STUDIES ON THE MORPHOLOGY AND FUNCTION OF THE OVIDUCT IN THE DOMESTIC FOWL.

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Thesis presented by

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CONTENTS.

PART I.

OBSERVATIONS ON THE DEVELOPMENT OF THE OVIDUCT IN THE DOMESTIC FOWL WITH SPECIAL REFERENCE TO THE FORMATION OF THE OCCLUDING PLATE.

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C O N T E N T S.

1. Introduction.

Balfour and Sedgwick (1879) first suggested that in the chick the funnel of the oviduct is derived from the pronephros, and the Müllerian duct is split off from the Wolffian duct. Most of the early workers subscribed to their views. There seems to be no doubt that in elasmobranchs the Müllerian duct is derived from the Wolffian duct (Kerr, 1919) and the early observers were influenced to a great extent in adopting Balfour and Sedgwick's interpretation with reference to other animals. Subsequent investigators (vide Goodrich, 1930), however, have satisfactorily established that in Amniotes a strip of thickened coelomic epithelium (the tubal ridge) on the antero-lateral face of the Wolffian body is responsible for the formation of the Müllerian duct. The anterior part of the latter originates as a groove-like invagination of the tubal ridge which grows caudad between the Wolffian duct and the tubal ridge. Now in the chick the growing caudal end of the Müllerian duct reaches the cloacal region on the seventh day (Lillie, 1927) but the exact time and the mode of fusion with the cloaca has received scant attention from previous workers. Greenwood (1935) was able to demonstrate that up to the onset of puberty in the female fowl there is an interrupted continuity between the lumen of the oviduct and the cloaca. His experiments suggested that the subsequent perforation of the duct is under the control of a hormone of the ovary. However, it has not been possible to obtain from the literature any reference to the developmental history of this oviducal occlusion.

In/

In view of the above, the present investigation was undertaken as a prelude to an experimental analysis of the phenomenon of perforation of the oviduct in the domestic fowl. An opportunity has also been taken in course of this study to throw some light on the ultimate fate of the tubal ridge and the course of the Müllerian duct in relation to the Wolffian duct in its developmental phases.

2. Material and Method.

Eggs from the pure-bred Brown Leghorn hens of the Institute flock were incubated in an electric "Petersime" incubator. The earlier embryos (4 to 7 days old) were carefully taken out of the incubated eggs and fixed in toto. Older embryos (8 to 20 days old) on the other hand, after removal from the shell, were dissected under a binocular dissecting microscope and sexed by the careful examination of their gonads (Greenwood, 1925). The embryos of the female line were sorted out by this method and finally prepared for histological investigation.

A number of fixatives, including Zenker's fluid, Orth's Formol-Muller, 10% Formol-Saline, Aqueous and Brasil Duboscqu's modification of Bouin's fluid were tried, but only the last two gave uniformly good results. It was found necessary to decalcify the older embryos (8 to 20 days old) and for that purpose overnight immersion in Jenkin's fluid proved successful. The serial sections were obtained by the usual method and were stained by Ehrlich's haemotoxylin followed by alcoholic eosin.

3. Observations.

The development of oviduct in the fowl shows that the organ/

organ is originally paired and that the right one ultimately degenerates and only a small rudiment of it is normally left in an adult hen (Domm, 1927; a, b). For that reason only the development and differentiation of the left duct was studied. The later embryonic stages are variable to some extent and this variability has made it seem best to describe the typical individual embryos at a particular stage of development rather than to attempt to make average observations on a given age group. The embryos successively described should represent successive stages; that is, they are so arranged that each one shows on the whole an advance in oviducal development over the preceding one, although in relation to the development of other body organs it may be less advanced.

Particulars regarding the embryonic stages such as age and length are presented in Table I.

Table I /

TABLE I. Successive stages of the chick embryos employed in this study.

Stages.	Age in days.	Length in mm.
A	4	12
B	5	19
C	6	27
D	7	30
E	8	33
F	9	36
G	10	41
H	11	46
I	12	50
J	15	71
K	16	81
L	17	85
M	18	97
N	19	99
O	20	103

Stage A. The Anlage of the Müllerian duct is laid down at this stage as a longitudinal band of thickened coelomic epithelium (the tubal ridge) on the antero-lateral face of the Wolffian body (text fig.1). It lies immediately external to the Wolffian duct and measures 12.5 microns at its greatest width. The cells constituting the tubal ridge are of the columnar type.

Stage B/

Stage B. The tubal ridge has extended caudad down to the cloacal region. A slight difference in thickness between the cranial and the caudal portions of the ridge is noted, the latter being slightly thinner than the former.

A groove-like invagination of the thickened anterior portion of the tubal ridge occurs at this stage. The lips of this groove approach each other and fuse together forming a short tube which lies along the antero-lateral surface of the Wolffian body between the tubal ridge and the Wolffian duct being slightly dorsal to the latter (text fig. 2). This tube has a very narrow lumen in its cephalic portion but its caudal end is blind being a solid cord of epithelial cells. It measures 37.5 microns in diameter and its cephalic portion consists of a single layer of radially arranged columnar epithelial cells. At the cranial end the lips of the tube do not meet and as a consequence it remains open. Four similar openings, arranged successively but very close to one another, are also seen along the tube at a short distance behind the first one.

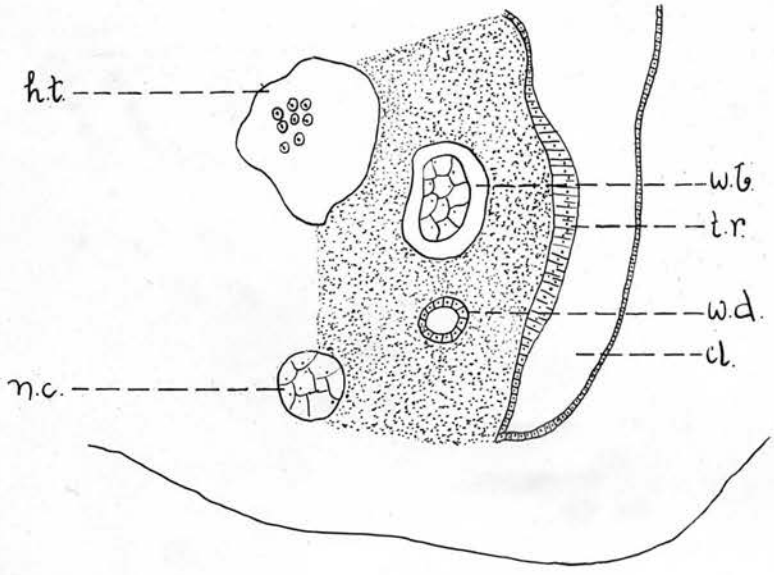
Stage C. At this stage only one opening, the future coelomic opening of the oviduct is seen at the cranial extremity of the Müllerian duct, the four others having been closed up. The Müllerian duct has grown caudad down to the posterior end of the Wolffian body and its lumen has also extended. The caudal end of the duct, however, remains solid as in the previous stage. Mitotic figures are frequently seen in the epithelial cells throughout the length of the duct.

The mesenchymatous cells from the adjoining areas of the Müllerian duct are seen migrating round it. Cells are also seen/

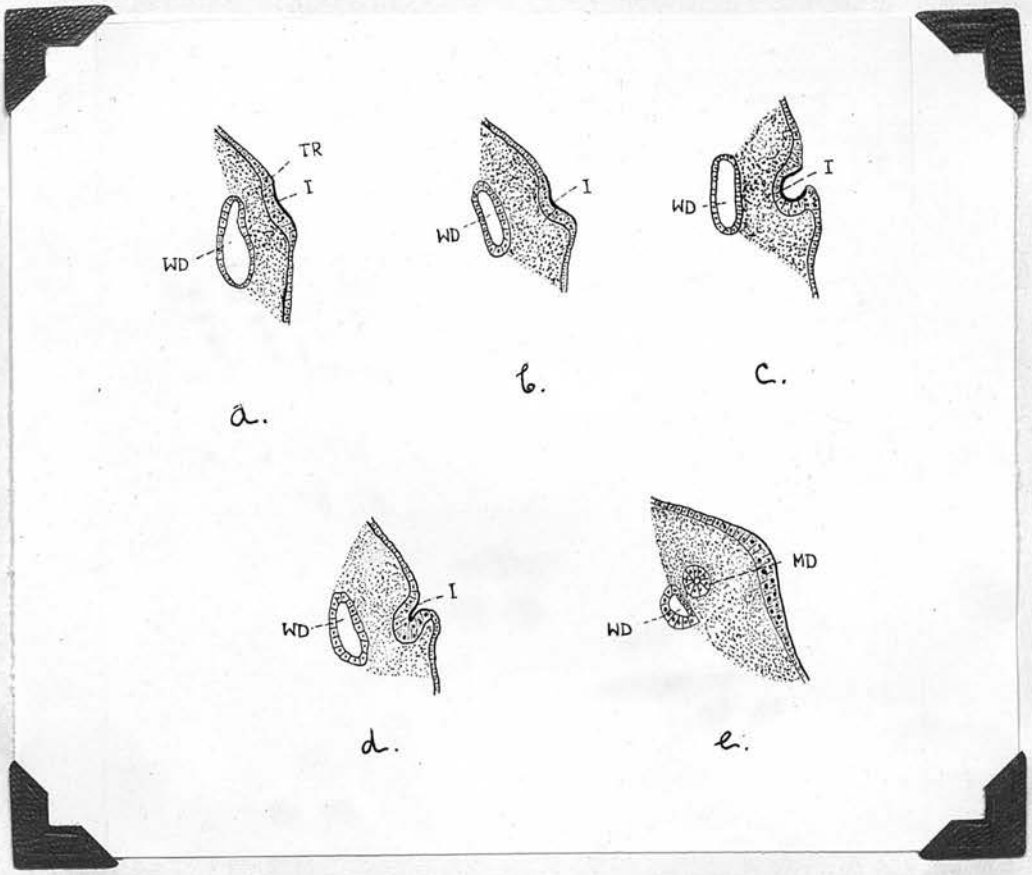
seen to proliferate from the cranial portion of the tubal ridge round the duct (text fig. 3). No cell proliferation from the caudal portion of the tubal ridge is observed at this stage.

In the cephalic region it is seen that the Müllerian and the Wolffian ducts occupy the entire lateral surface and the gonad the median surface of the Wolffian body which thus functions as the urinogenital ridge (Lillie, 1927). It has three divisions: (1) the sexual division, containing the gonad, involves about the anterior half of the Wolffian body (2) the non-sexual division, involves the rest of the Wolffian body (3) behind the Wolffian body itself the urinogenital ridge is the mesenchymatous caudal prolongation of the non-sexual division which forms a sort of connecting ridge between the latter and the lateral diverticulum of the cloaca. (This diverticulum appears at about the 60th hour of incubation and at about 72th hour it establishes a connection with the Wolffian duct. (Lillie, 1927).) In transverse section it appears as a projection from the dorsal body wall and contains only the Wolffian duct at this stage (text fig. 4). The first two divisions of the urinogenital ridge may be termed together as the cephalic portion and the third division as the caudal portion.

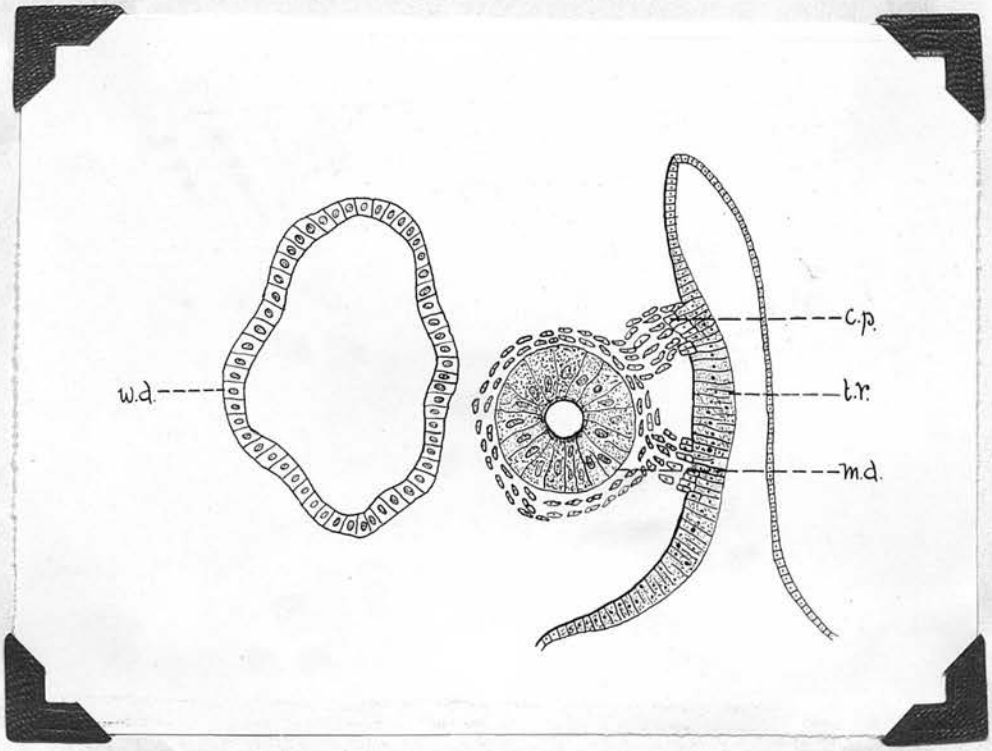
In the sexual division of the urinogenital ridge the Müllerian duct occupies an external and dorsal position to the Wolffian duct (text fig. 5). In the non-sexual division, however, it has changed its course and lies externally but on the same level with the Wolffian duct (text fig. 6).



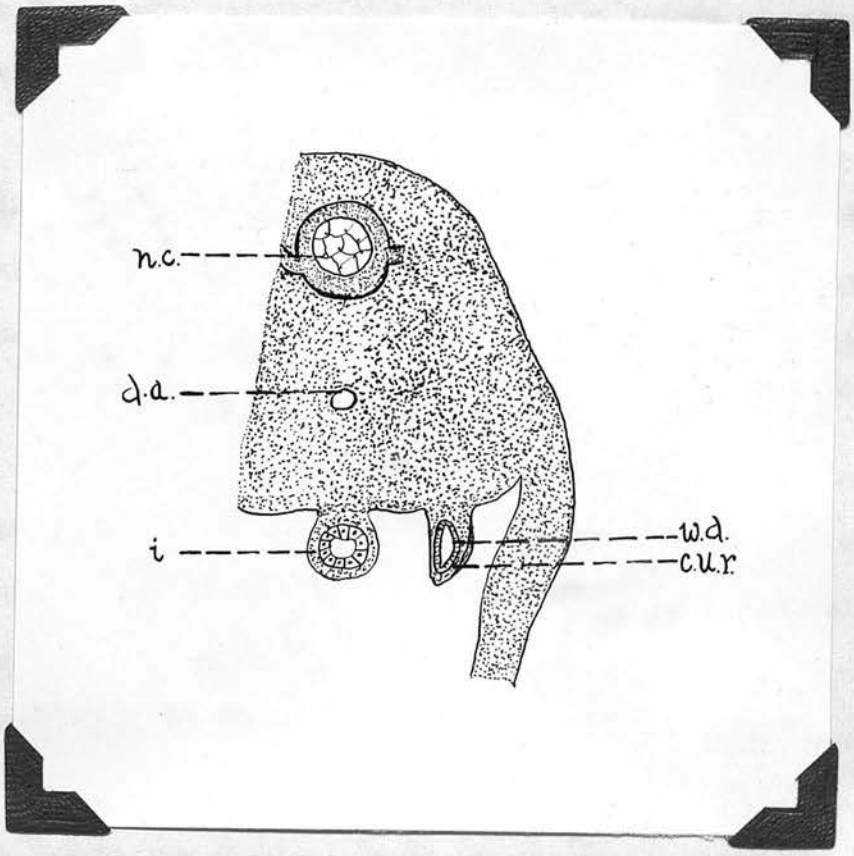
Text - fig.1 - Transverse section through the region of the heart of a 4 days old chick embryo showing the tubal ridge (x30).



Text - fig.2 - Five consecutive transverse sections through the cranial portion of the tubal ridge of a 5 days old chick embryo showing the formation of the Müllerian duct (x16). For further explanation see the text.



Text - fig.3 - Transverse section through the cranial region of the Müllerian duct of a 6 days old chick embryo showing the cell proliferation from the tubal ridge round the Müllerian duct (x50). Other parts not represented in the figure.



Text - fig.4 - Transverse section through the region of the intestine of a 6 days old chick embryo showing the caudal portion of the urinogenital ridge (x25).

Stage D. The Müllerian duct has entered into the caudal portion of the urinogenital ridge and has reached the vicinity of the cloaca. The caudal end of the duct, however, is solid as in the previous stage.

The cell proliferation from the tubal ridge round the Müllerian duct is not restricted to the cranial region only, but is seen to be extended throughout the entire length of ridge at this stage.

Throughout its entire course in the caudal portion of the urinogenital ridge the Müllerian duct lies ventral to the Wolffian duct (text fig. 7). In the cranial region, however, its positions in relation to the Wolffian duct are similar to those in the previous stage.

In this stage it is seen that the cloaca has become compressed in an antero-posterior direction (Lillie, 1927) and an extension from its dorsal wall forms a bridge-like connection with the caudal portion of the urinogenital ridge. Only the Wolffian duct, however, is continuous with the cloaca through this extension.

Stage E. Unlike the previous stage the entire course of the Mullerian duct in the caudal portion of the urinogenital ridge is not uniform in relation to the Wolffian duct. It is seen that in the cloacal region it occupies a lateral but more or less internal position to the Wolffian duct though further cephalad it occupies a ventral position to the latter as in the previous stage. This change in position of the Mullerian duct at this stage is presumably due to slight tilting of the caudal portion of the urinogenital ridge towards the mesial side in the cloacal region.

Tracing/

Tracing caudad from the cephalic extremity of the Müllerian duct it can be seen that in the sexual division of the urinogenital ridge it lies external and dorsal to the Wolffian duct (text fig.8.a); in the non-sexual division it is external but on the same level with the latter (text fig.8.b). In the caudal portion of the urinogenital ridge the Müllerian duct is ventral to the Wolffian duct (text fig.8.c) for some distance, and finally in the cloacal region it occupies, as stated above, a lateral but more or less internal position to the Wolffian duct (Pl. fig.1., text fig.8.d).

The process of cell proliferation from the tubal ridge which started in Stage C is now complete and, as a result, it ceases to exist. The epithelium of the site previously marked by the thickened tubal ridge appears flat like the adjacent peritoneal epithelium.

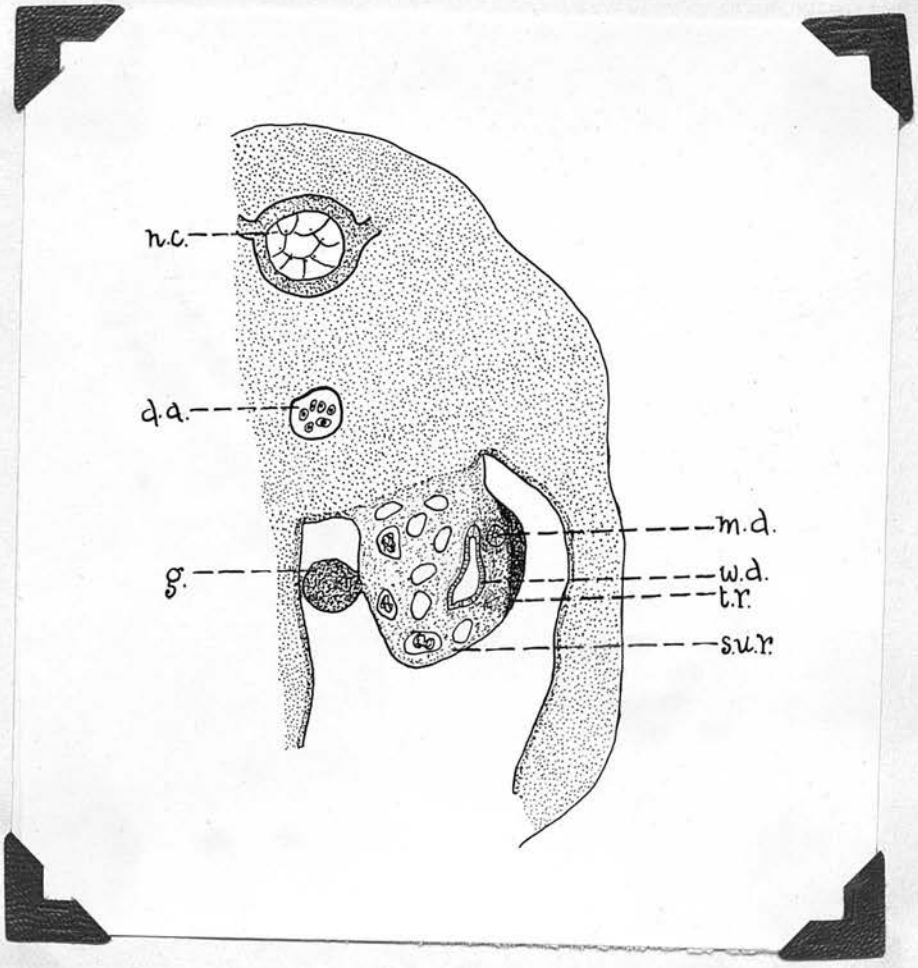
At this stage the fusion of the Müllerian duct with the cloaca takes place. The progressively growing duct passes through the bridge-like extension of the cloacal wall referred to in the previous stage and eventually penetrates into the cloacal wall (Pl. , figs. 1 to 5). The caudal end of the duct comes to rest on the epithelial lining of the cloacal lumen (Pl. fig.6). The lumen of the duct extends down into the solid cord-like portion and only a single layer of epithelial cells is left at its caudal end. This layer ultimately becomes continuous with the epithelial wall of the Müllerian duct (Pl. fig.7). The mesenchymatous cells of the cloacal wall migrate in between the caudal end of the latter and the epithelial lining of the cloacal lumen.

Owing/

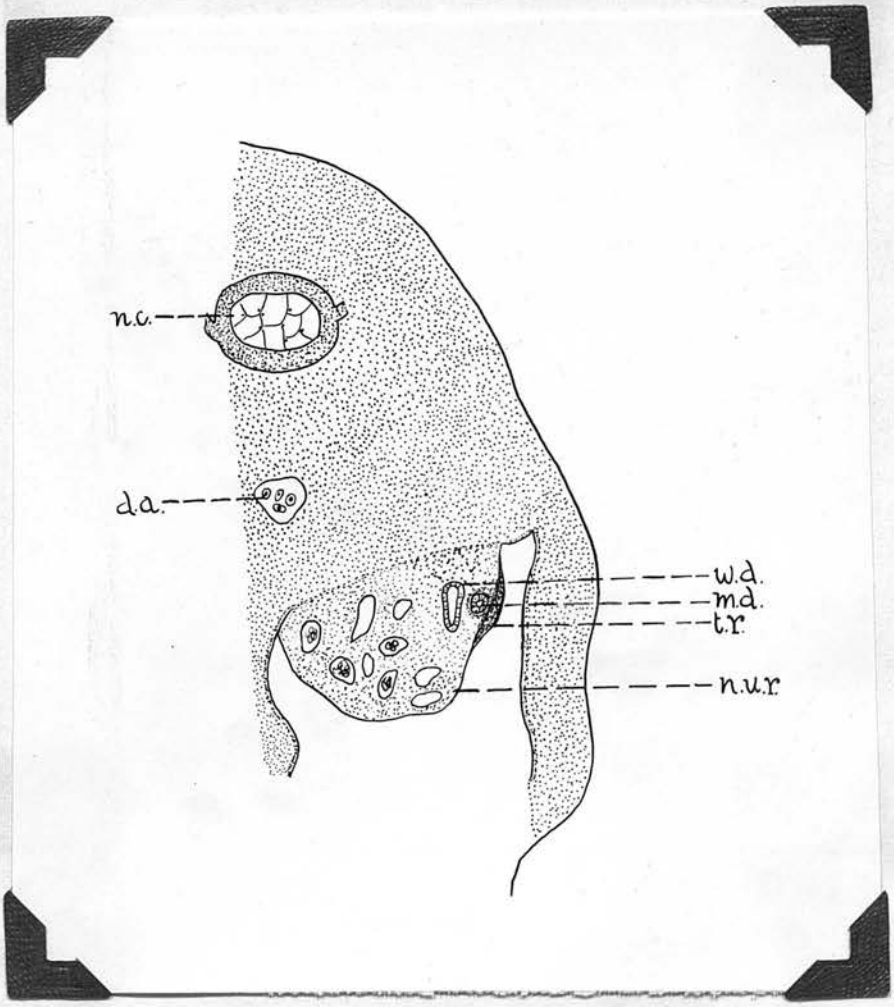
Owing to this mode of fusion a short terminal portion of the Mullerian duct lies hidden within the cloacal wall in the embryonic life of the chick. In an adult hen also a short caudal portion of the oviduct lies within the cloacal wall. Morphologically this portion may be regarded as the cloacal portion of the vagina.

Stages F to H. The wall of the Mullerian duct has increased in thickness and a slight increase in diameter of the duct is also noted in these stages. More mesenchymatous cells have invaded the region between the caudal end of the Mullerian duct and the epithelial lining of the cloacal lumen and have formed a distinct zone between the two.

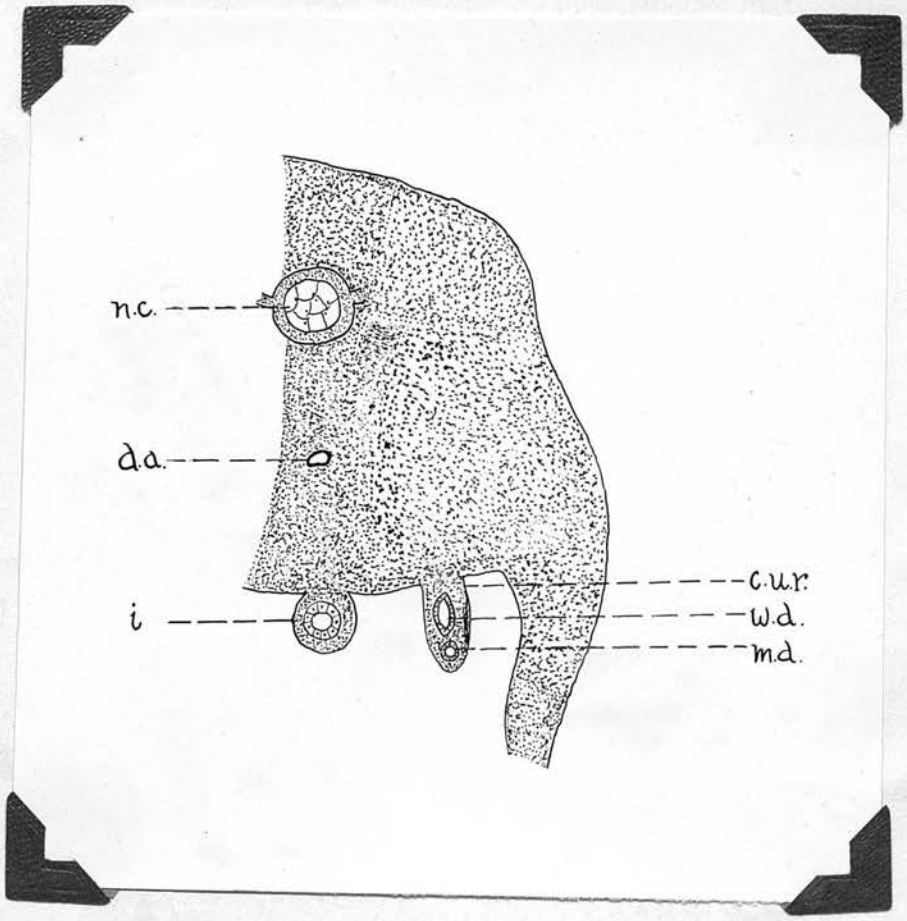
No further changes in the course of the Mullerian duct are noted in these stages; its relation to the Wolffian duct is the same as in stage E.



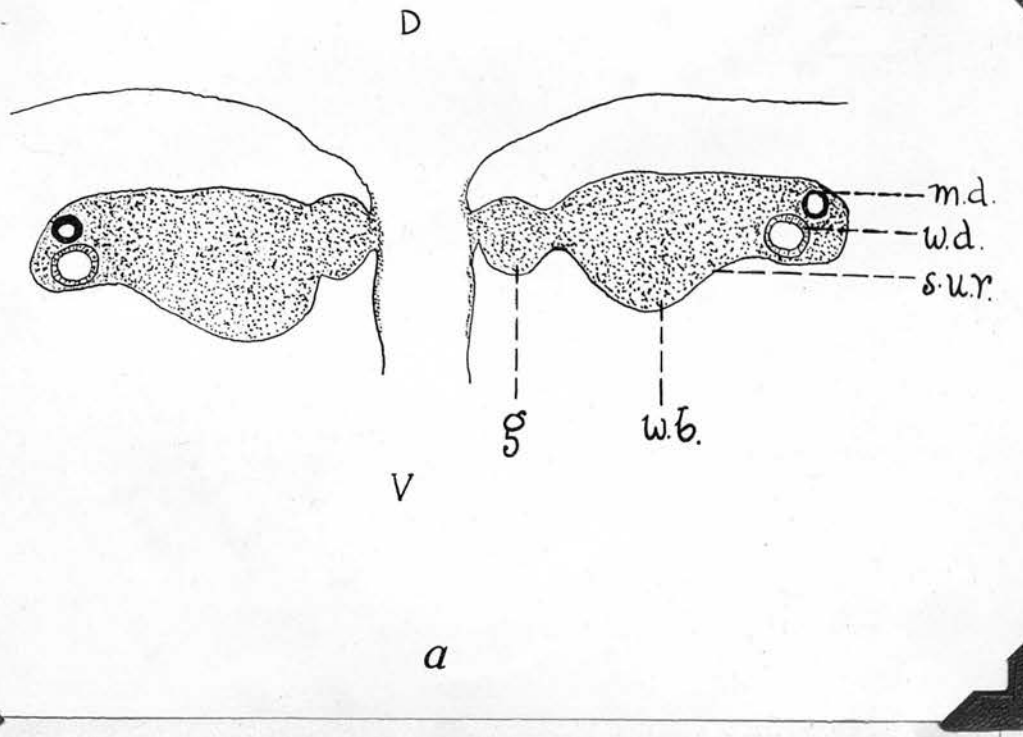
Text - fig.5 - Transverse section through the sexual division of the urinogenital ridbe of a 6 days old chick embryo showing the relative position of the Wolffian and the Müllerian ducts. (x20).



Text - fig. 6 - Transverse section through the non-sexual division of the urinogenital ridge of a 6 days old chick embryo showing the relative position of the Wolffian and the Müllerian ducts (x20).



Text - fig. 7 - Transverse section through the region of the intestine of a 7 days old chick embryo showing the caudal portion of the urinogenital ridge (x20). Note the relative position of the Wolffian and the Müllerian ducts.



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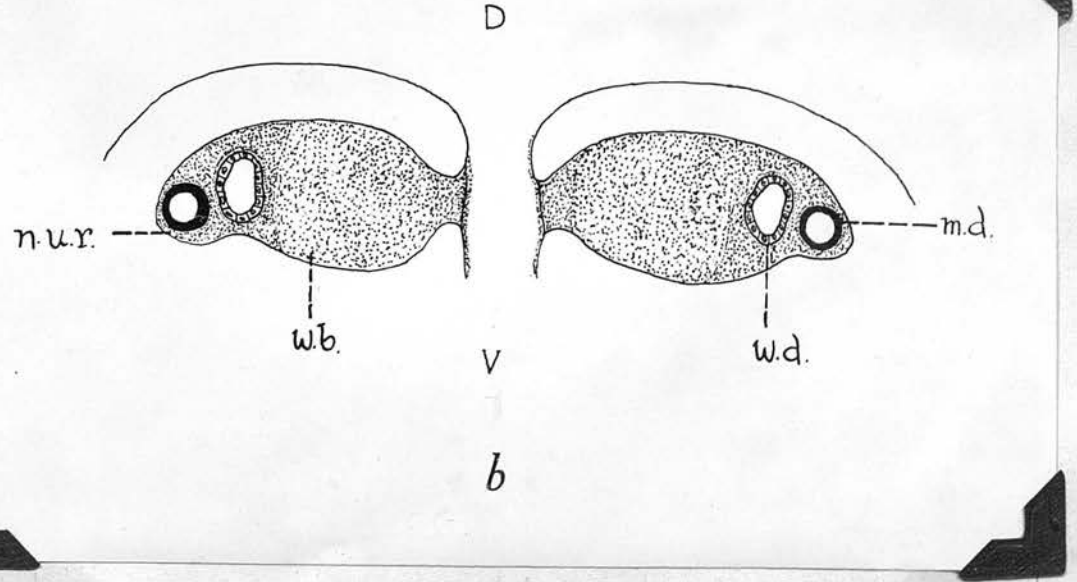
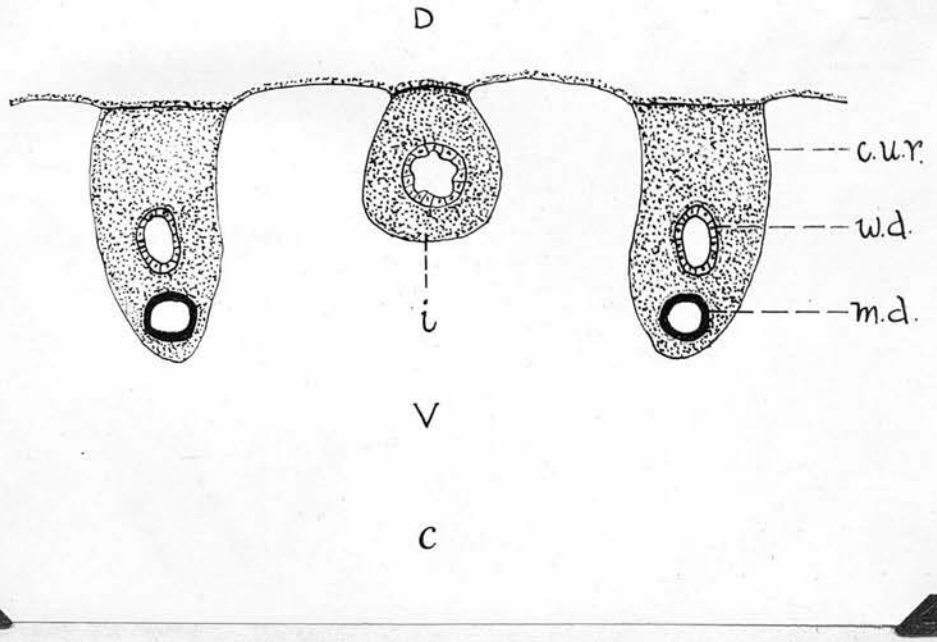
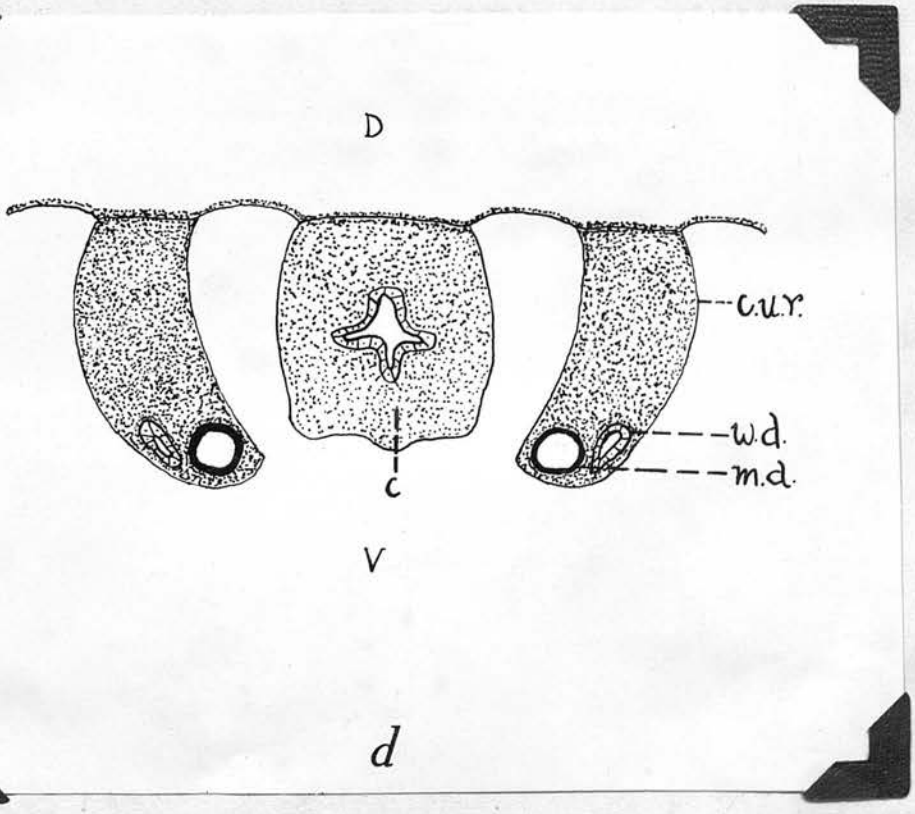


b

Text - fig.8 - Diagrammatic representation of the transverse sections through the successive regions of the urogenital ridge of a 8 days old female chick embryo showing relative positions of the Wolffian and the Müllerian ducts. For further explanation see the text.





Text- fig. 8 (continued)

Stage I. The Müllerian duct shows differentiation into ostium, magnum and the primordium of the shell gland. The mesenchymatous zone between the caudal end of the Müllerian duct and the epithelial lining of the cloacal lumen, is greatly thickened at this stage.

Stage J. The Müllerian duct has further differentiated. Both the Wolffian body and the Wolffian duct, as usual, have considerably degenerated leaving traces along their former sites. The thickened mesenchymatous zone together with the epithelium of the Müllerian duct and of the cloacal lumen forms an occluding plate (Pl. fig.7) which prevents the entrance of the Mullerian duct into the cloaca.

Stages K to O. Only traces of the Wolffian body and the Wolffian duct are visible. The former is, however, mostly replaced by the permanent kidney. The left ovary has increased enormously in volume and the Mullerian duct has assumed more or less the adult pattern.

72 days old chick. Pl. figs. 8 and 9 depict the sagittal section through the occluding plate which consists of a zone of connective tissue supplied with blood-vessels and is bordered, as stated above, by epithelium on its two antero-posterior sides.

4. Discussion.

There has been much controversy concerning the backward growth of the Müllerian duct. Bornhaupt (1883) suggested an independent backward growth of the duct. Later on Janosik (1883), V.Mihalcovics (1885-86) and Lillie (1927) also subscribed to this view/

view. Waldeyer (1870) pointed out that the Müllerian duct grows at the expense of cells contributed by the tubal ridge. However, Balfour and Sedgwick (1879) maintained that the posterior growth of the duct is effected by the cells proliferated from the Wolffian duct. The observations recorded in the present work point to an independent caudad growth of the Müllerian duct by the division of its own cells and not by the cells contributed by the tubal ridge or the Wolffian duct. A partial fusion of the Müllerian duct with the Wolffian duct has been suggested in the case of the duck (Burger, 1894a,b) but no such phenomenon is recorded in the case of the chick.

A variable number of evanescent openings have been recorded at the cranial end of the Müllerian duct in the chick only to disappear in subsequent development (Lillie, 1927). It is also interesting to note that in the duck as many as fifteen similar openings have been observed (Burger, 1897 a, b) but the present studies record only four such openings and their real significance is not yet understood.

Balfour and Sidgwick (1879) maintained that in the chick the ostium is derived from the pronephric nephrostomes. But a careful examination of the serial sections through the pronephros fails to corroborate this view, and it can safely be said that whatever may have been its phylogenetic origin the ostium has no ontogenetic connection with the pronephric nephrostomes. This is also the view expressed by Lillie (1927).

Previous workers on the chick (vide Lillie, 1927) as well as/

as on other Amniotes (vide Goodrich, 1930) failed to observe the ultimate fate of the tubal ridge. In the Urodela (Hall, 1904) the tubal ridge proliferates cells to help in the process of formation of an outer mesenchymatous jacket of the Müllerian duct. In the case of chick a similar fate of the tubal ridge is observed. Curtis (1910) suggested that the central ligament of the oviduct originates from cells contributed by the tubal ridge. This suggestion needs substantiation and the present author has taken an opportunity to study the embryology of the oviducal ligaments (to be published later).

In Teleostean fishes the growing caudal end of the Müllerian duct eventually fuses with the lips of the so-called "Abdominal pores" of the cloaca (Kerr, 1919). In the frog, MacBride (1892) has stated that the caudal end of the Müllerian duct touches and eventually fuses with the cloacal wall, but from the Pl.15, fig.14 of his paper it appears that the Müllerian duct fuses with a diverticulum-like structure from the cloacal wall. In the Marsupial Trichosurus vulpecula the Wolffian and the Müllerian ducts of one side fuse to form a single tube which enters the urinogenital sinus (Buchanan and Fraser, 1918). In the case of man the two Müllerian ducts fuse with each other to form a common duct which presses against a median projection (Müller's Tubercle) from the wall of the urinogenital sinus (Arrey, 1940). In the case of chick it has been shown here that the growing Müllerian duct penetrates through a bridge-like extension of the cloacal wall on the 8th day (Stage E) and comes to rest on the epithelial lining of the cloacal lumen. Unlike

man/

man and Trichosurus the Müllerian ducts in the chick, however, remain independent of each other and of the Wolffian ducts.

Lillie (1927) has stated that in the sexual division of the urinogenital ridge the Müllerian duct lies dorsal and external to the Wolffian duct, but he has made no mention of its course in either the non-sexual or the caudal portions of the urinogenital ridge. The present studies have shown that in the non-sexual division the Müllerian duct is external but on the same level with the Wolffian duct while in the caudal portion it occupies a ventral position to the latter for some distance and finally in the cloacal region it becomes more or less internal to the Wolffian duct (vide Stages C to E). It is interesting to note that after 8th day no further changes in the course of the Müllerian duct take place.

Lillie (1927) has given no description of the caudal portion of the urinogenital ridge. The present studies, however, have shown that it is the mesenchymatous caudal prolongation of the non-sexual division which connects the latter with the cloaca. In the marsupial Trichosurus vulpecula the caudal portion of the urinogenital ridges swings mesad to the mid-plane and fuse to form the genital cord. As a result of this fusion the Müllerian duct passes from a ventral position to the mesial side of the Wolffian duct (Buchanan and Fraser, 1918). In man, the caudal portion of the two urinogenital ridges swings as in Trichosurus to form the genital cord and a similar change of position of the Müllerian/

Müllerian duct in relation to the Wolffian duct takes place (Arrey, 1940). In the case of the chick there was no previous report of tilting of any part of the urinogenital ridge. It has been shown here that the caudal portion of the latter tilts slightly to the mesial side in the cloacal region on the 8th day and consequently in this region the Müllerian duct is manoeuvred to a more or less internal position to the Wolffian duct (see above). Unlike man and Trichosurus, however, the two urinogenital ridges in the chick never fuse to form the genital cord.

Gasser (1874) first pointed out that in the case of the domestic fowl the oviduct does not open into the cloacal lumen until after the hen is six months old. Greenwood (1935) showed that in immature female fowls the junction between the oviduct and the cloacal lumen is occluded by a membrane which eventually becomes perforated and finally destroyed during the process of laying the first egg. With the destruction of the occluding membrane at its mouth, the oviduct becomes continuous with the cloacal lumen. In the case of lower vertebrates (Kerr 1919) has stated that the opening of the Mullerian duct into the cloaca is "Commonly delayed till a comparatively late stage --- often till a period shortly before the sexual maturity". In immature rat and mouse the external orifice of the vagina is occluded by a "Plate" (Cameron, 1940). In the case of virgin human females also the entrance to the vagina is guarded by a membrane or the hymen (Arrey, 1940; Hamilton et al, 1945). The occluding plate of an immature female fowl is, thus, analogous to that of the/

the mammals. It is also interesting to note that it appears similar in structure to the hymen of a virgin human female.

5. Summary.

(1) Successive stages in the development of the Müllerian duct and its mode of fusion with the cloaca are described.

(2) The tubal ridge is concerned in the process of formation of the outer tunic of the Müllerian duct.

(3) The course of the Müllerian duct in relation to the Wolffian duct in its different developmental phases is described and discussed.

(4) The Müllerian duct instead of opening into the cloaca ends blindly, owing to the interruption of an occluding plate.

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7./

7. Explanation of Lettering.

WB, w.b. Wolffian body.

WD, w.d. Wolffian duct.

h.t. Heart.

cl. Coelome.

TR., T.r. Tubal ridge.

n.c. Notochord.

I. Invagination.

MD, m.d. Müllerian duct.

c.p. Cells proliferating from the tubal ridge round the Müllerian duct.

d.a. Dorsal aorta.

i. Intestine.

u.r., c.u.r. Caudal portion of the urinogenital ridge.

g. gonad

s.u.r. Sexual division of the urinogenital ridge (Wolffian body)

n.u.r. Non-sexual division of the urinogenital ridge (Wolffian body).

D. Dorsal side.

V. Ventral side.

c.d. Bridge-like extension from the cloacal wall.

c.e. Cloacal epithelium

c.l. Cloacal lumen.

m.b. Junction of the caudal portion of the urinogenital ridge with the cloacal extension.

o.w. Opening of the Wolffian duct.

m.z. Mesenchymatous zone.

o.p. Occluding plate.

e.m.d. Epithelial layer at the caudal end of the Müllerian duct.

b.v. Blood-vessel.

c.z. Connective tissue zone.

8. Description of Plates.

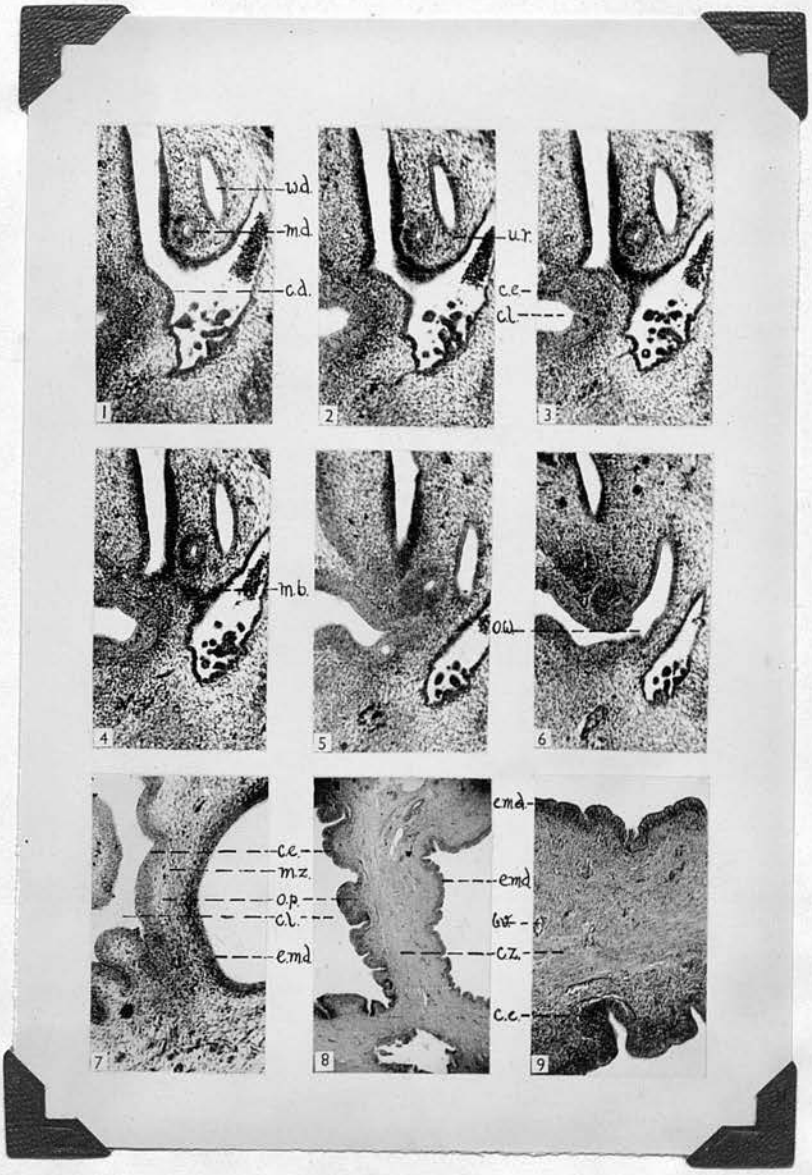
Figs. 1 - 6. Photomicrograph of six successive transverse sections through the cloacal region of an 8 days old female chick embryo showing the joining of Müllerian duct with cloaca (x80).

Fig.7. Photomicrograph of the sagittal section through the cloacal region of a 15 days old female chick embryo showing the occluding plate (x70).

Fig.8. Photomicrograph of the sagittal section through the occluding plate of the oviduct of a 72 days old chick (x35).

Fig.9. Magnified view of a portion of the occluding plate as shown in Fig.8.(x70).

PLATE



PART II.

STUDIES ON THE LIGAMENTS OF THE OVIDUCT IN THE DOMESTIC FOWL.

----oOo----

C O N T E N T S.

----oOo----

1. Introduction.

It is well known that the oviduct in the domestic fowl is held in position by two ligaments, one ventral and the other dorsal which form very conspicuous structures in an adult hen. Unfortunately the developmental history of these structures has received scant attention from the previous workers.

In the present paper an attempt has been made to investigate the embryonic development of the ligaments. Attention has been paid to the morphology and histology of the ligaments in immature chicks and their growth and differentiation with the approach of sexual maturity. An opportunity has also been taken in the course of this study to throw some light on the function of the ligaments.

2. Review of the Literature.

Curtis (1910) was the first to give a comprehensive account of the morphology of the ligaments in adult hens. She also gave a very brief account of the morphology of the ligaments in young chicks and further suggested that the tubal ridge (vide Lillie, 1927) is responsible for the formation of the ventral ligament. Hewitt (1939) also described the morphology of the ligaments in adult hens.

Curtis (1910) and later on Kaupp (1918) pointed out that the oviduct and its attached ligaments elongate considerably with the onset of sexual maturity.

Curtis/

Curtis (1910) showed that the ligaments terminate anteriorly in such a manner that they form a sort of "Pocket" which guides the egg into the funnel portion of the oviduct. She also suggested that the musculature of the ligaments helps, to a great extent, in maintaining the normal peristalsis of the oviduct. Asmundson (1931) pointed out that the shape of the egg is determined mostly by the activity of the muscles on the wall of the isthmus and the uterus and the ligaments exert no influence on the egg shape. Hewitt (1939), on the other hand, observed that the ligaments influence the egg shape and recently Harper and Marble (1945) subscribed to his view.

3. Material and Method.

Eggs from the pure-bred Brown Leghorn hens of the Institute flock were incubated in an electric "Petersime" incubator. The embryos among the female line were sorted out by the careful examination of their gonads (Greenwood, 1925) and sections were prepared by the method described in a previous paper (Kar, unpublished).

For histological study, the entire oviduct with its attached ligaments was dissected out and fixed in toto. 10% Formol-Saline proved to be useful for the latter purpose. After the usual process of dehydration and clearing small pieces of oviduct with the ligaments in situ were embedded in paraffin and transverse sections were prepared. The latter were stained by Mallory's triple stain or Ehrlich's haemotoxylin followed by alcohol/

alcohol eosin. For the study of collagenous fibres small pieces of the ligaments from fresh unfixed material were stained by methylene blue and mounted in glycerine.

Only the development and differentiation of left ventral and left dorsal ligaments were studied.

4. Observations.

A. Embryonic development of the ligaments.

The method of description of the embryonic stages followed here is the same as in a previous paper (Kar, unpublished). Particulars regarding the embryonic stages such as age and length are presented in table I.

Table I.

Successive stages of the chick embryos treated in the study.

Stages.	Age in days.	Length in mm.
I	8	33
II	9	36
III	10	41
IV	11	46
V	12	50
VI	15	71
VII	17	85
VIII	20	103
IX	21	105

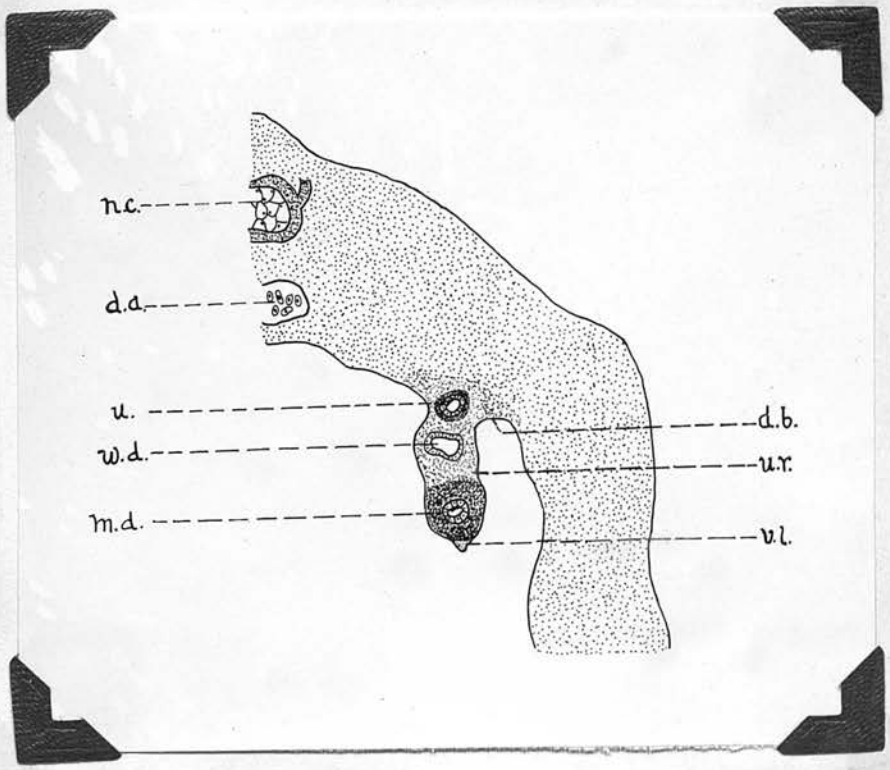
(a)/

(a) The Ventral ligament.

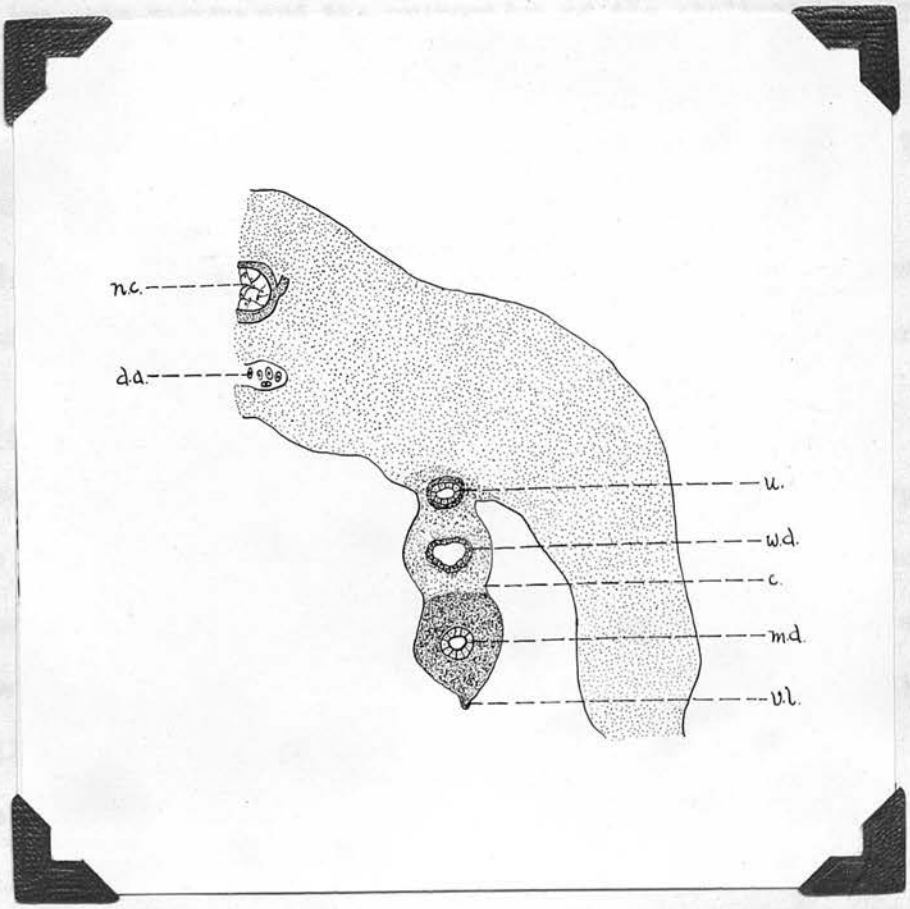
Stage I. - The Müllerian duct is seen as an epithelial tube surrounded by a thick outer tunic of concentrically arranged mesenchyme cells. The thickened epithelium of the tubal ridge appears flat and similar to the adjacent peritoneal epithelium. On the mid-ventral surface of the Müllerian duct a swelling is observed which extends from the posterior end of the non-sexual division of the urinogenital ridge for a short distance only caudad. In transverse section this swelling appears as the mid-ventral bulging of the peritoneal epithelium covering the urinogenital ridge and is loosely filled with mesenchyme cells (text - fig. 1). It measures about 3 microns in diameter.

Stage II. - At this stage the swelling on the mid-ventral surface of the Müllerian duct is seen up to the ostium region cephalad and down to the cloacal region caudad. In the cranial region the mesenchymatous cells from the outer tunic of the Müllerian duct migrate into the swelling and, as a result, it becomes closely packed with mesenchyme cells (text - fig.2). In the caudal region, however, no cell migration is observed and the swelling remains loosely packed with mesenchyme cells as in the previous stage.

Stages III and IV. - The process of cell migration from the outer tunic of the Müllerian duct is extended down to the caudal region and, as a result, in this area also the swelling becomes closely packed with mesenchyme cells. In transverse section it appears/



Text - fig. 1 - Transverse section through the caudal portion of the urinogenital ridge of a 8 days old female chick embryo showing the Anlage of the ventral ligament (x30).



Text - fig. 2 - Transverse section through the caudal portion of the urinogenital ridge of a 9 days old female chick embryo showing the Anlage of the ventral ligament (x35). For further explanation see the text).

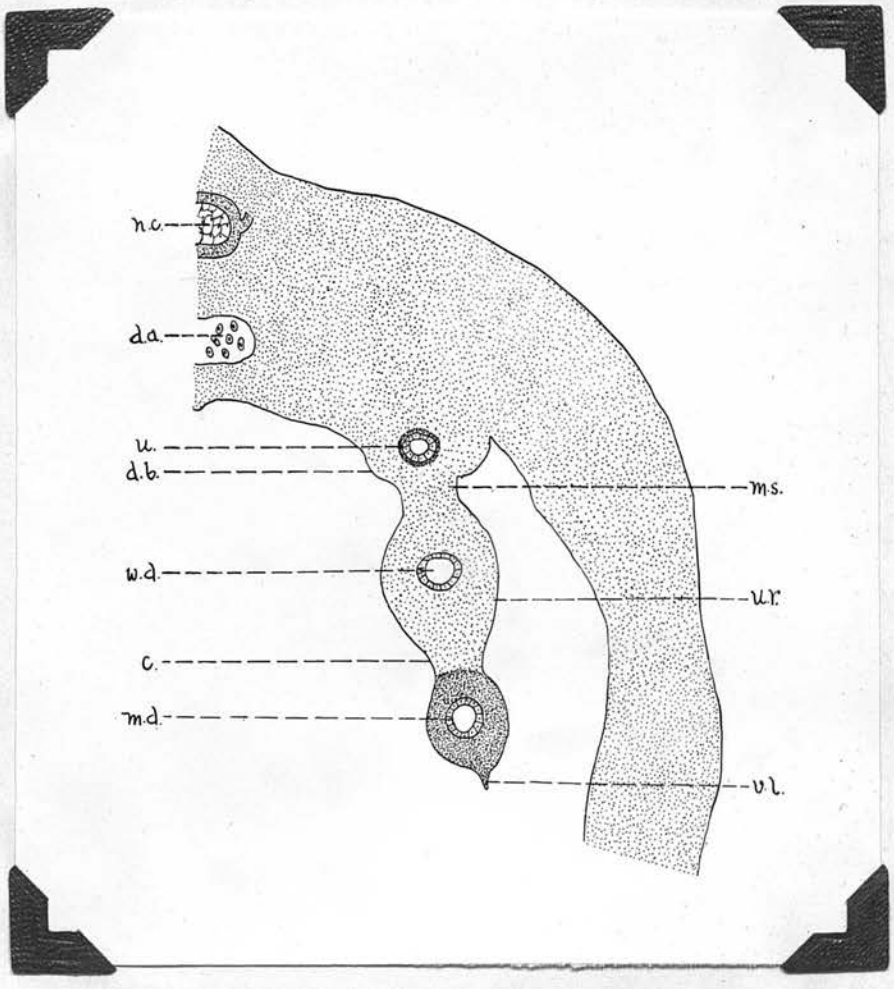
appears as the mid-ventral protuberance from the outer mesenchymatous tunic of the Müllerian duct covered by the peritoneal epithelium of the urinogenital ridge (text - fig.3; Pl. , fig.1).

Stage V.- The left Müllerian duct has differentiated into the ostium, the magnum and the primordium of the shell-gland. The mid-ventral swelling of the Müllerian duct or the ventral ligament exhibits considerable increase in diameter and in the cephalic region its free end appears somewhat curved (Pl. fig.2). Near the caudal region of the primordium of the shell gland the future ventral ligament appears as a spherical cord.

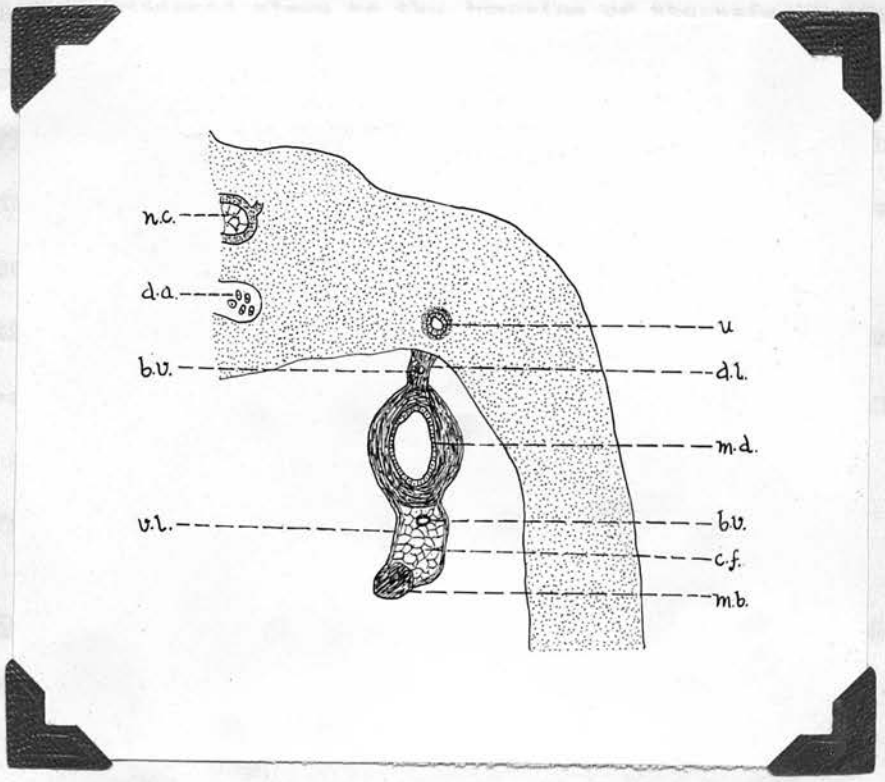
Stages VI and VII. - The central ligament is supplied with blood-vessels and blocks of muscular tissue of the plain type are seen near the free end (Pl. , fig.5). Patches of muscular tissue continuous with that of the Müllerian duct are also seen along the two lateral margins of the ligament (text - fig.4). Collagenous fibres are seen throughout its extent except in the shell gland region where it is only a muscular cord without any collagenous fibres.

Stages VIII and IX. - The free ventral margin of the ligament appears like a muscular cord and the two lateral margins are marked by the presence of thin patches of muscle which are continued round the Müllerian duct. The rest of the ligament, however, is filled with collagenous fibres. In the caudal region of the shell gland the ventral ligament is a muscular cord without any collagenous fibres as in the previous stages (Pl. , fig.6).

(b)/



Text - fig. 3 - Transverse section through the caudal portion of the urinogenital ridge of a 10 days old female chick embryo (x30). For further explanation see the text.



Text - fig. 4 - Transverse section through the magnum region of the Müllerian duct of a 17 days old female chick embryo showing the ligaments (x30).

(b) The dorsal ligament.

Stage I. - Except in the cloacal region, the Müllerian duct occupies a ventral position to the Wolffian duct in the caudal portion of the urinogenital ridge and is surrounded by a thick outer tunic of mesenchyme cells. The Wolffian duct is surrounded by a rather thin layer of mesenchyme cells (text - fig.1). The ureter is situated close to the junction of the urinogenital ridge with the dorsal body wall. The anterior end of the Wolffian body and the Wolffian duct have degenerated and the ostium region of the Müllerian duct remains attached to the dorsal body wall by a strip of mesenchyme. (Pl. , fig.3). Further caudad i.e. in the remaining sexual and the whole of the non-sexual portions of the Wolffian body (urinogenital ridge) the Müllerian duct occupies its usual position in relation to the Wolffian duct.

Stage II. - In the caudal portion of the urinogenital ridge a slight constriction is observed in its middle region (text - fig. 2). In the cloacal region the portion of the urinogenital ridge dorsal to the Wolffian duct also becomes constricted and appears as a narrow mesenchymatous strip which remains attached to a diverticulum-like portion of the dorsal body wall (text - fig.5). The ureter is situated in the latter region and is surrounded by a thick mesenchymatous outer tunic. In the ostium region the Müllerian duct remains attached to the dorsal body wall by the mesenchymatous strip referred to in the previous stage. In the entire cephalic portion of the urinogenital ridge (Wolffian body) the Wolffian duct has completely degenerated and disappeared.

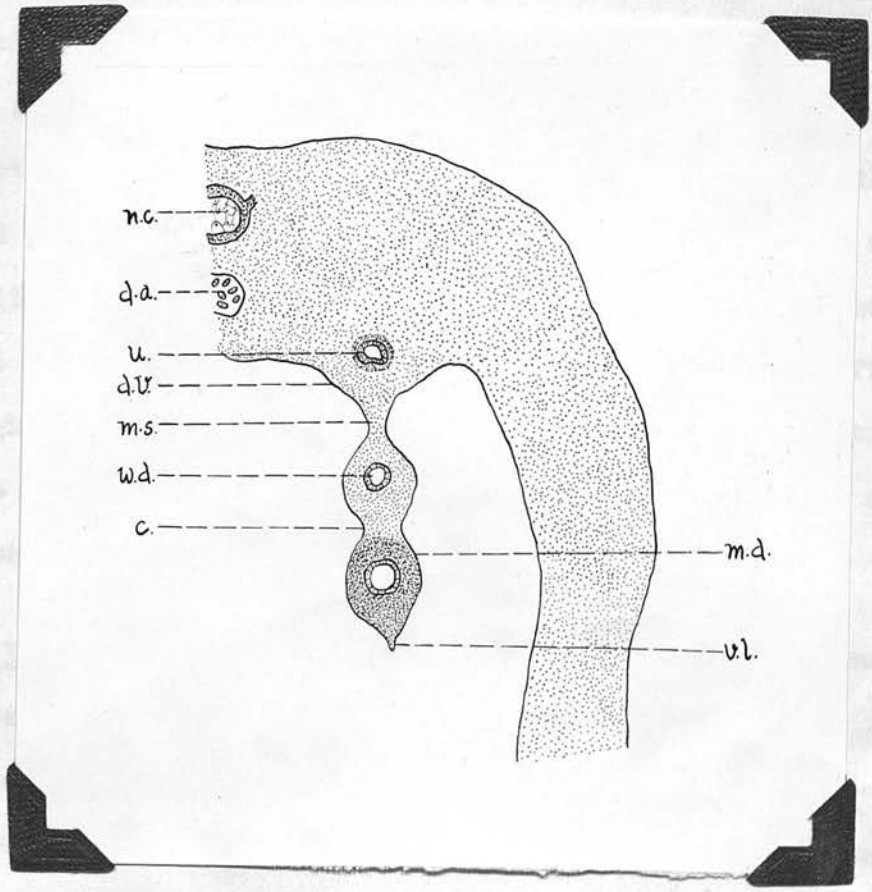
Caudad/

Caudad to the ostium region, however, the Müllerian duct remains attached to the Wolffian body as a mesenchymatous strip which appears as the caudal prolongation of the similar strip at the ostium region.

Stages III to IV. - The constricted middle region of the caudal portion of the urinogenital ridge appears narrower in stage III (text - fig.3). In stage IV it appears to be distinctly divided into a ventral and a dorsal portion, and the two remain connected by the constricted strip-like portion (Pl. ,fig.1). In the cephalic region of the urinogenital ridge, however, no marked change is noted.

Stage V. - In the caudal region the strip-like portion connecting the ventral with the dorsal portion of the urinogenital ridge appears narrower than at the previous stage. This strip becomes vascularized at this stage. The remaining portion of the Wolffian duct is in a stage of rapid degeneration and its epithelial lining is invaded by mesenchyme cells. The strip of mesenchyme which connected the caudal portion of the urinogenital ridge to the diverticulum-like projection of the dorsal body wall in the previous stages, ceases to exist at this stage and consequently the former remains directly attached to the latter. (Pl. , fig. 2). The major portion of the Wolffian body (corresponding to the entire sexual and the major part of the non-sexual division of the urinogenital ridge) has degenerated and the Müllerian duct remains attached to the dorsal body wall (Pl. , fig. 4).

Stage VI /



Text - fig. 5 - Transverse section through the caudal portion of the urinogenital ridge of a 9 days old female chick embryo in the region of the cloaca (x30). For further explanation see the text.

Stage VI. - The dorsal region of the caudal portion of the urinogenital ridge has merged with the adjoining body wall. The diverticulum-like projection of the dorsal body wall observed in the previous stages appears more or less triangular at this stage and the Müllerian duct remains attached to the apex of this projection by the constricted strip-like portion of the urinogenital ridge (Pl., fig.5) The ureter is situated inside this triangular projection. Portions of the Wolffian duct have completely degenerated but traces of the duct, however, are still visible. Only a small rudiment of the Wolffian body (corresponding to a rudiment of the non-sexual division of the urinogenital ridge) is left. Throughout its entire course, the Müllerian duct remains attached to the dorsal body wall by a continuous sheet of mesenchyme or the dorsal ligament.

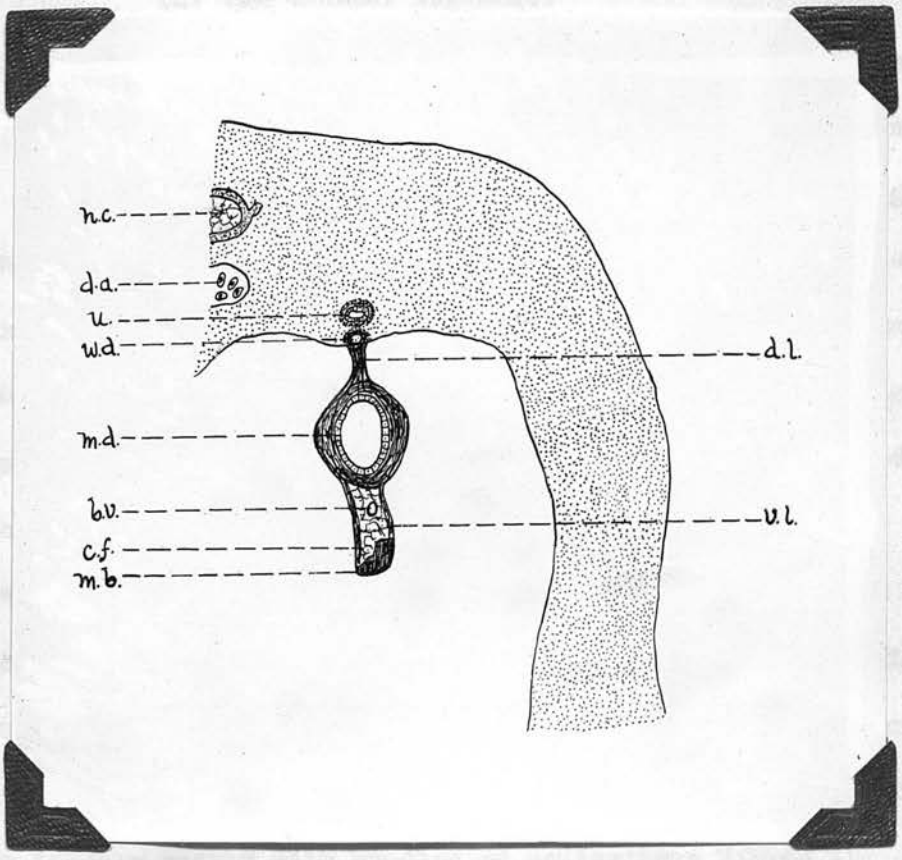
Stage VII. Muscular tissue of the plain type and blood-vessels are observed throughout the dorsal ligament but very few collagenous fibres are seen. Traces of the Wolffian duct are left only in isolated locations along the caudal region. The triangular projection of the dorsal body wall referred to above is no longer seen at this stage. The latter appears flat like the adjacent regions (text - fig.4).

Stages VIII and IX. - The dorsal ligament extends from the fourth thoracic rib down to the cloacal region. It is made up mostly of muscular tissue with few collagenous fibres interposed. Traces of the Wolffian duct can still be seen in the caudal region (text/

(text - fig. 6). In the cephalic region a small rudiment of the Wolffian body is left (vide Stage VI).

Appendix to the development of the dorsal ligament.

It is a well established fact that the Wolffian duct eventually degenerates in the female chick embryos but the details of this process are not mentioned in the literature. Lillie (1927) suggested that it degenerates along with the Wolffian body. From a review of the above it is evident that the process of degeneration of the Wolffian duct is closely associated with the embryonic development of the dorsal ligament. The process starts with the degeneration of only the anterior end of the duct on the 8th day (Stage I) but afterwards goes on with great rapidity in a caudad direction with the result that it ceases to exist in the entire cephalic portion of the urinogenital ridge by the 9th day (Stage II). In the caudal portion of the urinogenital ridge the degeneration of the Wolffian duct is slow and irregular, that is, not in a cranio-caudad direction as in the cephalic region. The major portion of the duct in this region, however, totally disappears by the 20th day but traces are left in some isolated locations even at hatching time. It is interesting to note that at the extreme cranial end the Wolffian duct degenerates along with the Wolffian body as suggested by Lillie but further caudad the former disappears earlier (Stage II). During the process of degeneration the epithelial wall of the duct is invaded by cells of mesenchymatous nature (Pl., fig.7), but the actual transformation of one kind of cell into the other is not seen. Incidentally, in the male chick embryos similar histological/



Text - fig. 6 - Transverse section through the Müllerian duct of a 20 days old female chick embryo immediately cephalad to the shell gland showing the ligaments and trace of the Wolffian duct (x30).

histological changes take place in the epithelial wall of the Müllerian duct which eventually degenerates after the 8th day (Lillie, 1927).

B. Morphology and histology of the ligaments in immature chicks.

(a) The ventral ligament.

The ventral ligament is a thin membrane which extends along the mid-ventral region of the oviduct from the funnel and ends as a thin muscular cord in the caudal end of the shell-gland. It remains attached to the oviduct by its dorsal margin while the ventral margin lies free in the body cavity. A transverse section through the ventral ligament in the magnum region (Pl. , fig.8) shows that the muscle fibres are concentrated in the free and in the attached margins of the ligament. The lateral margins, however, are bordered by comparatively thinner muscle fibres which are continued round the outer musculature of the oviduct. The musculature of the lateral margins anastomose with each other by very thin muscular trabeculae. The spaces between trabeculae are rather loosely packed with bundles of collagenous fibres which appear as finely dotted areas separated from one another by broken angular lines. Triangular fibroblast cells are situated in the spaces between the collagenous fibres. The cytoplasm of these cells takes up deep stain and each contains a spherical nucleus.

The caudal end of the ventral ligament, as previously stated, is a muscular cord and does not contain any collagenous fibres./

fibres. It finally breaks up into very thin bundles of muscle fibres which extend down to the utero-vaginal junction (text - fig. 7, a to c).

(b) The dorsal ligament.

The dorsal ligament remains attached to the oviduct by its ventral margin while the dorsal margin is attached to the body wall. It extends from the caudal end of the oviduct up to the fourth thoracic rib. The bulk of the ligament is made up of muscle fibres with very little connective tissue interposed. A transverse section through the dorsal ligament in the magnum region shows that the musculature of the lateral margins are continued round the outer musculature of the oviduct. Near the attached end a small area of the ligament is filled with collagenous fibres (Pl. , fig.9).

C. Growth and differentiation of the ligaments.

Like the oviduct its ligaments also exhibit two distinct growth phases in the post-hatching period. The first covers a considerable period of time (up to about the 20th week of age) during which the ligaments exhibit slow but steady growth (Isogony). This period is followed by one of extremely rapid growth (Heterogony) (vide Table II).

In this period of sudden growth the ligaments become highly vascularized and their musculature also becomes very thick. The ventral ligament is seen to be closely packed with bundles of collagenous fibres. It might be mentioned that this period of heterogonic/

Table II.

Summary of the measurement of the oviduct and the ligaments in the post-hatching period.

Age in weeks.	Number of Birds.	Average length of the oviduct in cm.	Average length in cm.		Average diameter in cm. (in the magnum region)	
			Ventral ligament	Dorsal ligament	Ventral ligament.	Dorsal ligament
6	2	8	7	10	0.8	1
10	3	8.3	7.2	10.5	1	1.3
13	2	9	8	11	1.5	1.8
15	3	10	9	11.5	1.6	2
18	2	10.5	9.5	12	2	2.5
20	2	11	10	13	2.2	2.8
21	2	25	20	30	4.5	5.2

heterogonic growth does not alter the relation of the ligaments which exists at hatching time, that is, the ventral ligament extends from the posterior end of the funnel down to the caudal end of the uterus and the dorsal ligament from the caudal end of the oviduct up to the fourth thoracic rib. It is interesting to note that the growth phases similar to those of the ligaments and the oviduct are exhibited by the comb and wattles (Greenwood, 1935). For a considerable period of time in the post-hatching period they show isogonic growth, then, with the onset of sexual maturity (after about the 20th week), follows a period of heterogonic or sudden growth.

D. Ligaments in mature chicks.

(a) The ventral ligament.

The ventral ligament is a fan-shaped structure which remains attached to the oviduct by its dorsal margin while the ventral margin lies freely in the body cavity. It measures about 6 cm. in diameter in the magnum region. At the cranial end the ligament remains attached to the posterior elongation of the funnel. Caudad to the funnel the free ventral margin of the ligament is a muscular cord from which bundles of fibres radiate on either side dorsally and become continuous with the outer muscle layer of the oviduct (Curtis, 1910). These muscular bundles are thicker than those of immature chicks and frequently anastomose with each other. The irregular-shaped compartments formed by these anastomosing muscular bundles are closely packed with numerous bundles of collagenous fibres.

The/

The caudal portion of the ventral ligament terminates as a solid muscular cord, without any collagenous bundles over the posterior blind sac of the uterus. This solid cord breaks up into bundles of fibres which pass on to the ventral and ventro-lateral sides of the convoluted anterior portion of the vagina (text - fig.7, d x). These muscular bundles are very conspicuous in a mature bird and have been referred to as the utero-vaginal musculature (Greenwood and Blyth, 1938).

(b) The dorsal ligament.

The dorsal ligament maintains a line of attachment to the dorsal body wall from the caudal end of the oviduct up to the fourth thoracic rib. It measures about 7 cm. in diameter in the magnum region. The ventral margin of the ligament, however, remains attached to the oviduct. Only the cranial portion of the dorsal ligament is spread out in the form of a "Fan" (Curtis, 1910). In the utero-vaginal region it is more or less straight. The dorsal ligament is made up mostly of muscular tissue with very little connective tissue interposed. The muscular bundles of the ligament originate from the mid-dorsal margin and pass ventrally and finally continue round the outer muscle layer of the oviduct (Curtis, 1910).

E. Function of the Ligaments.

It has been shown above that the caudal end of the ventral ligament terminates as a solid muscular cord over the posterior blind sac of the uterus and finally breaks up into thick/

thick bundles of fibres. The latter radiate on to the ventral and ventro-lateral sides of the peculiar "S" anterior portion of the vagina. As in the other regions of the oviduct thick bundles of muscle fibres from the dorsal ligament continue round the utero-vaginal region. The muscular bundles from the cord-like end of the ventral ligament, together with those of the dorsal ligament, are responsible for the maintenance of the vaginal convolutions. In fact, the latter can be straightened out only after the removal of the musculature of the ligaments. Greenwood and Blyth (1938) observed that a number of experimental pullets derived from eggs injected with oestrin at an early stage of incubation, laid shell-less eggs. On post-mortem examination of the oviduct it was noticed that the peculiar "S" convolutions were absent at the anterior end of the vagina and the latter appeared practically straight. On the basis of this observation they suggested that "The function of the peculiarly convoluted anterior portion of the vagina is to prevent the premature passage of the egg through the shell-gland before it has received its calcareous deposit. The absence of adequate vaginal flexures allowed of no resistance to the passage of the eggs through these regions normally characterized by well-developed musculature, the action of which determines the movement of the egg, and were therefore conducted without pause through shell gland and vagina to the exterior.[*]

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[*] It might be mentioned in this connection that during post-mortem examination I happened to come across a bird with an egg in the shell-gland which was, however, without any calcareous deposit. Obviously the bird was killed immediately after the migration of the egg from the isthmus into the shell-gland. An attempt to push the egg from the latter down into the vaginal Portion/

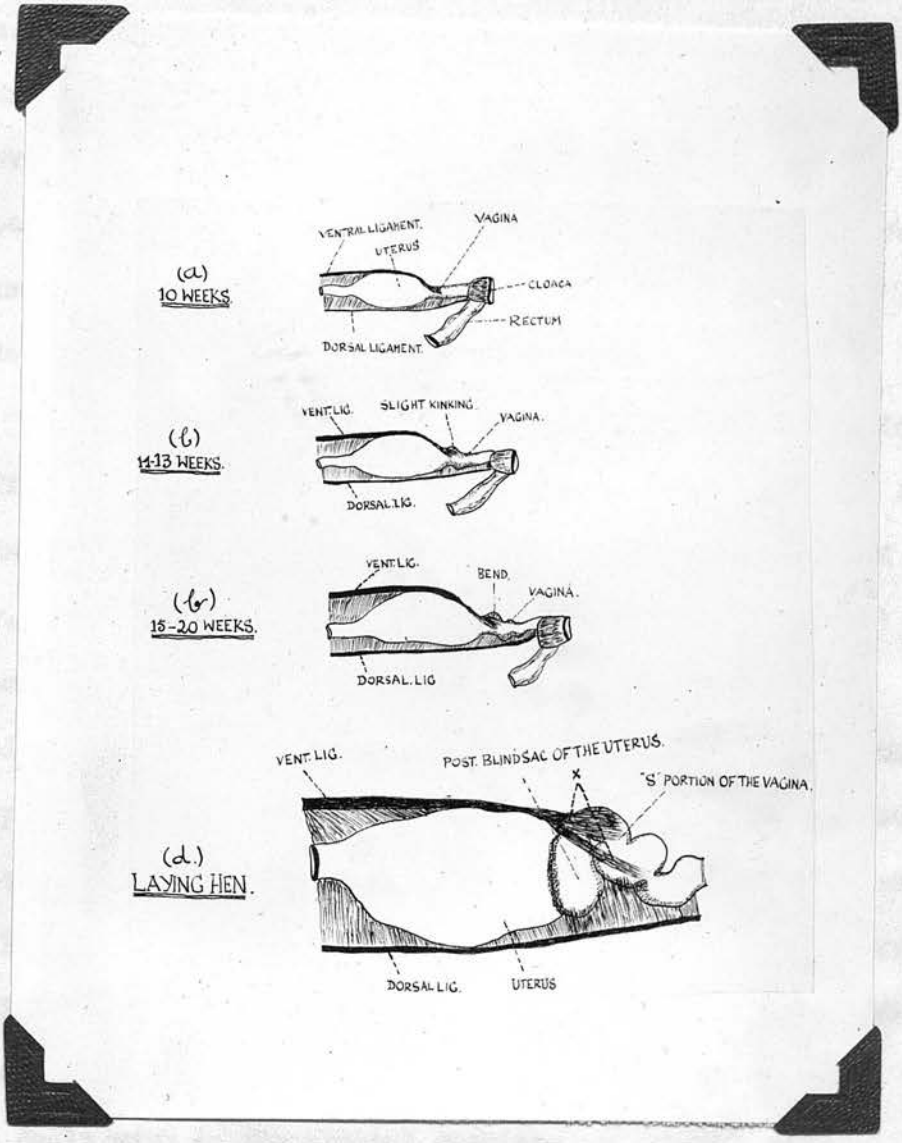
portion, however, proved futile. Next, the musculature of the ligaments were removed and the convoluted anterior end of the vagina was straightened out. Then by repeated application of gentle pressure at the isthmo-uterine junction it was found possible to bring the egg into the vagina. Incidentally, during laying the pointed end of the egg is normally extruded first but the eggs which project deeply into the posterior blind sac of the uterus may be turned with the blunt end caudad during the act of laying. (Olsen and Byerly, 1932). It is not unlikely that musculature of the ligaments plays an important role in the turning of eggs, the cause of which, however, is still unknown.

Examination/

Examination of the vaginal portion of chicks of varying age reveals certain peculiarities which suggest that a close relationship exists between the development of the vaginal convolutions and the growth and differentiation of the ligaments. In chicks up to the 10th week of age the vagina appears as a straight tube which runs from the caudal end of the uterus down to the cloaca (text - fig. 7, a). In chicks 11 to 13 weeks old, a slight kinking is observed in the anterior portion of the vagina (text - fig. 7, b). This kinking eventually takes the form of a sharp bend (15 to 20 weeks) (text - fig., 7, c). Finally, after about the 20th week, the anterior portion of the vagina exhibits convolutions characteristic of a laying hen (text-fig.7, d). It might be recalled here that up to the 20th week of age the ligaments are rather inconspicuous structures with poorly developed musculature. After this period the ligaments exhibit sudden growth. They become very conspicuous with their highly-developed musculature which in the vaginal region maintains its convolutions.

5. Discussion.

Curtis (1910) suggested that the strip of thickened coelomic epithelium or the tubal ridge which gives rise to the Müllerian duct is also responsible for the formation of the ventral ligament. However, it has been shown in a previous paper (Kar, unpublished) that the tubal ridge proliferates cells to help in the process of formation of the outer mesenchymatous tunic of the Müllerian duct. This process of cell proliferation is completed/



Text - fig. 7 - Diagrammatic representation of the utero-vaginal region of the oviduct and the ligaments in chicks of varying ages. For further explanation see the text.

completed by the 8th day, as a result of which the tubal ridge ceases to exist and its thickened epithelium appears flat and similar to the adjacent peritoneal epithelium. The present investigation, therefore, does not substantiate Curtis' suggestion. Lillie (1927) has made no mention of the fate of the caudal portion of the urinogenital ridge. The present studies, however, have shown that its mid-ventral region is greatly responsible for the formation of the caudal portion of the ventral ligament while the mid-dorsal region eventually becomes the caudal portion of the dorsal ligament.

Curtis (1910) observed that "During the first four or five months after hatching the growth of the oviduct and its ligaments is about proportional to the growth of the rest of the body. With the approach of functional activity (egg laying) the isthmus, albumen portion and funnel of the oviduct elongate considerably. This elongation includes the enclosing peritoneum", Kaupp (1918) also expressed a similar view. Both these authors, however, have not made any detailed study of the growth phases of the ligament. Further, they have not described the histology of the ligaments in immature chicks and their differentiation with the approach of sexual maturity. All these points, however, have been dealt with in the present studies.

Curtis (1910) and Hewit (1939) have made no mention of the relationship between the convoluted anterior end of the vagina and the ligaments. However, it has been suggested that the musculature of the ligaments is responsible for the maintenance of the vaginal convolutions. The latter, according to Greenwood and Blyth (1938) help to keep the egg in the shell-gland/

gland for adequate calcareous deposit.

Within recent years the hormonic activities of the ovary have been satisfactorily established (vide Allen, 1939). It is maintained that the head furnishings and the oviduct in the fowl are under the hormonic control of the ovary (Pezard, 1927; Domm, 1931; Greenwood and Blyth, 1930 and 1932; Greenwood, 1935; Juhn et al, 1932; Champey, 1931). Since the ligaments are intimately connected with the oviduct and show the same growth phases it is not unlikely that they are hormonically controlled. However, this statement should be regarded as tentative, subject to revision on the acquisition of experimental data.

6. Summary.

1. The embryonic development of the ligaments have been studied.
2. The morphology and histology of the ligaments in immature chicks have been described.
3. The growth phases of the ligaments and also their differentiation with the approach of sexual maturity have been dealt with.
4. It is suggested that the ligaments are under the hormonic control of the ovary.
5. It has been suggested that the ligaments are responsible for maintaining vaginal convolutions.

7./

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8. Explanation of Lettering.

n.c. Notochord.

d.a. Dorsal aorta.

u. Ureter.

w.d. Wolffian duct.

c. Constriction.

m.d. Mullerian duct.

v.l. Ventral ligament.

d.b. Dorsal bodywall.

u.r. Caudal portion of the urinogenital ridge.

b.v. Blood vessel.

m.b. Blocks of muscular tissue.

c.f. Collageneous fibres.

m.s. Mesenchymatous strip.

d.v. Diverticulum-like projection of the dorsal body wall.

d.l. Dorsal ligament.

w.b. Wolffian body.

o.d. Oviduct.

m.t. Muscle strands.

m. Muscular tissue.

m.c. Mesenchymatous cells.

9. Description of Plates.

Fig.1. Photomicrograph of the transverse section through the caudal portion of the urinogenital ridge of an 11 days old female chick embryo. (x30). For further explanation see text.

Fig.2. Photomicrograph of the transverse section through the caudal portion of the urinogenital ridge of a 12 days old female chick embryo showing the ventral and the dorsal ligaments (x30).

Fig.3. Photomicrograph of the sagittal section through the cephalic region of the Müllerian duct of an 8 days old female chick embryo showing the mesenchymatous strip (x30).

Fig.4. Photomicrograph of the transverse section through the cephalic region of the Müllerian duct of a 12 days old female chick embryo showing the dorsal ligament (x30).

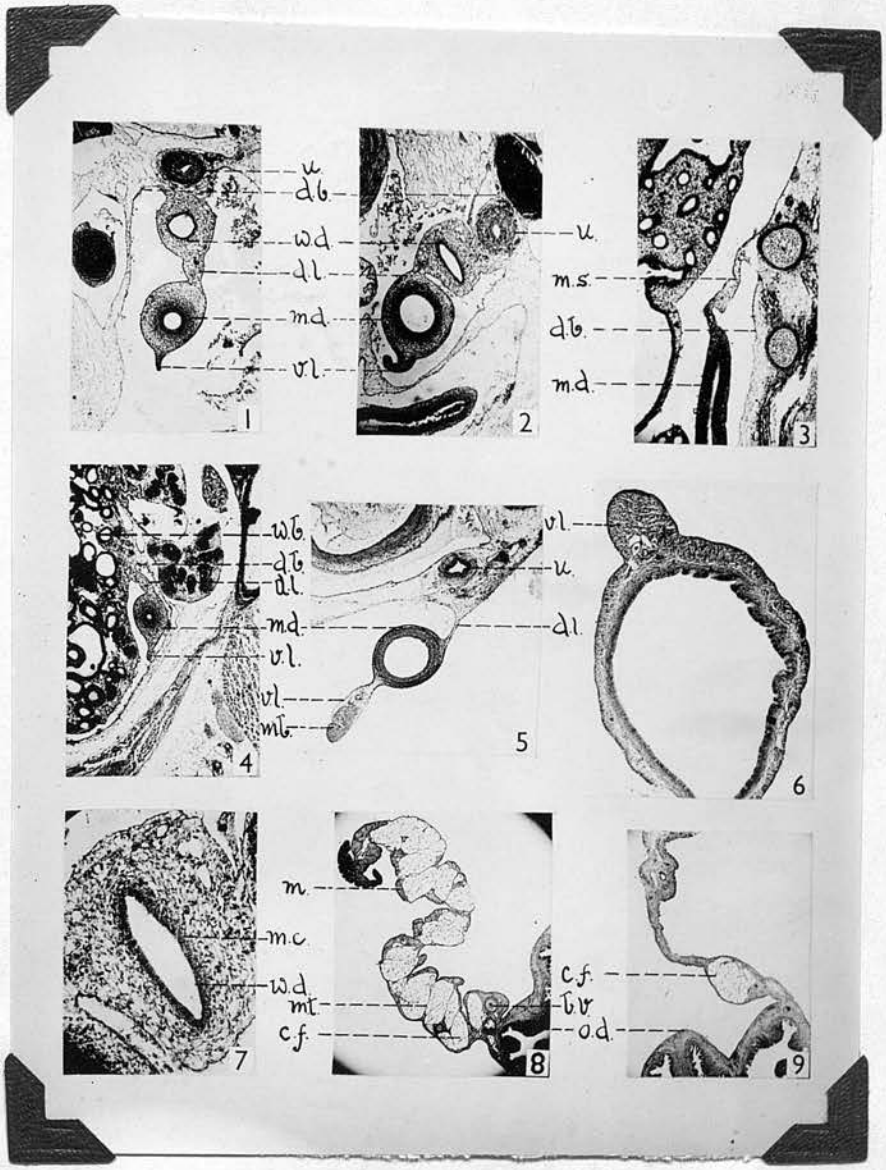
Fig.5. Photomicrograph of the transverse section through the Müllerian duct of a 15 days old female chick embryo showing the ligaments (x30).

Fig.6. Photomicrograph of the transverse section through the shell-gland of a 20 days old female chick embryo showing the ventral ligament (x35).

Fig.7. Photomicrograph of the transverse section through the caudal portion of the urinogenital ridge of a 12 days old female chick embryo showing the Wolffian duct (x80).

Figs. 8 and 9. Photomicrographs of the transverse sections through the ventral and dorsal ligaments respectively of a 80 days old female chick (x20).

PLATE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PART III.

SOME RESPONSES OF THE IMMATURE FEMALE FOWL TO INJECTION OF DIETHYLSTILBOESTROL WITH SPECIAL REFERENCE TO THE OVIDUCT.

CONTENTS

SOME RESPONSES OF THE IMMATURE FEMALE FOWL TO INJECTION OF DIETHYLSTILBOESTROL WITH SPECIAL REFERENCE TO THE OVIDUCT.

1. Introduction.

A great amount of work has been done on the oestrogenic influence of Stilboestrol in mammals but, unfortunately, its effect on birds has not received, as yet, the detailed attention it deserves. However, it has demonstrated that this synthetic oestrogen stimulates the immature oviduct of the domestic fowl (Herrick, 1944; Wolff, 1939; Munro and Kosin, 1943).

The present paper deals with certain experiments which show, in some detail, the responses obtained with varying amounts of Stilboestrol when injected into immature female fowls at different ages and for varying periods of time. Special attention in this study, however, has been paid to the effects elicited by this oestrogen on the oviduct.

2. Procedure.

For the experiments reported in this paper, pure-bred Brown Leghorn chickens from the Institute flock were used.

Stilboestrol (pp' - Dihydroxy-AB-diethyl-Stilbene (B.D.H.)) was dissolved in 70 per cent alcohol (4 mg. to 0.2 c.c. of alcohol). This alcoholic solution was diluted with 1.8 c.c. of/

of 0.9 per cent. physiological saline solution with the result that the final solution contained 2 mg. of Stilboestrol per c.c. The appropriate amounts of the hormone impregnated alcohol + saline solution were then injected to give the required total dose at the end of each injection period (vide Table I). The injections were made into the pectoral muscle of the chicks.

The chicks were allotted in groups for the injection of various levels of hormone and weighed at the commencement of each experiment. There was great variation in size of the birds available which made it seem best to keep record of the body weights of only those birds of an age group which were of uniform size at the beginning of the injection period (vide Table I).

The comb was measured at the beginning and end of each experiment and at other intervals when injecting was continued for longer periods. The maximum length plus height was used as measure of comb size (Gallagher and Koch, 1930).

The birds were weighed and killed on the day following the last injection. The ovaries were dissected out, weighed and fixed in Bouin's fluid. A small incision was made in the vaginal portion of the oviduct through which a specially made glass probe was inserted and, by eversion of the mouth of the oviduct, it was clearly seen whether the occluding plate was perforated or intact. After this operation the mouth of the oviduct with the occluding plate (or its remnants) in situ were carefully severed and fixed in Bouin's fluid. No grades of oviducal perforation have been made and even where a small opening was found in the occluding

plate /

plate it has been included under the category of perforated oviduct in Table II. The oviduct was finally dissected out, weighed, the ligaments measured and fixed in Bouin's fluid. Sections of the ovary, oviduct and the occluding plate were prepared by the usual methods and stained by Ehrlich's haematoxylin and followed by alcoholic eosin or Mallory's. Small pieces of the ligaments were stained by Borax Carmine and whole-mount preparations were made for microscopic study of the muscular bundles.

3. EXPERIMENTAL RESULTS.

A. EXTERNAL APPEARANCE.

Body weight:- In the age groups 1 to 25 days (Table I, groups 1 to 6), the injection of Stilboestrol had, in general some suggestion of a slight depressing effect on growth although number of birds involved was too small to provide adequate data on this point. In the age groups 30 to 90 days (Table I, groups 7 to 11) on the other hand the injection of hormone seemed to be associated with increased body weight as compared with the controls. In the oldest age group (Table I, groups 12 and 13) Stilboestrol produced no obvious effect on the body weight.

With regard to the depressing effect of Stilboestrol on growth, the results obtained here are not out of keeping with those obtained with rats by Freudenberger and Clausen (1937) and with chicks by Asmundson, Gunn and Klose (1937). However, Munro and Kosin (1940) and Herrick (1944) found no evidence of retardation of growth due to injection of oestrogens in young chicks.

It/

It is generally believed that the retarding effect of growth after oestrogen injection, if it occurs, is due to suppression of hypophyseal activity by administered oestrogen (Allen, 1939). Matthews, Schwabe and Emery (1942) found that the depression of growth by oestrogen is more pronounced in castrates than in normal female rats.

The chicks in which Stilboestrol treatment enhanced body weight (Table I, groups 7 to 11) exhibited a thick subcutaneous deposit of adipose tissue on autopsy. The fattening action of synthetic oestrogens, however, is well known and these are now used commercially for increased fat deposition and the resultant tenderizing of flesh in chickens. Thayer, Japp and Penquite (1944 and 1945) reported that dimethyl ether derivative of Stilboestrol was more potent orally for producing fatty deposit in the young pullets than was free Stilboestrol. Black and Booth (1946) also suggested oral use of Stilboestrol for fattening purposes. Lorenz (1945), on the other hand, concluded that subcutaneous implantation of Stilboestrol pellets produced superior fattening action in chickens than oral administration of other synthetic oestrogens. In the course of the present studies it was seen that the injection of Stilboestrol as a suspension in an alcohol + saline solution also produced thick fatty deposits in young chickens. It is suggested that a study of the comparative rate of fat deposition by Stilboestrol (a) when orally administered with feed, (b) when implanted subcutaneously in pellet form, and lastly (c) when injected in the form of suspension as mentioned above, might yield useful results.

COMB/

TABLE I.

SUMMARY OF THE WEIGHT OF CHICKS AND THE MEASUREMENT OF COMBS.

Group No.	Dosage	Age when injection started.	Age when killed.	Injection period.	No. of birds.	Total quantity of Stilboestrol injected.	Average weight of birds when injection started.	Average weight of birds when killed.
	Mgm.	Days	Days	Days		Mgm.	Gm.	Gm.
1	Not injected	1	11	-	2	-	44	52
1a	0.4 daily	1	11	10	2	4		
1b	1 daily	1	11	10	2	10	44	50
1c	2 daily	1	11	10	2	20	44	50
2	Not injected	5	15	-	2	-	54	64
2a	0.6 daily	5	15	10	2	6		
2b	1 daily	5	15	10	2	10	54	56
2c	1.5 daily	5	15	10	2	15		
2d	3 daily	5	15	10	2	20		
3	Not injected	10	31	-	2	-	68	160
3a	0.4 daily	10	31	20	2	8		
3b	1.2 daily	10	31	20	1	24		
3c	2 daily	10	31	20	1	40	68	100
4	Not injected	21	26	-	1	-	48	95
4	2 daily	21	26	5	1	10	48	90
5	Not injected	21	64	-	1	-	100	600
5a	0.4 daily	21	64	43	1	17.2	100	472
5b	1 daily	21	64	43	1	43		
5c	3 daily	21	64	43	1	129	100	300

Table I (Continued)

Group No.	Dosage	Age when injection started.	Age when killed.	Injection period.	No. of birds.	Total quantity of Stilboestrol injected.	Average weight of birds when injection started.	Average weight of birds when killed.
	Mgm.	Days	Days	Days		Mgm.	Gm.	Gm.
6	Not injected	25	31	-	2	-	112	116
6a	0.4 daily	25	31	5	1	2		
6b	1 daily	25	31	5	2	5		
6c	3 daily	25	31	5	2	15	112	112
7	Not injected	30	45	-	2	-	220	436
7a	0.4 daily	30	45	15	2	6	220	448
7b	1 daily	30	45	15	2	15		
8	Not injected	38	88	-	1	-	300	768
8a	2 weekly	38	88	50	1	16		
8b	4 weekly	38	88	50	1	32	300	836
9	Not injected	62	72	-	1	-	456	628
9a	1 daily	62	72	10	1	10		
9b	3 daily	62	72	10	1	30	456	700
10	Not injected	68	79	-	2	-	563	698
10a	1 daily	68	79	11	2	11		
10b	1.2 daily	68	79	11	2	13.2	563	764
11	Not injected	90	101	-	1		880	972
11a	1 daily	90	101	11	1	11		

TABLE I (Continued)

Group No.	Dosage	Age when injection started	Age when killed	Injection period.	No.of birds	Total quantity of Stilboestrol injected	Average weight of birds when injection started.	Average weight of birds when killed.
	Mg.	Days	Days	Days		Mgm.	Gm.	Gm.
11b	2 daily	90	101	11	1	22	880	1000
11c	4 daily	90	101	11	1	44		
12	Not injected	105	115	-	1	-	880	1000
12a	4 daily	105	115	10	1	36	880	1002
13	Not injected	105	122	-	1		885	1014
13a	2.5 daily	105	122	17	1	42.5		
13b	3 daily	105	122	17	1	51	890	1013

COMB. - The comb of the injected birds appeared small and pale as compared with those of the uninjected birds. Asmundson, Gunn and Klose (1937) reported a retarding effect on the comb of immature chicks injected with oestrin. Munro and Kosin (1940), on the other hand, observed that the comb of the female chicks was not affected by oestrogen injection. Dorfman and Grenulich (1937) concluded that the injection of oestrogens into female chicks will not accelerate comb growth. Herrick (1944) and Breneman (1940), on the other hand, reported stimulation of comb size in immature female chicks after injection with oestrogens.

Cloaca and Pelvis - The cloaca and pelvis of chicks injected with higher levels of hormone differed from those of the uninjected ones. The cloacae of the former were noticeably larger and more vascular than those of the uninjected chicks. The pubic bones of the treated chicks were farther apart, a response also reported for mature birds by Bates, Lahr and Riddle (1935), after injection with gonadotropic hormone. The birds injected with lower levels of Stilboestrol, however, showed less response. The results agree with those obtained by Asmundson et al (1937) with immature female chicks injected with oestrin.

B. OVARY.

The injection of Stilboestrol for 5 or 10 days into chicks 1, 5 or 21 days old (Table II; groups 1, 2 and 4), had no effect on the ovarian weight. In the most of the groups (Table II; groups 2, 3 and 5 to 12), on the other hand, the injection of Stilboestrol elicited a slight inhibitory effect on the ovary.

In/

In the oldest experimental chicks (Table II, group 13), the ovarian weight was considerably less than that of the uninjected control. The ovarian weight of the controls increased with age except in the case of birds killed when 31, 45, 64 and 88 days old in which the weight of the ovaries were found to be the same (Table II; groups 3, 7, 5 and 8).

The results obtained here appear to be in agreement with those reported by Juhn, D'Amour and Gustavson (1930), Asmundson, Gunn and Klose (1937) and Munro and Kosin (1940). The situation, however, has not been entirely clarified with regard to the effect of oestrogens on the ovarian weight. The earlier investigators report that extraneous oestrogens invariably depress the weight of the ovary and they conclude that this depression is due to the suppression of the gonad stimulating function of the anterior pituitary which secondarily inhibits the ovarian growth (vide Allen, 1939). On the other hand, Ellison and Burch (1936) observe that large doses of oestrogens will cause hypertrophy of the ovaries in the normal rat. Subsequent investigations (Freudenberger and Clausen, 1937; Mazer, Israel and Alpers, 1936; and Emery, 1937), however, tend to qualify the earlier idea of a depressing effect of extraneous oestrogens on the ovary. They further report that there is a time factor in the response of the ovary to the administered oestrogens. The older the animal is, the more susceptible it becomes to the inhibitory effects of oestrogens.

C. /

TABLE II.

SUMMARY OF WEIGHTS OF OVARIES AND OVIDUCTS.

Group No.	Dosage	Age when injections started.	Injection period.	Average weight of ovary.	Average weight of oviduct.	Oviduct Imperforate (I)	Oviduct Perforate (P)
	Mgm.	Days	Days	Gm.	Gm.		
1	Not injected	1	-	0.03	0.02	I	-
1a	0.4 daily	1	10	0.03	0.5	I	-
1b	1 daily	1	10	0.03	0.8	I	-
1c	2 daily	1	10	0.03	1	I	-
2	Not injected	5	-	0.05	0.02	I	-
2a	0.6 daily	5	10	0.05	0.8	I	-
2b	1 daily	5	10	0.04	1	I	-
2c	1.5 daily	5	10	0.05	1	I	-
2d	2 daily	5	10	0.04	1.5	-	P
3	Not injected	10	-	0.4	0.2	I	-
3a	0.4 daily	10	20	0.3	2.7	-	P
3b	1.2 daily	10	20	0.2	2.8	-	P
3c	2 daily	10	20	0.2	3.1	-	P
4	Not injected	21	-	0.2	0.1	I	-
4	2 daily	21	5	0.2	0.5	I	-
5	Not injected	21	-	0.4	0.3	I	-
5a	0.4 daily	21	43	0.2	3.2	-	P
5b	1 daily	21	43	0.2	4.2	-	P
5c	3 daily	21	43	0.2	5	-	P

TABLE II (Continued)

Group No.	Dosage	Age when injections started.	Injection period.	Average weight of ovary.	Average weight of oviduct	Oviduct	
						Imperforate (I)	Perforate (P)
	Mgm.	Days	Days	Gm.	Gm.		
6	Not injected	25	-	0.4	0.3	I	-
6a	0.4 daily	25	5	0.4	0.6	I	-
6b	1 daily	25	5	0.4	0.8	I	-
6c	2 daily	25	5	0.2	1.5	I	-
7	Not injected	30	-	0.4	0.6	I	-
7a	0.4 daily	30	15	0.3	1.5	-	P *
7b	1 daily	30	15	0.2	2.1	-	P *
8	Not injected	38	-	0.4	0.5	I	-
8a	2 weekly	38	50	0.2	2.1	-	P
8b	4 weekly	38	50	0.2	2.8	-	P
9	Not injected	62	-	0.5	0.4	I	-
9a	1 daily	62	10	0.2	2.1	I	-
9b	3 daily	62	10	0.2	4.4	-	P
10	Not injected	68	-	0.6	0.5	I	-
10a	1 daily	68	11	0.4	6.3	I	-
10b	1.2 daily	68	11	0.4	7	-	P
11	Not injected	90	-	0.7	0.6	I	-
11a	1 daily	90	11	0.5	4.8	I	-
11b	2 daily	90	11	0.5	6.5	-	P *
11c	4 daily	90	11	0.4	7.5	-	P *

Table II (Continued)

Group No.	Dosage	Age when injections started.	Injection period.	Average weight of ovary.	Average weight of oviduct	Oviduct	
						Imperforate (I)	Perforate (P)
	Mgm.	Days	Days	Gm.	Gm.		
12	Not injected	105	-	0.8	6.5	I	-
12a	4 daily	105	10	0.6	9	-	P
13	Not injected	105	-	5.5	16	I	-
13a	2.5 daily	105	17	0.8	15.5	-	P*
13b	3 daily	105	17	1.5	16.	-	P

P = cases where no remnant of the occluding plate was left.

P* = cases where remnant of the occluding plate was left at the mouth of the oviduct.

C. OVIDUCT.

Macroscopic observations. - The oviduct of injected birds was highly vascular in contrast to that of the controls.

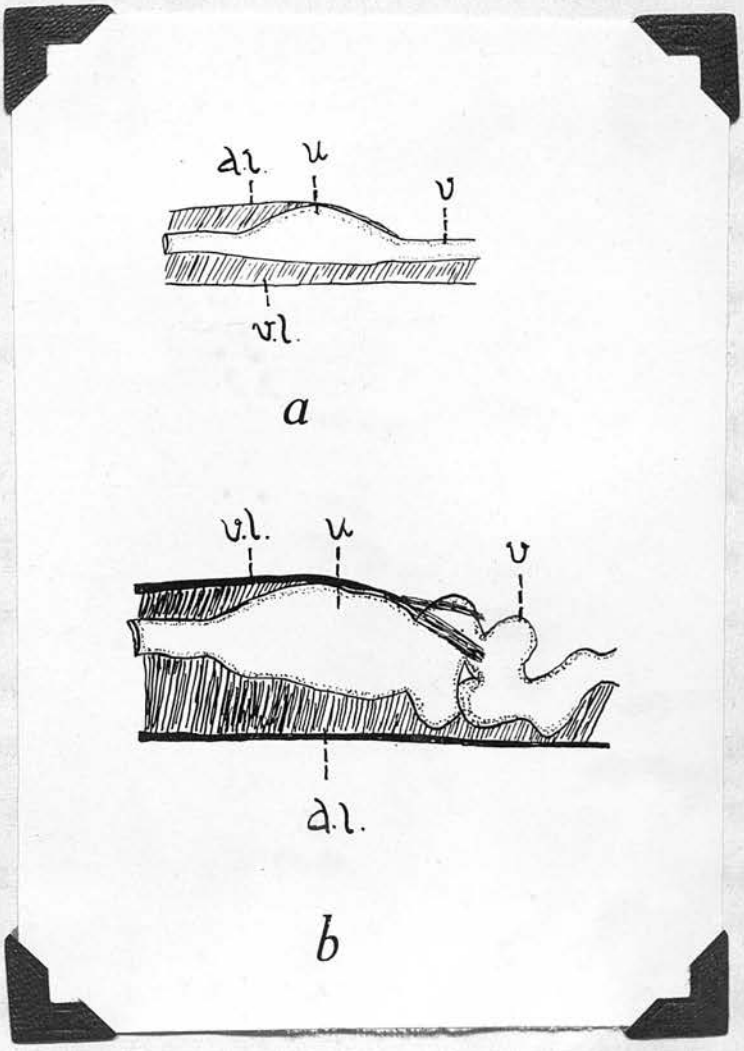
The vaginal portion of the oviduct was straight in the uninjected controls of the groups 1 to 7 and 9 (Table I). In the groups 8 and 10 (Table I) the uninjected birds showed slight kinking in the anterior portion of the vagina; while in the oldest group (Table I, groups 12 and 13) the control birds exhibited a sharp bend in the anterior portion of the vagina. In contrast to these, the injected birds showed conspicuous vaginal convolutions (text - fig.1). Among the birds injected for 5 or 10 days only those which received the highest level of hormone showed the characteristic "S" convolutions of the vagina while lower hormone levels produced only a sharp bend in the vaginal region. In all those birds injected for longer periods the vaginal flexures were like those of an adult hen.

Microscopic observations. - Microscopical changes similar to those observed by Juhn, D'Amour and Gustavson (1930), Raspopova (1935), Asmundson, Gunn and Klose (1937) and Herrick (1944) followed the injection of Stilboestrol. There was general growth of the oviduct with an increase in the muscle layers, connective tissue stroma and the epithelial layer. This growth of the oviduct, however, was less in the chicks injected for 5 or 10 days than those injected for 20 or more days. The oviducts of the latter had well developed tubular glands and the cells of the epithelial/

epithelial layer were crowded together giving them a high columnar appearance. The nuclei of these cells were centrally located but in some places were either apically or basally situated forming two apparent layers. It might be mentioned in this connection that similar pseudo-stratification of the nuclei is also seen in the epithelial layer of the oviduct of a laying hen (Richardson, 1935). In contrast to the injected birds, the tubular glands were absent or poorly developed in the oviducts of uninjected birds and the epithelial layer was low. The gross histological differences between the oviduct of an uninjected 64 day old chick and the oviduct of the same age previously injected for 43 days are depicted in Pl.1, figs. 3 and 4. In addition to the histological differences between the oviduct of an uninjected control and an injected bird there were indications of secretion into the lumen of the oviduct of the latter (Pl.1, fig.4) whereas there were no such indications in the oviduct of the uninjected bird nor any indications of secretion observed in the case of birds injected for 5 or 10 days (Pl.1, figs. 1 and 2).

Weight. - The weight of the oviduct increased after 10 days of injection with Stilboestrol into day old and 5 days old chicks. Five daily injections also increased the weight of the oviduct but only the highest dose had the greatest effect (Table II). Less hormone, however, had little effect on the oviduct weight in so short a time. Ten-day injections into older birds also increased the weight of the oviduct except in the case of the oldest group (Table II, groups 12 and 13) in which the injection of hormone elicited little effect on the oviduct weight because the latter

was/



Text - fig.1a - Semi-diagrammatic representation of the utero-vaginal region of the oviduct of a 31 days old uninjected female. Note the straight vaginal region and inconspicuous ligaments.

Text - fig.1b - Semi-diagrammatic representation of the utero-vaginal region of a 31 days old female injected 20 days with 2 mg. of Stilboestrol daily. Note the vaginal convolutions and the conspicuous ligaments.

was already growing at maximum rate under normal ovarian stimulation. Longer periods of injection, however, resulted in greater increase in the weight of the oviduct (Table II, groups 3, 5, 7 and 8).

Perforation. - None of the birds injected for 5 days had perforated oviducts (Table II, groups 4 and 6). Careful examination of the mouth of the oviduct showed that the occluding plate was intact without any indication of perforation. In day old chicks injected for 10 days and killed, when 11 days old the oviduct was also found to be occluded (Table II, group 1). Among chicks injected for 10 days and killed when 15 days old only the highest level of hormone elicited the perforation of the occluding plate (Table II, group 2). These were the youngest chicks in which the injection of Stilboestrol caused perforation of the occluding plate whereas normally, according to Greenwood (1935), it does not become perforated until the birds are about 140 days old. In 62 days old chicks injected for 10 days and killed, when 72 days old (Table II, group 9) only the highest hormone level initiated perforation of the occluding plate as in group 2. Similar results were also obtained with birds injected for 11 days and killed when 79 or 101 days old (Table II, groups 10 and 11). In the oldest group (Table II, groups 12 and 13), all the injected birds, however, had oviducts continuous with the cloaca. Prolonged injection for 15 days or more caused perforation even with smaller levels of hormone. Thus chicks injected for 20 days daily with 0.4 mg. of hormone had perforated oviducts (Table II, group 3). Similarly/

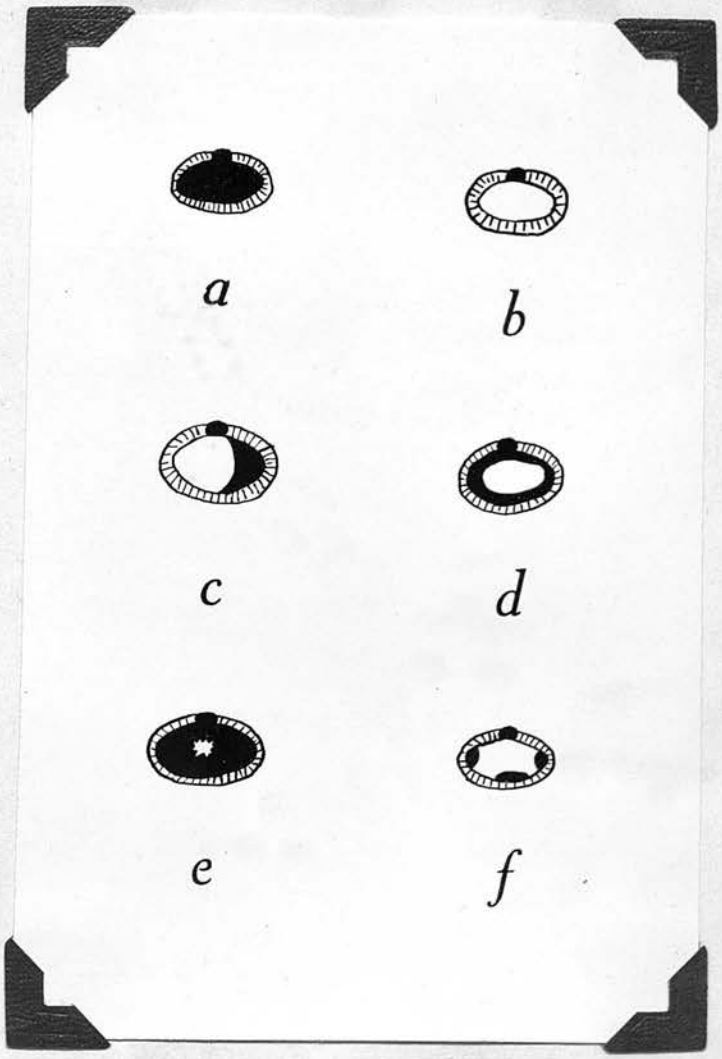
Similarly chicks injected for 15 days daily with the same hormone level exhibited perforated oviducts (Table II, group 7). The uninjected controls of all the age groups in the series, however, had occluded oviducts.

The occluding plate of the oviduct is a disc of connective tissue supplied with blood vessels and surrounded with epithelium on the antero-posterior sides (Kar; unpublished a). The anterior epithelial layer is the continuation of the epithelial layer of the oviduct while the posterior layer is the continuation of the epithelial lining of the cloacal lumen. In chicks injected for 5 days no histological changes could be detected in the occluding plate. In day old chicks injected for 10 days the highest level of hormone (see Table II, group 1) caused vacuolization of the posterior epithelial layer of the occluding plate (Pl.2, figs. 1 and 2). Similar vacuolization of the posterior epithelial layer was also noticed in the uninjected control killed when 115 days old (Table II, group 12). In birds injected for 10 days with lower hormone levels (see Table II, groups 1, 2 and 9), the occluding plate was similar to that of the control birds in histological details. The posterior epithelial layer of the occluding plate was lost in chicks injected for 10 days daily with 2 mg. of hormone and killed when 15 days old (Table II, group 2d) (Pl.2, figs. 3 and 4). Such was also the case with the bird injected for 11 days daily with 1 mg. of hormone and killed when 101 days old (Table II, group 11a). The uninjected control killed at an age of 122 days (Table II, group 13), showed similar conditions of the occluding plate minus the posterior epithelial layer

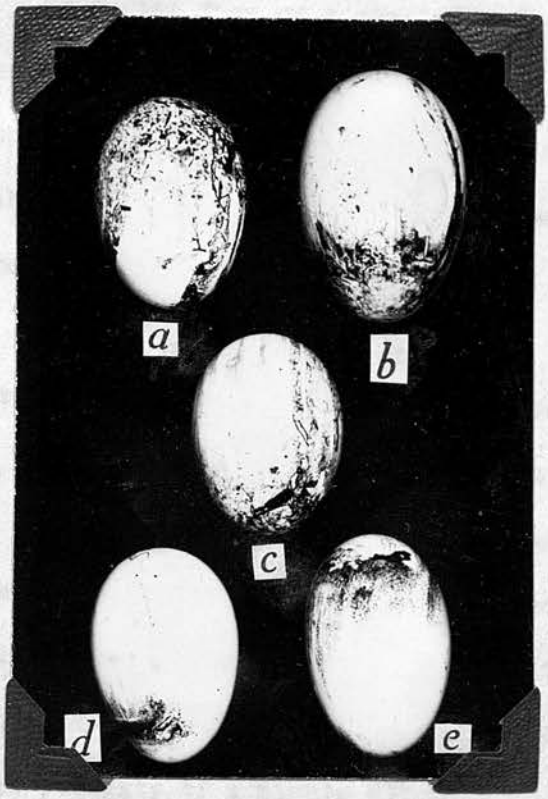
Pl.2/

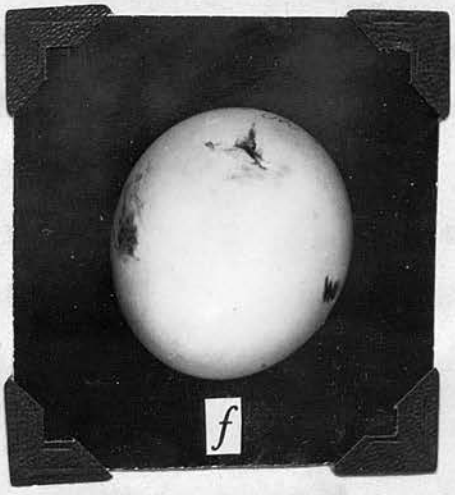
(Pl.2, fig.5). In the majority of cases where the injection of Stilboestrol elicited perforation, no remnant of the occluding plate was left (Pl.2, figs. 6 and 7). In a few cases, however, a remnant of the plate was left in the mouth of the oviduct (see Table II). Microscopic examination of the remnant revealed a dissoluted condition (Pl.2, fig.8). Microscopic examination of remnant of the occluding plate in normal pullets also showed similar conditions (Pl.2, fig.9).

The macroscopic appearance of the mouth of the oviduct of the injected birds in which a remnant of the occluding plate was left are depicted in text - fig. 2. In the first case the remnant was found to be in the form of a semilunar piece left on one side of the oviducal opening (text fig. 2c). In the second case the remnant encircled the entire mouth of the oviduct (text - fig.2d). In the third case the major portion of the occluding plate was persistent except for an irregular-shaped piece from the centre (text - fig.2e). In the last case three small pieces of a plate were left (text - fig. 2f). Examination of the mouth of the oviduct of normal pullets varying in age from 140 to 150 days old also revealed the persistence of similar remnants of the occluding plate. It is evident that the passage of the first egg exerts considerable pressure on the remnants of the occluding plate which, as stated before, persist in a state of dissolution. The blood vessels supplying the plate are ruptured as a result of this pressure, causing haemorrhage which leaves an impression on the portion of the shell in contact with the remnant of the plate. Thus an egg coming in contact with a semilunar piece of the occluding/



Text - fig. 2 - For explanation see the text. All figures are diagrammatic. Fig.a represents the typical appearance of the mouth of an occluded oviduct. Fig.b represents the typical appearance of the mouth of a perforated oviduct where no remnant of the occluding plate is left.





laid by

Text - fig. 3. - Photograph of the first pullet eggs showing different forms of blood smear on the shell.

occluding plate as in text - fig. 2c. exhibits an incomplete ring-like smear of blood on its shell. (text - fig.3d). Similarly the blood smear may be in the form of a complete ring (text - fig.3e) or isolated patches (text - fig.3f) while an egg forcing its way through the irregular-shaped opening in the centre of the occluding plate as shown in text - fig. 2e becomes smeared with blood practically over the entire surface of the shell (text - fig.3a to c).

Greenwood (1935) observed that "In the majority of the birds examined in which the duct was occluded the obstruction was found to be in the nature of a rather thin membrane at one side of which and close to the wall of the oviduct a small but well marked concrescence was present. The first indications of perforation of the membrane were seen near this point. In the earliest cases of perforation this consists of a minute circular orifice at the base of the papilla; this rapidly enlarges until only a remnant of the occluding membrane persists encircling the inner wall of the oviduct". Examination of normal pullets confirmed this observation but a second mode of perforation was also seen both in experimental chicks and normal pullets, according to which the perforation started in the centre of the plate either in the form of a small orifice or several small orifices which rapidly enlarged until only a small remnant of the plate was left.

Ligaments.- The ligaments of the uninjected birds were inconspicuous structures with poor vascular supply and thin muscular bundles. The ligaments of the injected birds, on the other hand, were highly vascular and had thick bundles of muscle fibres (Pl.1, figs. 5 and 6). The average length and diameter of the ligaments was also

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greater in the injected birds than those of the controls (Table III).

Incidentally, four laying hens were injected with varying doses of Testosterone propionate (dissolved in Arachis oil) for different periods of time. The first bird (2) was injected for 10 days daily with 0.5 mgm. of hormone and the second one (M220) for the same period but with 2 mgm. of hormone daily. The third (S94) and the fourth (R2183) birds were injected for 30 days daily with 1 and 2 mgms. of hormone respectively. All the birds were killed following the last injection. None of the birds laid during the injection period though before the commencement of injection they were regular layers. It is obvious that this cessation of laying was due to the antagonistic action of the male sex-hormone which interfered with the normal functions of the ovary. When examined post-mortem the oviduct of the hens M220 and R2183 were found to be like that of immature chicks. The ligaments were also inconspicuous structures with thin muscular bundles and scant vascular supply. There was decrease in length as well as in diameter of the ligaments (Table IV). The ovary of all the injected birds was found to be affected and there was also a definite regressive effect on oviduct weight (Table IV). The oviduct of all birds, however, were continuous with the cloaca. This experiment shows clearly the dependence of not only the oviduct but also of the ligaments on the ovarian hormone.

It is well known that during the moulting period the ovary ceases to function and a marked regression of the sexual characters such as the comb and oviduct takes place (Greenwood, 1936). The examination of the ligaments of moulting hens shows that these structures also regress considerably during this period (Table V/

TABLE III.

SUMMARY OF THE MEASUREMENT OF THE LIGAMENTS.

Group No.	Dosage	Age when injection started.	Injection period.	Average length in cm.		Average diameter in cm. (in the magnum region).	
	Mgm.	Days	Days	Ventral ligament.	Dorsal ligament.	Ventral ligament.	Dorsal ligament.
1	Not injected	1	-	3	5	0.2	0.3
1c	2 daily	1	10	4	6	0.8	1
3	Not injected	10	-	5	8	0.6	1
3c	2 daily	10	20	6	9	1	1.5
7	Not injected	30	-	6	9	0.7	2
7a	0.4 daily	30	15	7	10	2	2.5
9	Not injected	62	-	7.5	11	1	2.8
9b	3 daily	62	10	8	12.5	1.5	3.3

(Table V). The muscular bundles of the ligaments become very thin and the vascular supply becomes poor. The oviduct of all the moulting birds examined, however, was found to be perforated. It may be mentioned here that the first egg after the moulting period is very often smeared with blood. In the guinea-pig the occluding membrane grows over the vagina after each period of oestrus (Cameron, 1940). It was, therefore, suspected that the occluding plate in the fowl also regenerated during moulting and just before the conclusion of the moult it underwent dissolution, leaving traces which caused the blood smear on the first egg. However, a large number of autopsy records during the various stages of moult could not confirm that suspicion. Even the injection of large doses of Testosterone propionate reported in the foregoing pages failed to regenerate the occluding plate although the ovary and the oviduct were much regressed (vide Table IV). It is, therefore, concluded that the inactivity of the ovary during moulting does not in any way cause the regeneration of the occluding plate. The blood smear on the egg, therefore, must be due to some unknown causes.

4. /

TABLE IV.

THE EFFECT OF TESTOSTERONE PROPIONATE ON THE OVARIES, OVIDUCTS AND THE LIGAMENTS IN LAYING HENS.

Record No. of birds.	Dosage	Total quantity of Testostrone propionate injected.	Length of the injection period.	Weight of the ovary.	Weight of the oviduct.	Length of the oviduct.	Length in cm.		Diameter in cm. (magnum region)	
	Mgm.	Mgm.	Days	gm.	gm.	cm.	Ventral ligament.	Dorsal ligament.	Ventral ligament.	Dorsal ligament.
1	uninjected	uninjected	-	35	50	50	44	54	6	7
2	0.5 daily	5	10	30	45	40	35	45	5.5	6.6
M220	2 daily	20	10	3.6	10	18	16.5	20	2	3
S94	1 daily	30	30	2.3	44.2	35	30	36	4	5
R2183	2 daily	60	30	2	6.7	15	12	17.5	2	3

TABLE V.

DATA ON THE OVARY, OVIDUCT AND LIGAMENTS OF MOULTING HENS.

No.of birds examined.	Average weight of the Ovary.	Average weight of the Oviduct.	Average length of the Oviduct.	Average length in cm.		Average diameter in cm. (in the magnum region)	
	gm.	gm.	cm.	Ventral ligament.	Dorsal ligament.	Ventral ligament.	Dorsal ligament.
6	4.5	6	15	14	18	2.5	3.6

4. DISCUSSION.

It is a well established fact that the immature oviduct of the domestic fowl hypertrophies under the influence of exogenous oestrogens (Herrick, 1944; Munro and Kosin, 1940 and 1943; Wolff, 1936). This hypertrophy occurs in chicks varying widely in age as demonstrated by the experiments of Juhn, D'Amour and Gustavson (1930), Asmundson, Gunn and Klose (1937) and in this experiment. However, the degree of stimulation of the oviduct, both quantitative and qualitative, generally agrees with the ascending levels of hormone injected and the extent of the injection period.

The experimental induction of perforation of the oviduct in chicks appeared, in general, to be dependent on three factors (1) the age of the chicks, (2) the level of the hormone administered, and (3) the length of the injection period. Thus d ay old and 5 days old chicks injected for the same period of time and with the same hormone levels differed with respect to the perforation of the oviduct (vide Tables I and II). Again among the latter only the highest hormone level elicited perforation and such was also the case in general with chicks of all ages injected for 10 or 11 days. Prolonged injection, however, was effective in eliciting perforation of the duct in chicks irrespective of age and the hormone level. It is obvious, therefore, that the first two factors were somewhat interdependent whereas the time element was independent of either the age or the dosage. The results obtained in this investigation are somewhat at variance with those obtained by Asmundson, Gunn and Klose (1937) who reported perforation of the oviduct in 21 days old chicks injected with oestrin for

21 and 42 days. According to their findings 20 days' injection of oestrin failed to perforate the oviduct. In the present studies, on the other hand, even 10 days injection with Stilboestrol elicited perforation of the oviduct. These differences, perhaps, can be explained on the basis of differences in the potency of the hormone used in the two studies in the sense that Stilboestrol is several times more effective than oestrin (Sondern and Sealey, 1940).

Qualitatively, the perforation of the oviduct in the baby chicks due to Stilboestrol treatment appears to be the culmination of a gradual process viz. the vacuolization of the posterior epithelial layer of the occluding plate and its subsequent disappearance and the final dissolution of the plate. Thus the vacuolization of the posterior epithelium of the plate in day old chicks and its disappearance in 10 and 90 days old birds may be regarded as the initial stages in the process of gradual dissolution of the plate at the conclusion of prolonged treatment. Moreover, the perforation of the oviduct and the appearance of secretions in its lumen after prolonged Stilboestrol injection makes it appear probable that the oviduct of these birds is capable of functioning in every way like that of mature birds. The results appear to be comparable to those obtained by Allen and Doisy (1924) with immature female rats. In the normal pullets also the perforation of the oviduct appears to be a gradual process and the changes similar to those observed with baby chicks due to Stilboestrol treatment occur in the occluding plate. Thus the vacuolization of the posterior epithelial layer of the plate

in/

in a 115 days old pullet or its disappearance in a 122 day old pullet may be regarded as the initial stages of dissolution of the plate which takes place when the birds are about 140 days old (Greenwood, 1935).

Coming to the question of causes leading to the perforation of the oviduct in the baby chicks it appears that the exogeneous oestrogen exerted a suppressive influence over the occluding plate through the blood vessels which prevented further vascular supply, the gradual dissolution of the plate then following due to the hyperplasia of the oviduct itself. Since the dissolution of the occluding plate in the baby chicks may be regarded as experimental initiation of the similar process which occurs in normal pullets with the onset of puberty it is logical to conclude that this phenomenon of perforation of the oviduct in the domestic fowl is under the oestrogenic activity of the ovary. The present studies, therefore, substantiate Greenwood's (1935) hypothesis.

It was suggested in a previous paper (Kar, unpublished b) that the ligaments are under hormonic control of the ovary. From the present investigation it appears that this is the case.

5. APPENDIX.

Normally, the yolk sac slips into the body cavity on the 19th day of incubation by a complex process (vide Lillie, 1927), and is eventually absorbed with great rapidity as a result of which its weight is reduced from 5.34 gm. on 12 hours after hatching/

TABLE VI.

DATA ON THE WEIGHTS OF THE PERSISTENT YOLK SACS IN INJECTED CHICKS.

Group No.	Dosage	Age when injection started.	Injection period.	Average weight of the yolk-sac.
	Mgm.	Days	Days	Gm.
1	Not injected	1	-	No yolk-sac
1c	2 daily	1	10	2.2
2	Not injected	5	-	No yolk-sac
2c	1.5 daily	5	10	0.1
2d	2 daily	5	10	1
3	Not injected	10	-	No yolk-sac
3c	2 daily	10	20	3.2
4	Not injected	21	-	No yolk-sac
4a	2 daily	21	5	0.01
5	Not injected	21	-	No yolk-sac
5c	3 daily	21	43	0.001
6	Not injected	25	-	No yolk-sac
6c	3 daily	25	5	0.01.

hatching to 0.05 gm. on the sixth day of post-hatching life (Virchow, 1891 quoted by Lillie, 1927). Byerly (1932) observed that the yolk-sac grows steadily from the first day onward to reach a maximum weight of about 3.5 gm. on the 15th day of incubation, after which its weight falls to approximately 2.5 gm. at hatching. It is interesting to note that in young chicks injected with high doses of Stilboestrol the yolk sacs were persistent. The uninjected controls, however, showed no yolk sacs. The relevant data is summarized in Table VI. It is well known that undesirable gastro-intestinal reactions follow the use of Stilboestrol in large doses (vide Grollman, 1942). Since the yolk sac is intimately connected to the intestine it is not unlikely that this oestrogen exerted a harmful influence over the intestine which was secondarily extended over the yolk sac and as a result the rate of absorption of yolk was considerably slowed down in these chicks.

6. SUMMARY.

1. Young Brown Leghorn female chickens were injected for 5 to 50 days with Stilboestrol and killed when 11 to 122 days old. There appeared to ensue a depressing effect on growth in younger chicks; in the older ones, on the other hand, this oestrogen appeared to enhance growth.
2. In younger chicks the injection of Stilboestrol was without any effect on the ovary. In older chicks, on the other hand, Stilboestrol produced a slight depressive effect on the ovary.
3. The oviduct responded considerably after injection of Stilboestrol. In many cases Stilboestrol treatment caused perforation of the oviduct and secretion by the glands of the duct.

4. The ligaments were enlarged due to injection of Stilboestrol and their muscular bundles also became thicker.
5. It is concluded that the perforation of the oviduct and the ligaments are under the oestrogenic activity of the ovary.
6. Large doses of Stilboestrol in some cases interfered with the normal process of rapid absorption of yolk and as a result the yolk sac was abnormally persistent.

7. /

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8/

8. EXPLANATION OF LETTERING.

d.l. Dorsal ligament.

v.l. Ventral ligament.

u. Uterus.

v. Vagina.

m.b. Muscular bundles.

o.d. Oviduct.

o.p. Occluding plate.

m.ov. Mouth of the oviduct.

9. /

9. DESCRIPTION OF PLATES.

PLATE I.

Fig.1. Photomicrograph of the transverse section through the oviduct of 15 days old uninjected female chick (x10).

Fig.2. Photomicrograph of the transverse section through the oviduct of 15 days old female chick injected 10 days with 2 mg. of Stilboestrol daily (x10).

Fig.3. Photomicrograph of the transverse section through the oviduct of 64 days old uninjected female chick (X10).

Fig.4. Photomicrograph of the transverse section through the oviduct of 64 days old female chick injected 43 days with 1 mg. of Stilboestrol daily (x10) Note secretion which is collected at the tips of the folds.

Fig.5. Photomicrograph of the whole-mount preparation of a portion of the ligament of 31 days old uninjected female chick (x20). Note thin muscular bundles.

Fig. 6. Photomicrograph of the whole-mount preparation of a portion of the ligament of 31 days old female chick injected 20 days daily with 2 mg. Stilboestrol (x20). Note thick muscular bundles.

PLATE II.

Fig.1. Photomicrograph of the sagittal section through the occluding plate of 11 days old uninjected female chick (x70).

Fig. 2. Photomicrograph of the sagittal section through the occluding plate of 11 days old female chick injected 10 days with 2 mg. Stilboestrol daily (x70). Note vacuolization of the posterior epithelial layer.

Fig.3/

Fig. 3. Photomicrograph of the sagittal section through the occluding plate of 15 days old uninjected female chick (x20).

Fig.4. Photomicrograph of the sagittal section through the occluding plate of 15 days old female chick injected 10 days with 2 mg. Stilboestrol daily (x20). Note the absence of the posterior epithelial layer.

Fig. 5. Photomicrograph of the sagittal section through the occluding plate of 122 days old uninjected female chick (x20). Note the absence of the posterior epithelial layer.

Fig.6. Photomicrograph of the sagittal section through the occluding plate of 72 days old uninjected female chick (x70).

Fig. 7. Photomicrograph of the sagittal section through the mouth of the oviduct of 72 days old female chick injected 10 days with 3 mg. Stilboestrol daily (x70). Note the absence of the occluding plate.

Fig. 8. Photomicrograph of the sagittal section through the remnant of the occluding plate of 101 days old female chick injected 11 days with 4 mg. Stilboestrol daily (x20). Note the dissoluted condition of the remnant of the occluding plate.

Fig. 9. Photomicrograph of the sagittal section through the remnant of the occluding plate of 145 days old pullet (x18). Note the dissoluted condition of the remnant of the occluding plate.

PLATE I.

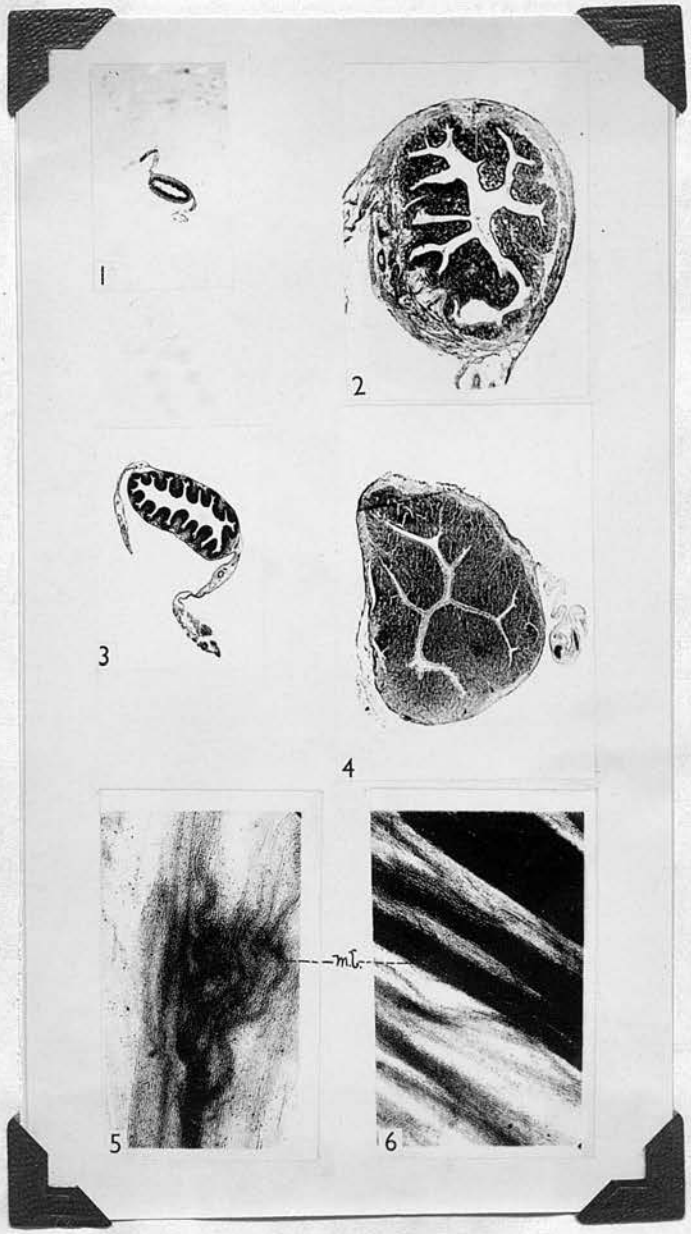
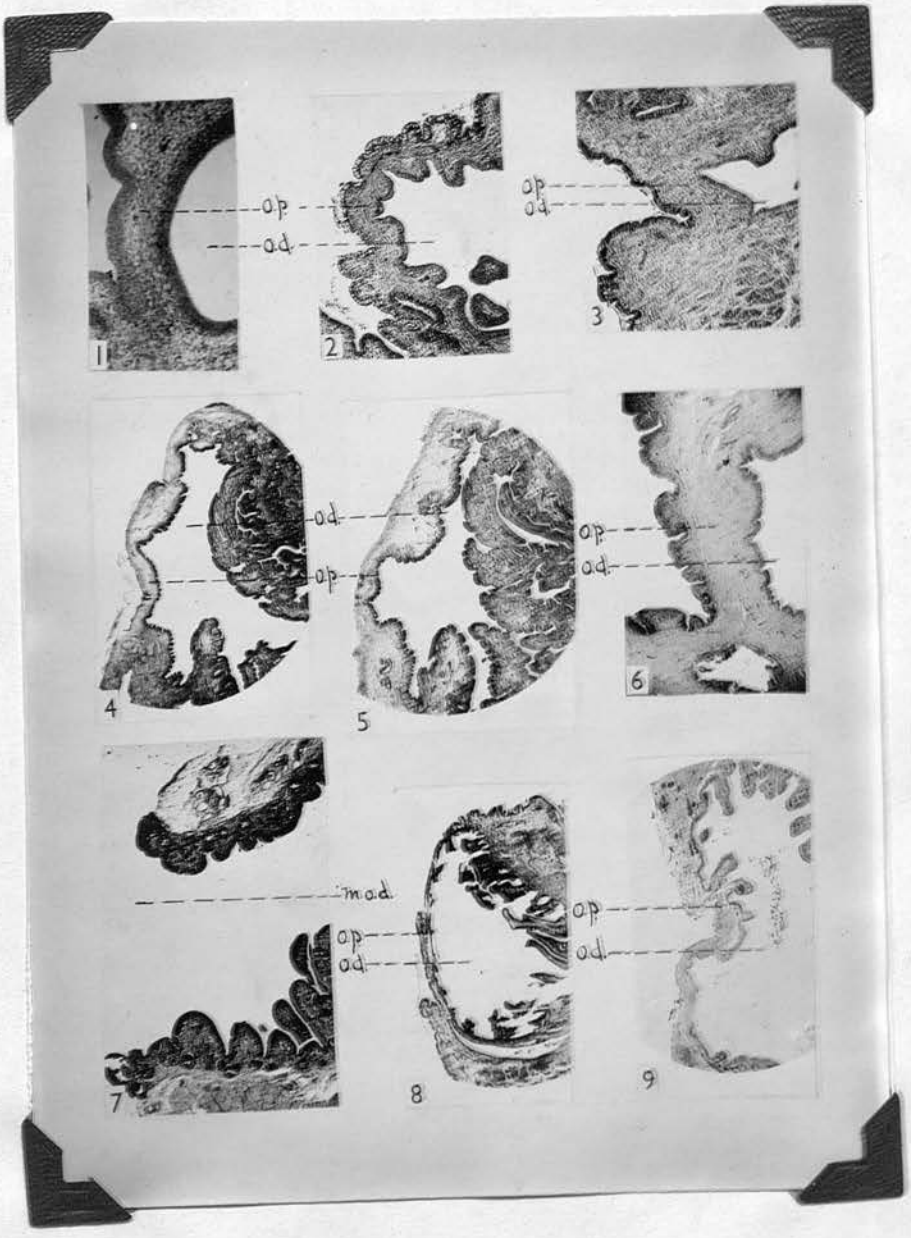


PLATE II.



PART IV.

FATE OF THE YOLK-STALK IN THE DOMESTIC FOWL

CONTENTS

FATE OF THE YOLK-STALK IN THE DOMESTIC FOWL.

1. INTRODUCTION

It is well known that in the chick the yolk sac remains connected to the intestine by a thick walled tubular stalk which is generally referred to as the Yolk-stalk. The fate of the yolk-sac in the chick, however, has received adequate attention (vide Lillie, 1927; Byerly, 1932) but, unfortunately, it has not been possible to obtain any reference from the literature as to the fate of the stalk. In view of this lack of specific information the present investigation was attempted to throw some light on the fate of the yolk stalk in the domestic fowl.

2. MATERIAL AND METHOD

The birds used in this investigation came from the pure-bred Brown Leghorn flock of the Institute. Birds of both sexes varying in age from day-old chicks to 2 years old adults were examined. In all cases the yolk-stalk was measured, its location noted and finally fixed in Bouin's fluid for histological study. Sections were prepared in the usual way and stained with Mallory's.

3. OBSERVATIONS

In all the birds examined it was seen that the yolk-stalk was present as a finger-shaped projection on the anterior portion of the duodenum (Pl. , fig.1). This was true of birds varying in/

in age from day-old chicks up to adults and irrespective of sex. The average length of the stalk was about 5 mm. The average width, on the other hand, was about 1 mm. in younger chicks (5 days to approximately about 3 months old), while in the older birds (3 months to adult birds) it was about 3 mm., slightly greater than in young chicks.

Transverse section of the yolk-stalk of a 5 days old chick shows that its gross histological features agree with those of the intestine. Thus there are serous and muscle layers, connective tissue stroma and mucosa lined by glandular epithelium but without villi (Pl. , fig.2). The lumen of the stalk is full of yolk which appears to be in the process of absorption by the glandular epithelium of the mucosa. It is interesting to note that at this stage the mucosa of the intestine also does not exhibit any foldings. Pl. , figs. 3 and 4 depict longitudinal and transverse sections through the yolk-stalk of four months old pullet. It is apparent that the histological picture resembles that of the intestine. Unlike that of the younger chicks, the mucosa of the yolk-stalk exhibits considerable foldings lined by glandular epithelium which resemble the intestinal villi. The lumen of the stalk is continuous with that of the intestine at the attached end. The free end of the stalk, however, is occluded by a continuation of its different layers. In the adult birds the yolk stalk is even more conspicuous, in the sense, that it has prominent serosa, muscle layers, connective tissue stroma and mucosa with villi-like foldings (Pl. , figs. 5 and 6). The lumen of the stalk is persistent and continuous with that of the intestine/

intestine. Furthermore, indications of secretions are seen in some isolated locations along the sub-mucous region of the stalk which are surrounded by glandular epithelium.

4. DISCUSSION

It is evident that the yolk-stalk persists permanently in the duodenal region of the intestine in the domestic fowl. In point of fact the presence of this structure may be regarded as a constant feature of this region of the intestine in birds of all ages and of both sexes. Apart from connecting the yolk-sac to the intestine (Lillie, 1927), no other function has been ascribed to it. Lillie (1927) on the contrary, has stated that "A narrow lumen remains in the stalk of the yolk-sac throughout, and even after incubation, but the yolk does not seem to pass through it into the intestinal cavity. The walls of the yolk-sac, excepting the part derived from the pellucid area, are lined with a special glandular and absorbing epithelium, which digests and absorbs the yolk and passes it into the viteline circulation, through which it enters the hepatic portal circulation and comes under the influence of the hepatic cells". The present studies, however, show that the glandular epithelium of the stalk may also take part in the process of absorption of the yolk. Moreover, the presence of secretions in the submucosa of the stalk in adult birds, makes it appear probable that the glandular epithelium of the yolk-stalk is capable of functional activity throughout life. A detailed investigation, however, is necessary for the elucidation of the real significance of the persistence of this structure/

structure in the domestic fowl.

5. SUMMARY

1. The stalk of the yolk-sac persists permanently as a finger-shaped projection of the duodenal region of the intestine in the domestic fowl.

2. Histological structure of the yolk-stalk is described and it has been shown that it helps in the process of absorption of the yolk.

6. REFERENCES TO LITERATURE

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7. EXPLANATION OF LETTERING

y.s. Yolk stalk.

i. Intestine.

8. /

8. DESCRIPTION OF PLATE

Fig.1. Photograph of the persistent yolk stalk of an adult hen about two years old (half actual size).

Fig.2. Photomicrograph of the transverse section through the yolk stalk of 5 days old male chick (x18). Note the presence of yolk in the lumen.

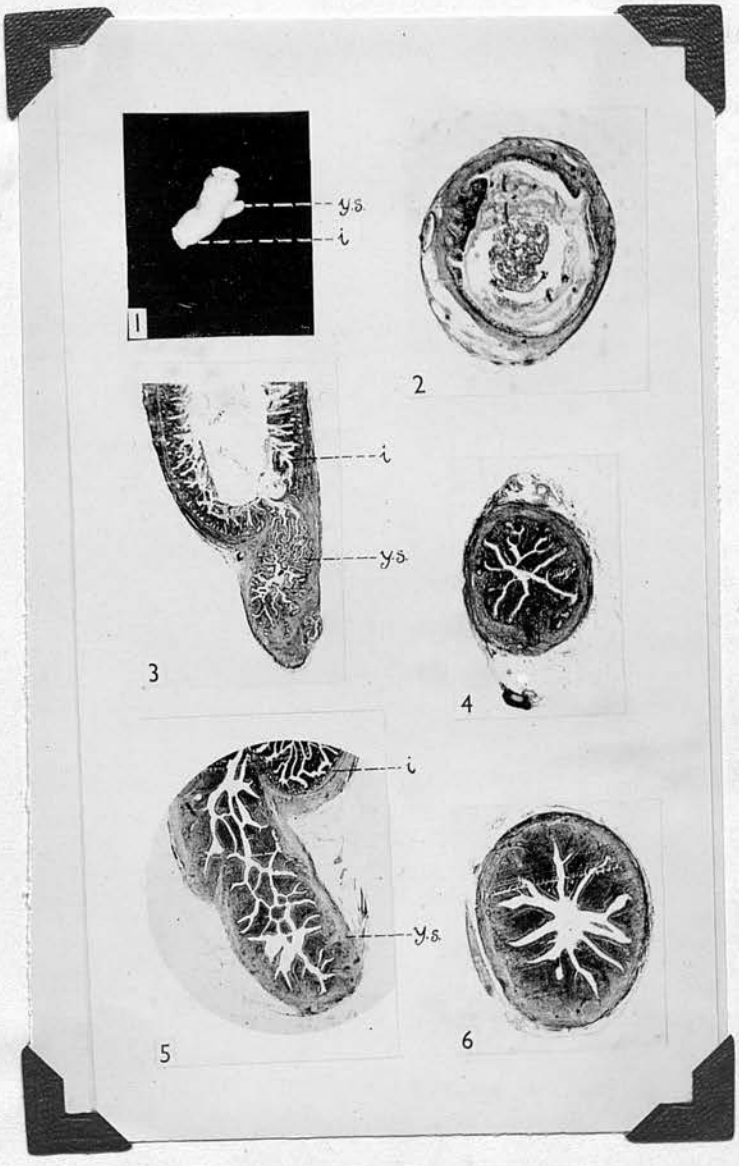
Fig.3. Photomicrograph of the longitudinal section through the yolk-stalk of a four months old pullet (x8).

Fig.4. Photomicrograph of the transverse section through the yolk-stalk of a four months old pullet (x18).

Fig.5. Photomicrograph of the longitudinal section through the persistent yolk stalk of an adult hen about two years old (x8).

Fig.6. Photomicrograph of the transverse section through the persistent yolk stalk of an adult hen about two years old (x8).

PLATE



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LIST OF PREVIOUS PUBLICATIONS BY THE AUTHOR

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