2}$ as $x \to \infty$, (iii) its intermediate range gives a good fit with experimental data from some particular species. This class includes relationships suggested by de Winton & Haldane (1935), Kosambi (1944) and by Carter & Falconer (1951). They are of little theoretical interest and therefore require no further discussion here.

The second class consists of theoretical relationships derived from some particular model. Various models have been used; but the cytological and genetical evidence obtained in the last twenty-five years has shown that, in the very diverse biological material examined, crossing-over almost certainly occurs at the four-strand stage of meiosis. Any relationship which is to be biologically plausible today must, therefore, be based on a four-strand model.

Theoretical relationships have been put forward by Haldane (1919), Jennings (1923), Winge (1935), Fisher *et al.* (1947), Fisher (1948), Srinath (1949), Owen (1949, 1950), and in the present paper. All meet, as they must, the requirement that $y/x \to 1$ when x is small; where small map distances are involved, therefore, the differences between them are slight. It is only where map distances are large that divergences may become established which could be capable of experimental verification. The nature of the relationship for large map distances, therefore, is the main point of interest; and here there are two possibilities which call for special examination.

First is the possibility that the recombination fraction may, in a finite linkage group map, be limited to values not greatly exceeding 25 %; and, secondly, the possibility that the recombination fraction may oscillate round the 50 % value. Both are the theoretical consequences of type interference; the former would occur where there is a great excess of two-strand pairs, the latter when four-strand pairs are in excess. These statements are true whether position interference is also present or not.

An excess of two-strand chiasma pairs appears to have been reported for only one species, namely *Neurospora crassa* (Lindegren & Lindegren 1939). The evidence came from analysis of the spores from individual asci heterozygous at several loci; the excess was slight. None of the chromosomes of this species, however, has a linkage-group map exceeding 40 cM (Houlahan, Beadle & Calhoun 1949); it would therefore be scarcely possible to detect the depression of recombination between the end-markers, even if all chiasma pairs were two-strand.

Recombination exceeding 50 %, due to an excess of four-strand chiasma pairs, can likewise be expected to exist only in a genetically long chromosome. But the detection of such excess recombination by genetic methods entails the use of

marker genes; it is therefore not sufficient that the total potential length of the linkage group map should be long; the genetically known mapped part must also be long. In material which is poorly mapped the defect of the mapped length of a linkage group from its potential length, as determined from the mean chiasma frequency of the bivalent, may be considerable. In the domestic fowl, for example, the longest known linkage group map is about 76 cM (Warren 1949); but one bivalent in this species has a mean chiasma frequency of 8·56 (White 1947) and must, therefore, correspond with a linkage group of potential length 428 cM. Furthermore, where there is no interference across the centromere, the optimum condition for finding recombination in excess of 50 % is not merely when the total known linkage group map is long, but when that part of it which corresponds with one chromosome arm is long, of the order of 100 cM or more. The present paucity of our knowledge of species with long mapped linkage groups therefore limits the material in which excess recombination might profitably be sought.

Well-mapped material, for which there are long maps corresponding with individual chromosome arms, includes several species of *Drosophila*. The acrocentric *X*-chromosome of *D. subobscura* has a mapped length of 150 cM (Spurway 1945) and that in *D. virilis* 170 cM (Chino 1936); the latter species has also an acrocentric autosome with a mapped length of 210 cM, and *D. pseudoobscura* has a metacentric *X*-chromosome with a mapped length of 180 cM (Beers 1937). However, linkages significantly in excess of 50 % do not appear to have been reported for any of these species. They are thus in accordance with *D. melanogaster* which, though it shows position interference, gave little or no evidence of type interference in experiments with attached *X*-chromosomes (Weinstein 1936). For *Habrobracon* the data of Whiting (1950) indicate 49·5 % recombination between two loci separated by 81 cM, again showing no evidence of type interference.

Among plants, maize has one linkage group with a mapped length of 156 cM (Rhoades 1950), but it corresponds with a chromosome in which the two arms are nearly equal; no single arm in the other maize chromsomes has a mapped length exceeding 75 cM. Linkage exceeding 50 % has not been reported in maize.

Fairly long linkage groups occur in the fowl; the longest has a mapped length in excess of 76 cM, but the observed recombination between the present end markers, *fray* and *frizzling*, does not significantly exceed 50 % (Warren 1949). The data do, however, indicate recombination significantly in excess of 50 % for several other pairs of 'independent' markers; thus *pink eye* shows 57 % recombination with *feathered legs* ($\chi^2_1 > 8$ testing independent segregation). Unfortunately, the data are not in a form which would make it possible to detect disturbed segregations due to other causes than linkage, e.g. differential viability. The possibility of linkage exceeding 50 % was specifically discounted in the original publication, and a re-examination of fowl linkage data with this in mind might prove fruitful. Interference is known to occur in the species (Warren & Hutt 1936), and the effect of type interference, if present, would be enhanced by position interference.

The house mouse is the one species for which recombination significantly in excess of 50 % has been found in experiments carefully planned to minimize disturbances due to differential viability. Wright (1947) found recombination of about 56 %

between sex and each of two loosely linked markers, *waved*-2 and *shaker*-2. No significant linkage, either above or below 50 %, was found in the sex recombination of a third marker in the group, *Rex*. It would be more satisfactory if an intermediate marker were known, showing recombination of less than 50 % with sex and any one of the three markers in the group; but even in its absence, Wright's data appear to be difficult to interpret on any basis other than that of linkage. If this view be accepted, her data establish the existence of type interference in the mouse; furthermore, since type interference alone cannot account for recombination fractions exceeding 53·34 %, her data also point to the existence of some degree of position interference. They thus confirm the findings of Grüneberg (1935, 1936), who first established the existence of interference in the mouse by a study of coincidence. Wright's data are quite compatible with the uppermost curve in figure 3, i.e. with position interference of the strength assumed ($I = \frac{1}{2}$) and with the complete suppression of all but four strand chiasma pairs ($g_0 = -1$, $\lambda = 0$).

This raises a further question: is it likely that 'second-order' linkages of less than 50 % could be found, i.e. linkage between two markers whose separation is so great that their recombination fraction is given by a point on the curve approaching the first minimum. For $I = \frac{1}{2}$, $g_0 = -1$ and $\lambda = 0$, a recombination fraction of less than 47 % is expected for map distances of about 200 cM. It is known from the cytological observations of Crew & Koller (1932) and of Slizynski (1949) that the *average* potential length of the mouse's linkage groups is 130 cM and that the longest chromosome is about twice as long as the shortest; hence it is reasonable to suppose that maps of length 200 cM or more may exist in the mouse. An acrocentric chromosome with a map of this length might show 47 % recombination between the centromere and a marker at the distal end; a linkage such as this is not quite beyond the possibility of experimental detection.

The possibility would then exist that, where three markers A, B and C lay in that order, A and C might both show more than 50 % recombination with B, yet less than 50 % *inter se*.

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The estimation of total genetical map lengths from linkage test data.

by

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INTRODUCTION

It is commonly supposed that the total number of crossover units in the linkage map of an organism, if it could be completely mapped, would be equal to half the mean number of chiasmata formed in its meiotic nuclei. This is a deduction from three tenets of current cytological theory, namely, (i) that crossing over is the genetic expression of chiasma formation, (ii) that chiasma formation occurs at the four-strand stage, but only two of the strands take part in each chiasma, (iii) that sister-strand chiasma formation does not occur. Recently, however, the validity of these assumptions has been questioned. For example, Cooper (1949) concluded from his observations of gametogenesis in Drosophila melanogaster that chiasmata may be formed which do not entail genetic crossing over; and Schwartz (1952) found that his observations of anaphase bridges formed by a ring chromosome in Zea could best be interpreted on the basis of sister-strand crossing over. Chiasma counts may therefore form an unreliable basis for estimating total genetical map lengths. Furthermore, there are many species for which chiasmata can be counted only with difficulty, if at all. It follows that there is a need for some alternative method of estimating the total potential map length of an organism.

This paper suggests one possible method, based on the 'swept radius' concept.

THEORY

(i) Calculation of swept lengths

The 'swept radius', r, round a marker gene used in a linkage detection test has been defined (Carter & Falconer, 1951) as the genetical map length corresponding with the recombination fraction p_r, such that

$$p_r = \tfrac{1}{2} - k(Ni)^{-\frac{1}{2}}$$

N is the number of segregating progeny in the test; i is the mean amount of statistical information derived from each of them; and k is such that an observed recombination is considered significant evidence of linkage if it deviates from the free segregation value, $\frac{1}{2}$, by $k(Ni)^{-\frac{1}{2}}$ or more. Thus linkage detection is expected whenever the locus of a gene under test lies within a genetical map distance r of a marker gene locus. In other words, a length of the genetical map surrounding the marker locus, and within a distance r of it, is 'swept' for the locus of the gene under test.

How long is the swept length? This is known with certainty only when the position of the marker locus is known, relative to the ends of its linkage group map. Thus when a marker is at one end of its map, (Text-fig. 1a), the swept length is

$$m = r$$

When a marker is at the middle of its map (Text-fig. 1b) and the distance from the marker to either end of the map exceeds r, the swept length is

$$m = 2r$$

When the position of a marker is unknown, the actual length swept is also unknown; but the average length swept by one or more markers can easily be calculated on the assumption that all possible map positions are equally likely. Thus it has been shewn that when two markers, separated by a map distance h, lie in a group of map length ℓ, (Text-fig. 1d), the mean swept length is

$$m = (2r+h) - r^2/(\ell-h)$$

provided that $h \leq 2r$ and $(r+h) \leq \ell$ (Carter & Falconer, 1951). When there is only one marker (Text-fig. 1c), this reduces to

$$m = 2r - r^2/\ell$$

Mean values for various relative sizes of r, h and ℓ are given in Table 1. It is seen that the mean swept length always has two components: (i) a maximum value term, involving only r and h, which is the value the swept length would have if the linkage group were infinitely long; and (ii) an end correction term, involving r, h and ℓ, which represents the swept length lost when a marker is near the end of its map and the swept radius consequently reaches beyond the end.

(ii). Use of swept lengths to estimate total genetical map lengths.

~~How can knowledge of the map length swept in a linkage test be used to obtain an estimate of L, the total genetic map length of the organism?~~ The probability of discovering linkage between a new mutant and a marker is the same as the probability, P, that the locus of the new mutant lies in a swept part of the map. But this is simply

$$P = \frac{m}{L}$$

where L is the total genetical map length of the organism. Hence, if P and m are known, an estimate of L can be obtained from

$$L = \frac{m}{P} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

An estimate of P is given by the observed proportion of successes in a series of linkage detection tests. To calculate m, values are required for r, h and ℓ; h can be calculated from the observed recombination of linked markers by means of some mapping function, e.g. that proposed by Kosambi (1944); r can be calculated, in terms of N, i and k, from the equation

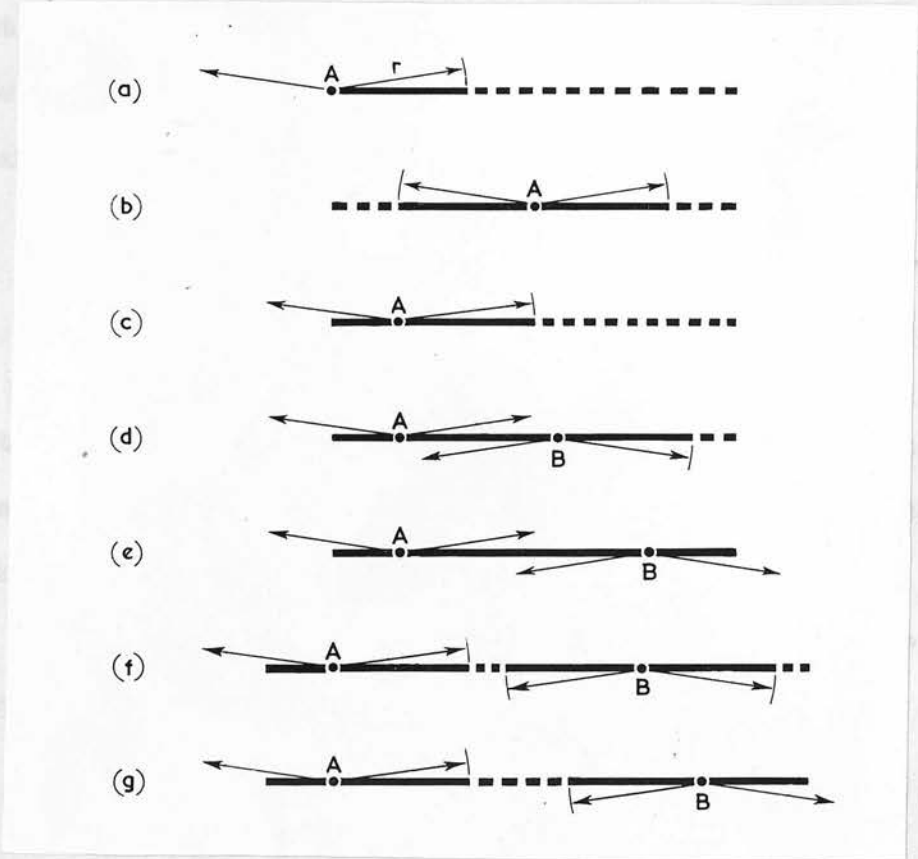
$$r = \tfrac{1}{4}\log_e\left[k^{-1}(Ni)^{\frac{1}{2}}-1\right]$$

(Carter & Falconer, 1951); N and i are known from the scale and type of the linkage test; and k is set arbitrarily. ℓ is thus the only remaining unknown.

If all the linkage groups were of equal map length, ℓ would be given by

TABLE 1. Mean values of the swept length for various relative magnitudes of the swept radius, r, marker separation, h, and linkage group map length, ℓ.

Number of markers	Marker positions	Conditions	Maximum swept length	End correction	Text-figure
1	Terminal	$r \leq \ell$	r	0	1a
1	Central	$2r \leq \ell$	2r	0	1b
1	Unknown	$r \leq \ell$	2r	r^2/ℓ	1c
2	"	$(r+h) \leq \ell$, $2r \geq h$	2r+h	$r^2/(\ell-h)$	1d
2	"	$(r+h) \geq \ell$, $2r \geq h$	2r+h	$(2r+h-\ell)$	1e
2	"	$(r+h) \leq \ell$, $2r \leq h$	4r	$r^2/(\ell-h)$	1f
2	"	$(r+h) \geq \ell$, $2r \leq h$	4r	$(2r+h-\ell)$	1g



Text-fig. 1. The relationship between linkage group map length, marker separation, swept radius and swept length. Each horizontal line represents the genetical map of a linkage group of length l. A, B, loci of marker genes, separated by a map distance h; r, swept radius. Full line, swept length of the map; broken line, unswept. See also Table 1.

$$\bar{l} = \bar{\bar{l}} = \frac{L}{n}$$

where n is the haploid number. It will now be shewn that the mean value $\bar{l}$ can be used, even when the linkage groups are not equal, for the purpose of calculating the end corrections in a test with a number of markers. Consider the mean length swept when there are two unequal groups of mean length l; let them have lengths $(\bar{l}+e)$ and $(\bar{l}-e)$. The probability that a marker will lie in one or other of these groups is in proportion to their lengths; the mean swept length of the two groups is therefore the weighted mean swept length

$$m_w = \frac{1}{2\bar{l}}\left[(\bar{l}+e)\left\{2r - \frac{r^2}{\bar{l}+e}\right\} + (\bar{l}-e)\left\{2r - \frac{r^2}{\bar{l}-e}\right\}\right]$$

$$= 2r - \frac{r^2}{\bar{l}}$$

But this is the same as the mean swept length when the groups are equal and of length $\bar{l}$. This argument may now be extended to cover all the linkage groups, taking them in pairs of mean length $\bar{l}$.

Equation (1) can now be solved for L by trial and successive approximation. The standard error of L is

$$\sigma_L = \frac{m}{p^2}\sigma_p \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

APPLICATION TO THE HOUSE MOUSE

To obtain a correct estimate of L, the total genetical map length of an organism, an unbiased estimate is required of P, the probability of success in a linkage test. This can be derived from the total data, negative as well as positive, of the tests of a number of new mutants against standard linkage detection stocks, where the scale of test had been decided in advance. Such data, however, are rarely available in the literature, for one of two reasons:

first, only successful tests may be published; second, linkage-detection tests often consist of serial experiments which are cut short or otherwise modified when linkage is found. Data taken from publications must therefore be examined critically for evidence of selection before they are used to give an estimate of P.

Standard linkage detection stocks of Mus musculus have now been in existence for several years (Snell, 1946; Carter & Falconer, 1951), and data are available at Edinburgh from tests completed on a number of mouse mutants. It should be possible, therefore, to estimate the total genetical map length of the mouse. Some of the mutants tested were sublethals or of low penetrance, and the swept radii in their tests cannot be assessed with confidence; they have therefore been disregarded. The 'good' mutants, ten in number, were all tested with a swept radius of at least 25 centimorgans round each marker; five of them were found to lie within this distance of a marker used in the tests. They were not all tested against the same test stocks, because some of the tests were undertaken before construction of the stocks was complete; this complicates computation of the swept lengths slightly, but it does not introduce bias, since all tests planned were completed. The working is shewn in Table 2, which lists the markers used and, for each marker, (i) the number of tests, (ii) the total maximum swept length, m_∞, and (iii) successive estimates of the total end correction, c. Linked markers are treated together, even if they were tested separately against the same new mutant. The total genetical map length available for the mutants under test was 10L, since ten mutants were tested; the total length swept was $(\Sigma m_\infty - \Sigma c)$, whence

$$P = \frac{\Sigma m_\infty - \Sigma c}{10L}$$

But five linkages were found, within the swept radius, so that P is estimated by

$$P = \frac{5}{10}$$

Hence L is estimated by

$$L = \frac{\sum m_o - \sum c}{5}$$

whence

$$\bar{l} = \frac{\sum m_o - \sum c}{5 \times 20}$$

since the mouse has a haploid number of twenty. The estimates obtained are $L = 14.1 \pm 4.5$ morgans and $\bar{l} = 70 \pm 22$ centimorgans.

An attempt was next made to extract further estimates of L and $\bar{l}$ from linkage test data in the literature. For the reasons given above it seemed probable that these would be to some extent selected; it was therefore necessary to try to correct this by excluding any bodies of data for which there was reason to suspect selection. As a first step, all recently discovered mutants were disregarded, since the results of a set of linkage tests are likely to be published sooner if successful than if unsuccessful; for this purpose a mutant was regarded as recently discovered if it was not mentioned in the 1943 edition of Grüneberg's Genetics of the Mouse. Thirty-three mutants qualified for consideration, and they were listed in chronological order of first publication. If each had been tested against all the mutants known at the time of its discovery, the total data would have yielded an unbiased estimate of P; it was therefore necessary to see to what extent these tests had been carried out, and whether the defections were likely to have introduced bias.

Linkage and independence test results were obtained from the 1952 edition of Genetics of the Mouse and the sources quoted therein. It appeared that

the swept radius had been at least twenty-five centimorgans in almost all the tests, so it was arbitrarily decided to set this as the acceptable scale of test. A two-way table was built up (Table 3) shewing, for each pair of mutants, whether they had been tested on this scale and, if tested, whether linkage was found within the swept radius. For a surprisingly large number of pairs a direct test had been made and published; for many more no direct test had been reported, but linkage closer than 25 centimorgans could be excluded because an extensive test had been made with a marker closely linked to one of the pair in question; and for still more there were unpublished data available in Edinburgh. There had been few reports of partial sex linkage tests; but it seemed safe to assume that the linkage would have been noticed if any of the mutants had been located within 25 centimorgans of the effective locus of sex. When Table 3 had been completed as far as possible, it was found that for almost all the remaining pairs there were good reasons why the tests had not been carried out. Thus hydrocephalus-1 is extinct; grey-lethal homozygotes die; pituitary dwarfs are sterile; rodless retina and thermotactic optimum require special methods of classification; Fused, harelip, light-head and ~~xxxxxxx~~ silvered require favourable genetic milieux for manifestation. Where segregation data had been reported for these mutants, they had often been obtained coincidentally, because markers happened to be present in the mutant stock. Either way, there was no reason to suspect selection of the data.

Of the thirty-three mutants listed, nine were found to lie within 25 centimorgans of a mutant discovered previously. The computation of L and $\bar{l}$ is shewn in Table 4; the estimates obtained are L = 16.9 $\pm$ 4.8 morgans and $\bar{l}$ = 84 $\pm$ 24 centimorgans; they are in good agreement with those obtained in

Table 2. Joint estimates, for both bodies of data, are computed in Table 5; they are L = 16.2 ± 3.5 morgans and $\bar{\ell}$ = 81 ± 18 centimorgans.

A third pair of estimates, independent of these, is obtained in Table 6 from the translocation test data of Snell (1946) and of Carter, Lyon & Phillips ( ), who tested, respectively, six and seventeen translocations (i.e. twelve and thirty-~~five~~ four chromosome breaks). Snell raised twenty progeny in each test (r = 0.237 for k = 2.5)/ Carter, Lyon & Phillips raised twenty-five (r = 0.275 for k = 2.5). Fifteen of the breaks were found to lie within the swept radius of a marker locus. The estimates obtained are L = 12.7 ± 2.7 morgans and $\bar{\ell}$ = 63 ± 14 centimorgans.

DISCUSSION

1. ACCURACY OF THE GENETICAL METHOD

Before a genetical estimate of the total map length of an organism is compared with a cytological estimate, it is as well to consider the accuracy of the genetical estimate. There are three main sources of possible error:-

(i) Non-validity of the equation L = m/P

(ii) Errors in the calculation of m:-

Use of an erroneous mapping function

Erroneous calculation of the end corrections.

(iii) Errors in the estimate of P:-

Bias in the data.

Sampling errors.

These will be considered in turn.

Non-validity of the equation L = m/P.

Equation (1) rests on the assumption that the loci of new mutants are distributed at random along the genetic map; and this is equivalent to assuming

that the gene loci and chiasmata are both distributed evenly ~~at random~~ along the chromosomes. The magnitude of any error likely to be introduced by the assumption of even ~~random~~ chiasma distribution may be examined by considering the effect on the probability of linkage detection of a change from even ~~random~~ to localised chiasma formation. Consider an organism with only one chromosome, which carries one marker gene and always forms one chiasma. Then its total genetic map length is 0.5 morgans, in the absence of sister-strand chiasma formation. Now suppose a new mutant were tested for linkage, the swept radius in the test being 0.25 morgans. With even ~~random~~ chiasma distribution the swept length is

$$m = 2r - r^2/L$$

$$= 0.375 \text{ morgans.}$$

Hence the probability of linkage detection is

$$P_r = m/L$$

$$= 0.75$$

Now suppose that chiasma formation were localised, so that it never forms in one half of the chromosome (though evenly ~~randomly~~ distributed in the other half). Then, by application of the argument used by Carter & Falconer (1951) for calculating mean swept lengths, the probability of linkage detection is found to be

$$P_\ell = \frac{1}{2}\left(\frac{3}{4}\right) + \frac{1}{4}\left\{\frac{\frac{3}{4}+1}{2}\right\} \quad \frac{1}{4}\left\{\frac{\frac{1}{2}+\frac{1}{4}}{2}\right\}$$

$$= 0.6875$$

Thus the change from the even ~~random~~ to this localised form of chiasma distribution has been followed by a reduction of the probability of linkage detection by about 8%; and therefore an estimate of L would be increased by this amount.

It seems likely that the form of non-random distribution considered here is more extreme than that which might occur in many biological systems, so it is probable that any error introduced by the assumption of ~~randomness~~ evenness will usually be slight. This should be true especially of species for which ~~a random~~ an even distribution of chiasmata has been reported from cytological observations, e.g. Mus musculus (Crew & Koller, 1932; Slizynski, 1934).

Errors in the calculation of m.

There are numerous functions which can be used for calculating the genetical map distance between a pair of linked loci (vide Carter & Robertson, 1952). However, for low recombination percentages, not exceeding about 25%, they differ very slightly. Kosambi's (1944) function has the advantage that conversion tables are available (Fisher & Yates, 1949).

Calculation of the swept length end corrections rests on the assumption that no error is introduced, on the average, by the use of the mean linkage group map length, $\bar{l}$, instead of the actual map length of each group, l. This has been justified where a single marker gene is used in each group, but it has not been shewn to be strictly accurate where linked markers are used. There is a possibility of some error when the marked segment covers a large part of the linkage group map.

Errors in the estimate of P.

The likelihood of bias in published linkage data has been considered above.

Sampling errors of P will often be fairly large, since the number of mutants tested for linkage will usually be farily small.

Thus the genetical method may be expected to give a reasonably accurate estimate of the total genetical map length, provided that bias is removed from the data, that chiasma formation is not extremely localised, and that widely

separated markers are not used in a large proportion of the tests. Sampling error is likely to be the biggest single cause of variation.

2. ESTIMATES BASED ON TESTS WITH TRANSLOCATIONS.

The estimate of the total genetic map length of the mouse derived from tests of gene mutations was 16.2 ± 3.5 morgans, that from tests of translocations 12.7 ± 2.7 morgans. The difference is not statistically significant; but there is reason a priori for expecting a lower estimate from the translocation tests, since it is known that apparent crossing over may be reduced in the region of a translocated chromosome between the centromere and the break ('interstitial region'). If a chromosome is acrocentric, with an even ~~With random~~ distribution along the chromosome of marker loci and of translocation breaks, a marker locus will lie as often on the centromere side of the break as on the opposite side; i.e., ~~if the chromosome is acrocentric,~~ the marker will lie with equal frequency in the interstitial segment and in the free arm. If the reduction of apparent crossing over in the interstitial segment is q, it follows that the average reduction in crossing over near the marker gene is $\frac{1}{2}q$, and the estimate of L will be reduced in proportion. Tests with translocations can therefore always be used to set a lower limit to the estimate of L; but to obtain a true estimate, the amount of reduction in crossing over must be known and allowed for. Thus if interstitial crossing over in the mouse translocation data of Table 6 had been reduced by half (as in some biological material), the estimate of L would be too low by a quarter; the adjusted estimate would then be 16.9 ± 3.6 morgans, which is in good agreement with that from gene mutation data, namely, 16.2 ± 3.5 morgans.

3. COMPARISON OF GENETICAL AND CYTOLOGICAL ESTIMATES

If there is no sister-strand chiasma formation, the total genetical map length

of an organism may be estimated as half the mean number of chiasmata initially formed in its meiotic nuclei; and this is assumed to be the same as the mean number of chiasmata visible at diplotene. Counts made at diakinesis or metaphase cannot be used to obtain a true estimate of L, since some chiasmata may have disappeared through terminalisation; they can, however, be used to set a lower limit to the value of L.

Until recently the only published counts of mouse diplotene chiasmata were those of Crew & Koller (1932). However, they did not in general analyse complete nuclei, so their data may be open to bias if the larger bivalents were more easily scored for chiasmata than the smaller. The one complete nucleus which they analysed, a spermatocyte, had 49 chiasmata, corresponding with a total map length of 24.5 morgans. Recently Slizynski (~~1954~~) has ~~published~~ made chiasma counts of the complete autosomal complements of 50 diplotene spermatocytes and of 70 diplotene XY bivalents; the estimates obtained of L are 19.2 morgans for crossbred stocks and 21.0 morgans for the CBA inbred strain. There is evidence from diakinesis chiasma counts (Huskins and Hearne, 1936) that CBA mice may have an abnormally high chiasma frequency, so the lower estimate is probably better representative of mice generally.

The genetical estimate of L, 16.2 $\pm$ 3.5 morgans, is in good agreement with the cytological estimate, 19.2 morgans, from which it differs by less than its sampling error. This agreement may be interpreted as support for the assumptions on which the cytological estimate is based. In particular, the data do not provide any evidence of sister-strand chiasma formation in the mouse; and they constitute a proof (if further proof were needed) that chiasma formation occurs at the four-strand, and not the two-strand, stage.

SUMMARY

The probability of success in a linkage-detection test of a new mutant depends on L, the total genetical map length of the organism; it should therefore be possible to use the observed frequency of success to obtain an estimate of L. A procedure is described, based on the swept radius concept, whereby this can be done. Applied to the house mouse, it gives $L = 16.2 \pm 3.5$ morgans; this agrees well with recent estimates based on cytological data and must be considered to supersede earlier cytological estimates viz. about 25 morgans. The method may be valuable as an alternative or adjunct to the method of chiasma counts.

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TABLE 2. Estimation of the total genetic map length of Mus musculus from linkage tests with new mutants at Edinburgh. Ten mutants tested; five found to lie within 25 centimorgans of a marker gene.

Marker genes	Number of tests	Marker separation*	Maximum swept length	End correction, successive estimates			
♂	10	0.000	2.500†	0.000†		0.000	0.000
b, f, Fu, Mi^{wh}, N, si, T, U, v, Va, wa-1	70	0.000	35.000	4.312		6.202	6.209
c^{ch}, p	10	0.145	6.450	0.719		1.116	1.118
d, se	10	0.000	5.000	0.616		0.886	0.887
W, s	1	0.602	10.000	0.152	Inter-	0.397	0.398
lx, W, s	9	0.787	10.665	2.457	mediate	5.238	5.247
Sd, a	9	0.669	9.000	1.630	stages	4.176	4.185
Sd, pa, a	1	0.669	1.169	0.181	omitted	0.464	0.465
Ca, bt	1	0.118	0.618	0.070		0.106	0.107
Re, wa-2	10	0.539	10.000	1.315		3.340	3.350
ru, je	10	0.735	10.000	2.240		5.300	5.310
fz, lu	10	0.580	10.000	1.440		3.750	3.760
Successive estimates of total end correction, all tests				15.132		30.975	31.036
" " " total swept length, all tests			101.402	86.270		70.427	70.366
" " " total genetic length per individual			20.280	17.254		14.085	14.073
" " " average linkage group map length			1.014	0.863	0.705	0.704	0.704

$$\sigma_L = \frac{m}{p2}\sigma_p = \frac{(70.366/10)}{(5/10)^2}\sqrt{\frac{5 \times 5}{10^3}} = 4.45 \text{ morgans}$$

Final estimates: $L = 14.07 \pm 4.45$ morgans; $\bar{l} = 70.4 \pm 22.3$ centimorgans

*Marker separations calculated from the recombination data quoted by Grüneberg (1952), by means of Kosambi's (1944) mapping function.

†The effective locus of sex is presumed to lie at the end of its linkage group map.

TABLE 3. Summary of independent and linkage data for mouse mutants mentioned in Genetics of the Mouse (1943 edition).

Linkage group and mutant		XX	I			II		III		V			VIII	X	IV		IX		VI		VII			XIII			XI
		♂	p	c	sh-1	d	se	s	hr	W	a	pa	Sd	b	v	r	si	my	T	Fu	N	Ca	f	wa-2	sh-2	Re	dw	ln	hp	hy-1	wa-1
I	c	*																												
I	p	*	†																											
II	d	*	*	*																										
III	s	*	*	*		*																								
III	W	*	*	*		*		*																						
V	a	*	*	*		*		*		*																				
VIII	b	*	*	*		*		*		*	*																			
X	v	*	*	*		*		*		*	*			*																
II	se	*	*	*		†		*		*	*			*	*															
IV	r	*	*	*		*	*	*		*	*			*	*															
	my	*	*	*		*	*	*			*			*	*															
IX	T	*	*	*		*	*	*		*	*			*	*	*		*												
VI	N	*	*	*		*	*	*		*	*			*	*	*		*	*											
	f	*	*	*		*	*	*		*	*			*	*	*		*	*		*									
III	hr	*	*	*		*	*	†		*	*			*	*	*		*	*		*		*							
I	sh-1	*	†	†		*	*	*	*	*	*			*	*	*		*	*		*		*							
VII	sh-2	*	*	*	*	*	*	*	*	*	*			*	*	*		*	*		*		*							
	dw	*	*	*	*	*	*	*	*	*	*			*	*				*		*		*		*					
IV	si	*	*	*	*	*	*	*	*	*	*			*	*	†			*		*		*		*					
V	pa	*	*	*	*	*	*	*	*	*	†			*	*			*	*		*		*		*					
XIII	lu	*	*	*	*	*	*	*	*	*	*	*		*	*			*	*		*		*		*					
	hp	*		*	*	*	*							*					*		*		*							
	hy-1	*		*	*	*	*	*	*	*	*	*		*	*	*			*		*		*		*		*	*		
XI	wa-1	*	*	*	*	*	*	*	*	*	*	*		*	*	*		*	*		*		*		*		*	*		
	gl	*		*	*						*																			
VII	wa-2	*	*	*	*	*	*	*	*	*	*	*		*	*			*	*		*		*		*		//	*		*
	TH	*		*	*										*															
	te	*		*	*	*	*	*	*	*	*	*		*																*
IX	Fu	*	*	*	*	*	*	*	*	*	*	*		*	*	*		*	†		*		*	*			*	*		*	*
VI	Ca	*	*	*	*	*	*	*	*	*	*	*		*	*	*		*	*	*	†		*	*			*	*			*
V	Sd	*	*	*	*	*	*	*	*	*	*	*		*	*			*	*	*	*	*	*	*				*			*
VII	Re	*	*	*	*	*	*	*	*	*	*	*	*	*	*			*	*	*	*	*	*	*	†			*			*
XII	je	*	*	*	*	*	*	*	*	*	*	*	*	*	*			*			*	*	*	*	*	*		*			*

*Mutants not linked or, if linked, separated by more than 25 centimorgans.
†Mutants linked, separation 25 centimorgans or less.

TABLE 4. Estimation of the total genetic map length of Mus musculus from linkage and independence data summarised in Table 3. Thirty-three mutants tested; nine found to lie within 25 centimorgans of a marker gene.

Marker genes	Number of tests	Marker separation*	Maximum swept length*	End correction, successive estimates *			
d	33	0.000	8.250	0.000		0.000	0.000
c, d, s, a, b, v, r, my, T, N, f,	200	0.000	100.000	12.500		14.740	14.800
p, c; sh-2, dw, ln, hy-1, wa-1	14	0.145	9.030	1.023		1.246	1.252
c, sh-1	5	0.038	2.690	0.325		0.386	0.387
p, c, sh-1	12	0.183	8.196	0.918		1.129	1.134
d, se	22	0.000	11.000	1.375	Inter-	1.621	1.628
W, s	9	0.602	9.000	1.417	mediate	2.295	2.322
W, hr, s	15	0.602	16.260	2.361	stages	3.825	3.870
pa, a	8	0.196	5.568	0.622	omitted	0.768	0.771
Sd, pa, a	2	0.669	2.338	0.378		0.644	0.650
T, Fu	3	0.043	1.629	0.196		0.233	0.234
N, Ca	3	0.022	1.566	0.192		0.227	0.228
sh-2, wa-2	4	0.331	3.324	0.374		0.484	0.487
Re, sh-2, wa-2	1	0.539	1.039	0.014		0.204	0.205
Successive estimates of total end correction, all tests				21.695		27.802	27.968
" " " total swept length, all tests			179.890	158.195		152.088	151.922
" " " total genetic length, per individual			19.988	17.577		16.898	16.880
" " " average linkage group map length			0.999	0.879	0.847	0.844	0.844

$$\sigma_L = \frac{m}{p^2}\sigma_p = \frac{(151.922/33)}{(9/33)^2}\sqrt{\frac{9 \times 24}{33^3}} = 4.80 \text{ morgans}$$

Final estimates: $L = 16.88 \pm 4.80$ morgans; $\bar{l} = 84.4 \pm 24.0$ centimorgans.

* See notes under Table 2.

TABLE 5. Joint estimation of total genetic map length of Mus musculus from the data used in Tables 2 and 4. Forty-three mutants tested; fourteen found to lie within 25 centimorgans of a marker gene.

Data	Maximum swept length		End correction, final estimate
Table 2	101.402		24.628
Table 4	179.890	Inter-	29.804
Successive estimates of end correction, all tests		mediate	54.432
" " " total length swept, all tests	281.292	stages	226.860
" " " total genetic length per individual	20.092	omitted	16.204
" " " average linkage group length	1.005	0.810	0.810

$$\sigma_L = \frac{m}{p^2}\sigma_p = \frac{(226.860/43)}{(14/43)^2}\sqrt{\frac{14 \times 29}{43^3}} = 3.52 \text{ morgans}$$

$L = 16.20 \pm 3.52$ morgans; $\bar{\ell} = 81.0 \pm 17.6$ centimorgans.

TABLE 6. Estimation of the total genetic map length of Mus musculus from linkage tests with translocations. Forty-six chromosome breaks tested; fifteen found to lie within the swept radius of a marker gene.

Marker genes and source of data	Number of tests	Marker separation*	Maximum swept length*	End correction, successive estimates*			
Snell (1946) (r = 0.237):							
♂	12	0.000	2.844	0.000		0.000	0.000
p, a, Ca, b, Fu, v, wa-1, 1n, f	108	0.000	51.192	7.355		9.558	9.536
se, d	12	0.000	5.688	0.817		1.062	1.060
W, s	12	0.602	11.376	3.012		5.292	5.304
sh-2, wa-2	12	0.331	9.660	1.364		2.218	2.218
Carter, Lyon & Phillips( )(r=0.275):							
♂	34	0.000	9.350	0.000		0.000	0.000
c^{ch}, a, b, T, Mi^{wh}, Ca, s	216	0.000	118.800	19.786		25.704	25.747
se, d	34	0.000	18.700	3.114		4.046	4.053
W, s	18	0.602	19.800	5.886		9.306	9.324
Successive estimates of total end correction, all tests				41.334		57.186	57.242
" " " total length swept, all tests			247.410	206.076		190.224	190.168
" " " total genetic length per individual			16.~~696~~ 494	13.738		12.682	12.679
" " " average linkage group map length			0.825	0.687	0.635	0.634	0.634

$$\sigma_L = \frac{m}{p^2}\sigma_p = \frac{(190.168/46)}{(15/46)^2}\sqrt{\frac{15 \times 31}{46^3}} = 2.69 \text{ morgans}$$

Final estimates: $L = 12.68 \pm 2.69$ morgans; $\bar{\ell} = 63.4 \pm 13.5$ centimorgans

* See notes under Table 2.

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STOCKS FOR DETECTING LINKAGE IN THE MOUSE, AND THE THEORY OF THEIR DESIGN

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(With Three Text-figures)

1. Introduction

The value of an animal as material for genetic research increases as the knowledge of its chromosome map is extended. The house mouse (*Mus musculus* L.) is in this respect by far the best known of the mammals, but its value is nevertheless still seriously limited by the incomplete knowledge of its chromosome map and by the lack of suitable marker genes. This limitation is felt particularly in the study of chromosome rearrangements and of mutation, and it has prevented the development of inversion stocks comparable with those upon which many of the *Drosophila* techniques depend. The recent success of Slizynski (1949) in describing the cytological picture of the mouse's chromosomes gives promise of a fruitful synthesis of genetical and cytological studies, and makes the completion of the genetical map all the more urgent.

Recognition of the need to add to the chromosome map has often led the discoverer of a new mutant in the mouse to test it for linkage with as many of the previously known genes as possible. In this way considerable progress in mapping the twenty chromosomes has been made, and already twelve linkage groups are recognized, though not all have suitable marker genes on them and it is far from certain that the twelve recognized groups represent twelve different chromosomes. There are now, however, so many genes known that it has become a formidable task to test a newly discovered mutant fully for linkage, and most investigators have been content with tests against a limited number of genes, the choice of which was often decided more by convenience than by deliberate planning. The probability of locating the new mutant is then, of course, rather small, and—which is more important if the new mutant is suitable as a marker—independence of all the known linkage groups cannot be asserted. It has been recognized in the past that this difficulty would be greatly lessened by the use of special testing stocks, and such stocks have been constructed (e.g. by Snell, quoted by Cooper, 1939); but the list of marker genes in the mouse and our knowledge of its genetical map have increased so much in recent years that a new set of stocks is now needed.

The main problem in the design of a set of mouse linkage-testing stocks lies in the choice to be made where two or more linked markers are available. This particular aspect of planning linkage tests appears not to have been given theoretical consideration anywhere in the literature. The purpose of this paper, then, is first to restate some of the general principles which underlie the planning of linkage tests, and to develop out of these a new

theoretical concept, the 'swept radius', by means of which the problem of linked markers can be handled; and then to describe how this concept has been applied to the design of some special linkage-testing mouse stocks. The section on theoretical principles rests very largely on the applications of Fisher's (1925) maximum likelihood method of estimation developed by himself, Mather (1935, 1936), Finney (1943, 1949) and others.

The application of these principles has led to the conclusions that, if suitably arranged, all the known genes that are desirable as markers for testing linkage could be contained in five stocks; and that, if only 100 offspring were raised in tests with each of these five stocks, the fraction of the total genetical map that would be covered would be of the order of 0·5–0·6. In other words, there would be at least an even chance of success in assigning the new mutant under test to a linkage group. This gives so great an improvement over what is at present possible with a comparable expenditure of labour that we think these

Table 1. *Values appropriate to the* 12 *common types of linkage-testing mating, of the swept radius* (m_r) *and the corresponding recombination fraction* (p_r); *and of the length swept* (m_s) *around a marker gene, when corrected for the end effect. Values are given for progenies of* 50 ($N=50$) *and of* 100 ($N=100$). *A significance level of* $2\frac{1}{2}\%$, *corresponding to* $k=2$, *is assumed; the average genetic map length is assumed to be* 130 *cm.*

	Mates			$N=50$			$N=100$		
Mating type	Coupling	Repulsion	i	p_r	m_r	m_s	p_r	m_r	m_s
Backcross	**AZ/az** × **az/az**	**Az/aZ** × **Az/aZ**	4·0	0·359	0·451	0·745	0·400	0·549	0·867
Intercross, semi-dominance at both loci	**AZ/az** × **AZ/az**	**Az/aZ** × **Az/aZ**	4·0	0·359	0·451	0·745	0·400	0·549	0·867
Intercross, semi-dominance at one locus	**AZ/az** × **AZ/az**	**Az/aZ** × **Az/aZ**	2·6̇	0·327	0·391	0·664	0·378	0·487	0·792
Mixed cross, semi-dominance at one locus	**AZ/az** × **aZ/az**	**Az/aZ** × **aZ/az**	2·0	0·300	0·347	0·601	0·359	0·451	0·745
Intercross, dominance at both loci	**AZ/az** × **AZ/az**	**AZ/aZ** × **Az/aZ**	1·7̇	0·288	0·328	0·573	0·350	0·424	0·723
Mixed cross, dominance at both loci	**AZ/az** × **aZ/az**	**Az/aZ** × **aZ/az**	1·3	0·255	0·281	0·502	0·327	0·391	0·664

stocks, when built up, will be worthy of general adoption as standard linkage-testing stocks. Moreover, the addition of a few new markers, requiring perhaps two or three more stocks, could cover nearly the whole of the mouse's genetical map, and the identification of the linkage group to which new mutants belong could become almost a certainty.

It should be made clear at the outset that these new stocks are designed for the special purpose of assigning a new or hitherto untested mutant to its linkage group. The accurate localization of the mutant with reference to the other genes of the group is a different problem for which the stocks are not specially suited.

2. Theoretical background

Types of mating used for detecting linkage

Twelve types of mating are commonly used to test a new mutant (**Z**, **z**) for linkage with a marker gene (**A**, **a**). They are derived from the three basic types, backcross, intercross and mixed cross, by various combinations of phase and dominance relationships and are listed in Table 1. All show the essential features of a linkage-testing mating, namely, that the frequencies with which the progeny are expected to fall into two or more phenotypic

classes can be expressed in terms of the proportion of recombinant gametes produced by the segregating heterozygote.

Statistical procedures for analysing linkage data

Statistical procedures for analysing the results of linkage-testing matings have been described by many authors. All the techniques operate on the same raw data, namely, observations of the numbers of the progeny of a linkage-testing mating which fall into each phenotypic class; and all have the same objects, namely, the estimation of the fraction, p, of recombinant gametes produced by a heterozygote **Aa Zz** and the estimation of the reliability of this estimate. It is not within the scope of this paper to compare the various techniques, and in what follows it will be assumed that the data will be treated by a maximum likelihood method, e.g. the procedure described by Finney (1949).

A feature of this method which is of importance in connexion with planning linkage tests is the concept of statistical information, I. This is a quantity which gives a measure of the accuracy of the conclusion drawn from an experiment, since it is related to the standard error, s, of the estimate of p, by the equation

$$s = I^{-\frac{1}{2}}. \tag{1}$$

For any given mating type I is proportional to the number of progeny raised; the amount of information per individual progeny, i, is therefore a relative measure of the efficiencies of different types of mating. Moreover, the amounts of statistical information obtained in tests with different types of mating can be added, thereby leading to an estimate of the accuracy of the conclusion drawn from several bodies of data jointly.

In its simple form the method is suitable only for application to data which show good, Mendelian, single-factor segregations; it is therefore necessary, in general, that the marker genes and new mutants under test shall not show greatly disturbed single-factor segregations, and in particular that the penetrance shall be complete or nearly so. In what follows it will be assumed that the single-factor segregations have been tested and found to be Mendelian.

The concept of swept radius

Suppose two marker genes, known to be located in different, independent linkage groups, were tested one against the other for linkage. The value found for the recombination fraction, p, would probably not be exactly equal to the free-segregation value, $\frac{1}{2}$; but the values of p found in a series of similar experiments would be distributed round the mean value $\frac{1}{2}$. The spread of the distribution would be determined by the amount of statistical information, I, contained in each experiment and therefore by the type of mating used and the number of progeny raised.

Now suppose that one of the marker genes were replaced by a new mutant at an undetermined locus. Since the test would be aimed at detecting departures from free segregation, it would be assumed *ex hypothese* that free segregation existed; the value found for p would therefore be expected to be distributed round the value $\frac{1}{2}$, again with a standard error determined by the type of mating and number of progeny raised. If the recombination fraction found in the experiment deviated from the value $\frac{1}{2}$ by less than ks (where k is a constant which sets the significance level), it would be concluded that the experimental results did not contain any significant evidence of linkage; or, in other words

that the new mutant did not lie within a 'swept radius' m_r of the marker gene, where m_r is defined as the genetical map-length corresponding with the recombination fraction p_r such that

$$p_r = \tfrac{1}{2} \pm ks, \tag{2}$$

or, making use of equation (1), and ignoring the possibility of linkage significantly exceeding 50%,

$$p_r = \tfrac{1}{2} - kI^{-\frac{1}{2}}. \tag{3}$$

In order to obtain a measure of the swept radius, m_r, it is therefore necessary to know the relationship between genetical map-length, m, and recombination fraction, p. It is known from experiment that when the recombination fraction between two loci is very small, the linear relationship proposed by Morgan holds and m may be equated to p; it is also experimentally found that p tends to the value $\frac{1}{2}$ for large values of m; but there is no general agreement about the exact form of the intervening curve (which, in any case, is not necessarily the same for all linkage groups and all species). Various forms for this relationship, some purely empirical and some based on theoretical considerations, have been suggested by Haldane (1919), de Winton & Haldane (1935), Kosambi (1944), Fisher, Lyon & Owen (1947), Owen (1949) and Srinath (1949). We have found that our own mouse-linkage data give a good fit, where large recombination fractions are concerned, with yet another relationship, namely,

$$m = \tfrac{1}{4}\,(\tanh^{-1} 2p + \tan^{-1} 2p). \tag{4}$$

This is one of a family of curves to which the Morgan, Haldane and Kosambi relationships also belong, since they are all integrals of the differential equation

$$\frac{dp}{dm} = 1 - (2p)^n. \tag{5}$$

The differences between them lie in the value chosen for n; the Haldane relationship is obtained for $n=1$, the Kosambi relationship for $n=2$ and the Morgan relationship for $n=\infty$; the new relationship is based on $n=4$.

These relationships differ widely where large recombination fractions are under consideration, but they are closely similar when p is small; when values of p exceeding, say, 40% are excluded, the curves differ only slightly and the exact form of the expression chosen to relate recombination fraction to map-length is of relatively little importance. Therefore practical convenience may be considered, and for the lower p values we have adopted Kosambi's formula, because it is easier than the new fourth power relationship to handle mathematically. Kosambi's equation is

$$\begin{aligned} m &= \tfrac{1}{2} \tanh^{-1} 2p \\ &= \tfrac{1}{4}\,[\log_e (1+2p) - \log_e (1-2p)]. \end{aligned} \tag{6}$$

When combined with equation (3) it gives the expression for the swept radius, namely,

$$m_r = \tfrac{1}{4} \log_e \left[\frac{I^{\frac{1}{2}}}{k} - 1 \right]. \tag{7}$$

Planning linkage tests

A number of problems which arise when planning tests for linkage, especially those involving linked markers, can be handled by means of the concept of swept radius.

(i) *The length swept in a backcross test.* Provided that the locus of the marker gene does

not lie within a distance m_r of the end of the genetic map of the linkage group in which it lies, the distance swept in the course of a linkage test will be $2m_r$. For a backcross linkage-testing mating $i=4$; the 'swept length' for such a mating is therefore

$$m_s = 2 \cdot \tfrac{1}{4} \log_e \left[\frac{(4N)^{\frac{1}{2}}}{k} - 1 \right].$$

In a practical test with mice 100 progeny might be raised and deviations exceeding twice the standard error might be considered significant. (This corresponds with a significance level of about $2\frac{1}{2}\%$, since only one tail of the distribution of p is included because linkages giving values of p far in excess of $\frac{1}{2}$ are not expected.) Putting $N=100$ and $k=2$ gives the swept length

$$m_s = 109{\cdot}8 \text{ cM}.$$

(ii) *The length swept in tests of other types.* The method of calculating swept length for other types of linkage-testing mating is similar to that used for backcrosses; the only difference lies in the value of i, which must be the value appropriate to the mating type (see Appendix I, Table 2).

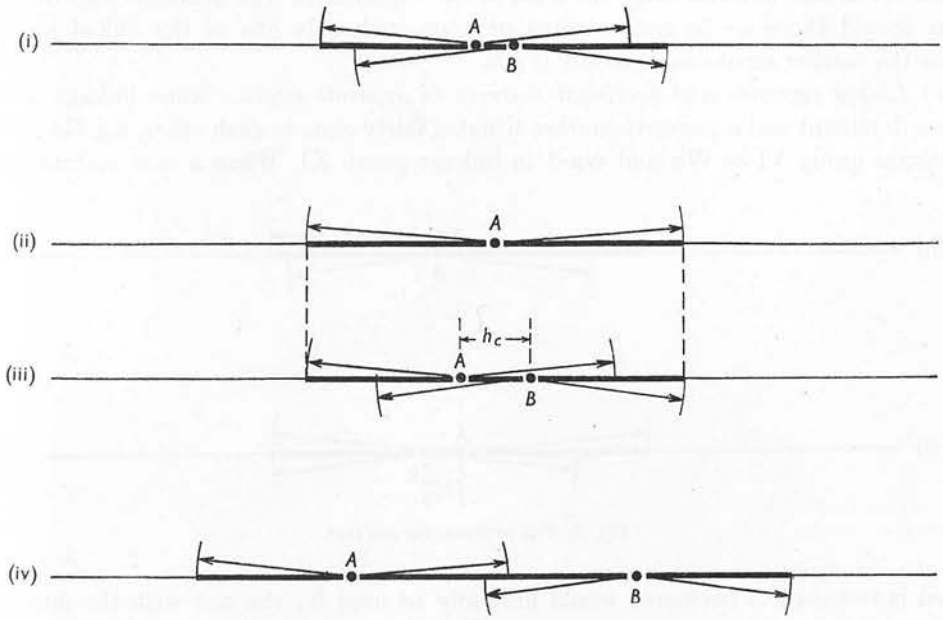

Fig. 1. For explanation see text.

(iii) *Alternative linked markers, both recessive or both dominant.* Sometimes a doubly marked chromosome, otherwise desirable, may not be available; or there may be strong interactions between two linked markers (e.g. **p** and **c**) which make their use in the same test undesirable. It may then be necessary to decide whether it is better to expend all the available effort on tests with one marker, or to divide the effort equally between separate tests with the two markers. It is clear that the former course would be better when the markers are closely linked, as **se** and **d** (Fig. 1(i), (ii)); and that the latter course would be better when they are loosely linked, as **s** and **lx** (Fig. 1(ii), (iv)). There must, therefore, be some critical separation of the two linked markers for which the two courses are equally good, and this critical separation, h_c, must be known before the correct decision can be

made. It can be found by equating the swept lengths attained by the two procedures (Fig. 1(ii),(iii)); the first will yield $2I$ units of information in tests with one locus; the second will yield I units of information in tests with each of two loci; therefore the critical separation is given by

$$2 \cdot \tfrac{1}{4} \log_e \left[\frac{(2I)^{\frac{1}{2}}}{k} - 1 \right] = h_c + 2 \cdot \tfrac{1}{4} \log_e \left[\frac{I^{\frac{1}{2}}}{k} - 1 \right],$$

whence

$$h_c = \tfrac{1}{4} \log_e 2 + \tfrac{1}{2} \log_e \left[\frac{I^{\frac{1}{2}} - k\sqrt{\frac{1}{2}}}{I^{\frac{1}{2}} - k} \right].$$

In all practical tests $I^{\frac{1}{2}}$ will be much bigger than k, so the last term will tend to vanish, since $\log_e 1 = 0$. Hence

$$h_c \simeq \tfrac{1}{4} \log_e 2$$
$$= 0{\cdot}173 \text{ or } 17{\cdot}3 \text{ cM.}$$

As I and k do not appear in this expression, this result holds good for all practical significance levels and however large the scale of the experiment. The available experimental effort should therefore be concentrated on tests with only one of the linked markers unless the marker separation exceeds 17 cM.

(iv) *Linked recessive and dominant markers in separate stocks.* Some linkage groups have a dominant and a recessive marker situated fairly close to each other, e.g. **Ca** and **bt** in linkage group VI or **Wh** and **wa-1** in linkage group XI. When a new mutant to be

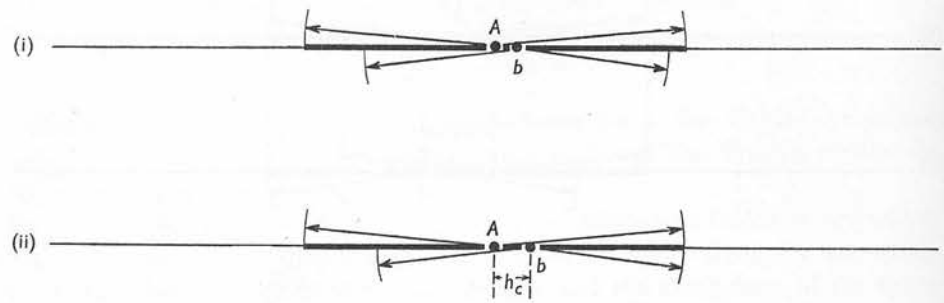

Fig. 2. For explanation see text.

tested is recessive, a backcross would normally be used for the test with the dominant marker, whereas an intercross must be used for the test with the recessive marker. The two markers would therefore require to be tested separately and would normally be kept in different stocks. If equal numbers of progeny were raised in each test, the radius swept round the dominant marker would be greater than the radius swept round the recessive marker, by virtue of the greater efficiency of backcrosses than intercrosses; and if the length of the segment between the markers were sufficiently small, it might happen that the part of the map swept in the test with the recessive marker lay entirely within the part already swept in the test with the dominant marker (Fig. 2(i)). The test with the recessive marker would then be superfluous. The critical marker separation, above which the test with the recessive marker would first begin to sweep a new part of the genetic map, is that separation for which the radius swept round the dominant marker is equal to the sum of the marker separation and the radius swept round the recessive marker

(Fig. 2(ii)). For a backcross $i=4$ and for an intercross with complete dominance $i=\frac{16}{9}$; the critical separation is therefore given by

$$\tfrac{1}{4}\log_e\left[\frac{(4N)^{\frac{1}{2}}}{k}-1\right]=h_c+\tfrac{1}{4}\log_e\left[\frac{(16N/9)^{\frac{1}{2}}}{k}-1\right],$$

whence

$$h_c=\tfrac{1}{4}\log_e\tfrac{3}{2}+\tfrac{1}{4}\log_e\left[\frac{4N^{\frac{1}{2}}-2k}{4N^{\frac{1}{2}}-3k}\right].$$

Here also the last term tends to vanish for all practical values of k and N, leaving

$$h_c\simeq\tfrac{1}{4}\log_e\tfrac{3}{2}$$
$$=0{\cdot}101 \text{ or } 10{\cdot}1 \text{ cM}.$$

Hence a test with the recessive marker would be superfluous unless its locus were more than 10 cm. from that of the dominant marker.

(v) *Linked recessive and dominant or semi-dominant markers in the same stock.* A problem related to the last arises when a single stock is available carrying both a recessive and

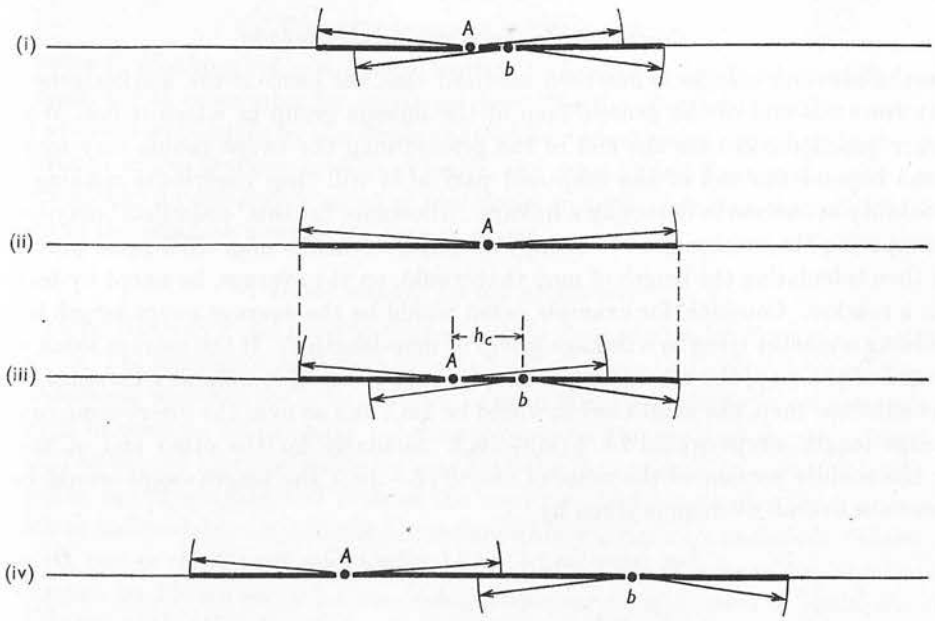

Fig. 3 For explanation see text.

a dominant or semi-dominant marker linked to it, e.g. **pa** and $\mathbf{A^w}$ or **hr** and $\mathbf{W^v}$. When such a stock is used for tests with a recessive new mutant, two courses are open, namely, (*a*) the triply-heterozygous F_1 could be mated to an animal from the new mutant stock, thereby constituting a backcross test involving only the new mutant and the dominant or semi-dominant marker; (*b*) triply-heterozygous F_1 could be mated *inter se*, thereby constituting a triple intercross testing the new mutant against both markers. The former procedure is clearly desirable when the marker separation is very small, since a backcross is more efficient than an intercross (Fig. 3(i),(ii)); the latter procedure is better when the separation is large (Fig. 3(ii),(iv)). The critical marker separation will be that for which the same map length is swept by either method (Fig. 3(ii),(iii)).

When one marker is fully dominant, so that $i=\frac{16}{9}$ for the intercross, the critical separation will be given by

$$2.\tfrac{1}{4}\log_e\left[\frac{(4N)^{\frac{1}{2}}}{k}-1\right]=h_c+2.\tfrac{1}{4}\log_e\left[\frac{(16N/9)^{\frac{1}{2}}}{k}-1\right],$$

whence

$$h_c\simeq\tfrac{1}{2}\log_e\tfrac{3}{2}$$
$$=0{\cdot}203 \text{ or } 20{\cdot}3 \text{ cM.}$$

Therefore it is better to backcross the triply heterozygous F_1 to the new mutant stock, thereby neglecting the recessive marker, unless the marker separation exceeds 20 cm.

When the two markers are a recessive and a semi-dominant, the analysis is slightly different; for an intercross with semi-dominance, i is $\frac{8}{3}$ instead of $\frac{16}{9}$, and the critical separation is found to be

$$h_c\simeq\tfrac{1}{8}\log_e\tfrac{27}{8}$$
$$=0{\cdot}152 \text{ or } 15{\cdot}2 \text{ cM.}$$

Therefore it is better to use the triple intercross when the marker separation exceeds 15 cM.

Corrections for finite genetic map lengths

In the previous section it has been assumed that the locus of the marker gene is well away from the end of the genetic map of the linkage group in which it lies. When the marker gene locus is near the end of the genetic map the swept radius may sometimes extend beyond the end of the map and part of it will then contribute nothing to the probability of success in detecting a linkage. Allowance for this 'end-effect' may be made by supposing the marker gene to occupy all positions in the map with equal probability, and then calculating the length of map that would, on the average, be swept by tests with such a marker. Consider, for example, what would be the average swept length in a test involving a marker lying in a linkage group of map-length L. If the marker locus were at one end of the map, the actual length swept would be m_r; if it were at a distance m_r from the end of the map, the length swept would be $2m_r$; and so over the intervening range the average length swept would be $\frac{1}{2}(m_r+2m_r)$. Similarly for the other end of the map. For the middle portion of the map, of length $(L-2m_r)$, the length swept would be $2m_r$. Hence the overall average is given by

$$m_s=\frac{\frac{1}{2}m_r(m_r+2m_r)+2m_r(L-2m_r)+\frac{1}{2}m_r(m+2m_r)}{L},$$

i.e.

$$m_s=2m_r-\frac{{m_r}^2}{L}. \tag{8}$$

A similar argument can be applied to the more general case when there are two linked markers, separated by a map-distance h, and the swept radii are f round one marker and g round the other. Provided that the separation is not so great that the swept radii fail to overlap, this leads to

$$m_s=(f+g+h)-\frac{(f^2+g^2)}{2(L-h)}. \tag{9}$$

When f and g are both equal to m_r, this reduces to

$$m_s=(2m_r+h)-\frac{{m_r}^2}{L-h}. \tag{10}$$

In each case the expression giving the swept length consists of two parts, namely, (*a*) the sum of the swept radii and the marker separation; (*b*) an end-correction involving L, which expresses the loss of swept length that occurs when the marker loci are near the end of the genetic map. L occurs only in the denominator of this end-correction and it therefore tends to vanish when L is large compared with m_r^2.

Before a corrected swept length can be calculated, using equations (8)–(10), it is necessary to know the value of L, the map-length of the linkage group in question. The average value of L can be obtained from cytological evidence, since it is equal to half the average number of chiasmata per bivalent. The latter has been found to be approximately 2·6 at diplotene in *Mus musculus* (Crew & Koller, 1932); hence the average value of L is 1·3, or 130 cM.

Sex is at present the only marker whose position relative to the end of the genetic map is known, its effective locus being presumed to lie at one end of the map (Fisher, Lyon & Owen, 1947). Thus the part of the map swept when sex is used as a marker lies wholly to one side of its locus, and the swept length is consequently equal to the swept radius.

3. THE NEW LINKAGE-TESTING STOCKS

The first step in planning the new linkage testing stocks was to decide which of the many known genes would be suitable for use as markers. The list of suitable markers was then reduced as much as possible by the elimination of redundant genes according to the principles outlined in the previous section. Finally, the genes chosen for use as marker were grouped together for the construction of the stocks in such a manner that all would be contained in the smallest possible number of stocks.

Genes suitable for use as markers

Three properties were considered to be essential for a gene to qualify for use as a marker in the stocks. The most important, without which the statistical calculations would become unduly laborious, was that the gene should give good Mendelian single-factor segregation. Genes with incomplete or irregular penetrance such as **Fu**, **py** and **si**, were therefore excluded. The second essential property was that the gene should be easy to classify at or before the age of weaning and without the need for special methods. On this ground genes such as rodless-retina, **r**, and the histocompatibility genes were excluded. Siderocyte anaemia (**f**), on the other hand, was considered to be suitable, in spite of the slight extra trouble caused by the necessity for classifying within forty-eight hours of birth. Finally it was considered essential that the gene should not seriously impair viability or fertility. Thus lethals such as grey lethal (**gl**) and steriles such as pituitary dwarf (**dw**) were clearly unsuitable. The inclusion of genes of the waltzer-shaker group seemed to be unavoidable, even though some of them reduce fertility quite considerably, because there are so many of them and some occupy important positions in known linkage groups. Every opportunity was, however, taken in later stages of the planning to eliminate as many as possible of these undesirable genes.

The genes that were considered by the above three criteria to be suitable for use as markers are listed in Table 2. They were selected from the list of genes of the mouse published by Law (1948), and they are arranged here by their linkage group. The genes shown in heavy type are those that were finally chosen, for the reasons described below, to be included in the stocks.

Choice of markers for stocks

Though all the genes* listed in Table 2 would be suitable for use as marker genes, not all merited a place in the linkage testing stocks. It was, of course, undesirable to have any gene in the stocks that would not contribute materially to the total swept length attainable with the stocks. On the grounds of redundancy, therefore, the list of markers was substantially reduced. The following genes were eliminated because they lie between two other markers whose swept radii together would cover the whole intervening segment: **W**; **pa**†; **pi**; **un**; **we**; **sh-2**. The following recessives were excluded because their swept lengths would be overlapped by those of adjacent dominant markers in the stocks: **bt**; **wa-1**. The following lie so close to another marker of the same type that the gain in swept length did not seem to be worth the cost of another gene in the stocks, and they were therefore excluded: **d**; **Ca**; **m**; **Ki**. The reasons for choosing one rather than the other of the adjacent pairs were the following: **se** rather than **d** because it does not mimic or interact with any other known gene; **N** rather than **Ca** because no mimics are known; **b** rather than **m** because classification is easier; **T** rather than **Ki** because it is better known genetically and was available in our laboratory.

Table 2. *Genes of the mouse that would be suitable for use as markers, arranged according to their linkage groups. Those chosen for the stocks are shown in heavy type*

Linkage group	Gene symbol
I	c^{ch}, $\mathbf{c^e}$, *c*; **p**
II	**se**; *d*
III	**s**; *W*, W^v; *pi*; **lx**
IV	(None)
V	*kr*; A^y, A^w, **A**, $\mathbf{a^t}$, *a*; *un*; *we*; *pa*; **fi**
VI	*bt*; *Ca*; **N**
VII	**wa-2**; *sh-2*; **Re**
VIII	**b**; *m*
IX	**T**; *Ki*
X	**v**
XI	*wa-1*; **Wh**
XII	**ru**; **je**
'Independent'	**f**; **fz**; **ln**; **Sd**; **Va**

The existence of multiple alleles provided a choice of genes at two loci. At the albino locus $\mathbf{c^e}$ was chosen because **c** masks all other colour genes and $\mathbf{c^{ch}}$ was found to be difficult to classify in presence of **ru** which had to be allocated to the same stock. At the agouti locus two alleles, **A** and $\mathbf{a^t}$, were chosen for inclusion in the stocks. The presence of two alleles was necessary in order to ensure that the linkage testing stocks would always contain an allele different from that associated with the new mutant to be tested. **A** and $\mathbf{a^t}$ were chosen because each exhibits partial dominance over the other and both are dominant to **a**. $\mathbf{A^w}$ was rejected because it has not this advantage of incomplete dominance, and $\mathbf{A^y}$ was rejected because it impairs fertility and is lethal when homozygous.

The basic principle underlying the elimination of markers described above was the existence of a more suitable marker more or less closely linked to the marker eliminated. Several of the markers finally chosen, however, have been very inadequately tested for linkage with the others, and it therefore became imperative to survey the published data

* A glossary of the gene symbols used in this paper will be found in Appendix II.

† **pa** has recently been shown to lie between **a** and **fi** (Carter, T. C., *J. Genet.*, in the press).

upon which rests the assumption that the known linkage groups and the supposedly 'independent' genes are really independent of each other. The results of this survey are contained in another paper. They show that the early known genes have been extensively tested, though there are several pairs among them that have not been tested against each other or that have been tested by only small numbers of progeny. Of the more recently discovered genes, **fi** and **wa-2** have been tested against only a few other genes, while **ru**, **fz**, **Sd** and **Va** are almost completely untested. The retention of these genes in the linkage-testing stocks will therefore be conditional on their remaining independent of the others after they have been adequately tested.

Arrangement of the marker genes in the stocks

After the number of possible markers had been reduced to the minimum in the manner described above, twenty-one were left and the next task was to arrange these in groups suitable for maintenance in the smallest possible number of stocks. Two important principles governed the manner of grouping the genes. The first was that dominant and recessive markers should be contained in different stocks, because these call for different types of mating when tests for linkage with a new recessive mutant are to be made. The second was that all the genes in a stock should be easily classifiable in all combinations with each other.

The observance of these two principles left some latitude of choice in the allocation of the genes. This was utilized first by taking advantage of any combinations of genes that happened to be already available in the laboratory, and second by allocating to different stocks genes with symbols having the same initial letter. This latter, somewhat trivial, consideration was intended to reduce the probability of clerical errors in recording.

By the application of the above principles the genes chosen as markers were allocated to five stocks in the manner shown in Table 3. The construction of these stocks was immediately started, and the markers that have been combined in each stock at the time of going to press are shown in the right-hand column of Table 3. No serious trouble has arisen from interactions or impaired viability, and no reason has yet arisen for doubting that the stocks will be capable of construction in the form planned.

Assessment of total swept length

The potential value of these stocks for the detection of linkage may be seen from an examination of the total swept length attainable by their use. The separate lengths swept by each marker or pair of linked markers can be calculated without difficulty in the manner described in § 2. These calculations have been made with the assumption that the stocks will be used in the way outlined in Appendix I; that is to say, when the new mutant under test is dominant all the data will come from backcross matings, and when the new mutant is recessive, the tests against dominant markers will be backcrosses, those against recessive markers will be intercrosses and those against sex will be mixed crosses. The swept lengths calculated for each marker or pair of linked markers are shown in Table 4. Values appropriate to dominant or recessive new mutants are given for tests based on 50 and on 100 classified progeny.

The map distances between the markers on doubly marked chromosomes have been calculated from the recombination fractions noted at the foot of the Table. Kosambi's formula was used for the short linkage groups and the new fourth power formula given on p. 310 where linkages exceed 40%. For linkage group VII, however, the map proposed

by Fisher, Lyon & Owen (1947) has been adopted; the effective locus of sex has accordingly been supposed to lie at a distance of 65 cM. from **Re**. On this supposition the length swept by the three loci will cover the whole of a map 130 cM. long. The greater part of the swept length from sex will, however, be overlapped by that from **Re**, and sex will therefore contribute little to the total swept length.

Table 3. *Constitution of the linkage-testing stocks*

Stock no.	Markers to be present	Combinations available at time of going to press
I	**Sd Va Re A**	All
II	**T Wh N** a^t	All
III	**p lx v se fz** a^t	(**p se fz**), (**v lx** a^t)
IV	**b ln fi s wa-2** a^t	(**b ln s wa-2** a^t), (**fi** a^t)
V	**ru** c^e **je f** a^t	All

Table 4. *Map lengths in cM. swept by the separate markers or pairs of linked markers in the stocks. N is the number of progeny classified*

			Swept lengths in cM.			
			New mutant dominant		New mutant recessive	
Linkage group	Markers	References	$N=50$	$N=100$	$N=50$	$N=100$
I	c^e **p**	(1)	87·1	98·2	70·8	83·7
II	**se**		74·5	86·7	57·3	72·3
III	**s lx**	(2)	127·2	129·9	100·9	125·9
V	**A fi**	(3)	104·8	112·1	98·5	107·2
VI	**N**		74·5	86·7	74·5	86·7
VII	**wa-2 Re** sex	(4)	130·0	130·0	130·0	130·0
VIII	**b**		74·5	86·7	57·3	72·3
IX	**T**		74·5	86·7	74·5	86·7
X	**v**		74·5	86·7	57·3	72·3
XI	**Wh**		74·5	86·7	74·5	86·7
XII	**ru je**	(5)	118·1	124·7	106·3	115·9
—	**f**		74·5	86·7	57·3	72·3
—	**fz**		74·5	86·7	57·3	72·3
—	**ln**		74·5	86·7	57·3	72·3
—	**Sd**		74·5	86·7	74·5	86·7
—	**Va**		74·5	86·7	74·5	86·7
Total swept length			1386·7	1548·6	1222·8	1430·0
Fraction of 2600·0			0·53	0·60	0·47	0·55

References: (1) 14% recombination (average of sexes) (Grüneberg, 1936).
(2) 8% recombination **s** to **hr** (Snell, 1931).
42% recombination **hr** to **W** (Gates & Pullig, 1945).
16% recombination $\mathbf{W^v}$ to **lx** (Carter, 1949).
(3) 33% recombination (Carter & Grüneberg, 1950).
(4) Map distances: sex to **Re** = 65 cM., **Re** to **wa-2** = 55 cM. (Fisher, Lyon & Owen, 1947).
(5) 45% recombination (Fisher & Snell, 1948).

The summation of the separate swept lengths to give an estimate of the total for the five stocks is shown at the foot of Table 4. If 100 progeny are classified in tests with each of the five stocks the total swept length will be 1549 cM. when the new mutant under test is dominant, and 1430 cM. when the new mutant is recessive. Since the mouse has 20 chromosomes with average map lengths of 130 cM., its total map length is 2600 cM. Therefore by raising 500 test progeny a dominant new mutant will be tested against 0·60 of the total genetic map, and a recessive new mutant against 0·55. These fractions therefore express the probability of success in assigning a new mutant to its linkage group by the use of the new stocks.

It must, however, be emphasized that the total swept length attained by the stocks cannot yet be estimated with precision, and the figures given above for the probability of success in detecting a linkage should be regarded as rough estimates. The most important source of uncertainty in the estimate is the possible existence of linkage between some of the markers now assumed to be independent. At least four of the markers in the stocks have been very inadequately tested for linkage, and it is possible that one or more of these will eventually be found to be linked, perhaps closely, with another marker. The total swept length would then be somewhat reduced. Other sources of uncertainty in the estimate of the total swept length and of the probability of detecting a linkage, which do not seem to be important enough to merit a detailed discussion, are: (i) the assumption that all the linkage-group maps are of equal length; (ii) the use of a possibly inaccurate relationship between recombination value and map-length; (iii) the adoption of a $2\frac{1}{2}\%$ significance level for the calculation of swept radii, and the assumption that a sub-significant indication of linkage, if found, would not be followed up by additional breeding tests.

Despite the uncertainty attendant on the attempt to estimate the total swept length, it is claimed that these stocks, when completed, will offer the best material for routine linkage tests that can at present be constructed. The discovery of new linkages and of new marker genes will, however, necessitate changes in the stocks, perhaps even before they are in fact fully constructed. But the necessary changes are unlikely to arise so fast that the stocks cannot form a useful basis for the construction and maintenance of a set of standard linkage-testing mouse stocks.

SUMMARY

1. The importance of mapping the chromosomes of the mouse is stressed, and the need for specially designed stocks for routine linkage testing is pointed out.

2. The theory of planning linkage tests is outlined, and the new concept of 'swept radius' is introduced. This makes it possible (*a*) to decide, on the basis of efficiency, between a number of alternative procedures that present themselves in the planning of linkage tests; and (*b*) to design special linkage testing stocks.

3. The composition of five specially designed linkage testing stocks is described. By the use of these stocks a gene can be tested against a little over half of the total genetic map, at the cost of raising about 500 test progeny, which is a considerable improvement over what is at present possible.

4. An outline is given in Appendix I of the procedure to be adopted for the use of the stocks.

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APPENDIX I

Procedure for the use of the linkage-testing stocks

Since the value of the stocks as a means of detecting linkage depends on their being correctly used, and the method of use differs according to the nature of the new mutant under test, a brief description of the methods intended is given here. In every case the test requires two generations. The first is always an outcross of an animal carrying the new mutant under test to an animal from each of the stocks. The animals from the stocks should have the mutant phenotype of all the markers in the stocks from which they come. The multiple heterozygotes, whose gamete formation is to be examined for evidence of linkage, occur in this F_1 generation. The second generation is obtained from the mating of a multiple heterozygote selected from the F_1 generation: but the type of animal to which it is to be mated depends on the character of the new mutant under test and on the character of the markers against which it is being tested. The type of animal to be mated to the multiple heterozygote under the different conditions is shown in Table 1. Three categories of new mutant require different treatment, namely, (i) dominants, (ii) recessives and (iii) recessives with sterile homozygotes. (Lethal recessives with homozygotes which die before other characters can be classified are not specifically considered, though the stocks can be used to test them for linkage.) The treatment of the first two categories is fully enough described by the Table, but the treatment of new mutants in the third category needs some more explanation. In the first place, the animal used for the outcross will, of course, have to be a heterozygote, and therefore only half the F_1 will carry the new mutant.

When a new mutant in this category is being tested against a recessive-carrying stock two courses are open; F_1 animals may be tested for the new mutant by mating to known heterozygotes, and suitable F_1 animals then mated *inter se*; or F_1 animals may be mated *inter se* at random, in which case the new mutant will be expected to segregate from only one mating in four. When a new mutant in the third category is being tested against a dominant-carrying stock two courses are again open. Multiply-mutant F_1 animals may either be mated to known heterozygotes from the new mutant stock or they may be mated *inter se*. The former course will usually be better, because the new mutant will be expected to segregate from one mating in two, compared with one in four intercross matings; only when the marker gene is semi-dominant, and the amount of information available per individual from an intercross is consequently twice the amount available from a mixed cross, will the two courses be equally good.

All the stocks test for linkage with sex if a male F_1 animal is used. The type of mating testing sex will be the same as that testing the marker genes in the stock except where this is an intercross: sex will then be tested by a mixed cross. The phase will depend on the sexes of the animals used in the first or outcross generation. If the animal carrying the

Appendix Table 1. *Type of animal to be mated to the multiply heterozygous F_1; and type of the resulting linkage-testing mating*

	Stock against which new mutant is to be tested	
Category of new mutant under test	Dominant-carrying (I, II)	Recessive-carrying (III, IV, V)
Dominant	Multiple recessive: i.e. wild-type for all markers in the stock, except **A**, a^t	Multiple recessive from the stock.
	Repulsion backcross	*Coupling backcross*
Recessive	Homozygote of new mutant	Multiple heterozygote: i.e. F_1 mated *inter se*
	Coupling backcross	*Repulsion intercross*
Recessive, with sterile homozygote	Heterozygote of new mutant	Multiple heterozygote: i.e. F_1 mated *inter se*
	Coupling mixed cross	*Repulsion intercross*

Appendix Table 2. *Scoring coefficients and amount of information per individual for different types of linkage-testing mating.* **A** *and* **B** *represent the dominant alleles of the two genes in the test,* **a** *and* **b** *their recessive alleles, irrespective of which are the mutant alleles. The genes are in coupling when* **A** *and* **B** *entered the test together from the same parent*

	Scoring coefficients Phenotypic class						
	AA B	**Aa B**	**AA b**	**Aa b**	**a B**	**a b**	Information per individual
Sign for Coupling	−	−	+	+	+	−	
Sign for Repulsion	+	+	−	−	−	+	
A semi-dominant:							
Intercross	4/3	0	4	0	4/3	4	8/3
Mixed cross (**A** intercrossed)	2	0	2	0	2	2	2
AA masks **B**,**b** distinction:							
Intercross	0	0	0	0	4/3	4	4/3
Mixed cross (**A** intercrossed)	0	0	0	0	2	2	1
AA inviable:							
Intercross	0		0		4/3	4	16/9
Mixed cross (**A** intercrossed)	0		0		2	2	4/3
A and **B** fully dominant:							
Backcross	2		2		2	2	4
Intercross	4/9		4/3		4/3	4	16/9
Mixed cross (**A** intercrossed)	2/3		2/3		2	2	4/3
aa masks **B**,**b** distinction:							
Backcross	2		2		0		2
Intercross	4/9		4/3		0		4/9
Mixed cross (**A** intercrossed)	2/3		2/3		0		1/3
Mixed cross (**B** intercrossed)	2/3		2		0		2/3
A indistinguishable from **B**:							
Backcross			$\pm 2/3$			∓ 2	4/3
aa indistinguishable from **bb**:							
Intercross	$\mp 4/9$				$\pm 4/7$		16/63

new mutant was a male, the phase will be coupling when the new mutant is dominant and repulsion when it is recessive. If the animal carrying the new mutant was a female the phases will be reversed.

When the breeding data have been obtained, it remains to examine the segregation of the

new mutant with each marker in order to detect deviations from free segregation; an estimate of the apparent recombination fraction and its standard error will also usually be required. We have found that this analysis can most conveniently be done by the procedure described below; it adds nothing new to the procedures given by Mather (1935), Finney (1943) and Fisher (1946), but does not follow any one in all details.

(i) The observed number of progeny in each of the recognizable phenotypic classes is multiplied by the 'scoring coefficient' appropriate to the class and to the type of mating. This product is the contribution of the class to the score for that type of mating. Scoring coefficients are listed in Table 2 for all the types of mating likely to be encountered in the tests. The signs of the coefficients, which depend on the phase of the multiple heterozygote under test, are shown at the heads of the columns in the Table: they are applicable only on condition that when the dominant alleles of the two genes enter the heterozygote together from the same parent the phase is said to be coupling, and when they enter separately from different parents it is said to be repulsion, irrespective of whether the mutant allele is dominant or recessive. The classes representing grand-parental combinations thus have negative coefficients, and a negative score favours linkage.

(ii) The score, D, for each type of mating is obtained by summing the contributions from the classes, due attention being paid to sign. Scores are summed over the mating types, if more than one type has been used, to give a total score, ΣD.

(iii) The number of progeny from each type of mating is multiplied by the amount of information per individual, i, appropriate to the type of mating; this is also listed in Table 2. The product is the amount of information, I, for the type of mating. If more than one type has been used, the amounts of information are summed to give a total ΣI.

(iv) The total score is divided by the total information to obtain a correction, which when added to $\frac{1}{2}$ (with due attention to sign) gives a first estimate, p_1, of the recombination fraction. Thus

$$p_1 = \tfrac{1}{2} + \frac{\Sigma D}{\Sigma I}.$$

(v) The standard error of p_1 is obtained by taking the square root of the reciprocal of the total amount of information

$$s_{p_1} = 1/\sqrt{(\Sigma I)}.$$

(vi) The significance of the deviation from free segregation is tested by comparing the correction $\Sigma D/\Sigma I$ with its standard error, which is the same as s_{p_1}; it is therefore a c-test:

$$c = \frac{1}{s_{p_1}} \frac{\Sigma D}{\Sigma I} = \frac{\Sigma D}{\sqrt{(\Sigma I)}}.$$

Alternatively the squared form may be used:

$$\chi_1^2 = \frac{(\Sigma D)^2}{\Sigma I}.$$

If linkage is found, the first estimate of the recombination fraction, p_1, may not be sufficiently accurate. A second estimate, based on revised scoring coefficients, will then be necessary; this may be found by the method and tables given by Finney (1949).

APPENDIX II

Glossary of gene symbols used in this paper and a later one, with a brief indication of the characters affected. References to detailed descriptions may be found in Law, L.W., 1948, Mouse Genetics News, No. 2, *J. Hered.* **39**, 300–8.

A	Agouti	coat colour
a	non-agouti	coat colour
a^t	tan	coat colour
A^w	White-bellied agouti	coat colour
A^y	Yellow	coat colour
b	brown	coat colour
bt	belted	white spotting
c	albino	coat and eye colour
c^e	extreme dilution	coat colour
c^{ch}	chinchilla	coat colour
Ca	Caracul	waved coat
d	dilute	coat colour
dw	dwarf	pituitary defect
f	flexed tail (siderocyte anaemia)	tail deformity and anaemia
fi	fidget	behaviour
Fu	Fused	tail deformity
fz	fuzzy	coat texture
gl	grey-lethal	bone deformity and coat colour
hr	hairless	loss of hair
j	jittery	behaviour
je	jerker	behaviour
Ki	Kinky tail	tail deformity
kr	kreisler	behaviour
ln	leaden	coat colour
lx	luxate	absence of tibia
m	misty	coat colour
N	Naked	loss of hair
p	pink-eye	coat and eye colour
pa	pallid	coat and eye colour
pi	pirouette	behaviour
r	rodless	defect of retina
Re	Rex	waved coat
ru	ruby	coat and eye colour
s	piebald	white spotting
Sd	Danforth's short tail	tail deformity
se	short-ear	reduced pinna
sh-1	shaker–1	behaviour
sh-2	shaker–2	behaviour
si	silver	coat colour
T	Brachyury	tail deformity
un	undulated	tail deformity
v	waltzer	behaviour
Va	Variant waddler	coat colour, spotting, behaviour
W	Dominant spotting (macrocytic anaemia)	white spotting
W^v	Dominant spotting, viable allele	white spotting and coat colour
wa-1	waved–1	waved coat
wa-2	waved–2	waved coat
we	wellhaarig	waved coat
Wh	White	coat colour

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A REVIEW OF INDEPENDENT SEGREGATION IN THE HOUSE MOUSE*

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In a previous paper a description was given of some new stocks for linkage tests in the mouse (*Mus musculus* L.) (Carter & Falconer, 1951). The planning of these stocks depended on a knowledge of the linkage relations of the markers chosen, and in particular on the assumption that the known linkage groups and the supposedly independent genes are really independent of each other. It was therefore necessary to survey the evidence on which the supposition of independence is based.

Much of this evidence comes from the early years of mouse genetics, when linkage was unknown in the mouse, and the very loose linkages now known to be common were not looked for; the independence of many pairs of genes was consequently deduced from evidence which would now be considered scanty. In a surprisingly large number of cases the published evidence has remained slender or non-existent; it is still true, as Grüneberg (1943*a*) has pointed out, that there are no records of a direct linkage test between two of the oldest known mouse mutants, waltzing and dominant spotting; nor have tests been reported between piebald spotting and brachyury, though the former is an ancient gene of the fancy and the latter has been known for more than two decades. On the other hand, the assumption of independence was often supported by later work, but until methods of combining linkage data were introduced the results of different workers could not be pooled and the independence thereby reliably established. Since there is no published survey in which the evidence for independence from all sources of data has been combined, except the purely qualitative one of Grüneberg (1943*a*), it may be useful to have the results of our compilation recorded.†

Sources of data

All the published records known to us of simultaneous segregations involving the genes included in the survey have been used, except a little of the very early work in which the phase of the matings was obscure or the numbers were very small. The survey did not, however, include all the known genes in the mouse, since it was intended primarily to cover the mutants considered suitable for general use as genetical markers. Some of the genes thus omitted have been fairly extensively tested for linkage, notably **pi** in linkage group III, **r** in linkage group IV, **dw** and **Tr**; as **si** was also omitted, having irregular penetrance, there was no representative of linkage group IV.‡

* A report to the Medical Research Council.

† Snell, G. D. in chapter 5 of *Biology of the Laboratory Mouse* (Blakiston, Philadelphia, 1941) has given a short summary of independence tests, though without combining data from different sources.

‡ A glossary of gene symbols used in this paper is given as Appendix II in Carter & Falconer (1951) **Y** denotes the locus of sex, maleness being dominant.

When the survey of the published data had been completed it was found that many important gene pairs had not been tested against each other; in particular, very few genes had been tested for partial sex-linkage. We therefore searched our own records for data on the pairs that had been inadequately tested, and have thus been able to fill some of the gaps. The data from our records are set out in detail in Table 3. They were all obtained incidentally from matings planned for other purposes; coupling and repulsion phases are therefore often ill balanced, and many matings were of an inefficient type.

Treatment of data

The analysis of the simultaneous segregation of each pair of genes was made by the method described in the previous paper (Carter & Falconer, 1951), in which references to the sources of the method are given. The results, together with references to the sources of the data, are given in Table 1A, where the gene pairs about which information is available are listed in alphabetical order. **se** and **d** were treated as though they were at the same locus, since their loci are very closely linked. All the information about each pair of genes has been combined to obtain a single estimate of the recombination fraction (p) and the standard error of this estimate. One body of data, however—that of Gates (1926)—has been treated separately; these data were obtained from a cross between *M. musculus* and *M. bactrianus* and, perhaps for this reason, some of the results are discordant with those of other workers. Thus in Gates's data **d** shows significant indications of linkage with **b**, **s** and **v**, the $\chi^2_{[1]}$ testing linkage being 10·3, 6·7 and 4·8 respectively. The results from Gates's paper are given separately in Table 1B, together with the estimates obtained by combining his results with the others contained in Table 1A.

Though many of the standard errors are very small, some of the observed recombination fractions may be biased because of the lack of balance between coupling and repulsion, by which alone disturbances due to viability interactions can be eliminated. For this reason the degree of balance between the phases for each gene pair was calculated. The columns headed 'Phase balance' in Tables 1A and 1B show the percentage of the total amount of information that is derived from coupling matings; so 50 indicates perfect balance, 100 indicates all coupling, and 0 indicates all repulsion.

All the single-factor segregations were examined for significant deviations from expectation. When disturbances were found the observed viability or penetrance was taken into account when calculating the recombination fraction. The only genes treated in this way were **sh–2**, **v** and **wa–2**, which are known to reduce viability; **f**, **Fu**, **s** and **W**, which are known to show incomplete penetrance; and the lethal **j**, which showed an excess of homozygotes, presumably because heterozygous parents were identified by the occurrence of homozygous offspring. The few disturbed segregations shown by other genes were accepted as the genuine sampling errors which were to be expected among so many samples. One intercross test of **p** against **d** (in Little, 1913) was excluded, however, because the segregation recorded (4 **pp** homozygotes in 74 mice) is so aberrant that we think a misprint or clerical error must have occurred.

The method of allowing for reduced viability or penetrance, when calculating the recombination fraction, was as follows. A new expectation was calculated for each phenotypic class on the assumption of a viability, v, or a penetrance, u, calculated from the data. From these expectations scoring coefficients and amount of information per individual were obtained in the usual way.

Table 1A. *Joint estimates of recombination fractions*

Loci	Recombination fraction (%)	Standard error	Phase balance	References
a b	50	0·6	100	15, 25, 30, 35, 36, 37
a bt	47	3·3	100	39
a c	49	2·3	62	46
a d	51	1·1	64	25, 30, 35, 36, 37
a f	57	4·2	100	8
a j	47	4·6	0	41
a je	46	5·4	0	31
a ln	50	4·4	100	38
a lx	49	2·6	37	3
a N	54	2·7	100	40
a p	51	0·7	83	15, 16, 37
a Re	49	3·0	27	13, 46
a s	48	1·0	60	16, 25, 43
a Sd	53	6·6	44	46
a sh-2	59	5·1	100	46
a T	49	3·2	0	46
a Va	48	7·7	0	46
a W	47	1·8	69	16, 43
a wa-1	51	1·2	100	2, 25, 30
a wa-2	53	3·3	72	46
a Y	51	1·4	69	25
b bt	54	3·8	100	39
b c	53	2·1	100	20, 22
b d	50	1·1	64	20, 25, 30, 35, 36, 37
b f	55	3·8	0	8
b fi	44	2·9	0	7, 46
b fz	48	7·5	100	46
b je	45	6·2	39	31
b ln	50	3·0	100	38
b lx	48	2·8	67	3
b N	54	2·8	100	40
b p	49	0·7	96	15, 35, 37
b Re	49	4·0	42	13, 46
b s	51	1·0	28	25, 44
b sh-2	51	3·7	71	10, 46
b T	50	4·8	100	9
b v	49	6·4	49	46
b Va	51	7·0	100	46
b W	50	1·8	63	44
b wa-1	49	1·2	100	2, 25, 30
b wa-2	49	3·1	100	46
b Wh	49	3·7	100	1
b Y	51	1·4	69	25
bt c	49	2·7	100	46
bt d	56	6·5	100	39
bt f	52	7·2	0	39
bt Fu	48	5·8	100	39
bt lx	56	6·2	0	3
bt p	53	6·1	0	39
bt v	57	6·5	23	46
bt wa-2	61	7·5	0	39
bt Wh	49	6·1	100	1
c Ca	47	3·2	63	46
c d	56	2·0	95	20, 46
c f	49	2·6	100	34
c fi	47	2·5	100	7, 33
c fz	50	4·1	13	46
c je	59	7·3	90	31, 46
c ln	50	8·2	0	38
c lx	46	2·8	55	3
c N	44	6·8	0	40
c Re	47	2·6	100	13, 46
c ru	46	7·7	87	46
c s	48	5·1	46	20, 46
c Sd	44	8·5	100	46
c sh-2	55	5·9	86	8, 46
c T	46	4·4	24	9, 46
c v	47	3·6	0	14

Table 1A (*cont.*)

Loci	Recombination fraction (%)	Standard error	Phase balance	References
c W	37	5·7	0	46
c wa-1	45	2·4	7	2, 46
c wa-2	54	4·9	0	46
c Wh	59	5·4	100	1
c Y	50	1·6	53	46
Ca j	57	6·4	32	41
Ca je	57	4·6	100	31
Ca lx	45	7·4	100	3
Ca Re	46	6·3	0	12
Ca Sd	59	3·8	0	19, 46
Ca v	41	7·1	72	46
Ca Wh	47	4·7	15	1
d f	46	3·2	0	8
d fi	50	7·8	100	33
d j	58	4·1	0	41
d je	45	4·8	0	31
d ln	54	2·6	0	38
d lx	49	3·3	36	3
d N	46	2·7	100	40
d p	46	2·1	92	35, 37
d Re	47	4·5	83	13, 46
d ru	61	8·8	100	46
d s	50	1·3	81	25, 44
d sh-2	56	2·5	0	28
d T	49	4·5	100	9
d v	44	4·9	57	46
d W	50	3·7	48	44
d wa-1	53	1·3	43	2, 25, 30
d wa-2	53	4·6	100	46
d Wh	41	4·9	100	1
d Y	54	3·9	100	25
f hr	47	5·7	0	8
f je	58	14·7	100	31
f ln	50	5·0	100	8
f lx	57	5·1	9	3
f N	49	5·9	100	8
f p	51	5·0	0	8
f s	51	10·9	0	8
f sh-2	58	10·8	0	8
f T	47	3·4	100	9
f v	59	8·8	0	8
f W	37	8·7	100	8
f wa-1	54	5·8	0	2
f Wh	46	4·9	100	1
f Y	52	4·9	0	8, 34
fi W	42	7·5	100	7
fi Y	53	2·9	0	46
Fu j	41	12·6	0	41
Fu lx	50	5·3	37	3
Fu Re	47	4·1	100	46
Fu Wh	39	10·9	0	1
fz lx	48	6·6	0	3
fz p	42	8·6	0	46
fz Wh	52	5·4	100	1
fz Y	51	3·5	56	46
hr ln	49	4·3	0	38
hr sh-2	56	6·6	0	10
hr T	44	5·9	100	9
j ln	55	6·1	0	41
j p	52	4·1	0	41
j s	53	6·7	0	41
j W	43	3·7	78	41
j wa-1	52	6·1	0	41
je ln	42	7·5	0	31
je lx	49	7·2	0	3
je p	51	4·8	0	31
je Re	51	6·1	100	31
je s	44	4·1	69	31

Table 1A (*cont.*)

Loci	Recombination fraction (%)	Standard error	Phase balance	References
je v	47	7·5	0	31
je W	57	5·8	100	31
je wa-1	44	7·5	0	31
je wa-2	52	7·9	0	31
je Y	50	4·2	0	46
ln lx	50	6·1	88	3
ln N	47	3·9	100	38
ln Re	53	7·1	100	13, 46
ln sh-2	53	7·3	0	10
ln T	50	4·9	100	9
ln v	45	6·3	0	38
ln W	49	3·4	100	38
ln wa-1	56	5·3	0	2, 46
ln Wh	51	3·1	100	1
ln Y	60	5·2	71	46
lx p	54	2·9	37	3
lx pa	43	5·2	0	3
lx Re	45	3·9	58	3
lx ru	59	6·7	0	3
lx Sd	44	6·7	100	3
lx v	55	5·5	37	3
lx Va	46	6·0	100	3
lx wa-1	41	6·3	0	3
lx wa-2	47	6·2	5	3
lx Y	49	1·4	35	3
N p	54	3·6	100	40
N s	56	4·3	100	40
N sh-2	53	4·8	100	10
N T	49	3·4	43	9, 46
N v	60	9·1	100	40
N W	47	7·0	0	40
N wa-1	65	11·2	100	2
N Y	52	4·1	50	46
p Re	47	5·6	46	13, 46
p ru	47	5·1	43	17
p s	49	1·2	13	16, 46
p sh-2	47	2·6	0	28
p W	51	2·1	68	16, 43
p wa-1	49	2·9	88	2, 30
p wa-2	48	3·8	47	46
p Wh	51	4·9	100	1
p Y	53	3·5	50	46
pa sh-2	56	7·9	0	10
pa T	47	5·1	100	9
Re s	52	3·6	32	13, 46
Re Sd	57	4·7	35	46
Re v	49	9·8	100	13
Re W	45	6·2	0	46
Re wa-1	62	9·8	100	13
Re Wh	51	3·8	0	1
ru T	46	2·4	100	17
ru Y	46	4·7	0	46
s sh-2	49	4·0	30	46
s v	50	8·0	25	46
s Va	47	3·5	100	11, 46
s wa-1	50	1·4	18	2, 25
s wa-2	50	3·2	30	46
s Wh	54	3·1	100	1
s Y	51	1·3	57	25
Sd T	57	4·5	0	19
Sd Va	39	4·7	12	46
Sd Y	45	3·3	47	46
sh-2 T	53	5·3	100	9
sh-2 v	79	16·3	0	10
sh-2 wa-1	53	5·9	0	2
sh-2 Wh	45	4·0	100	1
T v	48	4·9	100	9, 46
T W	52	3·5	83	9, 46

Table 1A (*cont.*)

Loci	Recombination fraction (%)	Standard error	Phase balance	References
T wa-1	46	3·1	100	2
T Wh	45	5·7	65	46
T Y	43	4·2	58	46
v wa-1	50	7·0	0	2
v Wh	48	3·1	100	1
v Y	58	5·3	50	46
Va W	55	3·7	0	11
Va Y	44	4·8	42	46
W wa-1	56	6·4	100	2
W Wh	44	5·1	0	1
W Y	50	4·4	55	46
wa-1 Y	49	1·4	66	25
wa-2 Wh	47	4·0	100	1
Wh Y	48	7·9	18	46

Table 1B. *Recombination fractions estimated from data from the* Mus musculus × M. bactrianus *crosses of Gates* (1926)

	Gates (1926) alone			All data combined		
Loci	Recombination fraction (%)	Standard error	Phase balance	Recombination fraction (%)	Standard error	Phase balance
a s	50	4·8	100	48	1·0	62
a v	56	4·8	100	56	4·8	100
b d	46	1·4	100	48	0·8	77
b p	53	2·4	100	49	0·7	96
b s	51	1·4	0	51	0·8	18
b v	50	1·4	0	50	1·4	2
c v	46	2·2	70	46	1·9	50
d p	50	2·4	100	47	1·6	96
d s	46	1·4	0	49	0·9	44
d v	47	1·4	0	47	1·3	4
p s	47	2·4	0	49	1·1	11
p v	48	2·4	0	48	2·4	0
s v	48	1·3	100	48	1·3	98

The results presented in Table 1A are summarized in the two-way Table 2A, where the genes are arranged by their linkage groups. For each gene pair there is thus a square in the table, and in it the closest admissible linkage is entered; this was obtained by deducting twice the standard error from the observed recombination fraction; the value given is the nearest unit per cent below the true value. The squares left blank are those of gene pairs that have not been tested against each other. Gene pairs known to be linked have the approximate recombination fractions entered in italics; these were obtained from the most easily available authority, generally Grüneberg (1943*a*); the references are given in Table 2B. Where there is a sex difference in recombination, the mean value of the sexes is given. In Tables 2A and 2B two further pairs of closely linked genes are treated as single loci, namely, **Ca–N** ($p=2\%$; Murray & Snell, 1945), and **Fu–T** ($p=4\%$; Dunn & Caspari, 1945).

Comments

Table 1A covers 206 gene pairs. If these do not include any linked pairs, and if the observed deviations of the recombination fraction from the free-segregation value are distributed normally, it is to be expected that about 5% of them (i.e. 10) will deviate from 50% recombination by twice the standard error or more. Actually nine such deviations are present, four being in excess of 50% and five below. The assumption that the deviations are normally distributed is, however, open to doubt on account of the disturbing

Table 2A. *Summary of independence data*

The entry in each cell is the closest linkage compatible with the data, and is obtained by subtracting twice the standard error from the observed recombination fraction. The entry for linked pairs (in italics) is the approximate recombination fraction. Roman numerals indicate the linknge groups.

	c	p	d, se	s	hr	W	lx	a	pa	fi	N, Ca	bt	wa-2	sh-2	Re	Y	b	T, Fu	v	j	Wh	wa-1	ru	je	f	fz	ln	Sd
p	*14*																											
d, se	52	41																										
s	37	46	47																									
hr	—	—	—	*9*																								
W	25	47	42	*46*	*42*																							
lx	40	48	42	*51*	*48*	*17*																						
a	44	49	48	46	—	43	43																					
pa	—	—	—	—	—	—	33	*19*																				
fi	42	—	34	—	—	26	—	*33*	*19*																			
N, Ca	41	46	40	47	—	33	30	49	—	—																		
bt	44	40	43	—	—	—	43	40	—	—	*12*																	
wa-2	44	40	44	44	—	—	35	46	—	—	—	46																
sh-2	43	42	50	40	43	—	—	48	40	—	43	—	*28*															
Re	42	36	38	44	—	32	37	43	—	—	33	—	*42*	*20*														
Y	46	45	46	48	—	41	46	48	—	46	44	—	*56*	*57*	*51*													
b	48	47	47	49	—	47	42	49	—	38	47	46	43	43	40	48												
T, Fu	37	—	40	—	31	45	39	43	36	—	42	36	—,	42	39	34	39											
v	39	—	34	33	—	—	44	—	—	—	36	44	—	46	29	46*	36	38										
j	—	43	49	39	—	36	—	38	—	—	44	—	—	—	—	—	—	15	*20*									
Wh	48	41	31	48	—	33	—	—	—	—	37	37	39	37	43	31	42	33	41	—								
wa-1	40	43	50	46	—	42	28	48	—	—	42	—	—	41	41	46	47	40	35	40	*9*							
ru	30	36	42	—	—	—	45	—	—	—	—	—	—	—	—	36	—	40	—	—	—	—						
je	44	41	35	36	—	45	34	35	—	—	47	—	36	—	39	41	32	—	31	—	—	29	*45*					
f	44	41	39	29	35	19	47	48	—	—	36	38	—	36	—	42	47	40	41	—	36	42	—	28				
fz	42	24	—	—	—	—	34	—	—	—	—	—	—	—	—	43	33	—	—	—	40	—	—	—	—			
ln	33	—	48	—	40	41	37	41	—	—	39	—	—	38	39	49	44	39	32	42	44	45	—	27	40	—		
Sd	26	—	—	—	—	—	30	39	—	—	51	—	—	—	47	38	—	47	—	—	—	—	—	—	—	—	—	
Va	—	—	—	39	—	47	33	32	—	—	—	—	—	—	—	34	37	—	—	—	—	—	—	—	—	—	—	29

* Cytological evidence of Painter, T. S. (1927, *Genetics*, **12**, 379–92), shows that the locus of **v** is not in the sex chromosome.

influence of viabilty interactions together with poor phase balance, and of the probable tendency for investigators to add to their data when the first tests showed deviations approaching significance. Therefore the customary significance tests cannot be applied with confidence here, and it is desirable to examine in detail the few cases where the deviation exceeds twice its standard error in order to see whether there are any additional grounds for regarding any of these as real evidence of linkage.

Five of the pairs (**b**/**fi**, **c**/**W^v**, **Ca**/**Sd**, **d**/**sh-2** and **Sd**/**Va**) can probably be dismissed because at least one gene of each pair is known to have some adverse effect on viability, and in every case the phase balance is bad. The pair **Ca**/**Sd**, however, shows good single-factor segregations, and more data on this pair are desirable.

Table 2B. *References for recombination fractions of linked genes*

Linkage group	Loci	References
I	**cp**	32
III	**hr lx**	3
	hr s	23
	hr W	29
	lx s	3
	lx W	3
	s W	23
V	**a fi**	7
	a pa	5
	fi pa	5
VI	**bt Ca, N**	39
VII	**Re sh-2**	21
	Re wa-2	4, 21
	Re Y	24
	sh-2 wa-2	32, 45
	sh-2 Y	45
	wa-2 Y	45
X	**j v**	41
XI	**wa-1 Wh**	1
XII	**je ru**	26

On the other hand, four pairs, namely, **c**/**d**, **p**/**d**, **c**/**wa-1** and **d**/**wa-1**, cannot be dismissed without further comment, on account of their striking interrelationships. Thus the statistical association of **d** with **wa-1** is supported by the similar association of **d** with **c**, because **c** itself shows association with **wa-1**. Furthermore, **p**, which is linked with **c**, also shows association with **d**. These interrelationships are further supported by the tests of **c** and **d** against **Wh**, which is closely linked with **wa-1**; both show deviations approaching twice their standard errors. The pairs of genes under discussion involve linkage groups I, II and XI. Altogether there are eight gene pairs concerned in tests between these three groups, and six of these pairs show deviations ranging between 1·6 and 3·1 times their standard errors. The recombination fractions, quoted with two more figures than in Table 1A, are set out below:

Groups	Loci	Recombination fraction	Deviation/S.E.	Phase balance
I/II	**c**/**d**	56·19±1·96	3·15	95
	p/**d**	45·61±2·10	2·09	92
II/XI	**d**/**wa-1**	53·20±1·27	2·51	43
	d/**Wh**	40·95±4·88	1·85	100
I/XI	**c**/**wa-1**	45·15±2·35	2·07	7
	c/**Wh**	58·82±5·42	1·63	100
	p/**wa-1**	49·19±2·91	0·28	88
	p/**Wh**	51·43±4·88	0·29	100

Table 3. *New independence data*

In most cases the locus with the alphabetically earlier symbol is put first, but this is sometimes reversed where one gene is epistatic to the other. In the column headed 'Loci', **A** represents the dominant allele at the locus listed first, **B** at the other, irrespective of whether the dominant allele is mutant or type. Sex is treated as though maleness were due to a dominant gene **Y**. In the column headed 'Mating type' the three letters indicate phase and mating type with respect to each locus. By coupling (*C*) is meant that the doubly heterozygous mate received both dominant alleles from the same parent, irrespective of which are mutant and which are type. *I* = intercross, *B* = backcross.

Loci			Phenotypes of progeny			
A or **a**	**B** or **b**	Mating type	**AB**	**Ab**	**aB**	**ab**
a	**c^{ch}**	*C I I*	219	60	62	20
		C B I	39	11	18	13
		C I B	104	124	44	33
		R B B	27	41	33	36
		R B I	43	14	53	17
a	**Re**	*C B I*	16	6	12	5
		R B B	22	26	41	39
		R B I	24	7	19	10
		R I B	50	58	20	20
a	**Sd**	*C B B*	3	6	8	8
		R B B	5	4	12	11
a	**sh-2**	*C I I*	101	38	40	8
		C B I	19	6	16	1
a	**T**	*R B B*	53	57	45	47
		R I B	45	48	22	22
a	**Va**	*R B B*	3	8	10	10
Va	**a**	*R B B*		9	5	8
a	**wa-2**	*C I I*	150	38	49	10
		C B I	59	23	55	21
		R B B	8	8	3	4
		R I I	26	6	8	2
		R B I	29	6	22	12
b	**fi**	*R I I*	197	79	95	21
b	**fz**	*C B B*	9	10	7	7
		C I I	7	2	1	1
fz	**b**	*C I I*	35	10	12	
b	**Re**	*C B B*	19	11	12	9
		C I B	13	16	5	7
		R I I	39	18	12	2
		R I B	18	12	7	6
b	**sh-2**	*C I I*	169	46	57	15
b	**v**	*C I I*	42	9	12	5
		R I I	46	10	11	4
b	**Va**	*C I B*	37	32	10	11
Va	**b**	*C B B*		18	4	10
b	**wa-2**	*C I I*	362	99	113	32
bt	**c^{ch}**	*C B B*	18	26	22	19
		C I I	356	103	95	34
bt	**v**	*C I I*	23	5	3	0
		R I I	53	15	14	7
		R B I	9	1	7	2
c	**Ca**	*C B B*	36	35	39	35
		C B I	18	3	12	4
		R B B	15	10	11	12
		R I I	22	11	3	0
		R I B	21	34	21	10
c^{ch}	**d**	*C B B*	10	20	17	16
		C I I	547	194	180	41
		C B I	106	32	112	33
		R B B	4	6	5	9
		R I I	19	4	2	6
d	**c**	*C B I*	25	40	17	
		C I I	39	14	25	

Table 3 (*cont.*)

Loci			Phenotypes of progeny			
A or a	**B or b**	Mating type	**AB**	**Ab**	**aB**	**ab**
c	**fz**	*C B I*	11	3	16	4
		C I B	10	12	3	1
		R B B	6	6	6	3
		R I I	107	35	30	12
		R B I	33	13	28	8
c	**je**	*C I I*	37	11	17	2
		R I I	10	1	0	0
c	**Re**	*C B B*	53	42	46	46
		C I I	91	27	23	12
		C I B	37	31	14	13
c^{ch}	**ru**	*C I I*	42	18	15	8
		R I I	9	2	1	0
c	**s**	*C I I*	57	16	23	
		C I B	13	17	6	
c	**Sd**	*C B B*	10	7	6	8
		C I B	2	4	2	2
c	**sh-2**	*C B B*	8	5	8	4
		C I I	20	9	8	2
		R I I	15	4	3	0
c	**wa-1**	*C I I*	41	12	15	3
		R I I	77	34	24	9
		R B I	52	21	78	23
c	**wa-2**	*R I I*	98	21	43	10
		R B I	31	3	31	13
c	**Y**	*C B B*	74	70	80	83
		C I B	201	228	62	66
		R B B	69	85	71	70
		R I B	163	127	61	59
Ca	**Sd**	*R B B*	5	4	0	5
Ca	**v**	*C I I*	39	11	4	3
		C B I	12	4	12	3
		R I I	17	5	9	0
d	**Re**	*C B B*	3	3	3	5
		C B I	9	2	13	7
		C I B	16	14	9	11
		R I B	22	25	7	10
d	**ru**	*C I I*	16	13	4	1
		C B I	24	8	15	4
d	**v**	*C B B*	3	2	1	4
		C I I	68	20	19	7
		R I I	53	21	22	6
d	**wa-2**	*C I I*	170	40	52	8
fi	**Y**	*R I B*	369	347	91	99
Fu	**Re**	*C B B*	40	34	29	30
		C B I	12	3	19	6
fz	**p**	*R I I*	45	17	12	2
	Y	*C I B*	133	114	49	43
		R B B	3	10	2	12
		R I B	64	78	20	25
je	**Y**	*R I B*	180	156	49	40
ln	**Re**	*C B B*	2	0	4	1
		C I I	4	1	5	1
		C I B	11	19	4	3
ln	**wa-1**	*R I I*	48	12	14	4
ln	**Y**	*C I B*	64	83	28	22
		R I B	35	33	4	9
N	**T**	*C B B*	30	25	19	23
		R B B	8	4	9	7
N	**Y**	*C B B*	21	15	22	16
		R B B	21	18	16	20

Table 3 (*cont.*)

Loci			Phenotypes of progeny			
A or a	B or b	Mating type	AB	Ab	aB	ab
p	Re	*C I I*	16	8	6	0
		C I B	32	31	5	4
p	s	*C B B*	13	21	23	17
		C I I	201	58	95	16
		C B I	63	18	69	6
		C I B	18	9	2	0
		R B B	10	4	10	10
p	wa-2	*C I I*	119	27	28	10
		R I I	116	31	48	14
p	Y	*C B B*	8	6	7	11
		C I B	86	75	30	25
		R B B	11	8	10	7
		R I B	82	67	20	31
Re	s	*C I I*	39	13	16	4
		C B I	33	11	37	8
		R B B	8	12	6	4
		R I I	45	12	14	2
		R B I	55	8	50	16
Re	Sd	*C B B*	9	11	11	9
		R B B	17	25	8	23
ru	Y	*R I B*	117	128	49	43
s	sh-2	*C I I*	64	22	16	4
		R I I	78	32	33	13
		R B I	40	18	48	13
s	v	*C I I*	11	4	5	2
		R I I	40	13	9	3
s	Va	*C B B*	47	50	40	43
s	wa-2	*C I I*	112	28	23	3
		R I I	175	52	47	15
		R B I	54	15	55	11
Sd	Va	*C B B*	4	4	2	4
		R B B	13	33	28	25
Sd	Y	*C B B*	25	26	20	37
		R B B	29	29	34	28
T	v	*C I I*	13	5	16	4
T	Wh	*C B B*	11	11	15	14
		R B B	4	10	8	5
T	Y	*C B B*	18	19	15	31
		R B B	12	14	18	15
v	Y	*C I B*	47	47	26	13
		R I B	48	56	13	16
Va	Y	*C B B*	15	7	14	15
		R B B	17	10	22	11
W^v	Y	*C B B*	13	19	18	20
		R B B	13	17	14	14

			B			b		
A	B	Mating type	AA	Aa	aa	AA	Aa	aa
c^{ch}	T	$cT/c^{ch}+ \times c+/c^{ch}+$	3	4	1	2	11	4
		$c^{ch}T/++ \times c^{ch}+/++$	9		2	13		4
T	c	$+c/T+ \times +c/T+$	—	40	11	—	10	10
W^v	c	*R I I*	30	58	19	7	39	
W^v	Re	*R I B*	9	15	9	2	14	4
		R B B	—	5	13	—	10	10
W^v	T	*C I B*	7	20	16	7	23	18
		C B B	—	19	30	—	39	38
Wh	Y	*C B B*	—	2	0	—	2	3
		R B B	—	6	6	—	6	9
		R I B	3	0	1	5	2	1

It is true that three of these cases of association show recombination fractions exceeding 50%; but this fact alone should not be considered as grounds for attributing the association to causes other than linkage, since Wright (1947), by carefully planned experiments in which the phases were balanced, has demonstrated the existence in the mouse of recombination fractions exceeding 50%. While all these cases except one have a poor phase balance, the single-factor segregations are nevertheless generally good, and the genes concerned are not known to have any serious effect on viability. Furthermore, the internal agreement of the class segregations within the bodies of data does not suggest that any of the deviations are primarily due to viability interactions, and therefore the poor phase balance may not detract seriously from the reliability of the estimates. It seems probable therefore that linkage or chance are the two most likely causes of these deviations from free segregation, but the decision between the two cannot be made with certainty on the basis of the present data.

Some of the individual bodies of data involved in the pairs discussed above have been commented upon by the original authors. Thus for the pair **d**/**wa-1**, Fisher & Mather (1936) originally found recombination very significantly exceeding 50%, but the addition of more data failed to confirm their first results. In the same year, however, Grüneberg published data on this pair and Burhoe on the pair **se**/**wa-1**, and all three bodies agree in indicating recombination exceeding 50% by an amount which is 2·5 times its joint standard error. This case warrants rather more weight than the others, since the phase balance in the data is good. Burhoe (1936) commented on the suggestion of loose linkage between **c** and **wa-1** contained in his backcross data, but he dismissed this for two reasons: he considered that the F_2 data, which he did not fully analyse, failed to agree with the backcross; and he expected that **wa-1**, if it were linked with **c**, should show closer linkage with **p**, which it was not found to do. However, our analysis shows his F_2 and backcross data to agree in favouring linkage; and his second reason has no obvious justification.

It should be noted that the tests of the **c** locus against **d** come almost entirely from our new $\mathbf{c^{ch}}$/**d** data, the only published data on this pair being ninety-four mice from intercrosses with **c** (the equivalent of eleven fully classified backcross progeny) recorded by Durham in 1908.

In view of the evidence discussed above, the possibility that groups I, II and XI are really parts of one linkage group must be seriously considered. The evidence, however, is not conclusive because the statistical significance cannot be satisfactorily assessed. It will be difficult to improve the evidence because most of the standard errors are already small, and to reduce these materially would require the breeding of very large numbers of mice. Two estimates, however, have comparatively large standard errors, namely, **Wh** with **c** and with **d**, and by improving the accuracy of these it may be possible to obtain decisive evidence.

How reliably can it be asserted that the known linkage groups are independent of one another? An attempt may now be made to answer this question, assuming provisionally that linkage groups I, II and XI are mutually independent. Of the twelve* groups, eight are represented in the survey by two or more marker genes, three by one marker and one group (IV) is unrepresented; therefore there are 171 pairs which require to be tested

* *Note added in proof.* Since the submission of this paper two important new linkages have been reported: **Sd** with **fi**; $p=25\%$, order **a** – **fi** – **Sd** (Wallace, M.E., 1950, *Nature*, **166**, 407); and **fz** with **ln**; $p=41\%$ (Dickie, M. M. & Woolley, G. W., 1950, *J. Hered.* **41**, 193–6).

before even the absence of close linkage can be asserted. Of these pairs, forty-six, or about one-quarter, are still untested. Much therefore remains to be done before it can be reliably asserted that all the known linkage groups are mutually independent. However, the important gene pairs which are untested include comparatively few genes. Thus thirty-one of the untested pairs involve one or other of three genes, namely, **fi**, **ru** and the lethal **j**; further testing of the first two, at least, which mark one end of groups V and XII respectively, is badly needed. The remaining fifteen untested pairs all involve one or more of **bt**, **T**, **v**, **wa-2** or **Wh**; **bt** and **Wh** are both closely linked to other markers which have been fairly extensively tested, but there is no such cover for **T** or **v**; and **wa-2**, which occupies an important position in linkage group VII, is only loosely linked to other tested markers. Completion of the outstanding tests of **fi**, **ru**, **T**, **v** and **wa-2** would fill all the important gaps.

SUMMARY

This paper is a review of all published data on the supposed independence of the more important marker genes in the house mouse, using modern statistical methods to combine data from different sources. New data have been added whenever they happened to be available and the published data were lacking or inadequate.

About three-quarters of the necessary tests of the mutual independence of the known linkage groups (except IV) have been made. The least well-tested genes in the groups are **fi**, **ru**, **T**, **v** and **wa-2**.

The evidence suggests that linkage groups I, II and XI may not be independent, and further investigation is needed.

We are indebted to Miss Rita Phillips and Mrs Susan Sobey for assistance with some of the classification of mice and abstraction of data in Table 3; to Miss Mary Lyon and Mr G. A. Clayton for new data on **s**/**Va** and **Va**/**sex** segregations; and to Mr W. S. Russell for assistance with the computing.

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INDEPENDENCE OF LINKAGE GROUPS I, II AND XI IN THE HOUSE MOUSE

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In a recent review of independent segregation in the house mouse (Carter & Falconer, 1952) evidence was brought to light which suggested that linkage groups I, II and XI might not show independent segregation; in particular, *albinism* (**c**), *blue dilution* (**d**) and *waved*-1 (**wa-1**) gave signs of very loose linkage. In each case the standard error of the recombination fraction was already small, and to make a further test, involving any appreciable reduction of these standard errors, would have entailed raising very large numbers of mice.

An alternative approach was to test other markers in the three groups. If linkage really existed, some other pairs might be more closely linked than the apparently very loosely linked loci **c**, **d** and **wa-1**; and the closer linkage would be easier to demonstrate. In group XI another marker was available, namely *White* (**Wh**), located some distance from **wa-1**. **Wh** had already been tested for linkage with **c** and **d** (Bunker & Snell, 1948), but neither test gave a very precise estimate of the recombination fraction; for the pair **Wh**/**d** linkage closer than 35% was not excluded.

In the hope of obtaining decisive evidence we have bred a further two thousand mice from three-point balanced backcrosses in which **Wh** segregated with markers in groups I and II. In group I *chinchilla* ($\mathbf{c^{ch}}$) was used, rather than its allele **c**, because it is not epistatic to **Wh**; in group II *short-ear* (**se**), rather than **d** (to which it is very closely linked), in order to avoid an eight-colour classification. Segregation was always in the male parent. Viability interactions were guarded against by raising equal numbers of young from each of the four possible phase combinations of heterozygote (see Fisher & Mather, 1936). Difficulty in classifying $\mathbf{c^{ch}}$ in the presence of **Wh** was anticipated but not realized in practice; on the non-agouti brown background used in almost all the matings, the hairs of the inner surface of the pinna were straw yellow in the phenotype **Wh** and ivory cream in the phenotype **Wh** $\mathbf{c^{ch}}$. As a precaution against systematic personal errors of classification the breeding was done in two independent, complete experiments, each carried out by one of us.

The results are given in Table 1. The two sets of data are consistent one with another. All three single-factor segregations are good, and there is no evidence of viability interactions, which in any case would be overcome by the balanced experimental design. The recombination fractions are given in Table 2, together with those from the previously published data for comparison. In the new data there is no suggestion of linkage between $\mathbf{c^{ch}}$ and **se**, nor between **se** and **Wh**. The deviation from independence in the segregation of $\mathbf{c^{ch}}$ and **Wh** is just significant at the 5% level; but it is in the opposite direction to that in the earlier data, and therefore does not constitute convincing evidence of linkage. Thus the new data fail to support the suggestions of linkage contained in the

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old, and in consequence the genetical independence of linkage groups I, II and XI must be assumed.

It may be pointed out, however, that the cytological independence of these three groups does not necessarily follow from their genetical independence. Crew & Koller (1932) found that the mouse has, on the average, 2·4 chiasmata per bivalent in spermatogenesis; this corresponds with an average genetic linkage group map of 120 cM. A map of this length could accommodate two short linkage groups showing nearly independent

Table 1. *Phenotype of progeny**

Genotype of segregating parent	Wh++	Wh c^{ch}+	Wh+se	Wh c^{ch}se	+++	+c^{ch}+	++se	+c^{ch}se	Totals	
Wh++/+c^{ch}se	53	37	39	45	53	58	46	55	386	522
	23	18	16	12	17	15	18	17	136	
Wh c^{ch}+/++se	43	57	45	51	64	49	42	57	408	540
	16	19	11	19	19	14	18	16	132	
Wh+se/+c^{ch}+	47	48	69	47	50	52	54	45	412	563
	25	19	22	19	15	12	12	27	151	
Wh c^{ch}se/+++	43	59	71	48	45	49	48	46	409	543
	15	16	19	16	19	17	16	16	134	
								Totals	1615	2168
									553	

* The data from the two independent experiments are shown separately, the upper figures being Carter's and the lower Falconer's data.

Table 2. *Recombination fractions*

	Previous data		New data		
Loci	R.F.	S.E.	R.F.	S.E.	χ^2
c with **d** or **se**	56·19	±1·96	50·83	±1·07	0·60
se with **Wh**	40·95	±4·88	49·03	±1·07	0·81
c with **Wh**	58·82	±5·42	47·83	±1·07	4·08

segregation; and if there were chiasma localization, it could carry three genetically independent groups. A clear distinction is therefore to be made between cytological chromosomes and genetical linkage groups. This distinction is no longer a purely theoretical one, since Slizynski (1949) has described the cytological chromosomes and given them numbers which, of course, do not correspond with the numbers of the linkage groups adopted by Dunn, Grüneberg & Snell (1940). The identification of the linkage group (or groups) to which each chromosome corresponds is a task for the future.

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COPY No 3

Gene-tagged chromosome translocations in eleven stocks of mice.

by

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(Short title for page headings: Translocations in the mouse)

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INTRODUCTION

Mus musculus is the most widely used laboratory rodent and, as such, has received a good deal of cytological and genetic study. Yet there have been few attempts to correlate the cytological and genetic findings; all the available cytogenetic information about the mouse can be summed up in one sentence. Linkage groups V and VIII are carried in two large autosomes (Snell, 1946; Slizynski, 1952), group X in a medium sized autosome (Gates, 1927; Painter, 1927) and group XX in the differential segment of the sex chromosome (Falconer 1953).

Cytological studies have shown that the haploid number is twenty, and genetically fourteen linkage groups are known, including a sex-linked group. Cytological studies have also shewn, however, that the chiasma frequency is high, its mean value in spermatocytes ranging from about 2.5 chiasmata in the sex bivalent and long autosomes to about 1.5 in the shortest autosomes (Crew & Koller, 1932; Slizynski, 1954). This implies that the corresponding linkage group maps must range from about 125 to 75 centimorgans. The fourteen known linkage groups are all shorter than this, most of them much shorter; so it is by no means certain that they are really mutually independent. It is quite possible that two or more of them may represent different parts of a single group. Furthermore, there must be many linkages still undetected among the hundred or so mutants known in the mouse.

Thus formal genetical methods are of limited value in working out the cytogenetic constitution of the mouse. Fortunately other methods are available; they depend on the use of genetically marked ('tagged') chromosome

translocations. There are both genetical and cytological methods by which tagged translocations can be used to establish the chromosomal independence of linkage groups in the mouse. The genetic method, used by Snell (1946), consists of mating together heterozygotes for a translocation which affects two known linkage groups. By the appropriate use of marker genes it is possible to identify the euploid zygotes formed by the fusion of two aneuploid but complementary gametes; and some of these types of zygote cannot be formed unless the marked linkage groups are normally carried in different chromosomes. This method has the disadvantage that a translocation cannot be used for this purpose until both affected linkage groups have been identified.

There are two main cytological methods. The first is to study meiosis in an animal heterozygous for two translocations, each involving at least one identified linkage group; and to observe whether the two structural changes are independent or have one or more chromosomes in common. From this the independence or otherwise of the linkage groups can be inferred. Second, if it is possible to identify individual chromosomes by their chromomeres at pachytene (Slizynski 1949, 1952), the translocations may be studied separately and compared with others involving the same or different linkage groups; thus the groups corresponding with particular pachytene chromosomes may be determined.

Tagged inversions, if available, would serve the same purpose; and, since an inversion affects only one chromosome and one linkage group, interpretation is straightforward. However, no inversion has yet been reported in the mouse; no genetic evidence of inversions affecting linkage

group III was found among more than three hundred tested mice from irradiated fathers, though the same sample yielded many translocations, (Carter & Phillips, unpublished data).

Any cytogenetic attack on the problems of linkage and independence in the mouse will therefore depend entirely on the availability of tagged translocations. This paper describes the induction by X-rays of a number of translocations and the identification of the linkage groups associated with eleven of them.

Though the experiments were intended only to provide tagged translocations, additional light was thrown on several problems of translocation behaviour.

EARLIER WORK ON TRANSLOCATIONS IN THE MOUSE

Pioneer work on radiation-induced translocations in the mouse was done by Snell (1933, 1935), followed by Hertwig (1940), Koller & Auerbach (1941) and Russell (1952). Snell detected his translocations by the 'semisterility' they caused. A mouse of either sex, if heterozygous for a translocation, produces abnormally small litters when mated to a normal mouse. This is because a translocation heterozygote forms two main types of gamete, with ~~euploid~~ ~~eupbid~~ and aneuploid chromosome complements respectively. Snell, Bodemann & Hollander (1934) found that about half the zygotes, presumably those which arose from aneuploid gametes, die during embryonic development and can be detected by examining gravid uteri. Hertwig (1940) considerably extended this work and shewed that, in several semisterile lines, the greater part of the aneuploid zygotes died soon after implantation in the uterine wall; a small proportion died before implantation and a few more in late embryogeny.

Thus the inheritance of a translocation can be followed by observing litter-size or by counting live and dead embryos in a gravid uterus.

The reduction in fertility, which is a measure of the proportion of aneuploid gametes, may not be exactly 50%. In Snell's stocks the fertility of semisteriles ranged from 38 to 62% in females and from 41 to 69% in males. Russell (1952) found two stocks in which the translocation heterozygotes were of three types; in addition to the usual semisterile individuals, some were nearly or completely sterile.

Snell and Hertwig were able only to infer that the semisterility which they studied was caused by translocations; but Koller & Auerbach (1941) obtained cytological confirmation of this by examining meioses in semisterile males. Genetic evidence to the same effect was obtained later by Snell (1946), using genetic tags.

A translocation in the mouse is inherited in a quasi-Mendelian manner. When a heterozygote is mated to a normal mouse, half of their viable progeny are normal and the other half are semisterile heterozygotes (Snell, 1935, 1946; Hertwig, 1940). If a semisterile animal is heterozygous for a marker gene in one of the translocated chromosomes, its progeny shew linkage between semisterility and the marker, as though the semisterility were due to a dominant gene located at the point of the chromosome break. If the linkage is sufficiently strong the marker may then be used as a tag for the translocation. If both translocated chromosomes are marked, the two markers shew linkage with each other, in addition to their usual linkages. Using these principles Snell (1946) found single markers for two translocations and two markers for a third; it was by means of the doubly marked translocation that he was able

to prove that linkage groups V and VIII are normally carried in different chromosomes.

Homozygotes for translocations were obtained by both Hertwig (1940) and Snell (1946) from matings between translocation heterozygotes. They were viable and fully fertile; when mated to normal mice all their progeny were semisterile heterozygotes.

METHODS AND STOCKS

(i) X-irradiation

Translocations were induced by X-rays in mature sperm carried in adult males. Each male to be irradiated was first immobilised by anaesthesia. Veterinary Nembutal (Abbott) was diluted elevenfold (v/v) with Ringer's saline and injected intraperitoneally, 0.1 ml. per 10 gm. body weight; fairly deep anaesthesia followed within five minutes. The male was then laid on his back and a rubber band passed over the lower abdomen; this held him in position and prevented retraction of the testes from the scrotum. Anterior to the band the body was shielded by lead.

Irradiation was from above, with the following constants: tube voltage 70 KV, current 7mA; filtration 0.5 mm. Al, HVL 0.8 mm. Al; dose-rate 180 r/min., total dose 700 r.

(ii) Linkage detection procedure

Nine linkage groups were selected for investigation, namely I, II, III, V, VII, VIII, IX, XI, and XX. The marker genes chosen were those which have the

least effect on viability and fertility, are fully penetrant, and are not mutually epistatic; they are listed in table 1. Two stocks of mice were built up; one, designated PCS, was homozygous for the five recessive markers (short-ear and dilution counting as one marker, being very closely linked); the other, YX, carried the dominant markers. Brachyury was necessarily kept heterozygous, being lethal when homozygous; Caracul and White were homozygous or heterozygous.

Males from the YX stock were irradiated and immediately placed with PCS females, three or four each. F1 males heterozygous for at least seven markers, including sex, and preferably for all nine, were tested for semisterility. Those found to be fully fertile or sterile were discarded. Proven semisteriles were mated to PCS females, thus constituting backcross matings segregating for semisterility and for seven to nine markers, all in the coupling phase. About twenty-five backcross progeny from each F1 male were tested for semisterility, and the segregation of semisterility from each marker analysed for linkage. This experimental design followed from a preliminary study based on the 'swept radius' concept (Carter & Falconer, 1951), which led to the following conclusions:-

(i) The F1 mouse should be heterozygous for the greatest possible number of marker genes.

(ii) It is more efficient to test his backcross progeny for semisterility than to look for linkages between the marker segregating.

(iii) If no linkage has been discovered by the time twenty-five or thirty backcross progeny have been tested, the translocation should be discarded.

TABLE 1. Linkage groups and marker genes used in the primary tests.

Linkage group	Dominant marker	Recessive marker
I		c^{ch} chinchilla
II		se, d short-ear, dilution
III		s piebald spotting
V		a nonagouti
VI	Ca Caracul	
VIII		b brown
IX	T Brachyury	
XI	Mi^{wh} White	
XX	d Sex	

While the PCS and YX stocks were being built up, linkage tests were started with nine other translocations. These had been obtained in an earlier experiment intended to yield inversions. In this experiment males of the CWX stock, carrying the linkage group III markers piebald spotting (s), macrocytic anaemia (W^v) and luxate (lx) were irradiated and mated to wild-type females of the CBA inbred strain. Some of the irradiated males carried also nonagouti (a) or tailless-lethal (t^L).

Once fairly close linkage had been found between semisterility and a marker, with recombination 20% or less, the marker was used as a tag in subsequent linkage tests. All tagged semisterilities were further tested against the other eight markers, the target in each test being 100 backcross young. Tests have now been started with markers in other linkage groups, but they are not yet complete and the results are not given in this paper.

(iii) Semisterility diagnosis

Semisterility was diagnosed by one or other of three methods, namely (a) autopsy of pregnant females, (b) progeny test, (c) litter-size. Methods (b) and (c) were used only rarely.

The presence of a small proportion of implanted but inviable embryos is a normal feature of a gravid mouse uterus. The mere presence of dead embryos is not, therefore, evidence that one parent is semisterile. Conversely, the absence of dead implantations does not constitute evidence that neither parent is semisterile, since this may be a chance distribution effect. A test based on the examination of a single gravid uterus may thus be inconclusive or misleading. At the beginning of the work, therefore, only males were tested for semisterility, and several females were used to test each male. A firm diagnosis was made only when the

findings were consistent; if there was any doubt, further tests were made. Females were sacrificed when visibly pregnant and the embryos had reached at least the 11½-day stage. Each implantation was classified as 'live embryo', 'dead embryo' or 'mole'. The ages of live and dead embryos were estimated by Grüneberg's (1943) 'external landmarks' method. Corpora lutea were not counted.

After 258 males had been tested by this method the records were analysed and a set of test criteria drawn up (Table 2) which enabled a diagnosis to be made, in many cases, on the evidence of the first uterus examined. These criteria were such that, applied to past records, a test using only one female would have led to a firm diagnosis of the fertility status of four males out of five, and 99% of these diagnoses would have been correct. Further test would have been required for the remaining one male in five. Thereafter the criteria were used in all semisterility tests based on embryo counts, and females as well as males were tested by this method. When the initial test was inconclusive, information about the fertility status was lost if the animal under test was female and, as such, had been killed to permit embryo counts; if male, a further test was made.

More extensive tests were made of the fertility status of the F1 males which had developed from irradiated sperm. In all cases at least two females were used, and more if the diagnosis was other then 'fertile'.

Semisterility diagnosis by progeny test was used only when the animal under test was female and was required for subsequent breeding. If any of her progeny by a normal male was found to be semisterile, it was concluded that

she also was semisterile.

Semisterility diagnosis by litter-size was used only for females required for breeding. The female was diagnosed as semisterile if her first three litters by a normal male totalled twelve young or fewer. Diagnoses based on litter-size evidence were usually confirmed by progeny test.

(iv) Translocation homozygous stocks.

When a tag had been found for a translocation, an attempt was sometimes made to obtain translocation homozygotes. This entailed mating translocation heterozygotes together, followed usually by selection of individuals homozygous for the tag; these, the putative translocation homozygotes, were tested for full fertility. A homozygous stock was then set up.

RESULTS

The experimental results are presented in two sections. The first deals with the breeding performance of the F1 males, i.e. those representing zygotes which developed from irradiated sperm. The second section deals with the linkage tests and breeding performance of the descendants of those F1 males which were diagnosed as semisterile.

1. THE F1 GENERATION

This section refers only to the main experiment in which the YY and PCS stocks were used. Comparable data are not available for the preliminary experiment with CWX and CBA mice. The fertility diagnoses of the forty-five tested F1 males are summarised in table 3.

(i) Fully fertile F1 males

Twenty-six F1 males were diagnosed as fully fertile; they sired sixty litters which were examined in utero and five which were allowed to come to term. At the time of autopsy 89% of all implanted embryos were still alive; this figure is in the normal range for mouse stocks. The mean number of live embryos per uterus at autopsy, 7.3, was close to the mean number of liveborn young at term, 7.2, suggesting that in the normal course of events there were few foetal deaths in the later stages of pregnancy.

(ii) Heritable semisterility

Eight F1 males shewed semisterility and transmitted it to their descendants. They sired 202 litters of which 37 were examined in utero. The mean number of implantations per uterus was 8.0, close to the corresponding number for males diagnosed as fertile, 8.2; this suggests that few of the zygotes died before implantation. Of the implanted zygotes, 38% were alive.

Six carried translocations which were subsequently found to be linked to marker genes used in the tests; no linkage was found for the other two and they were discarded.

(iii) Non-heritable semisterility

Table 4 summarises the breeding records of two F1 males, YF/101.1bd and YF/104.1fd. They were originally diagnosed as semisterile, on the basis of autopsy data; live-born litter sizes confirmed the diagnoses. Yet all seven sons of YF/101.1bd, and twelve of YF/104.1fd, were found to be fertile. In

TABLE 2. Semisterility test criteria

Dead implantations	Total implantations 1-5	6	7	8	9	10	11+
5+	S	S	S	S	S	S	S
4	S	S	S	S	S	S	S
3	I	I	I	I	I	I	I
2	I	I	I	I	I	I	F
1	I	F	F	F	F	F	F
0	I	F	F	F	F	F	F

F, fertile; I, test inconclusive;
S, semisterile.

TABLE 3. Fertility status of F1 males sired soon after their fathers had been exposed to X-rays (700r. in the testes).

Status diagnosed	Number
Fertile	26
Semisterile (heritable)	8
Semisterile (non-heritable)	2
Quasisterile	2
Sterile	7
Total	45

the case of YF/101.1bd the absence of semisterile sons might perhaps be due to chance deviation from a one-to-one ratio (P = 0.008), but this explanation is very unlikely in the case of YF/104.1fd (P = 0.00024). Neither male carried a t-series lethal, since both were short-tailed heterozygotes Tt. Linkage between an induced translocation and a t-series lethal, together with the known anomalous segregation of these lethals in males (Chesley & Dunn, 1936) cannot, therefore, be invoked to explain the absence of semisterile progeny of these males. Its origin remains unknown.

(iv) Quasisterility and sterility

Nine F1 males failed to sire any liveborn young, though all remained with three or more fertile females for at least eight weeks. Two of them produced copulation plugs but no ensuing implantations. Two more sired a few live embryos, but no evidence was found of young being brought to term; these two, YF/91.1bd and YF/106.1dd, were diagnosed as quasisterile.

Table 5 summarises their breeding performance. The number of implantations per uterus sired by YF/106.1dd was normal, but the proportion of live embryos was very low (12%). The number of implantations per uterus sired by YF/91.1bd was low, 4.2; this is about half the corresponding number for fertile and semisterile males, and suggests pre-implantation zygotic death.

TABLE 4. Total implantations (I), live embryos (L), sizes (S) and numbers (N) of litters sired by two F1 males shewing non-heritable semisterility.

Male YF/101.1b					Male YF/104.1f				
Litters in utero			Litters at term		Litters in utero			Litters at term	
♀	I	L	S	N	♀	I	L	S	N
1	8	6	1	1	1	8	3	1	0
2	7	5	2	1	2	10	7	2	1
3	8	6	3	7	3	12	7	3	7
4	7	3	4	5	4	7	4	4	1
5	7	1	5	5	5	9	7	5	3
6	6	2	6	1	6	8	7	6	3
Total	43	23	-	20	-	54	35	-	15
Mean	7.17	3.83	3.75	-	-	9.00	5.83	3.81	-

TABLE 5. Total implantations (I) and live embryos (L) sired by two quasisterile F1 males.

Male YF/91.1b Litters in utero			Male YF/106.1d Litters in utero		
♀	I	L	♀	I	L
1	2	2	1	9	1
2	4	3	2	9	2
3	12	6	3	6	0*
4	3	3	-	-	-
5	3	2	-	-	-
6	1	1	-	-	-
7	1	1	-	-	-
8	8	4	-	-	-
Total	34	22	-	24	3
Mean	4.25	2.75	-	8.00	1.00

*Copulation plug seen.

2. THE BACKCROSS AND SUBSEQUENT GENERATIONS.

This section refers to the eleven translocations, from both experiments, for which tags were found. A further nine semisterile lines were unsuccessfully tested and subsequently discarded, including the two in which semisterility proved to be non-heritable. Data referring to these are not reproduced.

(i) Linkage tests

Table 6 summarises the results of the initial tests for linkage between semisterility and marker genes whereby tags were found for eleven translocations. For translocations T1 to T8 the mice tested were progeny of the F1 male which had founded the stock; for the other translocations more remote descendants were used, as the necessary markers were not all present in the original cross. Table 7 summarises data on the segregation of semisterility from the translocation tag; it includes the results given in Table 6 as well as those obtained subsequently. Linkage between the translocation and its tag was close in all instances except that of T138, which shewed 19% recombination with d and se. The data of Table 7 probably overestimate recombination, since some of the apparent recombinants were females; these had to be killed before their fertility status could be diagnosed and were therefore not available for confirmatory tests. The occurrence of recombination between the translocation and its tag can be considered firmly established only for T2, T7, T138 and T264; for these translocations some of the recombinants were males and their fertility status diagnoses were confirmed by repeated tests.

The position of the translocation break relative to other markers in the

linkage group has been established only for T264. This translocation shows 4% recombination with W^v and 22% with lx, both in Linkage Group III; the break therefore lies either between lx and W^v, or on the far side of W^v with respect to lx. If the break were between lx and W^v, recombination between the break and W^v would imply recombination between lx and W^v, unless there were double crossing over. In fact, recombination between the break and W^v has been observed three times; on one occasion lx was not segregating, and on both of the other two lx remained with W^v. The order is thus established as lx, W^v, T264 break.

Table 8 summarises the results of tests for linkage between translocation tags and the remaining marker genes used in the experiment. They confirmed what had been suspected from the tests with semisterility, namely that Linkage Group V is involved in T7, as well as Group XI. In addition they disclosed two new linkages: T1 involves Group V as well as Group VIII, and T138 involves Group IX as well as Group II. These linkages are both very loose (table 9).

(ii) Segregation of tags.

The single-factor segregations of tags provide information about the segregation of the translocations. Data from ten of the eleven stocks are summarised in table 10. Data for T138 have been omitted, because this translocation is only loosely linked to d and se; their segregation would therefore be a poor reflexion of the segregation of the translocation.

With one exception, there is good, Mendelian segregation of the tag in all ten stocks. The exception is T190; here there is a statistically

TABLE 6. Tests for close linkage between marker genes and the eleven translocations for which tags were found.

Trans-location	Foundation F1 mouse	I		II		III				V		VI		VIII		IX		XI		XX	
		c^{ch}		d_2	se	s		W^v		a		Ca		b		T		Mi^{wh}		δ	
T1	YF/99.1dδ	8	16	19	7	10	16	-	-	14	9	11	15	26	0	-	-	16	9	16	10
T2	YF/100.1cδ	18	13	22	12	14	8	-	-	29	4	-	-	21	11	-	-	20	14	18	16
T5	YF/99.1cδ	-	-	22	10	15	17	-	-	30	0	16	16	16	16	-	-	19	13	17	15
T6	YF/103.1bδ	11	12	9	14	18	0	-	-	11	12	14	9	12	10	-	-	12	11	10	13
T7	YF/108.1bδ	15	5	-	-	9	8	-	-	16	4	8	12	7	11	8	12	20	0	12	8
T8	YF/130.1bδ	15	0	13	3	7	8	-	-	12	4	-	-	11	5	-	-	6	10	6	10
T83	CWF/13.1a♀	4	0	14	11	3	4	10	15	25	0	0	3	4	9	13	12	11	14	13	12
T138	CWF/52.1cδ	7	12	18	4	-	-	36	26	10	11	25	16	10	12	25	15	19	22	36	27
T190	CWF/59.1cδ	14	11	11	14	13	4	15	10	13	12	9	16	12	13	25	0	13	12	14	11
T264	CWF/135.1aδ	8	2	5	6	4	2	25	0	16	9	2	9	7	6	10	7	9	11	19	6
T281	CWF/156.1cδ	11	14	15	10	8	8	15	10	11	14	15	10	14	11	8	17	24	1	13	12

Each entry in the table gives the numbers of old and new combinations.

TABLE 7. Measurement of the linkage between eleven translocations and their tags. This table includes the data from table 6.

Translocation, Tr	Tag, Tg or tg	Backcross progeny: Tr Tg	Tr tg	+Tg	+tg	Total	Recombination (%): Mean	Upper limit (2σ%)	Remarks about recombinants
T1	$+^{b}$	48	0	1	29	78	1.3	7.1	♀ 9 implantations, all alive. Possible misclassification of +/b as she was also aa dd Mi^{wh}+
T2	$+^{a}$	37	3	5	41	86	9.3	17.4	Include re-tested ♂♂
T5	$+^{a}$	25	0	2	29	56	3.6	12.5	♀ 7 implantations, all alive ♀ 7 implantations, 6 alive
T6	$+^{s}$	24	1	0	17	42	2.4	12.9	♀ 8 implantations, 3 alive
T7	Mi^{wh}	23	2	1	22	48	6.2	17.3	Include re-tested ♂
T8	$+^{c}$	17	0	0	26	43	0.0	8.1	
T83	a	0	38	19	0	57	0.0	6.3	
T138	$+^{d}$	36	8	8	32	84	19.0	27.6	Include re-tested ♂♂
T190	t^{b}	0	70	31	1	102	1.0	5.5	♀ long-tailed, but not genetically proven; could be a normal overlap T+
T264	W^{v}	51	1	2	20	74	4.0	11.4	Include re-tested ♂♂
T264	lx	3	17	0	2	22	21.7	44.3	Include re-tested ♂♂
T264	$+^{lx}$	1	0	0	0	1			
T281	$+^{mi}$	1	30	29	0	60	1.7	8.8	♀ 9 implantations, 5 alive

TABLE 8. Tests for loose linkage between marker genes and the tags for eleven translocations.

Trans-location	Tag	I c^{ch}		II dse		III s				V a		VI Ca		VIII b		IX T		XI Mi^{wh}		XX d	
T1	$+^{b}$	24	27	102	116	55	61	-	-	142	75	85	91	-	-	16	20	111	97	110	133
T2	$+^{a}$	185	185	216	204	154	116	-	-	-	-	-	-	204	177	191	146	107	111	153	142
T5	$+^{a}$	54	45	131	107	62	73	-	-	-	-	134	141	126	121	78	95	131	96	112	103
T6	$+^{s}$	20	35	75	71	-	-	-	-	33	23	81	59	29	33	24	22	30	34	66	73
T7	Mi^{wh}	116	97	48	52	41	51	-	-	306	70	58	51	22	9	62	56	-	-	116	93
T8	$+^{c}$	-	-	59	43	27	29	-	-	57	45	-	-	48	52	26	32	17	18	57	54
T83	a	151	122	196	199	67	72	44	40	-	-	50	43	122	156	196	148	117	114	246	268
T138	$+^{d}+^{se}$	89	89	-	-	36	36	116	132	80	105	13	16	110	120	212	142	17	27	177	209
T190	t^{6}	81	98	108	100	10	14	32	29	104	112	97	102	83	90	-	-	55	55	129	151
T264	W^{v}	128	143	155	143	32	26	-	-	137	140	182	156	139	134	165	167	127	146	430	423
T281	$+^{mi}$	88	103	116	125	10	14	46	44	113	123	71	81	103	98	22	16	-	-	145	145

Each entry in the table gives the numbers of old and new combinations

TABLE 9. Measurement of the linkage between three translocation tags and the marker genes with which a second linkage was found. This table includes the data of Table 8.

Trans-location	Tag, Tg	Marker, M	Phase*	Backcross progeny					Recombination (%) and standard error
				Tg M	Tg m	tg M	tg m	Total	
T1	$+^{b}$	$+^{a}$	C	61	33	42	81	217	34.6 ± 3.2
T7	Mi^{wh}	$+^{a}$	C	147	33	37	159	376	18.6 ± 2.0
T138	$+^{d}$	T	C	80	58	57	103	298	40.1 ± 2.6
			R	9	19	10	18	56	

*C, coupling; R, repulsion.

significant excess of tagged individuals among the progeny of male heterozygotes. However, T190 is tagged by t^6, which is similar to t^o or identical with it (Dunn & Gluecksohn-Schoenheimer, 1950); and t^o segregates in a non-Mendelian manner in heterozygous males (Chesley & Dunn, 1936). The excess is therefore probably a property of the tag and not of the translocation.

(iii) Intercross matings and homozygous stocks

Intercross matings of heterozygotes were made for four of the five translocations of the preliminary series, namely T83, T138, T264 and T281: in each case fertile homozygotes were obtained. No intercrosses were made for T190, because the homozygote would be of the genotype t^6/t^6 and therefore inviable. In the translocation intercrosses for T281 only one parent carried the tag; the intercrosses for T83, T138 and T264 were intercrosses for the tags also. In the T264 intercrosses the translocation was in repulsion with W^v, i.e. it was tagged by $+^w$; coupling intercrosses would have provided translocation homozygotes which were homozygous also for W^v and, as such, sterile (Little & Cloudman, 1937). Translocation homozygotes were identified by fertility test of intercross progeny homozygous for the tag.

Table 11 shows the intercross segregation of the tag in the two stocks, T83 and T264, in which the tag is closely linked to the translocation; the intercross segregation of the tag therefore closely reflects the intercross segregation of the translocation. The T264 intercrosses shew a deficiency of W^vW^v homozygotes, which is usual (Little & Cloudman, 1937; Grüneberg, 1952). For both translocations the putative homozygotes constitute about a quarter of the intercross progeny

TABLE 10. Segregation of tags in the progeny of heterozygotes for ten translocations.

Trans-location	Tag	Male heterozygotes		Female heterozygotes		All heterozygotes	
		Tag	No tag	Tag	No tag	Tag	No tag
T1	$+^{b}$	129	141	37	22	166	163
T2	$+^{a}$	183	178	126	106	309	284
T5	$+^{a}$	102	103	53	54	155	157
T6	$+^{s}$	90	96	46	54	136	150
T7	Mi^{wh}	105	104	38	54	143	158
T8	$+^{c}$	103	115	8	10	111	125
T83	a	284	245	150	132	434	377
T264	W^{v}	422	431	64	52	468	483
T281	$+^{mi}$	168	122	-	-	168	122
Total:		1586	1535	522	484	2090	2019
T190	t^{6}	212	68	97	111	309	179
Grand total:		1780	1603	619	595	2399	2198

TABLE 11. Segregation of tags in translocation intercrosses.

Genotype of parents	Phenotype of progeny		
T83a/++ x T83a/++	+ 31		a 10
T264+/+W^{v} x T264+/+W^{v}	W^{v}W^{v} 2	W^{v}+ 22	++ 7

(iv) Intrauterine death

In the course of the linkage tests a large amount of information was obtained about the proportion of live and dead embryos in matings where one parent was a semisterile heterozygote or a fertile normal or homozygous segregant from a translocation stock.

For these purposes semisterility classifications based on embryo counts cannot be used, since this would constitute argument in a circle. Instead, only cases where the fertility status can be inferred from the segregation of a tag are considered here. No sufficiently close tag is available for T138, so this translocation is again excluded.

Table 12 gives the results obtained. As corpora lutea were not counted, it is not possible to determine the number of shed ova which failed to implant, and hence it is not possible to determine directly the pre-implantation relative embryonic viability. However, it is possible to determine both the post-implantation and the total relative viabilities (table 12), and the difference between them gives a measure of the pre-implantation viability. This difference was significant only for T6 male heterozygotes and T8 heterozygotes of both sexes; in these stocks the semisterile parents had only 30 to 40% as many live embryos as the fertile parents, and 10 to 14% of the total embryonic deficiency was accounted for by pre-implantation loss. It is not known whether this was due to failure of fertilisation or to death in early embryonic stages.

For seven of the ten stocks it is possible to compare the viabilities of embryos of male and female heterozygotes. This is not possible for the

other three stocks, either because no data are available from female heterozygotes (T83, T264), or because the gametic segregation in the male is upset by the linked tag (T190). In all seven stocks both the total survival and the post-implantation survival were lower for female heterozygotes; this appears to be due chiefly to differences in post-implantation viability. However, the total difference between the sexes, summed over all seven stocks, is less than twice its standard error; so the data, though suggestive, do not by themselves establish the existence of a sex difference.

One translocation, T6, shewed anomalous behaviour similar to that reported by Russell (1952). Of twenty-eight males tested for fertility, twenty fell into the usual two categories, semisterile and fertile. Of the remaining eight, all known from the presence of the tag to carry the translocation, four sired no embryos at all; the other four sired only twenty-five implantations in ten uteri, ten of the embryos being alive. Thus there was a very great reduction in pre-implantation viability, but little or no change in post-implantation viability. It is not clear whether T6 females shew the same effect, as fertility tests of females cannot be repeated.

Data on the fertility of homozygotes are available for only three translocations, namely T83, T264 and T281, and only for tested males. They did not differ appreciably from fully fertile normal segregants in the number and viability of embryos sired.

TABLE 12. Relative viability of embryos in matings of translocation stock animals to unrelated mice.

Trans-location	Sex	Fertility* status	Embryos† L	I	N	L/N	I/N	L/I	Relative viabilities: Post-implantation $(L/I)_s/(L/I)_f$	Relative viabilities: Total $(L/N)_s/(L/N)_f$
T1	♂	S	111	260	36	3.08	7.22	0.427		
		F	108	124	15	7.20	8.27	0.871	0.490 ± 0.039	0.428 ± 0.058
	♀	S	65	184	27	2.41	6.81	0.353		
		F	187	205	28	6.68	7.32	0.912	0.387 ± 0.040	0.361 ± 0.052
T2	♂	S	192	438	60	3.20	7.30	0.438		
		F	221	260	41	5.39	6.34	0.850	0.516 ± 0.031	0.594 ± 0.059
	♀	S	86	249	34	2.53	7.32	0.345		
		F	270	292	39	6.92	7.49	0.925	0.374 ± 0.033	0.366 ± 0.045
	♂	S	204	401	50	4.08	8.02	0.509		
		F	140	149	20	7.00	7.45	0.940	0.541 ± 0.053	0.583 ± 0.064
	♀	S	41	116	17	2.41	6.82	0.353		
		F	174	188	28	6.21	6.71	0.926	0.382 ± 0.127	0.388 ± 0.067
T6	♂	S	104	259	43	2.42	6.02	0.401		
		F	122	147	19	6.42	7.74	0.830	0.484 ± 0.041	0.377 ± 0.050
	♀	S	53	152	21	2.52	7.24	0.349		
		F	175	190	25	7.00	7.60	0.921	0.379 ± 0.043	0.360 ± 0.056
T7	♂	S	92	196	29	3.17	6.76	0.469		
		F	181	195	27	6.70	7.22	0.928	0.506 ± 0.040	0.473 ± 0.061
	♀	S	28	65	9	3.11	7.22	0.431		
		F	169	190	24	7.04	7.92	0.889	0.484 ± 0.070	0.442 ± 0.090
T8	♂	S	116	231	38	3.05	6.08	0.502		
		F	112	118	15	7.47	7.87	0.949	0.529 ± 0.036	0.408 ± 0.054
	♀	S	23	54	10	2.30	5.40	0.426		
		F	152	168	22	6.91	7.64	0.905	0.471 ± 0.075	0.333 ± 0.075
T83	♂	S	146	330	41	3.56	8.05	0.442		
		F	361	413	53	6.81	7.79	0.874	0.506 ± 0.064	0.523 ± 0.051
		H	131	136	19	6.89	7.16	0.963		
	♀		No data							
T190	♂	S	159	474	61	2.61	7.77	0.335		
		F	294	344	40	7.35	8.60	0.855	0.392 ± 0.068	0.355 ± 0.035
	♀	S	118	305	45	2.62	6.78	0.387		
		F	232	249	34	6.82	7.32	0.932	0.415 ± 0.074	0.384 ± 0.043

1264	♂	S	419	957	124	3.38	7.72	0.438	
		F	463	506	65	7.12	7.78	0.915	0.478 ± 0.039	0.475 ± 0.032
		H	75	81	11	6.82	7.36	0.926	
	♀	No data							
1281	♂	S	367	948	123	2.98	7.71	0.387	
		F	774	863	111	6.97	7.77	0.897	0.432 ± 0.043	0.428 ± 0.027
		H	58	64	9	6.44	7.11	0.906	
	♀	S	110	286	41	2.68	6.98	0.385	
		F	202	241	30	6.73	7.13	0.944	0.407 ± 0.077	0.398 ± 0.047

F, fertile; H, translocation homozygote; S, semisterile heterozygote.

T, total implantations; L, total live embryos; N, number of uteri.

DISCUSSION

The experiments reported here are of interest chiefly because they demonstrate the possibility of obtaining large numbers of tagged translocations, provided that an efficient experimental design is used. Nine linkage groups were used in the present work, and translocations were found involving seven of them. The translocations are now available for genetic and cytological study, and should provide a means of resolving a number of outstanding problems of linkage and independence in the mouse.

The information obtained about mouse translocation behaviour largely confirms what Snell (1946) had already found. In particular, our data confirm his conclusions that (a) translocations segregate one-to-one in heterozygotes, (b) the relative embryonic viability tends to be lower with female than with male heterozygotes, and (c) with some translocations, at least, the relative embryonic viability with female heterozygotes is significantly lower than 50%. We also confirm Russell's (1952) finding that occasional translocations throw anomalous sterile or nearly sterile individuals. Our translocation, T6, which shewed this behaviour is closely tagged by the normal allele of piebald spotting, s; and all the ss homozygotes tested (except one female) shewed normal, full fertility. Hence the anomalous behaviour cannot be explained by supposing that two translocations were present in this stock. It is clearly a characteristic of the translocation in question, but its mechanism remains obscure.

Few inferences can be drawn from translocation intercross segregation data, since there are thirty-six theoretically possible modes of gamete

formation when, as Koller (1944) has shewn, numerical non-disjunction may occur. However, considerations of symmetry shew that type and translocation homozygotes should always be formed in equal numbers. There are three bodies of data testing this point, namely Hertwig's (1940), Snell's (1946) for his T(I;?)c and ours for T264. They total 39 type and 26 translocation homozygotes; the ratio does not significantly differ from equality.

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SUMMARY

The cytogenetic problems of correlating linkage groups in the house mouse with their chromosomes, and of establishing the chromosomal independence of known linkage groups, call for the use of numerous genetically tagged translocations. Eleven new translocations have been induced and tagged; they involve linkage groups I, II, III, V, VIII, IX and XI.

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The problem of partial sex linkage in the mouse.

The chromosomes of *Mus musculus* have a high chiasma frequency[1] and for this reason very loose linkages are to be expected. Many of the problems of linkage and independence in this species may therefore have to be solved by cytogenetic methods rather than the breeding techniques of formal genetics.

Among them is the question whether linkage group VII is carried in the pairing segment of the sex chromosome. Partial sex linkage of two group VII mutants, *waved*-2, (*wa*-2) and *shaker*-2 (*sh*-2) was reported in 1947 by Wright[2]. However, there were two unusual features in her data, namely (a) recombination was in excess of 50%, and (b) *wa*-2 and *sh*-2 shewed the same recombination with sex, though they are not closely linked. As a result, the partial sex linkage interpretation has not been universally accepted. Carter & Phillips[3] repeated Wright's experiment, but failed to find any consistent evidence of sex linkage.

With the object of obtaining evidence on questions such as this we have induced a number of translocations in the mouse, using X-rays, and have identified linkage groups involved in eleven of them. One translocation, T8, involves groups I and VII. The linkage with group I was found by backcrossing animals heterozygous for T8 and for the group I marker *chinchilla* (*c*^{ch}) to chromosomally normal $c^{ch}c^{ch}$ homozygotes, and testing their progeny for the semisterility which indicates the presence of the translocation. No recombinants were found among forty-seven tested gametes. The *c*-locus was then used to 'tag' the translocation in further linkage tests, and was found to shew close linkage with *wa*-2 and loose linkage with *Rex* (*Re*), also in

group VII:-

Genotypes of parents	Phenotypes of progeny			
T8++/+c^{ch}wa-2 x +c^{ch}wa-2/+c^{ch}wa-2	++ 8	+wa-2 0	c^{ch}+ 1	c^{ch}wa-2 12
T8++/+c^{ch}Re x +c^{ch}+/+c^{ch}+	+Re 5	++ 11	c^{ch}Re 14	c^{ch}+ 3

Translocation T8 thus offers a means of settling the question whether linkage group VII is sex linked. The translocation and the sex bivalent should be cytologically recognisable in primary spermatocytes; it should therefore be possible to establish their chromosomal independence or interdependence.

This work was part of a programme carried out for the Medical Research Council.

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9th April, 1954.

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[1]Crew, F.A.E. & Koller, P.C. J. Genet. 26, 359 (1932).

[2]Wright, M.E. Heredity 1, 349 (1947).

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Cytological study of mice heterozygous for the translocation T8, mentioned above by Carter, Lyon & Phillips, shews that it does not involve the sex

chromosomes. The XY-bivalent is clearly recognisable in the accompanying microphotographs of diakinesis and metaphase nuclei of T8/+ males and, independent of it, the translocation figure. Analysis of the pachytene figures in the other two photographs has shewn that the translocated chromosomes are the medium-sized autosomes numbered 11 and 13 in the preliminary pachytene map of the mouse[1].

It follows that loci in linkage groups I and VII in the house mouse cannot be partially sex linked.

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1 and 2. Diakinesis and metaphase 1 in spermatocytes of T8/+ males, shewing the independent XY bivalent and translocation figure.

3 to 6. Microphotographs and camera lucida drawings of T8 in two spermatocyte pachytene nuclei. Autosome 11 is at the upper left part of each figure, autosome 13 lower right; the numbers refer to divisions on the preliminary pachytene map; C denotes the centromere.

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LINKAGE BETWEEN FIDGET AND AGOUTI IN THE HOUSE MOUSE

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INTRODUCTION

THE recessive gene fidget in the mouse (symbol *fi* ; Grüneberg, 1943) influences behaviour (head shaking, circling), the cornea and the skeleton (polydactylism and some other effects not yet described). Linkage tests of *fi* with tan, a^t, albinism, *c*, and blue dilution, *d*, all gave negative results ; there was no close linkage, but the possibility of loose linkage could not be excluded.

During 1949 further breeding records led us independently to suspect loose linkage between *fi* and a^t. The combined results, which are presented in this paper, establish its existence ; recombination is about 33·5 per cent. Work is now proceeding to determine the position of *fi* with respect to that of pallid, *pa*, which is also linked to a^t (Roberts and Quisenberry, 1935).

MATERIAL AND DATA

Two types of mating were used, namely repulsion intercrosses $a+/a^tfi \times a+/a^tfi$ and coupling intercrosses $A+/a^tfi \times A+/a^tfi$; but the progeny of one set of matings of the latter type were divided into three phenotypes with respect to the agouti locus, based on the appearance of the belly (light versus dark) as well as the back (agouti versus black).

The data are shown in table 1, which also reproduces those published previously (Grüneberg, 1943).

Several other mutants were also present in some of the matings ; they were brown, *b*, albinism, *c*, luxate, *lx*, pinkeyed dilution, *p*, and macrocytic anæmia, W^v. It was not possible to classify fully for pinkeyed dilution, owing to the simultaneous segregation of albinism ; nor was it possible to put any confidence in the classification of luxate, since the double heterozygote $+fi+lx$ was found to resemble the homozygote *lxlx* and the heterozygote $+lx$ often shows polydactyly similar to that seen in many fidgets. The segregations of *fi* with *b*, *c*, *p* and W^v are shown in table 3.

TABLE 1

Segregation of fi *and* a^t

Author and date	Mating *	Phenotypes and numbers of progeny							Segregation of *fi* †					Segregation of a^t † ‡				
								Total	*D*	*I*	χ^2	*DF*	*P*	*D*	*I*	χ^2	*DF*	*P*
Edinburgh :—		a^t +	a^t *fi*	*a* +	*a fi*													
Falconer, new data	I.R.	21	10	16	...	...	...	47	−9·3	250·6̇	0·348	...	...	−22·6̇	150·6̇	2·050	...	...
Carter, new data	I.R.	34	13	16	3	...	...	66	−2·6̇	352·0	0·020	...	...	−13·3̇	352·0	0·505	...	...
		AA +	*AA fi*	*Aa*^{t} +	*Aa*^{t} *fi*	a^ta^t+	a^ta^t *fi*											
Carter, new data	I.C.	16	2	45	13	16	10	102	−2·6̇	544·0	0·013	...	...	+32·0	816·0	1·255	...	...
Sum . .	...	...	...	...	...	...	...	...	...	...	0·381	...	...	...	...	3·810	...	...
Deviation .	...	...	...	...	...	...	...	...	−14·6̇	1146·6̇	0·215	1	>0·5	−4·0	1418·6̇	0·011	1	>0·9
Heterogeneity .	...	...	...	...	...	...	...	...	...	...	0·166	2	>0·9	...	...	3·799	2	>0·1
London :—		a^t +	a^t *fi*	*a* +	*a fi*													
Grüneberg, new data	I.R.	226	75	105	6	...	...	412	−117·3̇	2197·3̇	6·265	...	...	−42·6̇	2197·3̇	0·828	...	...
		A +	*A fi*	a^t +	a^t *fi*													
Grüneberg, 1943	I.C.	53	11	21	7	...	...	92	−26·6̇	490·6̇	1·449	...	...	+13·3̇	490·6̇	0·362	...	...
Sum . .	...	...	...	...	...	...	...	...	...	...	7·714	...	...	...	...	1·191	...	...
Deviation .	...	...	...	...	...	...	...	...	−144·0	2688·0	7·714	1	<0·01	−28·3̇	2688·0	0·299	1	>0·5
Heterogeneity .	...	...	...	...	...	...	...	...	...	...	0·000	1	>0·9	...	...	0·892	1	>0·3

* In the column headed " mating," C = coupling, I = intercross, R = repulsion.

† In the segregation analyses, *D* = discrepancy in the equation of estimation, *DF*= degrees of freedom, *I* = amount of information, *P* = probability.

‡ In all cases a^t entered with *fi* ; the signs have, therefore, been taken in the sense required to test the segregation of a^t from its allele, whether a^t was playing the dominant or recessive role.

ANALYSIS OF THE DATA

The data have been analysed by the methods described by Mather (1935, 1937), which are special applications of the maximum likelihood method of estimation (Fisher, 1925). In the linkage analysis, however, we have adopted the sign convention used by Fisher (1946), whereby linkage closer than the trial value is indicated by a positive instead of a negative score.

The single-factor segregation analyses (table 1) show a deficiency of fidgets, significant at the 1 per cent. level, in the London data ;

TABLE 2

Linkage of fi *and* a^t

Author and date	Mating	Analysis of linkage					
		D_{33}	D_{34}	I	χ^2	DF	P
Edinburgh :—							
Falconer, new data .	I.R.	+12·685	+13·241	55·613	2·893	...	...
Carter, new data .	I.R.	−7·343	−6·278	106·554	0·506	...	...
,, ,, .	I.C.	−9·672	−6·552	311·996	0·300	...	...
London :—							
Grüneberg, new data	I.R.	+26·266	+30·463	419·738	1·644	...	...
,, , 1943 .	I.C.	−27·912	−24·904	300·726	2·591	...	...
Sum . . .	...	...	...	...	7·934	...	...
Deviation . .	...	−5·976	+5·970	1194·628	0·030	1	>0·9
Heterogeneity .	...	...	...	...	7·904	4	>0·05

Recombination fraction $p = 33{\cdot}5 \pm 2{\cdot}9$ per cent. (standard error).

TABLE 3

Segregation of fi *with* b, c, p, W^v

Author	Mates	Phenotypes and numbers of progeny						
				++	+*fi*	*b* +	*b fi*	Total
Grüneberg	*b*+/+*fi*×*b*+/*b fi*	...	...	23	5	33	8	69
,,	*b*+/+*fi*×*b*+/+*fi*	...	...	84	29	30	7	150
				++	+*fi*	*c* +	*c fi*	Total
Falconer	*c fi*/++×*c fi*/*c fi*	...	...	19	3	16	10	48
,,	*c fi*/++×*c fi*/++	...	...	85	22	26	8	141
,,	*c fi*/++×*c fi*/*c*+	...	...	6	2	5	3	16
Grüneberg, new data	+*c fi*/*p*++×+*c fi*/*p*++	...	...	331 *112**	82 *35**	116	29	558
		W^vW^v +	$W^vW^v fi$	W^v+ +	W^v+*fi*	++ +	++*fi*	Total
Falconer	W^v+/+*fi*×++/+*fi*	...	...	11	2	11	4	28
Carter	W^v+/+*fi*×W^v+/+*fi*	8	...	20	5	14	5	52

* Figures in italics show the numbers of pinkeyed progeny within the non-albino classes.

the deficiency of a^t in the London data is not significant and the Edinburgh data show good segregations at both loci.

In analysing the linkage we assume that the deficiency of fidgets was entirely due to inviability of the homozygotes and that this

inviability was not dependent upon the genotype at the agouti locus. When these assumptions are made, the estimate of the recombination fraction is independent of the viability of the fidgets : the amount of statistical information available about the estimate is still measured by the rate of change of the discrepancy in the equation of estimation.

The linkage analysis is shown in table 2 : the five bodies of data are homogeneous and indicate linkage with a recombination fraction $33{\cdot}5 \pm 2{\cdot}9$ per cent. (standard error).

The data of table 3, which gives the segregations of *fi* with *b*, *c*, *p* and W^v, do not show any significant departures from free segregation.

SUMMARY

Fidget, *fi*, in the house mouse is linked to tan, a^t ; recombination is estimated at $33{\cdot}5 \pm 2{\cdot}9$ per cent. Its position with respect to pallid, *pa*, is not yet known.

Acknowledgments.—We are grateful to Dr D. S. Falconer for permission to quote some data from the Animal Breeding and Genetics Research Organisation's laboratory at the Institute of Animal Genetics, Edinburgh. One of us (H. G.) was assisted by a research grant from the Medical Research Council.

Note added 2nd January 1950.—We are grateful to Professor Fisher for pointing out, since this paper was written, that the linkage analysis of some of our data (the four class segregations) could have been facilitated by the use of table XIV_1, in the 1948 edition of Fisher and Yates' *Statistical Tables for Biological, Agricultural and Medical Research* (Edinburgh, Oliver and Boyd).

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THE POSITION OF FIDGET IN LINKAGE GROUP V OF THE HOUSE MOUSE

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Introduction

In 1935 Roberts & Quisenberry reported finding a fifth linkage group in *Mus musculus*; the mutant pallid, **pa** (Roberts, 1931), showed about 20·4 % recombination with the locus of agouti, **A**.

It has recently been shown that fidget, **fi** (Grüneberg, 1943), is also linked to agouti (Carter & Grüneberg, 1950); recombination was estimated at $33{\cdot}5 \pm 2{\cdot}5\,\%$. The position of **fi** with respect to those of **pa** and other mutants in this group was not established.

This paper presents new data on the segregation of **fi**, **pa** and **A**. They indicate that recombination between **fi** and **pa** is about 20 % and therefore establish that the three loci lie in the order **fi**, **pa**, **A**; and they enable revised estimates to be made of the **pa**/**A** and **fi**/**A** recombination fractions.

Material, methods and results

The greater part of the new data was obtained from *inter se* matings of triple heterozygotes $\mathbf{fi}+\mathbf{a}^{t}/+\mathbf{pa}\,\mathbf{A}$; $\mathbf{A}^{w}$ or $\mathbf{a}^{t}$ replaced **A** in the remainder.

In all these matings **fi** and **pa** were in the same phase and consequently there was a danger that the estimate of the **fi**/**pa** recombination fraction might be biased if there were low viability of the double recessive class. A second body of data, of a type which should be unaffected by mortality, was therefore obtained as a check; a number of singly recessive F_2 mice, all pallid females and chosen without reference to genotype at the agouti locus, were tested genetically for heterozygous **fi**.

The results of these tests were not treated by the usual method, but by an adaptation of the principles developed by Falconer (1949) in connexion with the estimation of mutation rates. The efficiency of test of each mouse was calculated from the number of progeny classified and the type of test; summation then gave the equivalent number of fully tested animals. The number of animals proved to carry **fi** is expected to constitute a fraction $2p/(1+p)$ of the fully tested total, where p is the recombination fraction; hence the two numbers could be used to provide an estimate of p. The method appears to be statistically better founded than the usual method of raising some arbitrary number of test progeny and concluding, according as some or none are fidgets, that the parent under test was $+\mathbf{fi}$ or $+\,+$; the arbitrary choice is eliminated and use is made of all test families, however small.

No difficulties of classification were experienced, except that a reliable distinction could not always be made between light-bellied and dark-bellied agouti pallid homozygotes; pallids were therefore classified as tan ($\mathbf{a}^{t}\mathbf{a}^{t}$) or agouti-backed (**AA**, $\mathbf{A}^{w}\mathbf{A}$, $\mathbf{A}\mathbf{a}^{t}$ or $\mathrm{A}^{w}\mathbf{a}^{t}$).

Joint tests of single-factor segregations and joint maximum likelihood estimates of recombination fractions were calculated by the methods described by Mather (1935, 1937).

The F_2 segregations are given in Table 1 and the results of genetic tests of pallid F_2 mice in Table 2.

Analysis of the data

(i) *Single-factor segregations.* These analyses are not reproduced. The segregations of **pa** and $\mathbf{a^t}$ agreed well with Mendelian expectation; there was some suggestion of an overall deficiency of fidgets, but not enough to be statistically significant at the 5% level.

Table 1. *Segregation of fidget, pallid and agouti*

Genotype of parents ♂	Genotype of parents ♀	Phenotype of progeny* ++$\mathbf{A^l}$	++**A**	+**paA**$^{\mathbf{b}}$	**fi**+$\mathbf{A^l}$	**fi**+**A**	**fipaA**$^{\mathbf{b}}$	++$\mathbf{a^t}$	+**paa**$^{\mathbf{t}}$	**fi**+$\mathbf{a^t}$	**fipaa**$^{\mathbf{t}}$
fi+$\mathbf{a^t}$/+**paA**	×**fi**+$\mathbf{a^t}$/+**paA**	118	14	87	29	7	1	43	2	33	—
fi+$\mathbf{a^t}$/+**paA**$^{\mathbf{w}}$	×**fi**+$\mathbf{a^t}$/+**paA**	24	—	14	9	—	—	8	—	2	—
fi+$\mathbf{a^t}$/+**paa**$^{\mathbf{t}}$	×**fi**+$\mathbf{a^t}$/+**paa**$^{\mathbf{t}}$	—	—	—	—	—	—	71	30	30	3

* The phenotype $\mathbf{A^l}$ ('light-bellied agouti') includes the genotypes $\mathbf{Aa^t}$, $\mathbf{A^wa^t}$ and $\mathbf{A^wa}$.
The phenotype $\mathbf{A^b}$ ('agouti-backed') includes the genotypes $\mathbf{Aa^t}$, $\mathbf{A^wa^t}$, $\mathbf{A^wa}$ and **AA**.
The phenotype **A** includes only the genotype **AA**.

Table 2. *Tests of F_2 pallid mice for heterozygous* **fi**

Progeny per test	Efficiency of test	Mice tested	Fully tested equivalent	Mice proved heterozygous
		Backcross tests		
10	0·999	2	1·998	1
9	0·998	6	5·988	1
8	0·996	4	3·984	—
7	0·992	5	4·961	2
6	0·984	4	3·938	1
5	0·969	3	2·906	1
4	0·937	2	1·875	2
		Intercross tests		
14	0·982	1	0·982	—
7	0·867	1	0·867	1
		Total	27·499	9

(ii) *Linkage of* **fi** *and* **pa** (see Table 3). The data from the genetic tests of pallid F_2 mice are homogeneous with the repulsion intercross data; it is therefore unlikely that there has been serious biasing of the joint estimate of the recombination fraction, $19{\cdot}0 \pm 3{\cdot}6\%$ (standard error).

(iii) *Linkage of* **pa** *and* **A**. Table 4 shows the joint analysis of the data of Roberts & Quisenberry (1935) and the new data. The joint estimate of the recombination fraction is $18{\cdot}7 \pm 1{\cdot}6\%$.

(iv) *Linkage of* **fi** *and* **A**. Table 5 shows the joint analysis of the data of Carter & Grüneberg (1950) and the new data. The joint estimate of the recombination fraction is $33{\cdot}1 \pm 2{\cdot}2\%$.

(v) *Order of the loci.* The estimate of the **fi**/**pa** recombination fraction disagrees significantly with a hypothetical value exceeding 40%, such as would be expected if the order were **fi**, **A**, **pa**; the observed value is in good agreement with expectation based on the order **fi**, **pa**, **A**.

Summary

New data are given on the segregation of the genes fidget (**fi**), pallid (**pa**) and agouti (**A**) in the fifth linkage group of the house mouse. They show that the loci must lie in that order. The three recombination fractions are estimated, from the data of Roberts &

Table 3. *Linkage of fidget and pallid*

Genotype of parents ♂	Genotype of parents ♀	Phenotype of progeny ++	Phenotype of progeny +pa	Phenotype of progeny fi+	Phenotype of progeny fipa	Analysis of linkage D_{18}	D_{19}	I	χ^2	D.F.	P
fi+a^t/+paA	×fi+a^t/+paA	175	89	69	1	−16·676	−19·102	242·609	1·504	—	—
fi+a^t/+paA^w	×fi+a^t/+paA	32	14	11	—	− 3·633	− 3·884	25·039	0·602	—	—
fi+a^t/+paa^t	×fi+a^t/+paa^t	71	30	30	3	+23·586	+21·176	241·043	1·860	—	—
Tests of F_2 pallids			Table 2			+ 4·136	+ 1·422	271·425	0·007	—	—
Sum		—	—	—	—	—	—	—	3·973	—	—
Deviation		—	—	—	—	+ 7·413	− 0·388	780·116	0·000	—	—
Heterogeneity		—	—	—	—	—	—	—	3·973	3	>0·2

Recombination fraction: $p = 18{\cdot}95 \pm 3{\cdot}58\,\%$.

Table 4. *Linkage of pallid and agouti*

Genotype of parents ♂	Genotype of parents ♀	Phenotype of progeny* A^y+	A^ypa	++	+pa	a^t+	a^tpa	Analysis of linkage D_{18}	D_{19}	I	χ^2	D.F.	P
				Roberts & Quisenberry (1935)									
A^y+/+pa	× +pa/+pa	96	25	19	84			+24·932	+ 9·357	1557·557	0·399	—	—
+pa/+pa	×A^y+/+pa	92	16	33	90			+50·271	+33·203	1706·769	1·481	—	—
				New data (Table 1)									
		A+	A^l+	A^bpa		a^t+	a^tpa						
fi+a^t/+paA	×fi+a^t/+paA	21	147	88		76	2	−44·037	−52·395	835·780	2·320	—	—
fi+a^t/+paA^w	×fi+a^t/+paA	—	33	14		10	—	− 3·084	− 3·305	22·074	0·431	—	—
Sum		—	—	—		—	—	—	—	—	4·631	—	—
Deviation		—	—	—		—	—	+28·082	−13·140	4122·181	0·191	—	—
Heterogeneity		—	—	—		—	—	—	—	—	4·440	3	>0·2

* See note under Table 1. Recombination fraction: $p = 18{\cdot}68 \pm 1{\cdot}56\,\%$.

Table 5. *Linkage of fidget and agouti*

Genotype of parents ♂	Genotype of parents ♀	Phenotype of progeny* A^b+	A^bfi	a^t+	a^tfi	Analysis of linkage D_{33}	D_{34}	I	χ^2	D.F.	P
				From Carter & Grüneberg (1950)							
—	—	—	—	—	—	+ 5·976	− 5·970	1194·628	7·934	5	—
				New data (from Table 1)							
fia^t/+A	×fia^t/+A	219	37	45	33	−18·958	−26·911	795·276	0·452	1	—
fia^t/+A^w	×fia^t/+A	38	9	8	2	+14·573	+13·104	146·439	1·446	1	—
Sum		—	—	—	—	—	—	—	9·832	7	—
Deviation		—	—	—	—	+ 1·590	−19·777	2136·748	0·001	1	—
Heterogeneity		—	—	—	—	—	—	—	9·831	6	>0·1

* See note under Table 1. Recombination fraction: $p = 33{\cdot}07 \pm 2{\cdot}16\,\%$.

Quisenberry on **pa/A** and those of Carter & Grüneberg on **fi/A**, as well as the new data, to have the following values: **fi/pa**, $19{\cdot}0 \pm 3{\cdot}6\%$; **pa/A**, $18{\cdot}7 \pm 1{\cdot}6\%$; **fi/A**, $33{\cdot}1 \pm 2{\cdot}2\%$ (standard errors).

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A New Linkage in the House Mouse: Undulated and Agouti

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A NEW LINKAGE IN THE HOUSE MOUSE: UNDULATED AND AGOUTI

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1. INTRODUCTION

WRIGHT (1947) has reported the occurrence of a new hereditary character in the house mouse (*Mus musculus*, L.); the affected mouse typically shows soft undulations of the tail and a tendency to a hunchback. She concluded that the inheritance of the character is unifactorial and recessive, and suggested the name "undulated" and the symbol *un* for the mutant gene concerned.

The present paper gives data which indicate that *un* is closely linked to the agouti locus; it therefore belongs to Linkage Group V (Dunn, Grüneberg and Snell, 1940). No data are yet available on its segregations with the other mutants in this group, namely pallid (Roberts and Quisenberry, 1935), wellhaarig and kreisler (Hertwig, 1942), nor with hydrocephalus-1 (Clark, 1936).

2. MATERIAL

The greater part of the material to which the data refer consists of four lines which are being set up in the Department of Genetics, Cambridge University. With a view to an extensive test of possible linkages of the new factor, undulated was introduced into these lines by Miss Margaret Wright during 1944 and 1945. It was intended to test it in a fifth line with a representative of Linkage Group V, namely wellhaarig; but this was delayed as wellhaarig was not then available outside Germany. Meanwhile the other four lines, which were not intended to segregate at the agouti locus, continued to do so; and during 1946 it became obvious that this continued segregation of the agouti alleles was associated with the segregation of undulated.

The fact that the greater part of the data arose in this way explains why most of the matings concerned were intercrosses rather than backcrosses; however, the linkage proved to be close, and the coupling intercrosses (which constitute the greater part of the data) are therefore almost as informative as coupling backcrosses.

Four of the agouti alleles were involved, namely tan (a^t), light-bellied agouti (A^w) and non-agouti (a) as well as agouti itself (A). Tan was acting as a dominant (to non-agouti) in the repulsion matings and as a recessive (to light-bellied agouti or to agouti) in the coupling matings.

All the mice were normally classified as early as possible, but the classifications were checked when the animals were between 18 and 21 days old.

3. DATA

The basic data are given in table 1. This shows the number of progeny in each of the phenotypic classes from matings of five types :

Coupling intercrosses with agouti segregating	$A\ Un/a^t un\ ♀ \times A\ Un/a^t un\ ♂$
Coupling intercrosses with light-bellied agouti	$A^w Un/a^t un ♀ \times A^w\ Un/a^t un\ ♂$
Coupling backcrosses with segregation in the female	$A^w Un/a^t un\ ♀ \times a^t un/a^t un\ ♂$
Coupling backcrosses with segregation in the male	$a^t un/a^t un\ ♀ \times A^w Un/a^t un\ ♂$
Repulsion intercrosses	$a\ Un/a^t un\ ♀ \times a\ Un/a^t un\ ♂$

In one of these bodies of data, namely the coupling intercrosses in which agouti and tan were segregating, a more complete classification is possible, by virtue of the incomplete dominance of agouti over tan ; details of this classification are not, however, given.

The data are unselected, save in the following respects :—

(i) Any animal which died before reaching the age of 14 days has been excluded. It was found that some animals which appeared at birth to have undulated tails later became fully normal ; acceptance of an unchecked early classification might therefore have led to biassed results. The age of 14 days was chosen because this was considered to be the earliest age at which a sure distinction could be made between undulated and normal animals.

(ii) Albinism was segregating in one of the lines : albinos, which could not be classified for the agouti phenotype, were excluded.

(iii) Fused was segregating in a few matings in one line. While there was no difficulty in classifying the fused progeny as such, difficulty was encountered in deciding whether these mice were also undulated or not ; they were therefore excluded.

4. ANALYSIS OF THE DATA

On inspection, the data give an impression of good unifactorial segregations of both of the loci concerned (undulated and agouti), but of close linkage between them. This impression is confirmed by statistical analysis, an outline of which is given in table 2 :—

(i) *Segregation at the undulated locus.* All five bodies of data show good unifactorial segregation at this locus.

(ii) *Segregation at the agouti locus.* The agreement between observation and expectation for the segregation at this locus is extremely good. As the data were unselected, except as indicated above, this must be attributed to chance.

TABLE 1

Mating type	Mating no.	Phenotypes of progeny			
		A Un	*A un*	*a^{t}Un*	*a^{t}un*
Coupling intercross . .	V/24	2	...	...	3
	V/25	14	1	...	2
	V/28	6	...	...	2
	V/29	4	...	...	2
	V/30	6	...	...	4
	Totals .	32	1	...	13
		A^{w}Un	*A^{w}un*	*a^{t}Un*	*a^{t}un*
Coupling intercross . .	*6/17	12	...	...	4
	*6/18	4	...	...	1
	6/30	36	...	2	13
	6/51	40	1	...	7
	*8/13	32	1	1	12
	*8/15	35	2	1	10
	*8/16	3	...	...	...
	8/34	17	...	...	9
	Totals .	179	4	4	56
Coupling backcross ; female segregation	6/48	5	...	...	2
	6/56	7	...	2	8
	8/42	1	...	...	1
	Totals .	13	...	2	11
Coupling backcross ; male segregation	6/53	7	...	...	11
	8/37	9	...	1	8
	VII/29	...	...	...	1
	VII/30	7	1	1	1
	Totals .	23	1	2	21
Repulsion intercross .		*a^{t}Un*	*a^{t}un*	*a Un*	*a un*
	*2/10	6	4	4	...
	*2/16	13	7	4	...
	2/23	7	7	3	...
	2/24	18	9	11	...
	*21/8	21	11	10	...
	21/21	15	15	4	...
	21/27	20	6	13	...
	V/18	7	2	4	...
	Totals .	107	61	53	...

The data given by Wright (1947) include details of some or all of the progeny of the matings here marked with an asterisk.

(iii) *Method of analysis of the linkage.* The "scoring method" developed by Fisher (1946), which is a variant of his

" maximum likelihood " technique, has been adopted ; its advantages include the comparative ease with which a joint test of significance and a joint estimate of the recombination fraction are obtainable from the five bodies of data.

(iv) *Significance.* The χ^2 testing independence of segregation, which has one degree of freedom, is

$$\chi^2 = 288{\cdot}7.$$

The corresponding probability is extremely small ; a hypothesis of independent segregation is therefore untenable.

(v) *Intensity of linkage.* The joint stimate obtained for the recombination fraction is

$$p = 3{\cdot}86 \text{ per cent.}$$

and its standard error is calculated to be

$$s = 1{\cdot}02 \text{ per cent. ;}$$

but Stevens (1942) has pointed out that it is incorrect to use a standard error to find fiducial limits in cases such as this where p is small compared with unity. The present results are not in a form suitable for the direct application of the correction tables he gives, and so the " equivalent backcross data " have been calculated ; *i.e.* a calculation has been made of the numbers of recombinant and non-recombinant progeny, which, if they had all been produced by backcross matings, would have yielded the same total score and the same total amount of information. Stevens's corrections have been applied to these figures, and the limits within which p probably lies (for a 5 per cent. significance level) are found to be

2·12 per cent. and 6·42 per cent.

(vi) *Homogeneity of the data.* For the purpose of obtaining a joint estimate of the recombination fraction, it has been assumed that the frequency of crossing-over in the male is the same as that in the female, and also that it is the same when A^w or a segregates with a^t as when A segregates with a^t. These assumptions are not contradicted by the data ; the heterogeneity χ^2 is not more than 2·523, which corresponds with a probability exceeding 0·5 since it has four degrees of freedom. The contributions to this χ^2 made by the backcross data alone amount to 1·835, which for one degree of freedom corresponds with a probability exceeding 0·17 ; and the contributions made by the coupling intercrosses amount to 0·589, which for one degree of freedom corresponds with a probability exceeding 0·3.

TABLE 2

	Coupling intercrosses A and a^t	Coupling intercrosses A^w and a^t	Coupling backcrosses: male segregating	Coupling backcrosses: female segregating	Repulsion intercrosses
Segregation at the undulated locus :					
Recessives observed	14	60	22	11	61
,, expected	11·50	60·75	23·50	13·00	55·25
χ^2	0·725	0·009	0·479	0·154	0·798
Segregation at the agouti locus :					
Recessives observed	13	60	23	13	53
,, expected	11·50	60·75	23·50	13·00	55·25
χ^2	0·261	0·009	0·005	0·000	0·122
Linkage : Testing independence ($p=0{\cdot}5$)					
$\Sigma\frac{a}{m}\frac{dm}{dp}$ (score)	−64·889	−292·889	−82·000	−44·000	−104·444
$n\Sigma\frac{1}{m}\left(\frac{dm}{dp}\right)^2$ (information)	81·778	432·000	188·000	104·000	392·889

Total score . . . . . −588·222
Total information . . . . . 1198·667
Independence : χ^2 . . . . . $(-588{\cdot}222)^2 \div 1198{\cdot}667 = 288{\cdot}658$

Estimating intensity ($p = 0{\cdot}0386$) :					
$\Sigma\frac{a}{m}\frac{dm}{dp}$	−22·688	−31·019	+31·954	+26·850	−4·687
$n\Sigma\frac{1}{m}\left(\frac{dm}{dp}\right)^2$	1183·702	6253·034	1266·502	700·618	221·824
χ^2	0·435	0·154	0·806	1·029	0·099

Total score . . . . . +0·410
Total information . . . . . 9625·680
Estimate of p . . . . . $0{\cdot}0386 + (0{\cdot}410 \div 9625{\cdot}680) = 0{\cdot}038643$
Standard error of estimate . . . . . $1 \div \sqrt{9625{\cdot}680} = 0{\cdot}010192$
Heterogeneity χ^2 . . $0{\cdot}435 + 0{\cdot}154 + 0{\cdot}099 + 1{\cdot}029 + 0{\cdot}806 = 2{\cdot}523$

Note.—m is the expectation (in terms of p), and a is the observed number, in each of the phenotypic classes of progeny. Σ indicates summation over these classes. $n = \Sigma a$.

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SUMMARY

1. Data are given which indicate close linkage between the agouti locus in the house mouse and the locus of Wright's mutant "undulated." Undulated therefore belongs to Linkage Group V. Its position with respect to pallid, wellhaarig, kreisler and hydrocephalus-1 is not known.

2. The data are abstracted from the records at the Department of Genetics, Cambridge University ; they are unselected, except that to

ensure correct classification no mouse is included which died before the age of fourteen days or which was also classified as fused ; albinos, being unclassifiable for agouti, are also excluded. The data are chiefly of the intercross type (coupling and repulsion) but some are of the backcross type.

3. The results indicate good Mendelian segregation of undulated and of the agouti alleles involved. There is a highly significant interaction between them, which leads (by Fisher's scoring method) to an estimated recombination fraction $p = 3{\cdot}86$ per cent. with fiducial limits (for a 5 per cent. significance level) $p = 2{\cdot}12$ per cent. and $p = 6{\cdot}42$ per cent.

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A MOSAIC MOUSE WITH AN ANOMALOUS SEGREGATION RATIO

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(With Plate 1 and One Text-figure)

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Introduction

Mosaic individuals, having different gene complements in different parts of the body, have been extensively studied in plants and in some invertebrate genera; but among mammals they are sufficiently rare for individual cases to be reported. They have usually been assumed to arise, in mammals, through somatic mutation, and other possible mechanisms have not often been considered.

The mosaic mouse described in this paper is of special interest because both germinal and somatic tissues were affected, and in such a way that somatic mutation is excluded as a possible cause.

Description of the mosaic mouse

The mosaic mouse was born in 1948 in the first of her parents' three litters. Her father was of the wild-type *CBA* inbred strain; her mother was a cross-bred heterozygote for the semi-dominant colour and macrocytic anaemia mutant **W^v** (Little & Cloudman, 1937).

The mosaic showed, over the greater part of her body, the characteristic phenotype of a **W^v**/+ heterozygote; there was a white brow spot, a large white belly patch, the agouti hairs of the dorsum were bleached and the ventral surface was silvered. Two regions, however, showed full, wild-type coloration. One covered the ears, right side of the neck and throat, right shoulder and upper arm; the boundary followed the midline on the dorsum of the neck and on the throat. The other wild-type region covered the tail, right rump, lumbar area, right hind leg and foot and the right side of the belly lateral and posterior to the white patch (see Pl. 1). The greater part of the wild-type coloration was on the right side of the mouse, but it extended to the left on the ears and in the lumbar region.

Breeding tests

The position of the posterior wild-type region suggested that the right gonad, or possibly both, might be involved; the mosaic was therefore mated to a wild-type male to test the segregation of **W^v**. Of the forty-one classified young, ten were wild-type and thirty-one were typical **W^v**/+ heterozygotes; the deviation from the expected 1:1 ratio is significant statistically ($P = 0{\cdot}00145$). None showed any sign of mosaicism. Classification presented no difficulties; there was very little variation in the expression of **W^v**, all the heterozygous young having a well-marked brow spot which was visible at the age of 5 days. Nevertheless, classification was always delayed until the other characteristic features were also visible, and it is very unlikely that any misclassification occurred. As a further check on the possibility of misclassification in the stock, the breeding records of the ancestors of the mosaic were examined; **W^v** had been introduced six generations earlier, and Table 1 shows

that there had been consistently good Mendelian segregation, apart from the usual shortage of W^v/W^v homozygotes from the intercross mating.

Five of the $W^v/+$ young of the mosaic were also tested for the segregation of W^v. They all showed good Mendelian segregation (Table 2); none of their progeny was a mosaic.

In addition to W^v, the mosaic may have carried any of **b**, **c**, **d** and **lx**; she was known to carry **a** and t^6. The latter is a lethal which is probably identical with t^0 (Dunn & Gluecksohn-Schoenheimer, 1950) and which was shown to be present in the stock when a spontaneous brachyury (**T**) mutation occurred in a great-grandparent of the mosaic, the female

Table 1. *Segregation of* W^v *in the ancestors of the mosaic*

	Genotype of mates		Phenotype of progeny		
Mating no.	♂	♀	W^v/W^v	$W^v/+$	$+/+$
Z/374	$W^v/+$	$+/+$	—	15	12
Z/410	$W^v/+$	$+/+$	—	9	4
Z/438	$W^v/+$	$W^v/+$	4	25	12
CT/145	$+/+$	$W^v/+$	—	15	12
CT/193	$+/+$	$W^v/+$	—	12	13
CT/363	$+/+$	$W^v/+$	—	3	7

Table 2. *Segregation of* W^v *in the progeny of the mosaic*

	Genotype of mates		Phenotype of progeny	
Mouse no.	♂	♀	$W^v/+$	$+/+$
CT/401.975*f*♂	$W^v/+$	$+/+$	12	13
CT/401.975*g*♂	$W^v/+$	$+/+$	17	13
CT/401.1107*e*♂	$W^v/+$	$+/+$	8	9
CT/401.1196*c*♂	$W^v/+$	$+/+$	5	8
CT/401.1196*d*♂	$W^v/+$	$+/+$	13	18

in mating CT/145 (Carter & Phillips, 1950). t^6 shows non-Mendelian segregation from its type allele in males, but there is no reason to suppose that the presence of t^6 caused the non-Mendelian segregation of W^v in the mosaic female; they are not known to be linked.

Cytological examination

When the mosaic had reached the end of her breeding life, she was killed and her ovaries were examined cytologically by Dr B. M. Slizynski. No clear figures were found on which a judgement about their chromosomal constitution could be based.

Discussion

The data establish that the mosaic female developed from a $W^v/+$ zygote, that parts of her soma were deficient of W^v, and that she bred as though her germinal tissues were partly deficient of $+^w$.

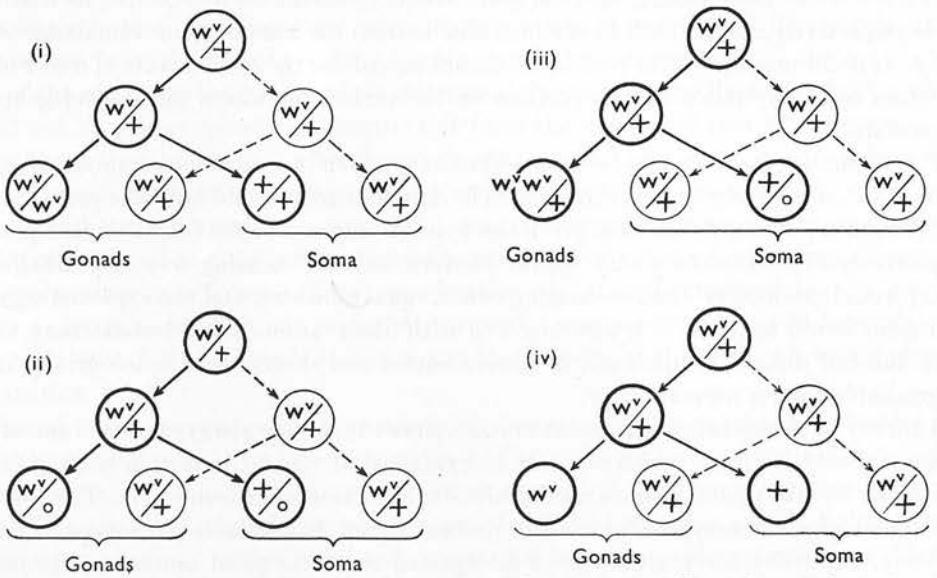
Somatic mutation could hardly have been the mechanism of formation, since two mutational steps would have to be postulated, W^v to $+$ in the soma and $+$ to W^v in the gonad (Text-fig. 1 (i)).

Deletion likewise fails to provide an explanation, since two deletions, in homologous chromosomes, would have to be postulated (Text-fig. 1 (ii)).

Non-disjunction of the W^v-bearing chromosome at an early cleavage division is a possible mechanism. It would lead to daughter cells having the constitutions $W^vW^v/+$ and $+/o$ respectively: the former would be supposed to have given rise to the germinal tissue, the latter to the fully coloured parts of the soma (Text-fig. 1 (iii)). Such an individual might be

expected to show a 2:1 segregation of W^v to +, if both gonads were fully affected, or a ratio nearer to equality if either gonad were partly or totally unaffected. The observed segregation, 31:10, does not significantly differ from a 2:1 ratio. Non-disjunction might be detectable cytologically, since part, at least, of the germinal tissue would be expected to carry 41 chromosomes instead of the usual 40.

Somatic reduction at an early cleavage stage (Text-fig. 1 (iv)) is a theoretically possible mechanism which must be considered, since it occurs occasionally in many plants (Huskins, 1948) and regularly in some animal tissues (Berger, 1941; Grell, 1946). Spontaneous haploid-diploid mosaic amphibian larvae have been reported (Fankhauser, 1941). On the other hand, haploid somatic tissues have not yet been reported in a mammal, and somatic departures from the diploid condition are usually in the direction of an increase rather than a decrease in ploidy. Somatic reduction at an early cleavage division of a $W^v/+$ zygote



Text-fig. 1. Five theoretically possible modes of formation of the W^v mosaic. (i) Two point mutations; or somati crossing-over. (ii) Two chromosome deletions. (iii) Non-disjunction. (iv) Somatic reduction.

would lead to haploid daughter cells of the constitutions W^v and +; the former might then give rise to one ovary, the latter to the fully coloured parts of the soma. Such an individual would have a diploid ovary producing W^v- and $+^w$-bearing ova in equal numbers and a haploid ovary producing only W^v-bearing ova; the expected segregation ratio, 3:1, agrees well with observation. Somatic reduction might be detectable cytologically, since some of the oogonia should show only 20 chromosomes.

Somatic crossing-over (Text-fig. 1 (i)) also gives a possible explanation; it requires consideration in the light of Stern's (1936) conclusion, referring to his analysis of *Drosophila* mosaics, that 'the mechanism of mosaic formation is not based on simple elimination of chromosomes but on processes of somatic crossing-over involving two strands of a four strand group'. Furthermore, Auerbach (1945) has shown that mustard-induced mosaics in *Drosophila* due to somatic crossing-over are about twenty times as common as those due to point mutation. It does not appear to have been considered in the past as a possible mechanism of formation of mosaic mammals, perhaps because somatic chromosome

pairing has not been reported in mammals. However, mammalian somatic chromosomes, widely regarded as cytologically difficult material, have received less study than the chromosomes of many invertebrate and plant species. Furthermore, as Stern (1936) pointed out, we do not know the reason why somatic cells in *Drosophila* occasionally undergo crossing-over, and therefore we are hardly justified in concluding that regular somatic chromosome pairing is a prerequisite condition. There is also the possibility of non-homologous crossing-over.

A feature of somatic crossing-over is the potentiality for forming twin spots. These will normally be seen only when each of the two homologous chromosomes carries a recessive marker gene distal to the point of crossing-over; each of the daughter cells may thus become homozygous for one of the recessive markers and therefore, if the markers are autonomous, show the recessive phenotype. A similar effect would occur when there is only one marker gene if it were semi-dominant; the twin spots, homozygous for the marker and its wild-type allele respectively, would both be distinguishable from the heterozygous remainder of the body. It is not necessary that both spots should be visible; the descendants of either of the daughter cells may not occupy a position on the surface, or where the marker gene can express itself.

The $\mathbf{W^v}$ mosaic mouse would be supposed to have arisen by crossing-over proximal to the locus of $\mathbf{W^v}$, at an early cleavage division. The daughter cells would have the constitutions $\mathbf{W^v/W^v}$ and $+/+$ and would have given rise to one ovary and to the fully coloured patches respectively. The affected ovary would produce only $\mathbf{W^v}$-bearing ova; the unaffected ovary would produce $\mathbf{W^v}$- and $+$-bearing ova in equal numbers; and the expected segregation ratio would again be 3:1, agreeing well with observation. The affected ovary would have the full diploid complement of chromosomes and therefore be cytologically indistinguishable from a normal ovary.

A survey of the literature on rodent mosaics shows that they always fall into one of two classes, namely, (*a*) those which can only be explained on a point-mutation basis, (*b*) those which can be interpreted as instances of somatic reduction or crossing-over. The common cases in which a heterozygote for a colour mutant shows the recessive phenotype in part of its body, and which have usually been interpreted as due to point mutation, can just as easily be placed in the second class; but a number of rodent mosaics also fall into this class which are very difficult to interpret on a point-mutation basis. Three are of particular interest:

(i) Pickard (1929, 1936) described a rabbit which was heterozygous for the recessive mutants *brown* and *Angora*. It carried seven mosaic patches; four were brown and were widely spread over the shoulders, flanks and rump; three were Angora, limited to a narrow strip along the vertebrae; apparently they did not overlap. It was considered to be a case of 'abnormal distribution of whole chromosomes during cellular division', in which 'the aberrant distribution of chromosomes had occurred twice during development, the chromosome bearing the gene for black being the first to be abnormal in its distribution. Later one chromosome bearing the gene for short hair became involved'. This hypothesis of somatic segregation by non-disjunction lacks the virtue of simplicity, since it was necessary to postulate two independent non-disjunction events; similar considerations exclude point mutation and deletion. Non-disjunction would still fail to provide a satisfactory hypothesis if Angora and brown were linked; for if they were in coupling the Angora and brown patches should have coincided; and if they were in repulsion there should have been patches

of Angora or brown but not both, since a trisomic patch would carry the dominant allele at both loci. Somatic reduction and somatic crossing-over remain as possible hypotheses. The latter would receive additional support if Angora and brown were linked; no direct test appears to have been made, but there cannot be close linkage, since there is no close linkage between brown and English spotting, to which Angora is closely linked (Castle, Feldman & Gates, 1926). Loose linkage is not excluded by the data.

(ii) Curtis & Dunning (1940) described a mosaic female rat which occurred in the F_2 generation from an *intense hooded* × *blue self* cross. The mosaic was hooded, the white region covering about 74 % of the coat; the pigmented region was partly (10 %) intense and partly (16 %) blue. Crossed to a blue male, she produced sixteen young, all blue. The interpretation adopted was that the mosaic failed, by chance, to receive the intense allele from either parent and was thus genetically homozygous blue; and that the intense region had arisen by new somatic reverse mutation. However, alternative interpretations based on somatic reduction or somatic crossing-over appear to fit the data at least as well. The mosaic would be supposed to have been originally a blue-intense heterozygote in which the gonads and part of the soma lost the intense allele; the complementary region, lacking the blue allele, would not be phenotypically distinguishable from the unaffected part of the soma.

(iii) Dunn (1934) described a black piebald mouse which carried on one shoulder a blue patch resembling the dilute colour usually associated with the genotype **dd**. One great-grandparent was known to have carried **d**; it was not known if the parents or grandparents carried it. Crossed to a **dd** homozygote, the mosaic sired twenty-four young, all intense. No explanation was given. Mutation, deletion and non-disjunction do not provide adequate hypotheses. Somatic reduction or somatic crossing-over in a **+/d** heterozygote, with the result that the gonads lacked **d** and the mosaic patch lacked $+^{d}$, would give an explanation.

None of these cases provides the means of distinguishing between somatic reduction and somatic crossing-over. The latter seems more likely if one accepts the old cytological dictum, now suspected of being honoured almost as much in the breach as in the observance (vide Huskins, 1948), that the somatic tissues of a diploid organism are all diploid. Cytological study of mosaic individuals holds out the only hope of providing conclusive evidence; but mammalian somatic chromosomes are notoriously poor cytological material.

Mosaic individuals may also throw light on some of the end-effects of morphogenetic movements in the mammalian embryo. This rests on the assumption that all anomalous patches in a mosaic descend in a direct cell-lineage from a single cell in which the mosaic-causing change occurred. One striking fact is that the anomalous tissues do not necessarily remain in continuous contact, at least on the surface of the body. In the W^{v} mosaic mouse there were two fully coloured regions, widely separated; Pickard's rabbit had seven patches and Wright & Eaton (1926) recorded several mosaic guinea-pigs with separated patches.

Finally, the occurrence of a W^{v} mosaic establishes that W^{v} is autonomous in its action on pigmentation; it is thus in accordance with 'the general rule, which is that pigmentation is strictly locally determined' (Russell, 1949).

SUMMARY

A mouse, heterozygous for the semi-dominant colour and anaemia mutant W^{v}, showed two patches of full pigmentation (i.e. lacking W^{v}); she bred as though her ovaries were partly deficient of the type allele $+^{w}$. Point mutation and deletion fail to provide satisfactory

explanations; non-disjunction provides a possible explanation; somatic reduction and somatic crossing-over, not previously considered as possible mechanisms of mammalian mosaic formation, would fit the facts well. They could also account for some other mosaic mammals which were hitherto difficult to explain. It is established that $\mathbf{W^v}$, like other mutants, is autonomous in its action on pigmentation.

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Ventral view of the **W**^{v} mosaic mouse, showing the region of full coloration on the right side of the abdomen and the right hind leg.

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WAVY-COATED MICE: PHENOTYPIC INTERACTIONS AND LINKAGE TESTS BETWEEN REX AND (*a*) WAVED-1, (*b*) WAVED-2

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(With Plate 11)

Introduction

There are five well-known mutants in the house mouse (*Mus musculus* L.) which produce a phenotype with curly hair and vibrissae. Three are recessive, namely, waved-1 (**wa-1**, Crew, 1933), waved-2 (**wa-2**, Keeler, 1935) and wellhaarig (**we**, Hertwig, 1942); two are dominant, namely, Caracul (**Ca**, Carnochan, 1937) and Rex (**Re**, Crew & Auerbach, 1939).

Genetic linkage tests have resulted in assigning them all to linkage groups; **wa-1** to group XI (Bunker & Snell, 1948), **wa-2** to group VII (Snell & Law, 1939), **we** to group V (Hertwig, 1942), **Ca** to group VI (Cooper, 1939) and **Re** to group VII (Falconer, 1947). **Re** and **wa-2**, both in group VII, are loosely linked (Falconer, 1947); no direct tests appear to have been made between **we** and the others, nor between **Ca** and **wa-2**; and the genetic independence of the others has not in all cases been established with a high degree of precision.

These five mutants do not appear to have received detailed comparative study for possible phenotypic differences; and where differences have been reported, genetic milieu was not excluded as a possible cause. Keeler (1935) noted that the mice of his **wa-2** stock, compared with Crew's **wa-1** mice, were more extremely 'marcelled', had shorter hair on the sides and belly and more curled vibrissae; but the two mutants were not segregating within litters, and he concluded that 'even these possible distinctions between the two mutants may be only apparent and not real'. Dunn (1937) reported that in Caracul mice 'the waving of the hair in the first pelage seems to be more marked than in either of the two previously reported recessive waved mutations'; but this also referred to between-stock rather than within-litter comparisons. Crew & Auerbach (1939) had litters in which **Re** and **Ca** segregated together, and they concluded that 'Rex is phaenotypically very similar indeed to if not identical with Caracul'; they also (1940) had litters in which **Re** and **wa-1** segregated simultaneously, but any differences in the phenotypes were not considered sufficient to form the basis of a classification. Similarly, Falconer (1947), who had **Re** and **wa-2** segregating within litters, concluded that 'the Rex and waved-2 phenotypes can be distinguished only with difficulty, if at all, and the double mutant phenotype is unknown'.

The mutant with which this paper is primarily concerned was obtained from a fancier in 1947. It is probably identical with **Re**. For the sake of clarity, however, the name 'Rexoid' (symbol $\mathbf{Re^d}$) is used in this paper and the term 'Rex' (**Re**) is reserved for the mutant derived from the stock described by Crew & Auerbach.

In the course of the investigations it appeared that in segregating litters the Rex-Rexoid compound differed in phenotype from the Rex heterozygote. Litters were therefore

examined which were expected to include both heterozygotes and homozygotes for **Re^d**, and it was found that the Rexoid homozygote differs from the heterozygote but resembles the Rex-Rexoid compound. This demonstration of the incomplete dominance of **Re^d** implied either that **Re^d** and **Re** were not identical, though perhaps alleles, or that **Re** is an incomplete dominant. This was put to the test by examining litters expected to contain both heterozygotes and homozygotes for **Re**; and it was found that these could also be separated phenotypically into two classes which were proved, by genetic test, to correspond with the heterozygous and homozygous condition. **Re** was thereby shown to be an incomplete dominant, notwithstanding earlier reports of its full dominance.

Differences observable with the naked eye were next found, again in segregating litters, between the Rexoid and waved phenotypes (**wa-1** and **wa-2**) and the Rexoid-waved compound phenotypes.

The objects of this paper are to describe the criteria by which these phenotypic distinctions were made, to present the evidence that they corresponded with genotypic differences, and to give the additional data on genetic independence and linkage which accrued in the course of the tests.

No investigations were made into the effects of hair-waving mutants on the microscopic structure of the coat; work in this field, using isogenic stocks, is now proceeding at this Institute and will be reported on at a later date by Dr D. S. Falconer and Mr A. S. Fraser.

MATERIAL AND METHODS

In 1947 Mr F. W. Coles, of Bingley, sent five young mice to Edinburgh for identification of the curly-hair mutant ('Rexoid') which they carried. They were all closely related and said to be descended from a single Rexoid female which had appeared, earlier in the year, in a stock in which curly-haired mice had not previously been seen. Mr Coles had established that the condition was inherited as a Mendelian dominant and concluded that it must represent a new mutation.

All five proved to be heterozygous for Rexoid and, as a prelude to tests for identity of locus with **Re**, **wa-1** and **wa-2**, outcrosses were made to homozygotes for each of these three mutants. The ensuing double heterozygotes were mated to the corresponding double recessive homozygotes obtained from cross-bred stocks available in the laboratory. All the stocks used in the first generation were therefore genetically heterogeneous, and no attempt was made to put any of the mutants on to a standard genetic background; most of the mates used in later generations were themselves the tested progeny of the earlier generations. The stock used for investigating the incomplete dominance of **Re** was mildly inbred, but it never reached more than four generations of sib-mating; several divergent sublines were used, and similar differences between homozygotes and heterozygotes were found in all sublines.

All the phenotypic differences described below were observed in living mice with the naked eye. They do not require the use of a lens, but some of them are best seen by silhouetting the mouse against an electric light.

Extensive genetic tests were made to determine the genotypes of individual mice with respect to the Rexoid locus. An animal under test was crossed with a wild-type test-mate, usually from the Strong *CBA* inbred strain; it was considered a homozygous mutant (or wild type) according as all (or none) of eight or more progeny were curly-haired. A few, known to be heterozygous at the **Re** locus, were also tested for genotype at the **wa-1** or

wa-2 locus by crossing to a waved homozygote; a mouse under test was considered to be homozygous at the waved locus if none of twenty-eight or more young was straight-haired.

Single-factor segregations were tested by the method described by Mather (1937). Recombination fractions required no special statistical treatment, since all the data were from double backcross tests. Errors due to differences of viability between the various phenotypes were minimized by obtaining balanced blocks of data in the coupling and repulsion phases (Fisher & Mather, 1936).

Results

(1) *Rexoid and Rex*

(i) *Phenotypic interactions and probable identity of locus*

The outcross mating **Re**^{d}+ ♂ × **Re Re** ♀ produced nine young, which were found to fall into two distinct classes, showing marked phenotypic differences at 2–3 weeks of age. In one class, consisting of four mice, the coat was very rough and the vibrissae heavily curved towards the mouth ('walrus'); the others had a somewhat smoother coat, with looser waves, and more widely spreading vibrissae.

When crossed with wild-type test-mates the two classes bred differently. The smoother coated mice produced about equal numbers of straight- and curly-haired young; they were therefore identified as Rexoid-free Rex heterozygotes. The rough-coated class produced none but curly-haired young, all similar, totalling 211. This identified them as Rex-Rexoid compounds and established that **Re** and **Re**^{d} must be either very closely linked or at the same locus. No subsequent finding has disproved the assumption that they are at the same locus.

(ii) *Incomplete dominance of Rexoid*

The existence of phenotypic differences between Rex-Rexoid compounds and Rex heterozygotes suggested that **Re**^{d} might be a new, incompletely dominant allele of **Re**. Litters from matings between **Re**^{d}+ heterozygotes were therefore examined; they also were found to contain two classes of curly-haired young, in a 2 to 1 ratio, similar to the two classes from the **Re**^{d} × **Re Re** cross. Eight of the rougher-coated class were genetically tested and all were found to be **Re**^{d}**Re**^{d} homozygotes. **Re**^{d} is therefore incompletely dominant, at least in this genetic milieu.

(iii) *Incomplete dominance of Rex*

Demonstration of the incomplete dominance of **Re**^{d} made it desirable to re-examine the dominance of **Re**, which had previously been thought to be complete. The progeny of some matings known to be of the types **Re**+ × **Re**+ and **Re**+ × **Re Re** were therefore examined, and they too were found to fall into two curly-haired classes, similar to those from the matings involving **Re**^{d} and presumed to correspond with the genotypes **Re Re** and **Re**+. The differences were most easily seen in mice between 10 and 21 days old, but classification was not difficult in the adult. The criteria used were:

(*a*) *Before* 11 *days*. At 7 days, the homozygote has strongly incurled, walrus-like vibrissae and its guard-hairs, especially on the neck and haunch, are curled forwards. The guard-hairs of the heterozygote look longer and nearly straight but are bent outwards just below the tip.

(*b*) *From* 11 *to* 20 *days*. The homozygote continues to show the more strongly curled vibrissae, and they often develop a marked ripple superimposed on the general curvature. The wave pattern of the coat appears in both homozygotes and heterozygotes between 11 and 13 days, but in the homozygote the pattern appears a little earlier, is tighter, and the coat has a rougher appearance. A crest, due to the dorsal hair tracts meeting in the midline of the sacrolumbar region, is sometimes seen in the homozygote, but it is transitory and disappears before 18 days. Homozygotes also show a characteristic parting of the hair tracts on the ventral abdominal midline, posterior to the umbilicus. The hair tracts in the corresponding regions of the heterozygote run more or less craniocaudad. The guard-hairs of the homozygote become rather ragged; those of the heterozygote remain long and fairly straight, with tips bent outward.

(*c*) *After* 3 *weeks*. The vibrissae of homozygotes remain more incurled and rippled than those of heterozygotes; the coat loses the wave pattern, but its appearance remains somewhat rougher in homozygotes.

The validity of classifications based on these criteria was examined by genetically testing twenty-nine mice; diagnosis was proved correct in twenty-eight cases (Table 1). The single faulty diagnosis was one of a number made on adult mice.

Table 1. *Tests of genotype at* **Re** *locus*

Genotype of progeny, diagnosed from phenotype	Mating type of parents: Backcross **ReRe** × **Re**+		Intercross **Re**+ × **Re**+		Double backcross **Re**^{d} and **wa-1**			Double backcross **Re**^{d} and **wa-2**		
	Genotype of progeny, proved by breeding test									
	ReRe	**Re**+	**ReRe**	**Re**+	**Re**^{d}	+	?	**Re**^{d}	+	?
ReRe	4	0	7	0	0	0	0	0	0	0
Re or **Re**^{d}	1	13	0	4	24	0	0	10	10	0
+	0	0	0	0	0	10	3	1	19	1
Totals	5	13	7	4	24	10	3	11	19	1

The descriptions given above apply equally to **Re** and **Re**^{d}. Because of this similarity and the lack of segregation in the litters from matings **ReRe**^{d} × + +, it is very probable that **Re** and **Re**^{d} are not merely at the same locus but are identical.

(2) *Rexoid and waved*-1

(i) *Phenotypic interactions*

Three curly-haired phenotypes were distinguished among the progeny of double backcrosses involving **Re**^{d} and **wa-1**; they corresponded with the genotypes **Re**^{d}+ +**wa-1**, **Re**^{d}+ **wa-1 wa-1** and **wa-1 wa-1**. No evidence was found for any heterozygous effect of **wa-1** in the presence of **Re**^{d}. Classification is most easily made between 13 and 20 days, though differences in the vibrissae exist soon after birth and last into adult life; it is more difficult to distinguish the **Re**^{d}-carrying classes from one another than from the waved-1 class.

The criteria of classification were:

(*a*) *Before* 11 *days*. The waved-1 class is distinguishable from the **Re**^{d}-carrying classes at 4 days by the shape of the vibrissae. In the waved-1 mouse these are regular, straight at the base and hooked forwards near the tip; in a **Re**^{d}-carrying mouse they look crumpled.

(*b*) *From* 11 *to* 20 *days*. The waved-1 class remains clearly distinguishable from the **Re**^{d}-carrying classes by the comparatively straight vibrissae, subterminally hooked, and

the low grade of coat pattern in the waved mouse. Distinction between the Rexoid and Rexoid-waved classes is possible at 12 or 13 days, when the pattern has formed in the coat; the Rexoid-waved class develop a sacrolumbar crest and ventral parting, not found in the Rexoid class. The Rexoid-waved-1 phenotype differs from that of Rexoid homozygotes in the degree of curling in the vibrissae, which is less extreme, and in the sacrolumbar crest, which persists at least until 3 weeks.

(*c*) *After* 3 *weeks*. The waved-1 class remains easily recognizable by the nearly straight vibrissae and the shape of the supraorbital sinus hairs, which resemble a shepherd's crook. The coat pattern disappears between 3 and 8 weeks, and with it the distinction between the Rexoid-waved and Rexoid classes.

In order to test the accuracy of the phenotypic diagnosis, thirty-seven animals chosen at random were tested genetically for the presence or absence of **Re^d**; the results are shown in Table 1. Three tests were not completed; all the remainder confirmed the diagnosis. Five mice, diagnosed as Rexoid-waved-1 and proved to carry **Re^d**, were further tested and proved homozygous **wa-1 wa-1**.

(ii) *Genetic independence*

The data are given in Table 2. They consist of two blocks from coupling and repulsion backcrosses (**Re^d** + / + **wa-1** × + **wa-1** / + **wa-1** and **Re^d wa-1** / + + × + **wa-1** / + **wa-1** respectively). The good Mendelian segregation of both **Re^d** and **wa-1**, with homogeneity between the two bodies, indicates that there were neither serious errors of classification nor reduced viability of the doubly mutant class. The recombination fraction is $50{\cdot}8 \pm 2{\cdot}5\,\%$; it therefore confirms the finding of Crew & Auerbach (1940) that the two mutants are genetically independent.

(3) *Rexoid and waved*-2

(i) *Phenotypic interactions*

Three curly-haired phenotypes were recognized among the progeny of backcrosses involving **Re^d** and **wa-2**; they corresponded with the genotypes **Re^d** + + **wa-2**, **Re^d** + **wa-2 wa-2** and **wa-2 wa-2**. No evidence was found for any heterozygous effect of **wa-2** in the presence of **Re^d**. Distinguishing between the three classes is less easy than with **wa-1**, but it is helped by a reversal of epistasy between 10 and 13 days; before 10 days the Rexoid-waved-2 and waved-2 classes are very similar, but after 13 days the Rexoid-waved class resembles the Rexoid class more closely. Classification is most easily done between 11 and 13 days, but it is still possible at 3 weeks. The criteria used were:

(*a*) *Before* 11 *days*. Differences in the vibrissae exist soon after birth; the Rexoid class is identifiable from 4 to 7 days by the more widely spreading vibrissae. From 7 to 10 days the waved-2 and Rexoid-waved-2 classes are characterized by their fuzzy appearance, due to the heavy forward curling of their guard hairs.

(*b*) *From* 11 *to* 20 *days*. Between 11 and 13 days the coat pattern develops in the **Re^d**-carrying classes. It appears slightly later and is at first rather tighter in the Rexoid-waved-2 than in the Rexoid class; it does not appear in the waved-2 class until between 14 and 16 days. A sacrolumbar crest and ventral abdominal parting appear in the Rexoid-waved-2 class at 12 or 13 days. The guard-hairs of the Rexoid-waved-2 class become long and bent outwards just below the tip, but they are rather more ragged than Rexoid guard-hairs. The guard-hairs of the waved-2 class remain more strongly curled forwards.

Table 2. *Segregation of* **Re^d** *and* **wa-1**

	Phenotypes of progeny					Segregation of **Re**			Segregation of **wa-1**		
Mating type	**Re^d**+	**Re^{d}wa-1**	++	+**wa-1**	Total	D	I	χ^2	D	I	χ^2
BC*	49	37	47	37	170	− 4·0	680	0·024	− 44·0	680	2·847
BR*	60	56	54	58	228	− 8·0	1112	0·058	0·0	912	0·000
Sum	—	—	—	—	—	—	—	0·082	—	—	2·847
Deviation	—	—	—	—	—	−12·0	1792	0·080	− 44·0	1592	1·216
Heterogeneity	—	—	—	—	—	—	—	0·002	—	—	1·631

Independence: $p=\frac{(37+47)+(60+58)}{(170+228)}=\frac{202}{398}$; $s_p=\sqrt{\frac{202\times196}{398^3}}$. Recombination fraction = 50·8 ± 2·5 %.

* B = backcross; C = coupling; R = repulsion.

Table 3. *Segregation of* **Re^d** *and* **wa-2**

	Phenotypes of progeny					Segregation of **Re^d**			Segregation of **wa-2**		
Mating type	**Re^d**+	**Re^{d}wa-2**	++	+**wa-2**	Total	D	I	χ^2	D	I	χ^2
BC	59	26	46	58	189	+38·0	756	1·910	− 42·0	756	2·333
BR	46	35	65	41	187	+50·0	748	3·342	− 70·0	748	6·551
Sum	—	—	—	—	—	—	—	5·252	—	—	8·884
Deviation	—	—	—	—	—	+88·0	1504	5·149	− 112·0	1504	8·340
Heterogeneity	—	—	—	—	—	—	—	0·103	—	—	0·544

Linkage: $p=\frac{(26+46)+(46+41)}{(189+187)}=\frac{159}{376}$; $s_p=\sqrt{\frac{159\times217}{376^3}}$. Recombination fraction = 42·3 ± 2·5 %.

Table 4. *Summary of phenotypes*

Age and character	Genotype					
	ReRe	**Re+**	**Re+wa-1wa-1**	**wa-1wa-1**	**Re+wa-2wa-2**	**wa-2wa-2**
days:						
Vibrissae	Heavily curled towards mouth	Spreading, crumpled	Spreading, crumpled	Curled forwards at tips	Curled towards mouth	Curled towards mouth
Dorsal guard-hairs	Curled forwards	Hooked outwards at tips	Hooked outwards at tips	Curled somewhat forwards	Heavily curled forwards, short, fuzzed	Heavily curled forwards, short, fuzzed
13 days:						
Vibrissae	Heavily curled towards mouth	Spreading	Spreading	Curled forwards at tips	Heavily curled towards mouth	Curled towards mouth
Dorsal guard-hairs	Ragged	Hooked outwards at tips	Hooked outwards at tips	Curled somewhat forwards	Ragged	Heavily curled forwards, short
Pattern	Well formed; may be some sacrolumbar crest	Forming	Forming; sacrolumbar crest present	None	Forming; sacrolumbar crest present	None
Belly hair tracts	Parting	Craniocaudad	Parting	Craniocaudad	Parting	Craniocaudad
20 days:						
Vibrissae	Heavily curled towards mouth, rippled	Spreading, little or no ripple	Spreading, little or no ripple	Curled forwards at tips	Heavily curled towards mouth, rippled	Curled towards mouth
Dorsal guard-hairs	Ragged	Hooked outwards at tips	Hooked outwards at tips	Curled somewhat forwards	Ragged	Rather ragged
Pattern	Rough, somewhat tight	Loose	Loose; sacrolumbar crest present	Slight	Rough; sacrolumbar crest present	Loose
Belly hair tracts	Parting	Craniocaudad	Parting	Craniocaudad	Parting	Craniocaudad

(*c*) *After* 3 *weeks*. Differences between the three classes slowly disappear.

In order to test the accuracy of diagnoses based on these criteria, thirty-one mice were tested genetically for the presence or absence of **Re^d**; one test was not completed and one proved the diagnosis to be wrong (Table 1). Of the mice proved to carry **Re^d**, five which had been diagnosed as Rexoid-waved were further tested and proved to be also homozygous **wa-2wa-2**.

(ii) *Linkage*

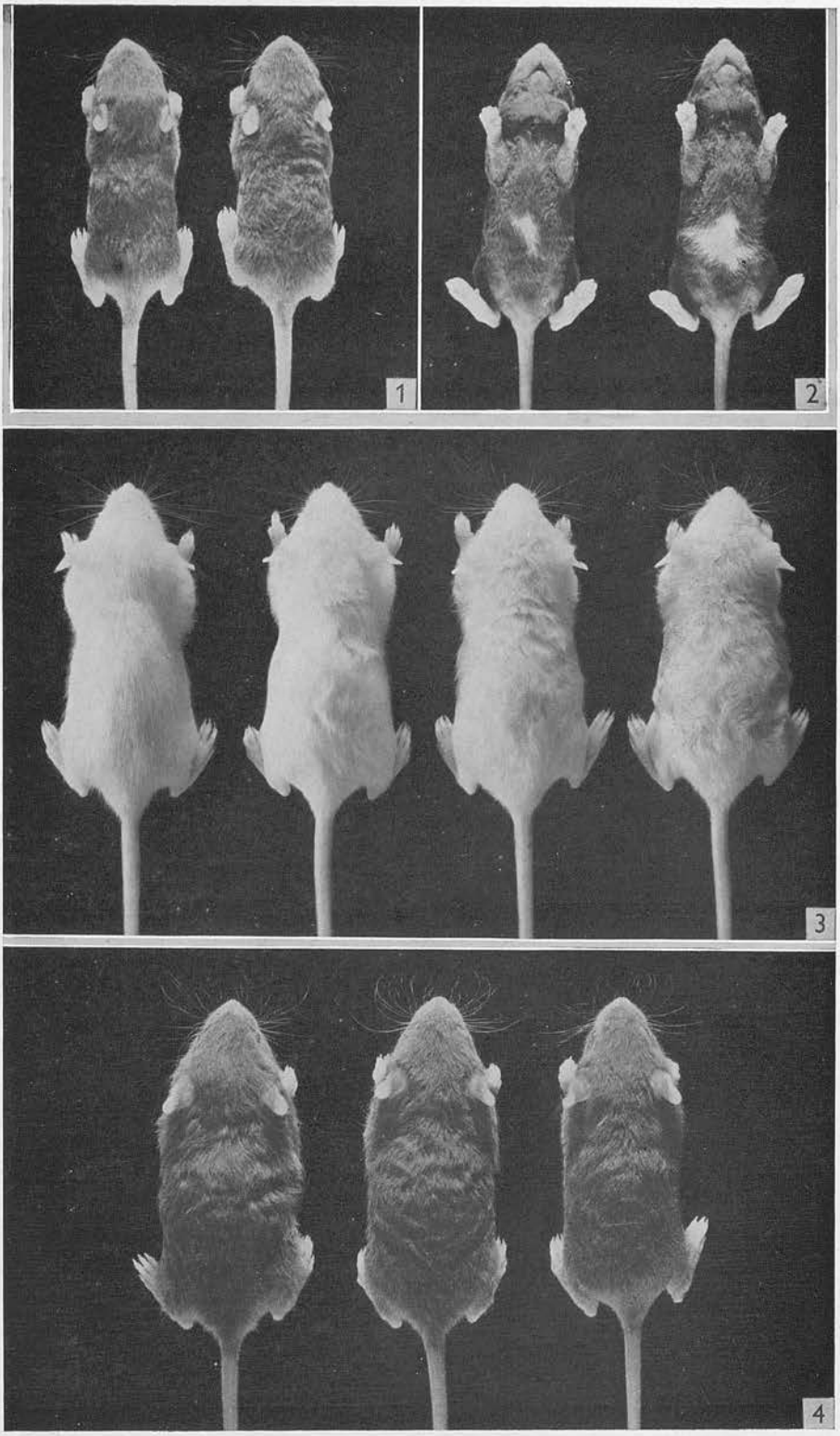
The data are given in Table 3. They consist of two balanced blocks in the coupling and repulsion phases, and refer exclusively to segregation in female gametogenesis. The single-factor segregation analyses show that the data are homogeneous in indicating deficiencies of both **Re^d** and **wa-2**, significant at the 5 and 1 % points respectively. In each block the Rexoid and waved classes contain approximately equal numbers, as expected, but the Rexoid-waved class is only about 55 % as large as the straight-haired class. It is therefore assumed that the disturbed single-factor segregations are both due to low viability of the doubly mutant type and that there have not been serious errors of classification. The recombination fraction obtained for the two bodies of data is $42{\cdot}3 \pm 2{\cdot}5$ %; this is somewhat larger than the value (37·5 %) predicted by Falconer (1947) by applying Kosambi's (1944) map-distance formula to the estimates then available for the **Re—sh-2** and **sh-2—wa-2** recombination fractions, but these estimates were not very precise. The present estimate agrees with Falconer's observed value (41 %).

SUMMARY AND CONCLUSIONS

A hair-waving mutant derived from mice bred by Mr F. W. Coles, and believed to have arisen by spontaneous mutation in 1947, has proved to be indistinguishable from Rex, both phenotypically and genetically. Observations made on heterogeneous material have shown that Rex is not completely dominant, and that there are characteristic phenotypes associated with four other genotypes involving hair-waving mutants, namely, **wa-1wa-1**, **Re**+ **wa-1wa-1**, **wa-2wa-2** and **Re**+ **wa-2wa-2**; no phenotypic differences were found between **Re**+, **Re**+ +**wa-1** and **Re**+ +**wa-2**. The phenotypic differences, which are summarized in Table 4, depend on the shape and amount of ripple in the vibrissae, the appearance of the guard hairs, the grade of waving pattern in the coat and the direction of the sacrolumbar and abdominal hair tracts. They vary greatly with age, being most marked at about 13 days, and tend to disappear in the adult. The mutants are to some extent additive and **wa-1** has less effect than either **wa-2** or **Re**, so that **Re**+ **wa-1wa-1** resembles **Re**+ more closely than **ReRe**, but the reverse is true of **Re**+ **wa-2wa-2**; the latter genotype probably impairs viability.

New segregation data confirm the genetic independence of **Re** and **wa-1** and lead to a new estimate ($42{\cdot}3 \pm 2{\cdot}9$ %) of the recombination fraction between **Re** and **wa-2** in female gametogenesis.

I am indebted to Dr C. Auerbach for a homozygote from her Rex stock and to Mr F. W. Coles for the stock of Rexoid mice and information about their origin.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EXPLANATION OF PLATE 11

Fig. 1. **Re^d**+ and **Re^{d}Re^d**, aged 12 days; dorsal view.
Fig. 2. **Re^d**+ and **Re^{d}Re^d**, aged 13 days; ventral view.
Fig. 3. Straight-haired, **wa-1wa-1**, **Re^d**+ +**wa-1** and **Re^d**+**wa-1wa-1**, aged 22 days; dorsal view.
Fig. 4. **Re^d**+ +**wa-2**, **Re^d**+**wa-2wa-2** and + +**wa-2wa-2**, aged 15 days; dorsal view.

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THE SEX DISTRIBUTION OF *WAVED*-2, *SHAKER*-2 AND *REX* IN THE HOUSE MOUSE.

By

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(Eingegangen am 14. September 1953.)

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Introduction.

The seventh linkage group of *Mus musculus* was first discovered by SNELL and LAW (1939); they found about 26% recombination between *waved*-2 (*wa*-2) and *shaker*-2 (*sh*-2), two mutants which affect, respectively, coat-texture and behaviour. FALCONER (1947) added to the group another coat-texture mutant, *Rex (Re)*; he reported about 20% recombination between *Re* and *sh*-2, and about 41% between *Re* and *wa*-2. The gene order was thus *Re*, *sh*-2, *wa*-2.

Later the same year M. E. WRIGHT (1947) reported anomalous sex-distribution in the progeny of males heterozygous for *wa*-2 and *sh*-2, and attributed it to partial sex-linkage. Her data shewed two unusual features, however, namely (i) both sex-recombinations were of the same intensity, though *wa*-2 and *sh*-2 are not closely linked; and (ii) they exceeded 50%, being about 56% in each case. WRIGHT used a factorial experimental design, which should have precluded bias due to viability differences between the classes of segregants; and the statistical significance of her results is beyond question. Further data on the segregation of *wa*-2 and *sh*-2, due to G. D. SNELL, were quoted by FISHER, LYON and OWEN (1947); they were consistent with WRIGHT'S results, though not of themselves sufficient to demonstrate recombination significantly exceeding 50%. FISHER, LYON and OWEN also gave some data on the sex-recombination of *Re*, culled from the records of experiments made for other purposes: the indicated recombination percentage, 51%, did not shew any significant departure from free segregation.

Recombination exceeding 50% is a theoretical possibility in a genetically long chromosome, provided that chromatid interference is present and is such that pairs of adjacent chiasmata involving all four chromatids are more common than those involving only two. Nevertheless, the partial sex-linkage interpretation of WRIGHT'S data has not been universally accepted (see, e.g., GRÜNEBERG 1952), partly because of the absence of any apparent sex-linkage of *Re*.

A few years ago it became essential, as an ancillary to experiments with translocations then being planned, to obtain further evidence on this question. The breeding records at EDINBURGH were therefore scanned for information on the sex-recombination of Group VII markers. None was available for *wa*-2 and *sh*-2; but for *Re* there were records of over 1500 mice, and these shewed $53{\cdot}0 \pm 1{\cdot}3$% recombination (Table 1A–D). Coupling and repulsion were ill-balanced, however,

so this estimate was open to bias; as evidence for partial sex-linkage it is therefore not necessarily as significant as the figures appear to suggest.

In the absence of decisive evidence it became necessary to undertake experiments with the specific object of testing Linkage Group VII markers for partial sex-linkage. Three independent experiments were undertaken, in each of which two of the three markers *wa*-2, *sh*-2 and *Re* segregated. Each experiment was on the same scale as WRIGHT's, and each used the same balanced, factorial design as hers: the experiment with *wa*-2 and *sh*-2 was, in fact, a repeat of hers. In this way two independent estimates were obtained of the sex-recombination of each of the three markers.

Meanwhile an attempt was made to assess the extent to which differential viability might have biased the estimate of the sex-recombination of *Re*. In the mouse, as in all mammals, sex segregates in the male, and therefore partial sex-linkage could not manifest in the progeny of females heterozygous for *Re*; it follows that any inequalities between the four classes of their progeny must be due to other causes, e.g. differential viability. These departures could therefore be used to estimate viability corrections to be applied to the segregation data from heterozygous males. A search of the records yielded information about more than 1100 progeny of heterozygous females.

The male segregation data had, of course, been divided into coupling and repulsion. For some reason which is now obscure the female segregation data were likewise divided according as the heterozygous female received *Re* from her father or her mother: the numbers of progeny from the two types of female were reasonably well balanced. A remarkable effect then came to light, namely that the female segregation data shewed sex-linkage-like inequalities in their progeny class-sizes; these inequalities were of opposite senses for the two types of female. The effect was statistically significant (Table 5A–D).

The records were thereupon searched for information on the sex-distribution of the progeny of females heterozygous for *wa*-2 and *sh*-2. The numbers from the two types of female were very unequal, but together they totalled over 1300: sex-linkage-like inequalities were present here also, and were even more strongly marked than in the *Rex* data (Table 6A–E).

This suggested that "pseudo-sex-linkage" was probably a real effect, and not merely a matter of chance distribution. If so, it had an important bearing on the question whether or not Group VII is partially sex-linked; for the sex-linkage hypothesis requires not only that linkage shall be shewn by segregating males, but also that it shall not be shewn by segregating females. If pseudo-sex-linkage really is present in females, then true sex-linkage must not be postulated to explain like observations in males.

Two further experiments were therefore undertaken, to test segregating females for pseudo-sex-linkage. The same factorial design was used as before. One experiment was a further repeat of WRIGHT's, save that *wa*-2 and *sh*-2 now segregated in the female parent. In the other experiment *Re* and *Ta* segregated in the female parent. *Ta*, *Tabby*, is a fully sex-linked marker (FALCONER 1952): this experiment therefore tested *Re* not only for pseudo-linkage with sex, but also for true sex-linkage (with *Ta*).

It will be seen that none of the experiments gave consistent evidence of true sex-linkage; but the female segregation experiments both gave some evidence of pseudo-sex-linkage.

Experimental Design.

The plan adopted for all the experiments was the balanced backcross, as used by WRIGHT (1947), following FISHER and MATHER (1936); its object is to provide unbiased estimates of the recombination fractions by arranging that errors introduced in the coupling phase, through reduced viability of some classes of progeny or other causes, shall be balanced by equal and opposite errors introduced in repulsion. In an experiment with three factors, e.g. *wa*-2, *sh*-2 and sex, this involves breeding from all four possible phase types of triple heterozygote, viz. $++\,♂/wa\,sh\,♀$, $wa+\,♂/+sh\,♀$, $wa\,sh\,♂/++\,♀$ and $+sh\,♂/wa+\,♀$. Successful elimination of bias requires that (i) the experiment shall be homogeneous, viability differentials in one phase being of the same magnitude as the corresponding differentials in the opposite phase; and (ii) the same primary number of zygotes shall be obtained from each type of heterozygote. Provided that these conditions are met, an unbiased estimate of the recombination fraction is still obtained even when some of the classes of progeny are missing. Misclassification, however, even if homogeneous, will usually introduce bias.

Methods of Analysis.

Four statistical tests were applied to the data from each experiment. The first two were to see to what extent the necessary conditions for eliminating bias were satisfied; the other two were analyses of the recombinations.

(i) *Single factor segregation test.* For each heterozygote type the single factor segregations were tested for departure from a 1-to-1 ratio; homogeneity of heterozygote types was tested by the 2xn-table method.

(ii) *Test of equality of complementary genotypes.* This test is intended to detect any serious viability differences. Complementary classes of zygote, e.g. $++\,♀$ and $wa\,sh\,♂$, will normally be formed in equal numbers; in the absence of viability differentials the numbers in complementary classes should therefore remain equal. They were tested by χ^2 for departure from a 1-to-1 ratio. For a joint test χ^2 was summed over the four pairs of classes to give χ^2 with four degrees of freedom.

(iii) *Two factor segregations.* Recombinants and non-recombinants were tested for departure from a 1-to-1 ratio (for sex recombination) or from the ratio estimated from the totals (for linked pairs); homogeneity of heterozygote types was tested by the 2xn-table method.

(iv) *Three factor segregations; test for sex-linkage.* This test was used by WRIGHT (1947). Consider, for example, the linked pair *wa*-2 and *sh*-2; they are to be tested jointly for linkage with sex. Assume *ex hypothese* that sex-linkage exists and that the gene order is *wa*-2, *sh*-2, sex. Suppose that recombination of *wa*-2 with *sh*-2 has frequency p, and recombination of *sh*-2 with sex has frequency q; and suppose that there is genetic interference, such that the fre-

quency of simultaneous recombination of *sh*-2 with both *wa*-2 and sex is reduced by an amount ε from the value pq expected in the absence of interference. Expected frequencies can then be written down (in terms of p, q and ε) for the non-recombinant gametes and for the recombinants in which *wa*-2, *sh*-2 or sex, respectively, have crossed over. For example, the heterozygote $++\,♂/wash\,♀$ gives:

Crossover type	*Classes*	*Frequency*
Non-recombinant	$++\,♂$ & $wash\,♀$	$a=(1-p)\,(1-q)-\varepsilon$
Sex	$++\,♀$ & $wash\,♂$	$b=(1-p)\,q+\varepsilon$
sh-2	$+sh\,♂$ & $wa+\,♀$	$c=p\,q-\varepsilon$
wa-2	$wa+\,♂$ & $+sh\,♀$	$d=p(1-q)+\varepsilon$

If *wa*-2 and *sh*-2 are not linked to sex, then $q=\frac{1}{2}$ and $\varepsilon=0$, and the class frequencies become $a=b=\frac{1}{2}(1-p)$ and $c=d=\frac{1}{2}p$; these are the usual expressions for the two-factor linkage of *wa*-2 with *sh*-2. Significant inequality of a with b, or of c with d, therefore constitutes evidence that sex is not independent of *wa*-2 and *sh*-2.

In WRIGHT's experiment c and d were equal (though a and b unequal). This requires that

$$pq-\varepsilon=p(1-q)+\varepsilon$$
$$\text{i.e.} \qquad \varepsilon=\tfrac{1}{2}p(2q-1)$$

For ε to be positive, i.e. if there is to be genetical interference in the normal sense, $(2q-1)$ must be positive, or $q>\frac{1}{2}$.

When, on the other hand, a and b are equal (c and d being unequal), it follows that

$$(1-p)\,(1-q)-\varepsilon=(1-p)\,q+\varepsilon$$
$$\text{i.e.} \qquad \varepsilon=\tfrac{1}{2}\,(1-p)\,(1-2q)$$

For ε to be positive this requires that $q<\frac{1}{2}$

Can $a=b$ and $c=d$ in the presence of partial sex linkage? This requires that

$$\tfrac{1}{2}p(2q-1)=\tfrac{1}{2}(1-p)\,(1-2q)$$

This condition is satisfied only by $q=\frac{1}{2}$ and $\varepsilon=0$: in other words, the locus of sex must be so remote from that of *sh*-2 that they segregate independently.

Similar results are obtained if the order is assumed to be *sh*-2, *wa*-2, sex.

Applied to experimental results, the test therefore consists of summing corresponding crossover types for all types of heterozygote, then testing the equality of a with b and c with d.

Stocks.

The stock of *Re* used was founded by a mouse from Sir RONALD FISHER's stocks received in 1947. *wa*-2 and *sh*-2 were introduced by a mouse obtained from Dr. D. S. FALCONER, also in 1947; he had obtained the stock a few months earlier from Sir RONALD FISHER. The stocks used in the present work were therefore closely related to those referred to by WRIGHT (1947) and by FISHER, LYON and OWEN (1947).

Classification of the phenotypes *Re*, *Rewa*-2 and *wa*-2 was based on the criteria given by CARTER (1951). Classification of *sh*-2 was delayed until about the age of weaning and was based on the onset of deafness as well as head movements. *Ta* could not be classified with confidence in *Re* females, so the *Re*+ *Ta*+ and *Re*+ ++ classes were lumped. Other classifications presented no difficulties.

Results.

Tables 1A, 5A and 6A give the segregation data, culled from the records at EDINBURGH, of mice which were bred for various purposes unconnected with these experiments. Tables 2A, 3A, 4A, 7A and 8A give the segregation data obtained in the factorial experiments. Following each table A, tables B, C, D and (where present) E and F give the results of the statistical tests.

(i) *Re* segregating in males. (Unbalanced data.) (Tables 1A—D.) Single-factor segregations are Mendelian and homogeneous. There is some evidence of

Table 1A. *Segregation of Rex and sex in males*.*

Genotype of father	Phenotypes of progeny				
	Re ♀	*Re* ♂	+ ♀	+ ♂	Total
Re ♂/+ ♀ ..	322	287	286	298	1193
+ ♂/*Re* ♀. .	77	116	83	75	351
Totals	399	403	369	373	1544

* These are the data, then unpublished, referred to by MICHIE (1952).

Table 1B. *Single factor segregations; analysis of* χ^2.

	Re and +			♂ and ♀		
	χ^2	DF	*P*	χ^2	DF	*P*
Deviation	2.332	1	>0.1	0.041	1	>0.8
Heterogeneity . .	1.682	1	>0.1	3.140	1	>0.05

Table 1C. *Equality of complementary genotypes; analysis of* χ^2.

χ^2	DF	*P*
2.373	2	>0.3

Table 1D. *Two factor segregations; analysis of* χ^2.

	Re and ♂		
	χ^2	DF	*P*
Deviation. . . .	5.723	1	<0.02
Heterogeneity. .	2.422	1	>0.1
Recombination .	53.04 ± 1.27%		

sex-linkage of *Re*, with recombination exceeding 50%; it is significant at the 5% but not the 1% probability level.

(ii) *Re* and *sh*-2 segregating in males. (Tables 2A–E.) Single-factor segregations are Mendelian and homogeneous. There is no evidence of sex-linkage of either *Re* or *sh*-2.

(iii) *Re* and *wa*-2 segregating in males. (Tables 3A–E.) Single factor segregations are Mendelian and homogeneous. There is no evidence of sex-linkage of either *Re* or *wa*-2.

Table 2A. *Segregation of Rex, shaker-2 and sex in males.*

Genotype of father	Phenotypes of progeny								
	Re + ♀	*Re* + ♂	*Resh* ♀	*Resh* ♂	+ + ♀	+ + ♂	*+sh* ♀	*+sh* ♂	Total
Re+♂/+*sh*♀ . .	15	28	6	6	4	5	16	25	105
++♂/*Resh*♀ . .	4	8	16	25	24	19	5	4	105
+*sh*♂/*Re*+♀ . .	19	22	5	8	2	8	22	18	104
Resh♂/++♀ . .	0	2	20	21	25	20	7	6	101
Totals	38	60	47	60	55	52	50	53	415

Table 2B. *Single factor segregations; analysis of* χ^2.

	Re and +			+ and *sh*-2			♂ and ♀		
	χ^2	DF	*P*	χ^2	DF	*P*	χ^2	DF	*P*
Deviation . .	0.060	1	>0.8	0.060	1	>0.8	2.952	1	>0.05
Heterogeneity	2.569	3	>0.3	0.711	3	>0.9	3.257	3	>0.3

Table 2C. *Equality of complementary genotypes; analysis of* χ^2.

χ^2	DF	*P*
3.851	4	>0.3

Table 2D. *Two factor segregations; analysis of* χ^2.

	Re and ♂			♂ and *sh*-2			*Re and sh*-2		
	χ^2	DF	*P*	χ^2	DF	*P*	χ^2*	DF	*P*
Deviation . . .	0.292	1	>0.5	0.292	1	>0.5	0.135	1	>0.9
Heterogeneity .	4.188	3	>0.2	1.768	3	>0.5	1.829	3	>0.5
Recombination	51.33 ± 2.45%			51.33 ± 2.45%			19.28 ± 1.94%		

* Expected values based on 20% recombination.

Table 2E. *Three factor segregations; sex-linkage test.*

Genotype of father	Crossover types					Equality test		
	oc	♂	*sh*-2	*Re*		χ^2	DF	*P*
Re+♂/+*sh*♀ . .	44	40	10	11	oc and ♂ . . .	0.361	1	>0.5
++♂/*Resh*♀ . .	35	49	8	13	*sh*-2 and *Re* .	0.000	1	>0.99
+*sh*♂/*Re*+♀ . .	37	44	13	10				
Resh♂/++♀ . .	46	40	9	6				
Totals	162	173	40	40				

(iv) *wa*-2 and *sh*-2 segregating in males. (Tables 4A–F.) Heterozygote types are homogeneous in giving an excess of *sh*-2 *sh*-2 homozygotes and a heavy excess of males. Complementary genotypes are very unequal. This is due, at least in part, to sex-ratio differences between the four phenotypes +, *wa*-2, *sh*-2

Table 3A. *Segregation of Rex, waved-2 and sex in males.*

Genotype of father	Phenotypes of progeny								
	Re+ ♀	*Re*+ ♂	*Rewa* ♀	*Rewa* ♂	++ ♀	++ ♂	+*wa* ♀	+*wa* ♂	Total
Re+♂/+*wa*♀. .	11	14	17	11	12	16	13	13	107
++♂/*Rewa*♀. .	7	6	10	17	20	11	12	16	99
+*wa*♂/*Re*+♀. .	18	15	3	13	10	7	16	13	95
Rewa♂/++♀. .	13	12	12	20	10	16	10	12	105
Totals	49	47	42	61	52	50	51	54	406

Table 3B. *Single factor segregations; analysis of* χ^2.

	Re and +			+ and *wa*-2			♂ and ♀		
	χ^2	DF	*P*	χ^2	DF	*P*	χ^2	DF	*P*
Deviation . .	0.158	1	>0.5	0 246	1	>0.5	0.798	1	>0.3
Heterogeneity	4.364	3	>0.1	1.334	3	>0.5	1.375	3	>0.5

Table 3C. *Equality of complementary genotypes; analysis of* χ^2.

χ^2	DF	P
1.818	4	>0.7

Table 3D. *Two factor segregations; analysis of* χ^2.

	Re and ♂			♂ and *wa*-2			*Re and wa*-2		
	χ^2	DF	*P*	χ^2	DF	*P*	χ^2 *	DF	*P*
Deviation . . .	2.522	1	>0.1	0.246	1	>0.5	0 027	1	>0.8
Heterogeneity .	0.946	3	>0.8	7.805	3	>0.05	6.592	3	>0.05
Recombination	53.94 ± 2.47%			48.77 ± 2.48%			43.60 ± 2.46%		

* Expected values based on 44% recombination.

Table 3E. *Three factor segregations; sex-linkage test.*

	Crossover types					Equality test		
	oc	♂	*Re*	*wa*-2		χ^2	DF	*P*
Re+♂/+*wa*♀. .	27	24	33	23	oc and ♂. . .	0.528	1	>0.3
++♂/*Rewa*♀. .	21	37	18	23	*Re* and *wa*-2 .	2.491	1	>0.1
+*wa*♂/*Re*+♀. .	31	31	23	10				
Rewa♂/++♀. .	30	28	25	22				
Totals	109	120	99	78				

and *wa*-2 *sh*-2 (Table 4F): within each phenotype the sex-ratio is homogeneous over the four heterozygotes types. Neither *wa*-2 nor *sh*-2 individually gives evidence of sex-linkage, but the joint test reveals inequality of the *sh*-2 and *wa*-2 crossover types, significant at the 5% (but not the 1%) probability level.

Table 4A. *Segregation of waved*-2, *shaker*-2 *and sex in males.*

Genotype of father	Phenotypes of progeny								
	++♀	++♂	+*sh*♀	+*sh*♂	*wa*+♀	*wa*+♂	*wash*♀	*wash*♂	Total
++♂/*wash*♀ . .	14	22	6	8	2	6	15	32	105
wa+♂/+*sh*♀ . .	8	9	14	18	13	20	6	14	102
wash♂/++♀ . .	15	18	17	8	1	4	14	27	104
+*sh*♂/*wa*+♀ . .	6	14	24	20	12	18	2	4	100
Totals	43	63	61	54	28	48	37	77	411

Table 4B. *Single factor segregations; analysis of* χ^2.

	+ and *wa*-2			+ and *sh*-2			♂ and ♀		
	χ^2	DF	*E*	χ^2	DF	*P*	χ^2	DF	P
Deviation . .	2.338	1	>0.1	5.375	1	<0.05	12.966	1	<0.001
Heterogeneity	7.140	3	>0.05	4.955	3	>0.1	2.510	3	>0.3

Table 4C. *Equality of complementary genotypes; analysis of* χ^2.

χ^2	DF	P
26·188	4	<0·001

Table 4D. *Two factor segregations; analysis of* χ^2.

	♂ and *wa*-2			♂ and *sh*-2			*wa*-2 and *sh*-2		
	χ^2	DF	*P*	χ^2	DF	*P*	χ^{2*}	DF	*P*
Deviation . . .	0.703	1	>0.3	2.046	1	>0.1	0.000	1	>0.99
Heterogeneity .	6.243	3	>0.1	1.176	3	>0.7	6.286	3	>0.05
Recombination	47.93 ± 2.46 %			53·53 ± 2·46 %			27.98 ± 2.21 %		

* Expected values based on 28% recombination.

Table 4E. *Three factor segregations; sex-linkage test.*

Genotype of father	Crossover types					Equality test		
	oc	♂	*sh*-2	*wa*-2		χ^2	DF	*P*
++♂/*wash*♀ . .	37	46	10	12	oc and ♂ . . .	0.122	1	>0.7
wa+♂/+*sh*♀ . .	34	31	22	15	*sh*-2 and *wa*-2 .	4.600	1	<0.05
wash♂/++♀ . .	42	32	21	9				
+*sh*♂/*wa*+♀ . .	32	42	16	10				
Totals	145	151	69	46				

Table 4F. *Heterogeneity of sex ratios between phenotypes* +, *wa*-2, *sh*-2 *and wa*-2*sh*-2.

Phenotype	Observed		Analysis of χ^2		
	♀	♂	χ^{2*}	DF	*P*
+	43	63	0.008	1	—
sh-2	61	54	6.895	1	—
wa-2	28	48	0.543	1	—
wa-2*sh*-2	37	77	3.440	1	—
Sum			10.887	4	—
Deviation	169	242	0.002	1	>0·95
Heterogeneity . . .			10.885	3	<0·02

* Expected values based on 41% females.

Table 5A. *Segregation of Rex in females.*

Genotype of mother	Phenotypes of progeny				
	Re ♀	*Re* ♂	+ ♀	+ ♂	Total
Re ♂/+ ♀. . .	123	143	127	121	514
+ ♂/*Re* ♀. . .	163	153	141	175	632
Totals	286	296	268	296	1146

Table 5B. *Single factor segregations; analysis of* χ^2.

	Re and +			♂ and ♀		
	χ^2	DF	*P*	χ^2	DF	*P*
Deviation	0.283	1	>0.5	1.260	1	>0.2
Heterogeneity	0.348	1	>0.5	0.033	1	>0.8

Table 5C. *Equality of complementary genotypes; analysis of* χ^2.

χ^2	DF	P
1·562	2	>0.3

Table 5D. *Two factor distributions; analysis of* χ^2.

	Re and ♂		
	χ^2	DF	*P*
Deviation	4.276	1	<0.05
Heterogeneity	0.103	1	>0.7
Dissociation	46.94 ± 1.39%		

(v) *Re* segregating in females. (Tables 5A–D.) Single factor segregations are Mendelian and homogeneous. There is some evidence of pseudo-linkage of *Re* with sex, with 47% dissociation; it is significant at the 5% but not the 1% probability level.

(vi) *wa*-2 and *sh*-2 segregating in females. (Unbalanced data.) (Tables 6A–E.) Single factor segregations are Mendelian and homogeneous. There is strong evidence of pseudo-linkage of both *wa*-2 and *sh*-2 with sex, with 45% dissociation in each case, significant at the 0.1% probability level.

(vii) *wa*-2 and *sh*-2 segregating in females. (Tables 7A–E.) There is a slight deficiency of *wa*-2 *wa*-2 homozygotes and marked heterogeneity between heterozygote types in this respect; the segregations of *sh*-2 and sex are Mendelian and homogeneous. There is no significant inequality of complementary genotypes. Neither *wa*-2 nor *sh*-2 individually shews significant pseudo-linkage with sex, but the joint test reveals inequality of the *wa*-2 and *sh*-2 crossover types, significant at the 5% probability level. The pseudo-linkage of *wa*-2 with sex, 45.4%, is of the same strength as in the unbalanced data (Table 6D); *sh*-2, however, shews no sex-association.

Table 6A. *Segregation of waved-2 and shaker-2 in females.*

Genotype of mother	Phenotypes of progeny								
	++♀	++♂	+*sh*♀	+*sh*♂	*wa*+♀	*wa*+♂	*wash*♀	*wash*♂	Total
++♂/*wash*♀ . .	13	11	9	2	3	4	10	8	60
wash♂/++♀ . .	275	243	72	67	83	76	189	292	1297
Totals	288	254	81	69	86	80	199	300	1357

Table 6B. *Single factor segregations; analysis of* χ^2.

	+ and *wa*-2			+ and *sh*-2			♂ and ♀		
	χ^2	DF	*P*	χ^2	DF	*P*	χ^2	DF	*P*
Deviation . .	0.537	1	>0.3	2.565	1	>0.1	1.769	1	>0.1
Heterogeneity	1.352	1	>0.2	0.006	1	>0.9	2.581	1	>0.1

Table 6C. *Equality of complementary genotypes; analysis of* χ^2.

χ^2	DF	*P*
8.793	4	>0.05

Table 6D. *Two factor distributions; analysis of* χ^2.

	♂ and *wa*-2			♂ and *sh*-2			*wa*-2 and *sh*-2		
	χ^2	DF	*P*	χ^2	DF	*P*	χ^{2*}	DF	*P*
Deviation . . .	11.514	1	<0.001	15.494	1	<0.001	0.063	1	>0.8
Heterogeneity .	3.191	1	>0.05	0.044	1	>0.8	1.598	1	>0.2
Dissociation . .	45.39 ± 1.35%			44.66 ± 1.35%			23.29 ± 1.15%		

* Expected values based on 23% recombination.

Table 6E. *Three-factor distributions; sex-association test.*

Genotype of mother	Crossover types					Equality test		
	oc	♂	*sh*-2	*wa*-2		χ^2	DF	*P*
++♂/*wash*♀ . .	21	21	5	13	oc and ♂ . . .	17.510	1	<0.001
wash♂/++♀ . .	567	432	148	150	*sh*-2 and *wa*-2.	0.316	1	>0.5
Totals	588	453	153	163				

(viii) *Re* and *Ta* segregating in females. (Tables 8A–E.) Single factor segregations are Mendelian and homogeneous; there is no evidence of viability differentials. *Re* does not shew linkage with the sex-linked gene *Ta*, nor pseudo-linkage with sex. *Ta* shews pseudo-linkage with sex, revealed by both the two-factor and the three-factor tests.

(ix) Summary of results. Recombination fractions, both from previously published data and from the data of Tables 1A–8A, are summarised in Table 9.

Table 7A. *Segregation of waved-2 and shaker-2 in females.*

Genotype of mother	Phenotypes of progeny								
	++♀	++♂	+*sh*♀	+*sh*♂	*wa*+♀	*wa*+♂	*wash*♀	*wash*♂	Total
++♂/*wash*♀ . .	15	24	3	9	6	5	17	25	104
wa+♂/+*sh*♀ . .	5	5	21	14	24	20	5	9	103
wash♂/++♀ . .	31	27	8	5	4	4	8	19	106
+*sh*♂/*wa*+♀ . .	8	10	18	25	10	19	9	4	103
Totals	59	66	50	53	44	48	39	57	416

Table 7B. *Single factor segregations; analysis of* χ^2.

	+ and *wa*-2			+ and *sh*-2			♂ and ♀		
	χ^2	DF	*P*	χ^2	DF	*P*	χ^2	DF	*P*
Deviation . .	3.846	1	>0.05	0.779	1	>0.3	2.461	1	>0.1
Heterogeneity	13.564	3	<0.01	6.781	3	>0.05	4.460	3	>0.2

Table 7C. *Equality of complementary genotypes; analysis of* χ^2.

χ^2	DF	*P*
7.853	4	>0.05

Table 7D. *Two factor distributions; analysis of* χ^2.

	♂ and *wa*-2			♂ and *sh*-2			*wa*-2 and *sh*-2		
	χ^2	DF	*P*	χ^2	DF	*P*	χ^2 *	DF	*P*
Deviation . . .	3.471	1	>0.05	0.038	1	>0.8	0.009	1	>0.9
Heterogeneity .	0.919	3	>0.8	2.462	3	>0.3	3.340	3	>0.3
Dissociation . .	45.43±2.44%			50.48±2.45%			23.80±2.09%		

* Expected values based on 24% recombination.

Table 7E. *Three factor distributions; sex-association test.*

Genotype of mother	Crossover types					Equality test		
	oc	♂	*sh*-2	*wa*-2		χ^2	DF	*P*
++♂/*wash*♀ . .	41	40	15	8	oc and ♂	0.912	1	>0.3
wa+♂/+*sh*♀ . .	41	38	14	10	*sh*-2 and *wa*-2	4.455	1	<0.05
wash♂/++♀ . .	50	35	12	9				
+*sh*♂/*wa*+♀ . .	35	37	19	12				
Totals	167	150	60	39				

Discussion.

We are faced with three questions. (i) Do the progeny of heterozygotes for Group VII markers shew sex-linkage-like departures from random segregation? (ii) If so, are these departures confined to the progeny of male heterozygotes, as would be expected for true sex-linkage? (iii) If they exist also in the progeny of female heterozygotes, what is their nature?

Table 8A. *Segregation of Rex and Tabby in females.*

Genotype of mother	Phenotypes of progeny								
	Ta+♂	++♂	*Ta Re* ♂	+*Re* ♂	*Ta*+♀	++♀	6-class totals	*Re* ♀	7-class totals
Ta Re♂/++♀ .	24	30	23	28	26	24	155	43	198
+*Re*♂/*Ta*+♀. .	25	32	22	24	22	34	159	58	217
Ta+♂/+*Re*♀. .	23	25	26	26	31	26	157	42	199
++♂/*Ta Re*♀ .	27	20	27	26	16	36	152	49	201
Totals	99	107	98	104	95	120	623	192	815

Table 8B. *Single factor segregations; analysis of* χ^2.

	Ta and +			*Re* and +			♂ and ♀		
	χ^2	DF	*P*	χ^2	DF	*P*	χ^2	DF	*P*
Deviation . .	2.441	1	>0.1	0.894	1	>0.3	0.001	1	>0.95
Heterogeneity	1.859	3	>0.5	0.637	3	>0.8	1.294	3	>0.7

Table 8C. *Equality of complementary genotypes; analysis of* χ^2.

χ^2 *	DF	*P*
3.120	3	>0.3

* The classes *Ta*+♂ and ++♂ lumped for comparison with the lumped classes *Ta Re* ♀ and +*Re* ♀.

Table 8D. *Two factor distributions; analysis of* χ^2.

	Ta and ♂			*Re* and ♂			*Ta* and *Re*		
	χ^2	DF	*P*	χ^2	DF	*P*	χ^2	DF	*P*
Deviation . . .	4.175	1	<0.05	1.680	1	>0.1	0.002	1	>0.95
Heterogeneity .	1.668	3	>0.5	1.397	3	>0.7	3.169	3	>0.3
Dissociation . .	54.09 ± 1.99 %			52.27 ± 1.75 %			50.08 ± 2.00 %		

Table 8E. *Three factor distributions; sex-dissociation test.*

Genotype of mother	Crossover types					Equality test		
	oc	♂	*Ta*	*Re*		χ^2	DF	*P*
Ta Re♂/++♀ .	47	30	54	24	oc and ♂. . .	4.891	1	<0.05
+*Re*♂/*Ta*+♀. .	46	25	56	32	*Ta* and *Re* . .	0.462	1	>0.3
Ta+♂/+*Re*♀. .	23	57	25	52				
++♂/*Ta Re*♀ .	20	63	27	42				
Totals	136	175	162	150				

It is clear that there was some disturbance of the sex distribution of *wa*-2 and *sh*-2 when they segregated simultaneously. All of three balanced experiments intended to test this distribution gave statistically significant indications of a disturbance. In WRIGHT's experiment the significance was high ($\chi^2_1 > 10$); SNELL's backcross data were consistent with WRIGHT's; and beside these may be set the unbalanced data gleaned from the breeding records at EDINBURGH

Table 9. *Summary of all data.*

Source of data	Phase balance †	No. of mice	Recombination percentages							
			♂ and *Re*	♂ and *sh*-2	♂ and *wa*-2	♂ and *Ta*	*Re* and *Ta*	*Re* and *sh*-2	*Re* and *wa*-2	*wa*-2 and *sh*-2
Male gametogenesis										
SNELL and LAW (1939) . . .	U	56	—	—	—	—	—	—	—	26.8 ± 5.9
FALCONER (1947).	U	38	—	—	—	—	—	18.4 ± 4.4	—	—
WRIGHT (1947)	B	453	—	56.7 ± 2.3**	56.1 ± 2.3**	—	—	—	—	31.1 ± 2.2
SNELL, quoted by FISHER, LYON and OWEN (1947) . .	U	143	—	54.5 ± 4.0	55.2 ± 4.0	—	—	—	—	30.1 ± 3.6
FISHER, LYON and OWEN (1947)	U	689	51.0 ± 2.0	—	—	—	—	—	—	—
New data, Table 1A	U	1544	53.0 ± 1.3*	—	—	—	—	—	—	—
New data, Table 2A	B	415	51.3 ± 2.5	51.3 ± 2.5	—	—	—	19.3 ± 1.9	—	—
New data, Table 3A	B	406	53.9 ± 2.5	—	48.8 ± 2·5	—	—	—	43.6 ± 2.5	—
New data, Table 4A	B	411	—	53.5 ± 2.5	48.0 ± 2·5	—	—	—	—	28.0 ± 2.2
Female gametogenesis										
SNELL and LAW (1939) . . .	U	256	—	—	—	—	—	—	—	25.0 ± 2.7
FALCONER (1947).	U	23	—	—	—	—	—	21.7 ± 8.6	—	—
FALCONER (1947).	U	122	—	—	—	—	—	—	41.0 ± 7.2	—
CARTER (1951)	B	376	—	—	—	—	—	—	42.3 ± 2.5	—
New data, Table 5A	B	1146	46.9 ± 1.4*	—	—	—	—	—	—	—
New data, Table 6A	U	1357	—	44.7 ± 1.3**	45.4 ± 1.3**	—	—	—	—	23.3 ± 1.1
New data, Table 7A	B	416	—	50.5 ± 2.4	45.4 ± 2.4	—	—	—	—	23.8 ± 2.1
New data, Table 8A	B	815	52.3 ± 1.7	—	—	—	—	—	—	—
New data, Table 8A	B	623	—	—	—	54.1 ± 2.0*	50.0 ± 2.0	—	—	—

† B balanced; U unbalanced. * Significant at the 5% probability level; ** significant at the 1% level.

($\chi_1^2 > 17$). In the two new balanced experiments (Tables 4A and 7A), however, the significance was low ($\chi_1^2 = 4.6$ and $\chi_1^2 = 4.45$). The evidence of disturbance is less convincing for *Re* when segregating by itself or with *Ta*; it is non-existent for *Re* when segregating with *wa*-2 or *sh*-2.

It is clear also that a sex-distribution disturbance is not confined to the progeny of male heterozygotes: in fact, the highest value of χ^2 found ($\chi_1^2 > 17$) referred to the segregation of *wa*-2 and *sh*-2 in females. On the other hand, a disturbance was not consistently present in either the female or the male segregation data: thus *sh*-2 gave a significantly high sex-recombination in WRIGHT's experiment ($56.7 \pm 2.3\%$), but Table 2A shews it segregating independently of sex ($51.3 \pm 2.5\%$); for female segregation, *sh*-2 shews significant association with sex in Table 6A ($44.7 \pm 1.3\%$), but not in Table 7A ($50.5 \pm 2.4\%$).

Taken together, the data cannot be said to support the sex-linkage interpretation. Two features lead to this conclusion, namely (i) the failure of *wa*-2 and *sh*-2 to give consistent evidence of sex-linkage in experiments intended to repeat and extend WRIGHT's work, and (ii) the presence of a sex-linkage-like disturbance in the female segregation data, where it cannot be due to sex-linkage. Affinity (MICHIE 1953), i.e. a tendency for centromeres of like origin to shew non-random segregation at meiosis, likewise fails to provide an explanation of pseudo-sex-linkage, where sex is segregating in one parent and marker genes in the other.

What, then, is the nature of the disturbance? One possible hypothesis is based on selective fertilisation. It requires, however, a number of assumptions which appear rather improbable, e.g. that a sperm carrying a marked chromosome segment derived from the male's father has an enhanced (or reduced) probability of fertilising an egg in which the homologous chromosome segment was derived from the female's father.

Another effect is suggested by the male segregation data of Table 4A, namely classification as males of animals which are genetically female, especially within the phenotype *wa*-2 *sh*-2; there are similar indications in the corresponding female segregation data (Tables 6A and 7A). This could hardly be due to widespread and homogeneous sexing errors, so it would be necessary to suppose that there may be some change in the appearance of the external genitalia associated with this phenotype. This, however, though it would bias the estimate of an existing linkage, could not produce the appearance of linkage where none in fact exists.

It seems unlikely that an early solution of the problem will be obtained from breeding data alone. Table 9 summarises the records of more than eight thousand mice, of which nearly three thousand were bred in balanced experiments; and yet no clear evidence has been obtained about the cause of the disturbed segregations. Elucidation may perhaps require a cytogenetic approach. For the present it can only be concluded that there is sometimes a disturbance in the sex-distribution of *wa*-2 and *sh*-2, and perhaps of *Re*, when segregating in either sex; that the available data fail to support a partial sex-linkage interpretation; and that the nature of the disturbance is unknown.

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Summary.

Wright (1947) reported partial sex-linkage of *wa*-2 (*waved*-2) and *sh*-2 (*shaker*-2) in the mouse, with recombination exceeding 50%. Three experiments are now reported, including a repeat of Wright's experiment, in which *wa*-2, *sh*-2 and a linked marker *Re (Rex)* were tested; no consistent evidence of sex-linkage was found. A linkage-like disturbance was found in the sex-distribution of the progeny of females heterozygous for *wa*-2 and *sh*-2, both in the records of breeding performed for other purposes and in an experiment specially intended to test this point. The cause of the disturbance is obscure; it cannot be due to sex-linkage. New data are given on the linkages of *wa*-2, *sh*-2 and *Re*.

Zusammenfassung.

Wright (1947) berichtet, daß die Gene *wa*-2 (*waved*-2) und *sh*-2 (*shaker*-2) partielle Geschlechtsgebundenheit zeigen, und daß die Rekombinationshäufigkeit mit dem Geschlecht 50% übersteigt. Drei Experimente, von denen eines eine Wiederholung des Wrightschen Experiments war, wurden ausgeführt, um die Beziehung von *wa*-2, *sh*-2 und dem mit ihnen gekoppelten Gen *Re (Rex)* zum Geschlecht zu prüfen. Sie ergaben keinen gesicherten Beweis für Geschlechtsgebundenheit. Eine einer Koppelung gleichende Störung der Geschlechtsverteilung fand sich in der Nachkommenschaft von Weibchen, die heterozygot waren für *wa*-2 und *sh*-2, und zwar übereinstimmend in einem eigens dazu angestellten Experiment wie in älteren, zu anderen Zwecken angestellten Kreuzungen. Die Ursache dieser Störung konnte nicht festgestellt werden; es kann sich aber nicht um Geschlechtsgebundenheit handeln. Neue Koppelungswerte für *wa*-2, *sh*-2 und *Re* werden gegeben.

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A search for chromatid interference in the male house mouse.

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(Short title for page headings: Chromatid interference in the mouse).

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INTRODUCTION.

When three linked genes A, B and C segregate together, the frequency of recombination between B and C is usually reduced among individuals in which B has recombined also with A (Sturtevant, 1913); this phenomen was investigated by Muller (1916), who named it cross-over interference.

It was supposed that cross-over interference is due to a tendency for chiasma formation to be suppressed in the neighbourhood of an existing chiasma. Evidence from cytological data of the existence of this phenomenon, chiasma interference, was found by Haldane (1931).

A possible alternative mechanism for cross-over interference was pointed out by Sansome (1933). Chiasma formation involves two strands of a four-strand bivalent (Janssens, 1909; Anderson, 1925) and cross-over interference would result if there were a tendency for one chiasma to involve strands other than those involved in an adjacent chiasma. Cytological confirmation of the occurrence of this phenomenon, chromatid interference, was obtained by Hearne & Huskins (1935).

Cross-over interference is thus the result of two quite distinct cytological phenomena affecting, respectively, the position and the strand-configuration of a new chiasma relative to an adjacent chiasma. There is no a priori reason for supposing that they operate with the same strength, or even the same relative strength, in all biological material; and therefore it is necessary to introduce them explicitly into any mathematical model used to investigate their genetical consequences. The genetical implications of chiasma interference were investigated by Haldane (1919, 1931) and those of chromatid interference by Weinstein (1928, 1936). The general equation relating/

relating recombination frequency to chiasma frequency and chromatid interference strength was given by Carter & Robertson (1952); but attempts to particularise require justification in the form of chiasma frequency and chromatid configuration observations of the species in question; and for most biological material chromatid configurations, at least, are at present technically difficult or impossible to observe.

However, with a species which is genetically well mapped, and for which chiasma counts have been made, it should be possible to reverse the argument and infer the strength of chromatid interference from genetical crossing-over and chiasma frequency data. In this paper data from Mus musculus males are analysed with this object; they lead to the conclusion that there is little or no evidence of chromatid interference in the male mouse.

THE CHROMOSOMES OF THE MOUSE.

The haploid number in Mus musculus is 20. The meiotic chromosomes of the male differ in size, the longest being about twice as long as the shortest (Crew & Koller, 1932; Makino, 1941; Slizynski, 1949). The sex chromosomes form one of the largest bivalents. Among the autosomes three size groups can be distinguished, consisting of four long, six medium and nine short autosomes (Slizynski, 1954). The autosomal centromeres appear to be subterminal when seen at mitosis in gonial cells (Makino, 1941), embryonic somatic tissues (Beatty & Fischberg, 1951) and ascites tumours (Levan & Hauschka, 1953). However, at meiosis some of them appear to have a short as well as a long arm (Koller, 1944; Slizynski, 1949).

Spermatocyte diplotene chiasma counts have been made by Crew & Koller (1932) and by Slizynski (1954). Crew & Koller analysed only one complete nucleus and a number of bivalents selected at random; for this reason it is difficult to estimate the chiasma interference in their data, and they will be disregarded here. Slizynski analysed fifty complete nuclei; but the sex chromosomes are in advance of the autosomes during meiotic prophase, and there is chiasma terminalisation, so a chiasma count of the sex bivalent is not comparable with counts of the autosomes of the same nucleus. For this reason, and also because of uncertainly about the position of the centromere in the sex chromosomes, only the autosomal chiasma frequencies are considered in this paper.

Slizynski's (1954) observations were made on two groups of mice; one consisted of males from the CBA inbred strain and the other was genetically heterogeneous/

heterogeneous. The CBA group shewed higher diplotene chiasma frequencies. As a high diakinesis chiasma frequency is characteristic of this strain (Huskins & Hearne, 1936), it is probable that a high diplotene frequency is also a strain characteristic. It was therefore assumed that the genetically heterogeneous group is better representative of mice generally, and the data from this group only are used in the analysis of chiasma interference. They are reproduced in Table 1.

TABLE 1. Slizynski's (1954) diplotene chiasma frequency data for genetically heterogeneous male mice.

Chromosomes	Bivalents with chiasmata							Total bivalents	Chiasmata per bivalent	Spermatogonial length (μ)
	0	1	2	3	4	5	Total			
Short (9)	0	207	154	8	0	0	539	369	1.461	3.989
Medium (6)	0	25	174	46	1	0	515	246	2.093	5.233
Long (4)	0	4	79	70	10	1	417	164	2.543	6.575

TABLE 2. Chiasmata expected in male mouse autosomes if there were no chiasma interference.

Chromosomes	Bivalents with chiasmata: 0	1	2	3	4	5 or more
Short						
Expected	85.634	125.085	91.356	44.481	16.252	6.192
Observed	0	207	154	8	0	0
Medium						
Expected	30.319	63.474	66.441	46.364	24.266	15.136
Observed	0	25	174	46	1	0
Long						
Expected	12.899	32.797	41.496	35.340	22.464	19.004
Observed	0	4	79	70	10	1

TABLE 3. Chiasmata expected in male mouse autosomes if there were one obligatory chiasma in each bivalent, but no chiasma interference.

Chromosomes	Bivalents with chiasmata: 0	1	2	3	4	5 or more
Short						
Expected	0.000	232.776	107.240	24.679	3.792	0.513
Observed	0	207	154	8	0	0
Medium						
Expected	0.000	82.420	90.125	49.276	17.960	6.219
Observed	0	25	174	46	1	0
Long						
Expected	0.000	34.989	53.977	41.635	21.410	11.989
Observed	0	4	79	70	10	1

gamete had occurred sequentially, proceeding distally from the centromere, which was assumed to have the interference properties of an exchange. The strength of interference assumed was such that the probability of an exchange occurring in a segment δu at a metric distance u from the preceding exchange is $4ue^{-2u}\,\delta u$. The frequency f_n with which there will be n exchanges in a telocentric chromosome entering a gamete was then shewn to be

$$f_n = e^{-2t}\left[\frac{(2t)^{2n}}{(2n)!} + \frac{(2t)^{2n+1}}{(2n+1)!}\right]$$

where t is the metric length of the chromosome, related to its map length, x, by the equation

$$x = t - \tfrac{1}{4} + \tfrac{1}{4}e^{-4t}$$

Now with a four-strand model x is related to the frequencies P_n with which a bivalent has 0, 1, 2 ... n ... chiasmata by the equation

$$x = \tfrac{1}{2}\sum_{n=0}^{\infty} n P_n \quad \ldots\ldots\ldots\ldots\ldots (1)$$

Furthermore, as Mather (1936) has shewn, the chiasma frequencies P_n can be inferred from the exchange point frequencies f_n, provided that the model is not complicated by chromatid interference. We have

$$P_0 = f_0 - f_1 + f_2 - f_3 + f_4 - f_5 + \ldots\ldots$$
$$P_1 = 2f_1 - 4f_2 + 6f_3 - 8f_4 - 10f_5 - \ldots\ldots$$
$$P_2 = 4f_2 - 12f_3 + 24f_4 - 40f_5 + \ldots\ldots$$

and so on. Table 4 shews the numbers of chiasmata expected in Slizynski's data on this basis. Once again there is obvious disagreement with observations.

TABLE 4. Chiasmata expected in male mouse autosomes if there were exchange point interference of strength $4ue^{-2u}$ and no chromatid interference.

Chromosomes.	Bivalents with chiasmata					
	0	1	2	3	4	5 or more
Short						
Expected	29.254	181.917	121.017	33.092	2.657	1.063
Observed	0	207	154	8	0	0
Medium						
Expected	-6.022	74.941	110.484	47.193	21.687	-2.283
Observed	0	25	174	46	1	0
Long						
Expected	-7.003	26.768	70.828	49.712	20.231	3.464
Observed	0	4	79	70	10	1

(iv). Mather's model.

Mather (1936, '37) suggested that chiasma formation is a sequential process, starting near the centromere and proceeding distally. The centromere was thought to have interference properties such that the proximal chiasma is distributed round some mean point at a 'differential distance' d from it. A second chiasma is then formed distal to the first, the distance between them being distributed round some mean value i ('interference distance'). A third chiasma then forms at a mean distance i distal to the second, and so on. The position of each new chiasma is related only to the position of the last chiasma formed before it.

Three simplifying assumptions will be incorporated in this model before testing it with Slizynski's (1954) data. They are:

(a) The centromere is effectively terminal in all mouse autosomes.

(b) The differential distance is equal to the interference distance.

(c) The distances between the centromere and the first chiasma, and between adjacent chiasmata, are distributed normally with standard deviation s.

Distances from the centromere will be measured in terms of some scale such that chiasma interference properties are uniform along it; they will therefore be proportional to cytological distances if the bivalent is uniform in its chiasma interference properties. The scale unit will arbitarily be chosen to be equal to the interference distance. The assumption that the first chiasma is distributed normally implies that it will sometimes be beyond the centromere, and therefore right outside the chromosome. However, these occasions will be very rare so long as d is greater than, say, $3s$; they/

they must be regarded as representing failure to form a bivalent. (One such event was, in fact, observed by Slizynski among the CBA mouse spermatocytes).

As it is assumed that there is no correlation between the length of one interchiasma and the next, the variance of their sum is equal to the sum of their variances. The position of the nth chiasma is therefore distributed with standard deviation $s\sqrt{n}$ round a point at a distance n units from the centromere. In text-fig. 1 the centromere is at 0 and successive chiasmata are distributed round the points 1, 2, 3 ... Consider the point Z at a scale distance z from the centromere. The frequency P_0 with which there will be no chiasma between 0 and Z is equal to the frequency with which the first chiasma lies distal to Z; it is therefore given by the vertically hatched area ${}^1R_z$ to the right of Z, so that

$$P_0 = {}^1R_z \quad \ldots\ldots\ldots\ldots \quad (2a)$$

Conversely the frequency with which there will be at least one chiasma between 0 and Z is given by the horizontal hatched area ${}^1L_z$ to the left of Z. Similarly the frequency with which there will be at least two chiasmata between 0 and Z is given by the diagonally hatched area ${}^2L_z$ to the left of Z. Therefore, by subtraction, the frequency with which there will be exactly one chiasma between 0 and Z is

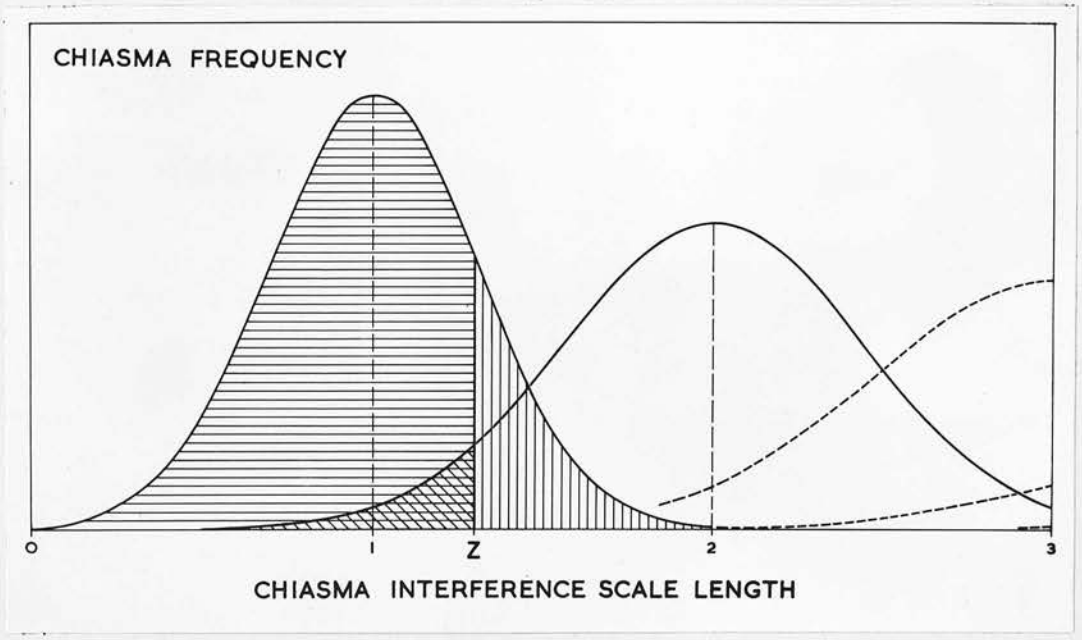
$$P_1 = {}^1L_z - {}^2L_z \quad \ldots\ldots\ldots \quad (2b)$$

Similarly

$$P_2 = {}^2L_z - {}^3L_z \quad \ldots\ldots\ldots \quad (2c)$$

and so on. If now the point Z marks the distal end of the bivalent, equations (2) give the frequencies with which it is expected to have 0, 1, 2 .. chiasmata; and its mean chiasma frequency is

m/



Text-fig. 1. For explanation see text.

$$m = \sum_{n=0}^{\infty} n P_n$$

P is a function only of the probability integrals L and R; these are functions of the scale length z and the standard deviations s, and their values can be obtained from tables of the normal probability integral, e.g. those of Lowan (1942). The values of z and s giving the best fit with the observational data can then be found by trial and successive approximation. Table 5 gives the expectations obtained by simultaneous fitting of the chiasma counts of all three groups of autosomes in Slizynski's (1954) data. The best fit is given by s = 0.3095. In computing these expectations account was taken of the fact that bivalent formation will fail when the expected position of the first chiasma lies beyond either end of the chromosome; equation (2a) was therefore replaced by

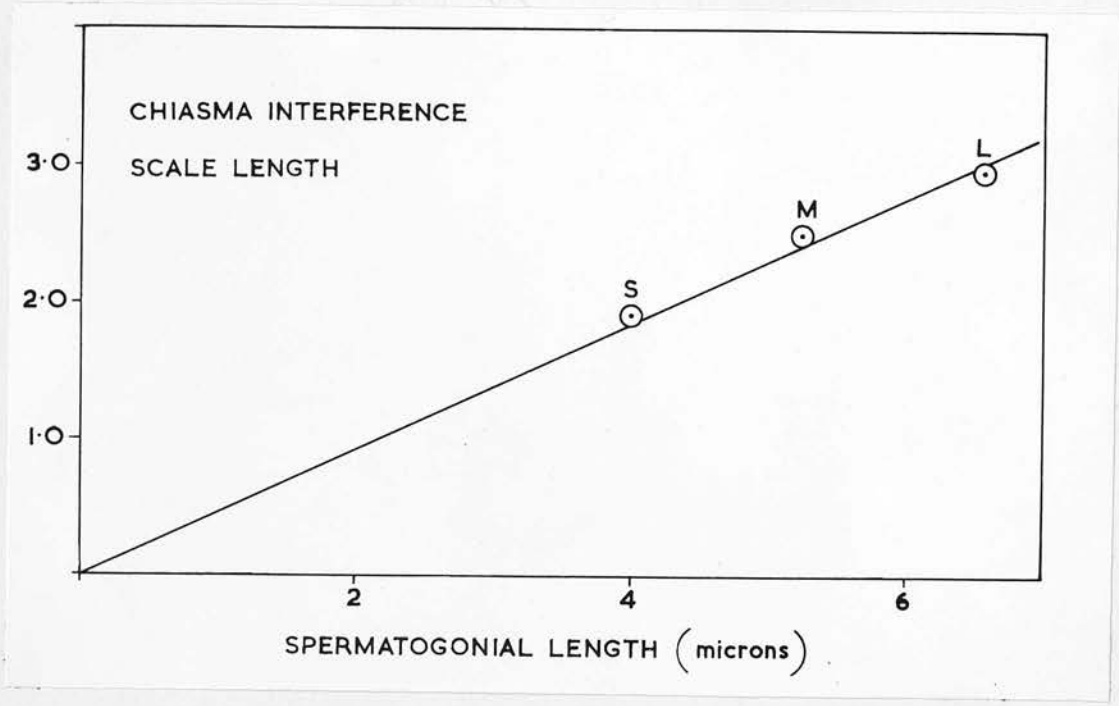
$$P_0 = {}^1R_z + {}^1L_0$$

This slight correction affected only the fourth significant figure. There is very good agreement between the expected and observed numbers of chiasmata, which encourages one to believe that this model fairly closely depicts the mechanism of chiasma interference in the mouse spermatocytes. Justification of the assumption that the differential and interference distances are equal is found in text-fig. 2, where the computed mean scale length of each group of autosomes, z, is plotted against the observed mean spermatogonial length (Slizynski, 1954). The points fall close to a straight line through the origin. This would be expected if d and i are equal and the chromosomes are homogeneous in their interference properties.

Text-fig. 3 shews the computed chiasma distributions and total chiasma density along the chromosomes. Near the centromere the chiasma density/

TABLE 5. Chiasmata expected in male mouse autosomes using Mather's chiasma interference model with s = 0.3095.

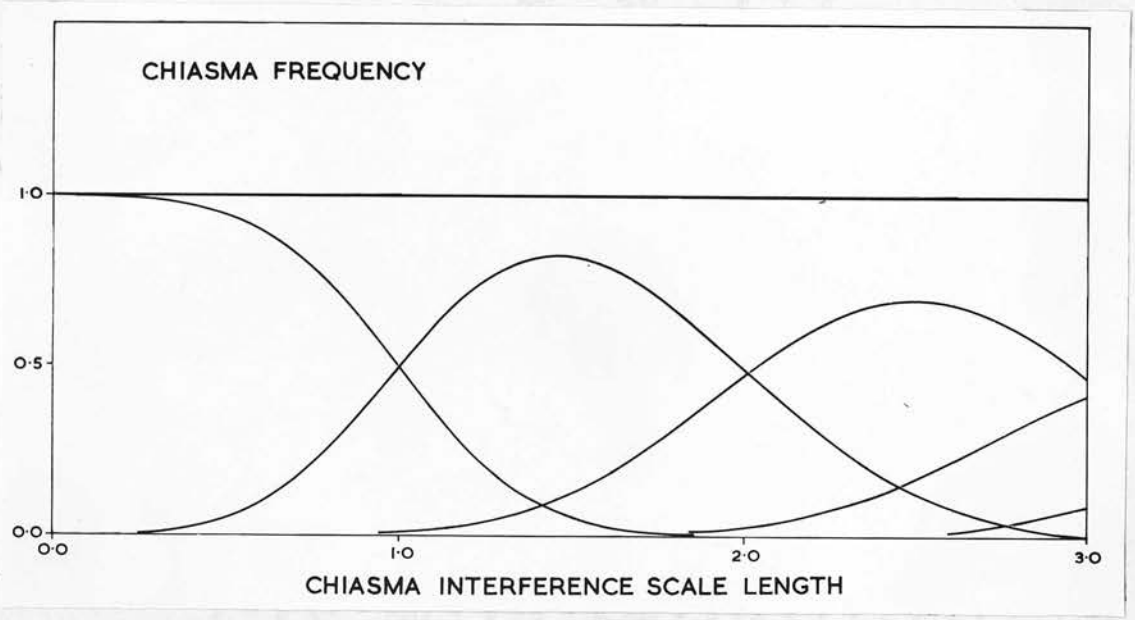
Chromosomes	Bivalents with chiasmata: 0	1	2	3	4	5 or more	z
Short							
Expected	0.707	206.326	153.390	8.424	0.155	0.000	1.933
Observed	0	207	154	8	0	0	
Medium							
Expected	0.153	26.792	171.243	45.576	2.189	0.047	2.538
Observed	0	25	174	46	1	0	
Long							
Expected	0.102	1.737	80.235	73.234	8.377	0.315	2.999
Observed	0	4	79	70	10	1	



Text-fig. 2. Chiasma interference scale lengths of three groups of mouse autosomes plotted against their mean cytological lengths in spermatogonia. Data of Slizynski (1954).

density is very variable, but elsewhere it settles down to unity. In regions remote from the centromere, therefore, one interference scale unit corresponds with 50 centimorgans of map length; but this relationship does not hold good in regions near the centromere.

Equations (2) give the frequencies with which 0, 1, 2 ... chiasmata are expected in a chromosome segment adjacent to the centromere. The corresponding frequencies for any other segment XY can be found in a similar way from the distributions of the first and successive chiasmata distal to X; these distributions must be found by mechanical integration on the basis of the known total chiasma density in the region proximal to X. Text-fig. 4 shews the distributions of the chiasmata distal to a point sufficiently remote from the centromere for the total chiasma density in its neighbourhood to be unity.



Text-fig. 4. Computed distributions of successive chiasmata and total chiasma density distal to a point remote from the centromere.

CHROMATID INTERFERENCE.

(i). Evidence from long linkage groups.

The recombination frequency, p, of any two chromosome markers A and B depends on (a) the frequencies P_0, P_1, P_2 ... with which 0, 1, 2 ... chiasmata are formed in the segment AB, and (b) the chromatid interference strength $(\alpha-\gamma)$, where α and γ are the probabilities of adjacent chiasmata having two- and four-strand configurations respectively. The fundamental equation is

$$p = \tfrac{1}{2}(1 - P_0) - \tfrac{1}{2}\sum_{n=1}^{\infty} P_{2n} \prod_{j=1}^{n} (\alpha_{2j} - \gamma_{2j}) \quad \ldots\ldots \quad (3a)$$

where $(\alpha_{2j} - \gamma_{2j})$ is the value of $(\alpha-\gamma)$ appropriate to the formation of the $2j$th chiasma (Carter & Robertson, 1952). In what follows it will be assumed that $(\alpha-\gamma)$ is constant within each bivalent, so that equation (3a) reduces to

$$p = \tfrac{1}{2}(1 - P_0) - \tfrac{1}{2} P_2 (\alpha-\gamma) - \tfrac{1}{2} P_4 (\alpha-\gamma)^2 - \tfrac{1}{2} P_6 (\alpha-\gamma)^3 - \ldots \quad (3b)$$

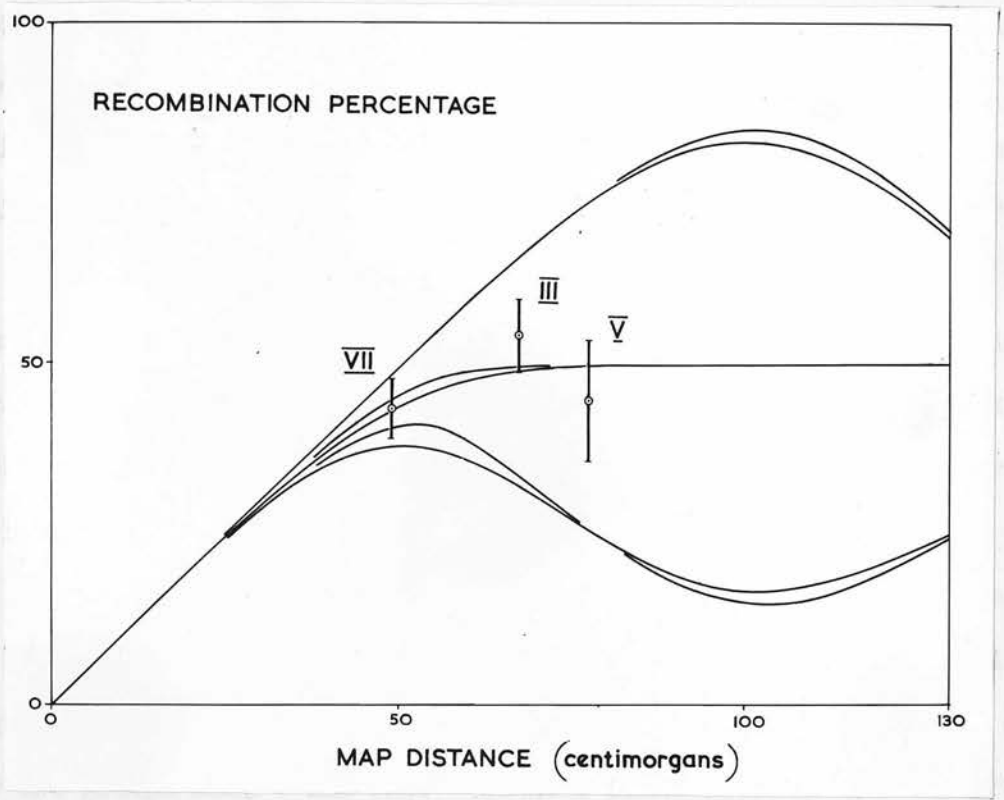
For the special cases of complete negative, zero, or positive chromatid interference (i.e. $\alpha-\gamma = +1, 0, -1$), equation (3a) reduced further to

$$p = \tfrac{1}{2}(1 - P_0) - \tfrac{1}{2} P_2 - \tfrac{1}{2} P_4 - \tfrac{1}{2} P_6 - \ldots\ldots\ldots \quad (3c)$$

$$p = \tfrac{1}{2}(1 - P_0) \ldots\ldots\ldots\ldots\ldots\ldots \quad (3d)$$

$$p = \tfrac{1}{2}(1 - P_0) + \tfrac{1}{2} P_2 - \tfrac{1}{2} P_4 + \tfrac{1}{2} P_6 - \ldots\ldots\ldots \quad (3e)$$

The relationship between the chiasma frequencies P and map distance x have already been given in equation (1). Text-fig. 5 shews p plotted against x for segments adjacent to the centromere and remote from it, and for these three limiting values of chromatid interference strength. It is apparent that the effect of proximity to the centromere is of minor importance compared with that of chromatid interference. It is also apparent that chromatid interference has little effect on recombination across segments shorter/



Text-fig. 5. Recombination percentage plotted against map distance, for the strength of chiasma interference found in Slizynski's data. The three sets of curves are based on the assumption of (upper set) complete positive, (middle) zero, and (lower) complete negative chromatid interference. The upper of the two curves in each set refers to segments adjacent to the centromere, the lower curve to segments remote from it. III, V, VII, observed recombination between the end markers of three linkage groups; the vertical bar indicates deviations of twice the standard error.

shorter than about 35 centimorgans, but for segments longer than 45 centimorgans its effect is important. With complete negative chromatid interference recombination never exceeds 42%; with complete positive chromatid interference it exceeds 50% for map distances between 50 and 150 centimorgans, reaching a maximum of 82% or more for segments of 100 centimorgans. The recombination observed across a long segment of known map length therefore provides a test for the presence of chromatid interference. The most sensitive test would be provided by recombination data from linkage groups about 100 centimorgans long; but no group of this length is yet known in the mouse, though Slizynski's (1954) chiasma frequency data for long autosomes imply that such groups exist.

There are three known linkage groups which exceed 45 centimorgans in the male mouse, and for which a lower limit can be set to the total map length by summing the frequencies of recombination across included subsegments; they are groups III, V and VII. Table 6 summarises the relevant recombinations. Only data referring to male gametogenesis should be used, but for the pa-fi subsegment in group V it was found necessary to use intercross data (Carter, 1952) and for the fi-Sd subsegment the sex of the segregating parent was not stated (Wallace, 1950). The observed recombination between the extreme markers is plotted in Text-fig. 5 against the minimum map length thus found. All three observations agree with expectation on the assumption that there is no chromatid interference and significantly disagree with complete positive chromatid interference; for the two longer groups observation disagrees also with complete negative chromatid interference. The observations for groups III and V deviate in opposite directions/

TABLE 6. Male recombination in long linkage groups in the mouse.

Author and date.	Markers.	Recombination.
Linkage group III.		
Carter (1951b)	s, W^v	0.484
" "	W^v, lx	0.189
	Sum:	0.673
Carter (1951b)	s, lx	0.543±0.027
Linkage group V.		
Carter & Phillips (1954)	Ra, a	0.220
Carter (1947), Fisher (1949), Owen (1953)	a, un	0.047
" "	un, we	0.045
" "	we, pa	0.023
Carter (1951a)	pa, fi	0.189*
Wallace (1950)	fi, Sd	0.250†
	Sum:	0.774
Carter & Phillips (1954)	Ra, Sd	0.447±0.045
Linkage group VII		
Snell & Law (1939), Wright (1947), Snell (quoted by Fisher, Lyon & Owen, 1947), Carter & Phillips (1953)	wa-2, sh-2	0.295
Falconer (1947), Carter & Phillips (1953)	sh-2, Re	0.192
	Sum:	0.487
Carter & Phillips (1953)	wa-2, Re	0.436±0.025

* Intercross segregation.

† Sex of segregating parent not stated.

directions from the curves for no chromatid interference, which evidently approximate closely to the best-fitting curve.

(ii). Evidence from genetical coincidence.

An alternative approach makes use of genetical coincidence data. When there are three linked markers, A, B and C, and attention is confined to chromatids in which a cross-over has occurred in the segment AB, it should be possible to predict the distribution of chiasmata distal to B, and therefore the frequencies with which there will be 0, 1, 2 ... chiasmata in BC. Once these are known, the expected frequency of recombination across BC can be calculated from equation (3). This can then be compared with the frequency actually observed.

Suppose A, B and C lie in a proximodistal sequence. Then the frequencies with which 0, 1, 2 ... chiasmata are to be expected in BC depend on the scale lengths of AB and BC and on the distribution of chiasmata in BC, subject to the proviso that only those chromatids shall be considered which cross over in at least one chiasma in AB. The scale lengths of AB and BC are, in general, unknown; in what follows it will be assumed that both segments are remote from the centromere, so that their scale lengths are equal to twice their map lengths. The analysis is therefore accurate only insofar as this assumption is valid. Provided also that AB is short, it may be assumed that the distribution of chiasmata in AB is uniform. The distribution of chiasmata in BC, subject to the stated proviso, can then be found by mechanical integration; and from it, by equation (2), the frequencies with which 0, 1, 2 ... chiasmata are expected in BC

The expected frequency of recombination across BC is, by definition

P/

$$p_e = P_0\, q_0 + P_1\, q_1 + P_2\, q_2 + \ldots\ldots\ldots \quad (4)$$

where q_n is the proportion of recombinants among the chromatids derived from bivalents with n chiasmata in BC. Values of q can be derived from the equations given by Weinstein (1948). Subject to the proviso that the chromatids under consideration must cross over in at least one chiasma in AB, they are

$$q_0 = 0 \ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (5a)$$

$$q_1 = \tfrac{1}{2} + \tfrac{1}{2}(\alpha-\gamma) \ldots\ldots\ldots\ldots\ldots\ldots \quad (5b)$$

$$q_2 = \tfrac{1}{2} - \tfrac{1}{2}(\alpha-\gamma) \ldots\ldots\ldots\ldots\ldots\ldots \quad (5c)$$

$$q_3 = \tfrac{1}{2} + \tfrac{1}{2}(\alpha-\gamma)^2 \ldots\ldots\ldots\ldots\ldots\ldots \quad (5d)$$

$$q_4 = \tfrac{1}{2} - \tfrac{1}{2}(\alpha-\gamma)^2 \ldots\ldots\ldots\ldots\ldots\ldots \quad (5e)$$

and so on. It follows that the expected frequency of crossing-over in BC, among chromatids which crossed over in AB, is

$$p_e = \tfrac{1}{2}(1-P_0) + \tfrac{1}{2}(\alpha-\gamma)(P_1-P_2) + \tfrac{1}{2}(\alpha-\gamma)^2(P_3-P_4) + \ldots \quad (6)$$

If P_0 be the observed frequency, equation (6) can be used for estimating $(\alpha-\gamma)$. If all P higher than P_4 can be neglected,

$$(\alpha-\gamma) = \frac{-(P_1-P_2) \pm \sqrt{(P_1-P_2)^2 - 4(P_3-P_4)(1-P_0-2P_0)}}{2(P_3-P_4)} \ldots \quad (7a)$$

When BC is short enough for all P higher than P_2 to be neglected,

$$(\alpha-\gamma) = \frac{2P_0-(1-P_0)}{P_1-P_2} \ldots\ldots\ldots\ldots\ldots\ldots \quad (7b)$$

When P_2 is negligible, compared with P_1 and P_0,

$$(\alpha-\gamma) = \frac{2P_0-P_1}{P_1} \ldots\ldots\ldots\ldots\ldots\ldots \quad (7c)$$

Thus far it has been assumed that A, B and C lie in a proximodistal sequence/

sequence. Considerations of symmetry shew that the order may be reversed without invalidating the argument, provided again that it does not refer to regions too close to the centromere.

Seven published bodies of multipoint backcross segregation data for the male mouse are summarised in Table 6.[7] Four of them refer to short BC segments in which the expected chiasma frequencies are very low and little or no double crossing-over was actually observed. They therefore contain very little information about chromatid interference. The expected and observed numbers of double cross-overs are shewn in Table 7,[8] expectations being based on the supposition that there is no chromatid interference; observation agrees well with expectation. The other three bodies of data refer to larger BC segments in which double crossing-over was both expected and observed (Table 8).[9] Once again observation agrees well with expectation on the basis of no chromatid interference (Table 9).[10]

TABLE 6.7 Multipoint backcross data for segregation in the male mouse.

Author and date.	Markers	Cross-over types of progeny						
		0	AB	BC	AB&BC	CD	BC&CD	AB,BC&CD
Grüneberg (1935, '36)	sh-1, c^e, p	805	29	112	0	-	-	-
Borger (1950)	a, pa, Sd	135	30	96	16	-	-	-
Carter (1951b)	lx, W^v, s	61	21	64	13	-	-	-
Mallyon (1951)	bt, Ca, N	567	73	18	0	-	-	-
Falconer & Snell (1952)	fr, sh-1, c^{ch}, p	26	7	1	0	2	0	0
Owen (1953)	a, un, we pa	2215	115	111	1	56	1	0
Carter & Phillips (1954)	Ra, a, Sd	54	14	41	14	-	-	-

TABLE 7.8 Observed crossing-over in a segment BC, among chromatids with a cross-over in an adjacent segment AB, and expected crossing-over on the supposition that there is no chromatid interference.

Author and date	Markers	Observed		Expected	
		AB	AB&BC	AB	AB&BC
Grüneberg (1935, '36)	sh-1, c^e, p	29	0	28.870	0.130
Mallyon (1951)	bt, Ca, N	73	0	72.751	0.249
Falconer & Snell (1952)	fr, sh-1, p	7	0	6.934	0.066
Owen (1953)	a, un, pa	115	1	115.821	0.179
" "	un, we, pa	111	1	111.964	0.036
Total, all experiments.		335	2	336.340	0.660

TABLE 9. Observed crossing-over in a segment BC, among chromatids with a cross-over in an adjacent segment AB, and expected crossing-over on the supposition that there is no chromatid interference.

Author and date	Markers	Observed		Expected		χ^2
		AB	AB&BC	AB	AB&BC	
Borger (1950)	a, pa, Sd	30	16	33.373	12.627	1.242
Carter (1951b)	lx, W^V, s	21	13	18.795	15.205	0.578
Carter & Phillips (1954)	Ra, a, Sd	14	14	19.076	8.924	4.238
Total, all experiments		65	43	71.244	36.756	6.058

TABLE 10. Analysis of χ^2 for data from Table 8.

Item	χ^2	DF	P
Total	6.058	3	0.2>P>0.1
Chromatid interference	1.608	1	0.3>P>0.2
Heterogeneity	4.450	2	0.2>P>0.1

DISCUSSION.

Chiasma interference is of common occurrence. Its effect is to decrease the variance of the number of chiasmata in a bivalent (Haldane, 1931); and this implies, inter alia, a decrease in the frequency of chromosome pairs which have failed to form at least one chiasma. It thus increases the frequency of bivalent formation; and since this is a necessary step in the formation of euploid gametes, chiasma interference may have an adaptive value.

Chromatid interference, on the other hand, has been reported on only a few occasions. For technical reasons it cannot be detected cytologically except in very favourable material, where individual meiotic chromatids can be followed (Hearne & Huskins, 1935; Huskins & Newcombe, 1941); and even then it is not possible to distinguish between two- and four-strand chiasma pairs, but only to compare their total with the total number of three-strand configurations.

Detection of chromatid interference must therefore usually depend on genetic methods. With organisms in which ordered tetrads are formed, analysis of the products of meiosis yields direct information about chromatid interference; it has thus been reported for various species of Neurospora (see Papazian, 1952, for a review of the literature). In higher organisms, where it is not possible to analyse complete tetrads, it is sometimes possible to draw inferences about chromatid interference from genetical observations of homozygosis in the progeny of chromosomally abnormal individuals; thus no evidence of its occurrence was found in ring-X and attached-X/

attached-X stocks of Drosophila melanogaster (L.V. Morgan, 1933; Weinstein, 1936). When these methods are not available it is necessary to search for recombination values shewing notable departures from 50%, either below it or above, for long map distances; or to combine chiasma frequency and genetical interference data, as in the present paper.

Recombination limited to 25% for long map distances, a consequence of complete negative chromatid interference, does not appear to have been reported. Recombination exceeding 50%, which requires either two-strand crossing-over or positive chromatid interference with four-strand crossing-over, was reported by M.E. Wright (1947) as occurring in Mus musculus. Her data came from experiments in which disturbances due to differential viability were carefully guarded against; when considered alone, their statistical significance is beyond question. However, attempts to repeat Wright's experiment yielded no consistent departure from free segregation (Carter & Phillips, 1953), so it cannot be regarded as a substantiated instance of recombination exceeding 50%. In view of this, and of the evidence adduced in the present paper, it seems unlikely that chromatid interference occurs to any marked extent in the male mouse.

If chromatid interference can be disregarded, it is legitimate to use recombination-frequency/map-distance curves based on chiasma interference data. The curves for segments adjacent to the centromere and remote from it are shewn in text-fig. 5; mean values, which should provide the best conversion for general use with male mouse data, are given in Table 10. A fairly close approximation is given by the fourth-power empirical curve used by Carter & Falconer (1951); the second-power empirical curve suggested by Kosambi/

TABLE 11. Conversion tables for recombination percentages (p) and map distances (m), for use with male mouse segregation data.

p to m

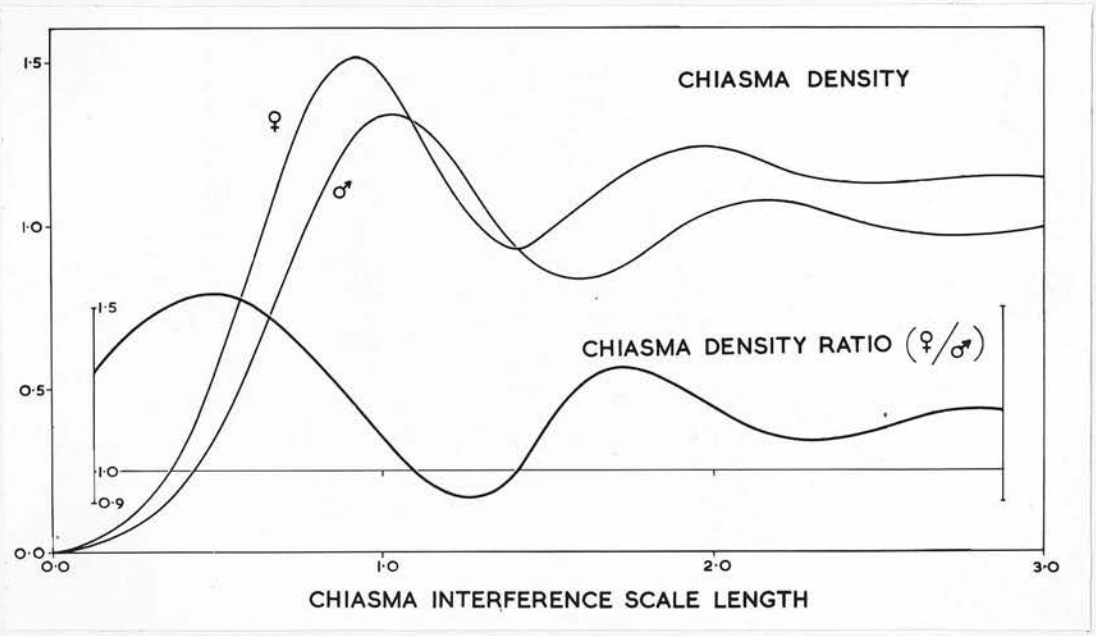
p	m	p	m
1	1.0	26	26.5
2	2.0	27	27.6
3	3.0	28	28.6
4	4.0	29	29.7
5	5.0	30	30.8
6	6.0	31	31.9
7	7.0	32	32.9
8	8.0	33	34.0
9	9.0	34	35.2
10	10.1	35	36.4
11	11.1	36	37.6
12	12.1	37	38.9
13	13.1	38	40.2
14	14.1	39	41.5
15	15.1	40	42.9
16	16.2	41	44.3
17	17.2	42	45.8
18	18.2	43	47.3
19	19.2	44	48.9
20	20.3	45	50.7
21	21.3	46	52.8
22	22.3	47	55.3
23	23.4	48	58.8
24	24.4	49	63.9
25	25.5	50	∞

m to p

m	p	m	p	m	p
1	1.0	26	25.5	51	45.2
2	2.0	27	26.5	52	45.6
3	3.0	28	27.4	53	46.1
4	4.0	29	28.3	54	46.5
5	5.0	30	29.2	55	46.9
6	6.0	31	30.2	56	47.2
7	7.0	32	31.1	57	47.5
8	8.0	33	32.1	58	47.8
9	9.0	34	33.0	59	48.1
10	9.9	35	33.8	60	48.3
11	10.9	36	34.6	61	48.5
12	11.9	37	35.5	62	48.7
13	12.9	38	36.3	63	48.9
14	13.9	39	37.1	64	49.0
15	14.9	40	37.8	∞	50.0
16	15.8	41	38.6		
17	16.8	42	38.4		
18	17.8	43	40.1		
19	18.8	44	40.8		
20	19.8	45	41.5		
21	20.7	46	42.2		
22	21.7	47	42.8		
23	22.7	48	43.5		
24	23.6	49	44.1		
25	24.6	50	44.6		

Kosambi (1944) deviates from them rather widely.

There are no chiasma frequency data for oogenesis in the mouse comparable with Slizynski's spermatogenesis data. From many observations of coincidence it is clear that cross-over interference in the female is much less strong than in the male, and oocyte chiasma counts (Crew & Koller, 1932) suggest that this is due to a lower degree of chiasma interference. The higher mean chiasma frequency in the female reported by Crew & Koller is in agreement with the general rule that recombination frequencies are higher in the female. However, there are two notable exceptions to this rule, namely Naked, Caracul and belted in Linkage Group VI, waved-2 and shaker-2 in Group VII These would be explained if, as a result of the higher mean chiasma frequency (and therefore shorter chiasma interference scale unit) in the female, the segments in question lay in this sex near a chiasma density minimum, though near a maximum in the male. (See text-fig. 6). This effect may be appreciable only near the centromere, where it will be strongest. It therefore suggests a possible means of finding the map position of the centromere, since the higher recombination in the male will be limited to a region between about 1.1 and 1.4 scale units (30 and 50 centimorgans) from the centomere. Its exact length will depend on the strength of chiasma interference in the female. Between this region and the centromere one would expect to find recombination very much higher in the female than the male, reaching a maximum at about 0.5 scale units (3 centimorgans) from the centromere. It is interesting to find that such a region appears to exist in Linkage Group VII, where the recombination between vestigial and Rex is much higher in females than males (Michie, 1952). This suggests that the/



Text-fig. 6. Hypothetical chiasma density distributions for male and female mice, and the ratio of the densities. Though the mean chiasma frequency is higher in the female, the density is higher in the male between 1.1 and 1.4 interference scale units (30 and 50 centimorgans) from the centromere. Closer to the centromere it is very much higher in the female.

the map-position of the centromere may be close to that of Rex.

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SUMMARY.

Mouse spermatocyte chiasma frequency observations by Slizynski shew chiasma interference which can be adequately represented by a model due to Mather. Some genetical implications of this model are analysed, assuming various strengths of chromatid interference, and are compared with genetical observations. This indicates that there is little or no chromatid interference in the male mouse. A new map length and recombination frequency conversion table is given, based on the observed chiasma frequencies.

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