<u>Studies on Structure and Function of the Ovary of the</u>

<u>Domestic Fowl (with reference to the Correlation of</u>

<u>Cell Changes with Physiological Activity).</u>

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by

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CONTENTS.

As a result of selection throughout the ages, the domestic
fowl has lost the distinct, limited reproductive period
characteristic of wild birds. In comparison with mammals, the
hen can be considered in active oestrous throughout the major
part of the year in so far as it is continuously ovulating.
This capacity for egg production has been increased by improved
breeding and management methods which aim to prevent wastages
and to allow normal processes to work maximally. In spite of
improvements in husbandry methods, however, there is still a
great loss of follicles by atresia in the chicken ovary. The
total number of follicles in the ovary of a chicken were
estimated by Pearl & Schoppe (1921), who counted 1,906
macroscopic and 12,000 microscopic ova in the ovary of a fully
laying hen. Later Faure-Fremiet & Kauffman(1928) estimated
the number of follicles in a 2 day old White Leghorn chick to
be 3 - 6 millions, but considered that only 1000 - 1500 were
potentially functional, the others being destined for degeneration.
Observations on the Edinburgh flock of Brown Leghorns show that
the greatest total number of eggs which has been laid by a
single hen is 1,515 (Greenwood 1949), although higher individual
records have been reported since that time in Danish flocks
(Davidsen 1954). The question of whether it is possible to
prevent this loss of eggs in the ovary, or whether it is a part
of a natural physiological phenomenon in ovarian activity are
interesting topics to which detailed microscopical examinations
could contribute useful information, and part of the present
work is devoted to an examination of this point.

Work extensively quoted in papers by Rowson (1951)
and Marden (1953) show that it is possible to successfully

induce superovulation in immature and mature mammals by the administration of a combination of gonadotrophic hormones, thus enabling one to obtain many more ova than would normally be provided. Attempts have been made to induce ovulations in immature birds and low egg producing hens by similar treatment (Nalbandov & Card 1946) but the results were not satisfactory. At present an experiment is in progress to observe the egg laying performance of Brown Leghorn hens kept under constant conditions of temperature, light and humidity (Greenwood 1955, personal communication). Results to date show that compared with the control birds, which are subject to usual seasonal fluctuations of temperature and light, the overall number of eggs eventually produced by the experimental birds is not significantly greater, thus confirming earlier work by Warren et al (1950) and Mueller et al(1951). These results could indicate that the phenomenon of atresia in the chicken ovary is a functional process and not a wastage.

Husbandry experiments and the indiscriminate administration of hormones to hens are likely to fail in the attempt to increase egg production, without a proper examination of the physiological limitations and functions of cells and other tissue elements of the target organ. An extensive study of the cellular changes and the activities of the different cell components in the ovary could be an essential basis for the proper appreciation of the results of experiments mentioned above, and for further investigations into the physiology of the chicken ovary. However, no detailed studies of the chicken ovary have been reported in the literature from the point of view of giving any idea of the relation between structure and function of the various component cell types of the organ. A survey of

the literature on the minute structure of the ovary reveals that only descriptive accounts of the morphology and embryonic derivation of isolated cell components exist. No attempt has been made to correlate the activities of the various parts, and investigate their functional relationships in the physiology of the gonad as a whole.

Bennett(1947) studied the ovary and testes of the fowl from hatching to sexual maturity reporting on the gross changes in the weight , length and breadth of the gonads with advancing age, and Romanoff & Romanoff (1949, p 180) similarly reported the changes in weight of the ovary from hatching to sexual maturity and throughout the laying cycle, but no attention was given to the cellular structure of the gonads. Early work on the histology of the ovary was confined mostly to a description of the embryonic gonad, the origin of germ cells and their role in the formation of follicles soon after hatching (D'Hollander 1904; Swift 1914; Firket 1914). Van Durme (1907, 1914) described the form of the nucleus and cytoplasm of the ovum during the process of oogenesis from the beginning of the intra-follicular growth period up to fertilisation. Brambell (1926) paid special attention to the distribution of mitochondria and Golgi elements in various parts of the follicle as it matured. He described the part played by the granulosa cell layer in the formation of the vitelline membrane. The Golgi elements of the granulosa cells were considered to be shed into the cytoplasm of the growing oocyte to help in the formation of yolk material. All the abovementioned workers devoted considerable attention to the yolk body of Balbiani (a structure capping the nuclei of intra-follicular oocytes) and its fate in subsequent yolk formation.

Extra-follicular cell components of the ovary have received little attention ,however, and those which were studied received conflicting terminology mainly due to a failure to study their functional relationships.

Van Durme (1914) maintained that cells from the medulla of the ovary migrated into the cortex during embryogenesis of the gonad and in appearance they resembled the interstitial cells of the mammalian ovary. Nests of clear cells in the cortex of the ovary, which appeared to be synonymous with Van Durme's medullary cells, were earlier termed interstitial cells by Ganfini (1908) and Poll (1911). Pearl & Boring (1917), however, stated that the term "interstitial cells" signified hormone secretory cells and should not be used indiscriminately. They described, as secretory elements, an abundance of interstitial cells with acidophile, cytoplasmic granules in the cortex of the mature ovary, which were absent from the ovaries of immature chickens. It appears that the lipid nature of ovarian secretions was not well established at that time and it was inevitable that cells with granules were to be considered as secretory in function. Pearl & Boring (1918) described a corpus luteum, consisting of vacuolated cells with shrunken nuclei, in discharged and atretic follicles, and it was believed to develop as a result of an inward migration of nests of clear cells from the theca interna. The clear cells were named "luteal cells", and a yellow pigment in the corpus luteum, which was neither fatty nor protein in nature, was homologised with similar material in the corpus luteum of the cow ovary.

Goodale (1919) considered that the interstitial cells described by Pearl and Boring (1917) were blood eosinophils, and in support of his contention pointed to their irregular distribution and presence in other glands like the thymus, pituitary etc.   In agreement with Poll (1911) and Ganfini (1908) he referred to nests of clear cells as interstitial secretory cells and was of the opinion that either proper stains were not available at that time to reveal the secretory granules of these cells or the secretions were not stored as granules.   Fell (1924) dealt with the histogenesis of the so-called/ luteal cells in the ovary and described their development from medullary cords in the embryonic and post-embryonic ovaries.   The cells appeared to be similar to those which were called interstitial or clear cells by Van Durme, Poll and Ganfini.   A cytological similarity of luteal cells to mammalian interstitial/ cells was admitted by Fell, and she reported that they appeared to be secretory in type rather than just constituents of adipose tissue. However, in view of the fact that the transformation of the luteal cells was always associated with degenerative structures (atresia) in the ovary, she concluded that they were not actually secretory in function but developed as a result of fatty degeneration of medullary cords.   Fell was unable to corroborate the hypothesis of Pearl & Boring (1918) which suggested that the luteal cells were homologous to the vacuolated cells inside atretic and discharged follicles or the mammalian corpus luteum.   The yellow pigment on which Pearl & Boring laid so much stress was shown to be haemosiderin derived from aborting blood vessels of the thecal layers of the follicles.   In a further communication,

Fell (1925) investigated the phenomenon of involution of discharged and atretic follicles to re-examine the hypothesis of Pearl & Boring (1918), regarding the homology of hypertrophied luteal cells with mammalian corpus luteum. She demonstrated, by employing special mitochondrial stains, that the luteal cells appearing as nests of clear cells in the theca interna of follicles and in the cortex were cytologically different from the vacuolated mass of cells which ultimately occupy atretic and discharged follicles. The latter were considered to develop as a result of hypertrophy and fatty degeneration of cells of theca interna and membrana granulosa. Luteal cells did not appear to play any part in the formation of the so-called corpus luteum. Davis (1942) investigated the regression of discharged follicles in the chicken, pigeon and Argentine Cow bird and found that unlike viviparous reptiles, there did not appear to be any proliferation of granulosa in avian post-ovulatory follicles and so he concluded that the latter were not homologous to the mammalian corpora lutea. In the present work, all the above-mentioned details about ovary cells have been re-examined using modern histochemical methods, and as a result the use of the term corpus luteum is not favoured.

Procedures of administering various hormones to birds and observing gross gonad and secondary sex character changes, or bioassays using extracts of various parts of the chicken ovary have been used in attempts to correlate structure and function in the chicken ovary. Riddle & Schooley (1944) chemically extracted avian post-ovulatory follicles, and were unable to find any progesterone with the sensitive McGinty test which is another example of the dangers of homologising

discharged follicles with the mammalian corpus luteum. Marlow & Richert (1940) detected oestrogens in the growing chicken follicle, and reported more oestrogenic activity in immature follicles than in mature ones. Taber (1948,1951) reported that FSH administration to young chicks caused hyperplasia of medullary cord cells and the formation of basophilic granules within them. She concluded that the cells were activated and responsible for androgen secretion as the combs grew at the same time. On administering oestrogens, which inhibited endogenous gonadotrophin secretion, changes in the appearance of the medullary cells were reported which were similar to those observed after withdrawal of exogenous gonadotrophic administration, namely the reversion of granular cells to lipoidal medullary cord cells.

In the present work, in an attempt to link structure and function, the chicken ovary has been studied continuously from the time of hatching to an age of several years. During these periods, changes in secondary sex characters have been observed along with varied cell activities in the ovary to ascertain where possible the source of secretions responsible for the development of the reproductive organs. Techniques of histo-chemistry, polarising and fluorescence microscopy, and other methods have been used to locate sites of secretory activity. Similar methods have been applied to mammalian gonads and other glands with some measure of success, but the chicken ovary has not been investigated in such a manner. Marza & Marza (1935-36) have used histochemical methods in a study of the chemical composition of the yolk of chicken eggs. They studied the growth of the egg, and reported changes in fat, lipid, iron, protein and glycogen contents

during the different phases of yolk formation, but they did
not examine extra-follicular structures of the ovary.
Marshall & Coombs (1952) studied changes of lipid distribution
in wild bird ovaries at different seasons of the year, using
Sudan black staining and the Schultz test to examine sites
of synthesis of cholesterol and its esters which were considered
to indicate the presence of sex hormone precursors.

Material and Methods.

Brown Leghorn fowl maintained at the Poultry Research
Centre, Edinburgh, were used throughout this work. Detailed
microscopical studies were made of the ovary and for the
purpose a number of birds, usually 3 or 4 in each age group,
were sacrificed to gain a fair idea of the appearance of the
gonad at successive stages in growth. Ovaries were taken
on successive days from 1 to 7, and thereafter every week
until approaching sexual maturity which varied from 22 to
32 weeks amongst birds used. During the latter period ,
owing to the variation in maturity age the birds were killed
more often to provide representative ovarian pictures of all
stages leading up to the time of laying the first egg. Birds
were also killed frequently during the peak period of egg
laying, the declining periods, the moulting period, and in
old age when eggs are laid at very irregular intervals.
The birds were killed by dislocation of the cervical vertebra;
ovaries were then dissected out and placed in appropriate
fixatives within a minute or two of the death. To investigate
and interpret the cellular activity in the ovary and its
association with reproductive phenomena in the female fowl,
the following methods of preparing and examining the gonad
were used to provide material for cytological study.

GENERAL HISTOLOGY.

The usual histological procedure of preparing paraffin embedded blocks was employed.   Sections were cut on a rocking microtome at 5-7μ thickness.   The following fixatives and stains were used.

a) Fixatives:

(1)   Orth's fluid (McClung, 1950.p.145) modified as follows:

Distilled water   100ml. ⎫
Potassium dichromate   2.5gms.⎬   Stock solution.
Sodium sulphate   1.0gm. ⎪
Saturated with mercuric chloride ⎭

To 10 parts of stock solution, 1 part of formalin was added before use.

(ii)   Carnoy-Lebrun fluid (McClung, 1950.p 56) modified to have the following composition:-

90% Ethyl alcohol   100ml.

Chloroform   75ml.

Glacial acetic acid   75ml.

Formalin   10ml.

Picric acid   3.5gms.

Mercuric Chloride 20gms.

(iii)   Susa.

(iv)   Flemming's fluid less acetic acid.

(v)   Champy's fluid.

In all cases fixation lasted for 12-24 hours, depending on the size of the tissue to be fixed and the penetrating powers of the fixative used.

b)   Stains:

Osmic acid was used to reveal Golgi elements and

and mitochondria, and for this purpose sections of tissue treated by fixatives containing osmic acid were stained in Heidenhain's iron haematoxylin followed by orange G, and a few excellently stained preparations were also obtained with Heidenhain's iron haematoxylin followed by Masson's trichrome stain. Sections from tissues fixed in non-osmic acid fixatives were routinely stained with Ehrlich's haematoxylin-eosin and Masson's trichrome stain to reveal general microanatomy, and connective tissue and muscle respectively .

HISTOCHEMISTRY.

1. Steroid compounds.

Since both androgens and oestrogens have been reported to be secreted by the chicken ovary (Domm 1929a, 1933; Taber 1948), it was thought desirable to attempt to identify the cellular source of these steroid hormones, and to follow changes in their distribution or formation with observed physiological changes in the egg laying activities of the hen. Several histochemical tests have been used for identifying ketosteroid compounds in the ovaries and adrenals of mammals, and similar tests were employed in the present work to investigate the cellular basis of ovarian steroid secretions. No single reliable method is available for localising ketosteroids in tissue sections, but a battery of reactions which are described below have been usefully employed to give good circumstantial evidence as to the sites of production of these compounds. Several tests depend on the fact that steroids are present at sites of lipid metabolism and deposition. All the individual methods have their protagonists and antagonists with regard to their specificity, and the subject has been adequately discussed

by Pearse (1953) and Deane & Seligman (1953). It is generally recognised that until a single specific method is developed , the application of the large number of tests on biological material is the only satisfactory procedure for localising the sites of steroid metabolism in organs. Gross chemical extraction tests of organs do not reveal the cells responsible for producing the substances.

For all the following tests, ovaries fixed in 10% Formol (neutralised with calcium carbonate) were cut on a freezing microtome at about 15 μ.

(a) <u>Sudan black B.</u> This is not a specific test for steroids, but is useful to reveal the sites of lipid-containing compounds. Compound lipids most likely to be associated with steroid compounds are revealed with this dye (Pearse 1953.p 176). Frozen sections were kept in a saturated solution of Sudan black B in 70% ethylalcohol for 5-10 minutes, rinsed in one or two changes of distilled water and mounted on slides in glycerogel.

(b) <u>Schultz Test.</u> Data has accumulated during recent years to suggest that sites of biosynthesis of oestrogenic hormone in the ovary are closely connected with the presence of cholesterol compounds. Cholesterol is considered a general precursor of steroid hormones in adrenal glands and ovaries (Bloch, 1945; Sayers, <u>et al</u> 1946; Everett, 1947; Claesson & Hillarp, 1947 ab; Claesson <u>et al</u> 1949; Rennels, 1951). Of all the histochemical tests , the Schultz test for cholesterol is the only test considered quite specific, and it was performed according to Glick (1949.p 41). Cholesterol positive areas were revealed by the development of a blue-green colour which changed into a brown colour after some time.

(c) <u>Fuchsin-Sulphurous acid staining (FSA).</u> This method is

a general stain for protein-polysaccharide compounds.
The method recommended by Hotchkiss (1948) was used both on
paraffin sections of tissues fixed in Orth's fluid, and on
frozen sections of formalin-fixed material.   Instead of
rinsing in sulphite water, after staining with decolourized
basic fuchsin, the sections were washed in distilled water
before mounting.

   It is generally considered that under certain circumstances
the aldehydes liberated from compound lipids are also revealed
by FSA reaction (Pearse 1953. p 137).   In the absence of a
positive reaction in paraffin sections, the method could be
usefully employed on frozen sections for locating the cells
containing the complex lipids,which are generally associated
with ketosteroids and soluble in the wax solvents.

(d) Ashbel & Seligman Method for Ketosteroids. This method
was developed by Ashbel & Seligman (1949), and Seligman &
Ashbel (1952) who claimed it to be specific for revealing
active carbonyl groups in tissue sections and thus sites of
ketosteroid metabolism.   Recently the method has been
criticised and shown to reveal sites where unsaturated fats are
oxidised by formalin during fixation and subsequent washing
(Karnovsky & Deane 1955).   However, Seligman (personal
communication) believes that even though this may be the case ,
it is highly probable that unsaturated fats are associated
with compounds which are immediate precursors of steroids.

   The procedure for the present work was similar to that
used by Seligman & Ashbel (1952) with the following modifications:-
The sections were washed in several changes of distilled water
for 4 to 6 hours to wash out formalin used for fixation.
The concentration of Naphthoic acid hydrazide employed was

0.15% and staining with it lasted for a period of 5 hours.
For coupling with the dye, diorthoanisidine, 0.1 gm of stain
was used in 40 ml of alcohol-buffer.   It was found that these
concentrations and timings gave the best result.   Fast Blue
B Salt (Hopkins & Williams product) was found to be better for
chicken ovary than tetrazotised diorthoanisidine.

(e) <u>Birefringence.</u>   It has been shown by Cain (1950) that many
lipids become crystalline in fixed preparations, and spherocrystals
showing black cross of polarisation are formed by steroid esters
and phosphatides.   To study the distribution of such birefringent
material in the ovary, unstained frozen sections were mounted on
slides in glycerine jelly and examined under polarised light.

(f) <u>Fluorescence.</u>   Detection of substances in tissue sections
by subjecting them to ultra-violet light irradiation and
observing their auto- or secondary fluorescence is another
interesting and useful method in histochemistry.   For secondary
fluorescence Volk & Popper (1944) recommended 0.1% aqueous solution
of Phosphine 3R as a suitable fluorochrome for revealing the
presence of lipids and cholesterol esters in UV light.   Fatty
acids, soap and cholesterol give a negative reaction.   The
advantage of staining sections in aqueous solutions of the dye
was that the finer droplets of lipids were not dissolved away.

In this study frozen sections were immersed in 0.1% aqueous
Phosphine 3R for 3 minutes.   They were then washed in distilled
water and mounted in 90% glycerine on quartz slides.   (It was
found that ultra-thin glass slides could also be used instead
of quartz).   Ultra-violet light of wave length 3700 A⁰ emitted
from a mercury vapour lamp was used for radiation.
Claesson & Hillarp (1947c) claimed that fluorescence in the
rat ovary was UV labile and revealed the presence of Vitamin A.

After Phosphine 3 R staining, sections of the chicken ovary were irradiated by UV light for 20-40 minutes without the loss of fluorescence and so the reactive material here could not be wholly due to Vitamin A. Moreover Nicander (1952) reported the loss of the fluorescing property of Vitamin A after exposure to formalin for 6-24 hours, a treatment which was given to ovaries in the present study without effect on fluorescence.

(g) Control tests. In all the above procedures (a) - (f) control sections were immersed in acetone for half an hour before applying the various tests. This solvent dissolves out pure steroids, but not phosphatides, so that the loss of a positive reaction after acetone pre-treatment would indicate a loss of steroids (Greep & Deane, 1949). In organs known to produce sex hormones, the presence of acetone-soluble substances, which give positive reaction with the above tests, is presumptive evidence of the presence of compounds similar to biologically active steroid hormones (Dempsey & Bassett, 1943).

2.   Peroxidase enzymes.

Large numbers of cells, with acidophile granules in their cytoplasm are found in the ovary of the adult hen especially in the cortical regions. In view of the conflicting reports about their significance, and their morphological similarity to blood eosinophils, it was thought that the benzidine test for peroxidase would be useful to determine whether or not they were blood leucocytes, since Jover (1954) showed that the granules of fowl oesinophils could be stained a green-blue colour with benzidine. For histochemical localization of peroxidase activity in the ovary sections, a method described by Glick (1949, p 90) was used, in addition to a modification of the method developed by Jover.

3.   Iron pigment.

Pearl & Boring (1918) considered the pigment in the chicken ovary to be similar to that in the corpus luteum of cow ovary.   Fell, disagreeing with this view, reported that the pigment was Haemosiderin derived from aborting blood vessels. To re-examine the nature and the mode of origin of this pigment the following tests for iron were applied:-

(a)   Prussian Blue test for iron (Glick, 1949. p 20).

(b)   Dinitroso-resorcinol test for iron (Glick, 1949. p 21).

(c)   Lillie's (1954) method for differential diagnosis of Haemosiderin, which depends on the solubility of formalin-fixed Haemosiderin in dilute oxalic acid, thus distinguishing it from other iron-containing pigments.

4.   Metachromasia.

Since cells with eosinophilic, cytoplasmic granules appear in the ovarian cortex at certain periods in the growth and activity of the ovary, it was thought useful to examine these cells for metachromatic activity when stained with toluidine blue. Mast cells are known to contain sulphated-mucopolysaccharides which show metachromatic activity in their granules and the cells are sometimes attracted by cell damage in tissues.   Thus it was not beyond speculation to assume that the aggregations of eosinophilic cells in the ovary might be associated with the tissue damage that occurs in the chicken ovary as a result of continuous ovulation and frequent atresia.

The method used for metachromatic staining was similar to that of Lison, (Glick, 1949. p 47).   The sections were stained with toluidine blue and then passed to a solution containing equal volumes of 5% ammonium molybdate & 1% potassium ferrocyanide for 5 minutes.   After washing in water the slides were transferred

to tertiary-butyl alcohol before dehydration and clearing in benzene and xylol.

5. Phospholipid compounds.

Since steroid hormones were most likely to be associated with complex lipid compounds in the gonadal tissue, Baker's acid haematin test was applied to frozen sections of ovaries to examine the location of phospholipids in certain cell types (Pearse, 1953. p 442). A persistent staining reaction with acid haematin, after pyridine extraction of tissue sections to remove phospholipids, is given by phospho-- proteins, other acid proteins (Baker, 1946) and possibly lipoproteins. This method was applied to ovary sections to further examine the nature of the eosinophilic granules in some cortical cells of the ovary, since together with histochemical tests for iron, it is possible to examine the relationships between pigment which appears in the ovary at certain times, degenerating red blood cells, and eosinophilic cells.

OPERATIVE PROCEDURES.

(a) Ovariectomy. It is well known that if the left ovary of the bird is removed, an organ develops on the right side which is purported to be responsible for the development of male secondary sex characters which follow the operation. If the secretions from the hypertrophied right gonad are responsible for androgen secretion, which modifies the secondary sex characters, it should be possible to find the cellular basis of this secretion by a histological and histochemical study of the gonad. To obtain this tissue, a few pullets were ovariectomised following the method explained by Domm (personal communication).

For all surgical operations intravenous injections of Nembutal were used for anaesthesia and in certain cases ether was

given to complete the process. There was a tremendous variation amongst birds with regard to their toleration of Nembutal and it is not possible to stipulate a dosage on a dose-weight basis, but in general, 0.7 ml. per 500 gms. body weight was found to be quite effective. Rigid antiseptic precautions were not taken during these operations, since birds have been reported to be fairly resistant to septicaemia. In all cases the poulards recovered quickly after the operation without any subsequent illnesses.

Ovariectomy in the chicken was an intricate operation, because of the intimate connection of the ovary to the left adrenal, and also its close proximity to the post-caval and left iliac veins. These factors made the complete removal of the gland difficult without causing haemorrhage. After performing a few initial incomplete ovariectomies, as revealed by transitory secondary sex character changes, it was possible to acquire considerable skill in successfully removing the entire gland. The operation was best performed on 6-8 week old birds when the ovary is not fully vascularised. The birds were prepared by withholding food for 24 hours prior to the operation, which resulted in emptying of the intestines and facilitated access to the ovary. Anaesthetized birds were secured to the operating table by laying them on the right side and tying the wings and legs with cords to the edges of the table as shown in fig. 1. All feathers in the thoracic region were plucked and the bared area cleaned with absolute alcohol. An incision was made in the skin above the space between the last two ribs and this was followed by cutting the intercostal muscles underneath. The latter incision extended from the ventral extremity of the ribs and as far towards the spinal column as possible. The ribs were then separated by a retractor and the body cavity exposed.

It was then necessary to puncture the abdominal air sac and remove the membrane covering the ovary to expose the organ (Fig. 2). Ovarian tissue was removed by gradually peeling it away from its underlying attachments with cotton wool pellets held with curved forceps. To ensure the final removal of all pieces of the ovary the attachment area was thoroughly seared by electro-cautery. In this latter step care had to be taken to prevent burning the skin, body wall and the abdominal viscera and also to avoid dehydration of the tissues. The latter was prevented by the use of cotton swabs moistened with physiological saline. Following removal of the ovary, the separated ribs and the skin were sutured separately and the birds allowed to recover and feed in a warm room. It was noted that a large percentage of birds recovered rapidly and started feeding within 1-2 hours of the operation. The operated birds were kept in a warm room overnight and then transferred to individual cages. The sinistrally ovariectomised pullets (poulards) started developing male plumage after 1-2 weeks, and the combs began to redden and enlarge after 4-6 weeks.

The poulards were allowed to grow and develop male plumage and head furnishings for 4-8 months and during this interval some of them were killed and the regenerated right gonad removed for histological and histochemical studies. In a few cases the right gonad was extracted by chemical means (p. 23) to remove possible androgens for assay by the chick comb method described by Jaap & Robertson (1953).

(b) Adrenalectomy. Apart from the ovary the adrenal glands are the only other organs known to produce steroids. Both oestrone and progesterone have been isolated from the adrenal cortex of mammals by Beall & Richstein (1938), and work of Woolley et al

(1939), Wade & Haselwood (1941) and Weinstein et al (1950)
suggest that adrenals exert considerable oestrogenic activity.
The close functional relationship between the gonad and adrenal
is thus well established, and the extensive work on this problem
was reviewed by Parkes (1945) and Zuckerman (1953).

In the present work adrenalectomy was performed to ascertain
whether or not the adrenals influenced the growth and differentiation
of plumage, comb and oviduct.   Adrenalectomy in birds was more
difficult than ovariectomy because of the close proximity of the
adrenal gland to the posterior vena cava, kidney and lungs.
The operation is further complicated by the vascular nature
of the adrenal and the fact that the left gland is partially
covered by the ovary.   In view of the observations of Sisson &
March (1935) that older rats survived adrenalectomies better than
young ones, a series of preliminary operations were performed on
birds of varying ages and it was found that birds survived
longest if operated/ at 12-16 weeks after hatching.

For right adrenalectomy the bird was anaesthetized and
opened as for ovariectomy except that the operation was done on
the right side.   After removing the peritoneal covering, the
gland was gently separated from its four vascular connections.
Firstly, to break the lateral vascular connection A (Fig. 3) a
small but hard pellet of cotton wool was placed laterally to the
adrenal between the anterior end of the kidney and posterior
border of the lung.   The pressure of the pellet and easing with
forceps breaks the blood vessels.   Next, to break the posterior
connection/ B a cotton wool pellet was placed between the posterior
vena cava and the anterior lobe of the kidney.   The greatest
difficulty was encountered when removing the vascular connection

at the anterior apical end C, beaause this part of the gland is rather drawn out and extended beneath the junction of the posterior border of the lung and post-caval vein. To separate the medial border of the adrenal gland B-C, which is adherent to the posterior vena cava, extreme care had to be exercised because haemorrhage as a result of puncture in the post caval vein was difficult to control. After severing the above vascular connections, it was quite easy to gently separate the gland from the underlying tissue by rubbing with cotton wool pellets and, provided the above-mentioned precautions were carefully observed, the entire gland could be removed without fragmenting it. To ensure complete destruction of the gland tissue, the entire pocket which housed the gland was finally electro-cauterized. If bilateral adrenalectomy was desired, the second operation on the left gland was performed 2-4 weeks later, and it was found then that injecting the unilaterally adrenalectomized bird with two priming doses of 2 mg. of Desoxycorticosterone acetate (DOCA) 24 hours and 48 hours before the second operation,was helpful in the subsequent recovery and activity of the bird. Removal of the left adrenal was similar in all essentials to that of the right, the only difference being that on the left side the part of the ovary covering the adrenal had to be separated from its attachment to bring the left adrenal into view. The separated flap of the ovary was not torn away, but after removal of the adrenal it was turned back to its original position. If bilateral adrenalectomy and ovariectomy was desired the ovary was also removed at this stage. After the second operation, the birds were transferred to a warm room where the temperature was controlled between 65-68° F. One

group with bilateral adrenalectomy and ovariectomy, and another with bilateral adrenalectomy only, were given an injection of 2mg. (DOCA) in propylene glycol, and 0. 9% salt water to drink. Two further groups of birds one with bilateral and another with unilateral adrenalectomy were not given any post-operative therapy.

HORMONE ADMINISTRATION.

It has been conclusively shown that the comb of the fowl enlarges only in response to androgens and not oestrogens. Similarly, the female plumage of the Brown Leghorn has been shown to be dependent on oestrogenic secretions from the ovary. In view of these observations it was decided to examine the possibility of correlating externally visible criteria of hormone activity with changes in the cellular structure of the gonad after administering different hormones to chickens.

(a) Gonadotrophin treatment. Pregnant Mare Serum (PMS) given to immature chicks resulted in precocious comb growth of the treated birds. (Nalbandov & Card 1946; Taber 1948, 1951), and the enlargement of the comb was shown to be dependent on secretions from the ovary (Domm 1933). Further the work of Domm (1937), Asmundson et al (1937), and Taber (1946, 1948) has shown that the reproductive organs of the pullet vary in their response-threshold to a given dose of hormone with increasing age.

For the present work serum gonadotrophin from pregnant mares (GESTYL —— Organon Laboratories) was intramuscularly administered to birds of three age groups, in concentrations given below (Table 1). The controls received the same volume of normal saline throughout the period of treatment. All the birds were killed a day after the last injection, and

observations of changes in the secondary sex characters recorded.
The ovaries were then recovered and fixed for histological and
histochemical study to observe and correlate the cellular changes
in the treated ovaries with the precocious comb and oviducal
growth.

	Pullets	Age at Beginning of Treatment.	Dose & Duration of Treatment.
Group 1.	D2789 D2744 N.W.B.	1 week " "	100 IU daily for 7 days.
Group 2	D1016 D1019 D 949	12 weeks " "	500 IU daily for 10 days.
Group 3	D1208 D1175 D1092	18 weeks " "	750 IU daily for 10 days.

TABLE 1.

(b) Oestrogen treatment. Exogenous oestrogen treatment has been
reported to inhibit the output of pituitary gonadotrophins.
Taber (1951) reported that regression in the comb following
oestrogen treatment in both male and female chicks indicated an
induced inhibition of androgen secretion as a result of pituitary
disturbance. Since oestrogens had been shown to inhibit androgen
secretion (as indicated by comb measurements) it was decided to
investigate cellular changes in the ovary correlated with decreased
androgen production. For such a study, oestradiol monobenzoate
dissolved in propylene glycol was injected daily into the breast
muscle of pullets of different age groups. Controls were
injected with corresponding volumes of propylene glycol for the
same period. Comb measurements and other external characters
were recorded before and after the treatment. All birds were

killed on the day following the last injection, and their ovaries
fixed for subsequent cytological study.   Dosage of hormone, the
duration of treatment and ages of the treated birds are given
below (Table 2) :-

	Pullets	Age at Beginning of Treatment	Dose & Duration of Treatment
Group 1	D 546 D 551	1 week	0.5 mg. in 0.5 ml. daily for 7 days.
Group 2	C9790 C9806	8 weeks	1 mg. in 1 ml. daily for 15 days.
Group 3	C8288 C8258	18 weeks	1.5 mg. in 1.5 ml. daily for 15 days.
Group 4	C 289 C 239	30 weeks	2 mg. in 2 ml. daily for 15 days.
Group 5	X1545 X2269	5 years	2 mg. in 2 ml. daily for 15 days.

TABLE 2.

CHEMICAL EXTRACTION, AND ASSAY OF HORMONE
SUBSTANCES FROM THE RIGHT GONAD OF THE POULARD.

In addition to cytological studies on the hypertrophied
right gonad which develops after ovariectomy, and which is
presumed to secrete the androgens responsible for the
development of male secondary sex characters in the poulard,
it was thought desirable to attempt to extract any active
principle from the gonad for androgen assay.   Allen et al
(1923-24) and Marlow & Richert (1940) reported considerable
oestrogenic activity in extracts of follicles and immature
gonads of the left side of the pullet, and so it was feasible
that detectable amounts of androgens might be recovered

similarly from the right gonads.

To extract the androgenic substances 4 right gonads from birds showing well developed comb and male plumage were removed 7 months after ovariectomy had been performed. The combined gonads weighing 2.5 gms. were cut into pieces, and ground with fine chemically pure sand and distilled water in a glass mortar. The resultant brei was then extracted with 50 ml. of a hot mixture of equal quantities of pure, redistilled chloroform and absolute ethyl alcohol. The mixture was heated and shaken on a water bath, and centrifuged at 3000 r.p.m. for 5 minutes. The alcohol-chloroform phase was then separated from the centrifuged mixture and the residue treated similarly once again. The resultant chloroform-alcohol mixture was finally evaporated to dryness on a water bath and the residue dissolved in 2 ml. propylene glycol for androgen assay. For the assay 0. 05 ml. of the supposed hormone extract was painted daily on the combs of one day old cockerels for a period of 7 days. The chick comb androgen assay described by Jaap & Robertson (1953) was used.

For a rough estimation of the strength of hormone, if any, in the extract, the combs of a control group of 1 day old cockerels were painted daily for 7 days with 0.05 ml. of propylene glycol containing 0.01 mg. of testosterone propionate. Another group was painted similarly with an extract from 2.5 gms. testes of cockerels aged 10 weeks and another with propylene glycol alone.

Results and Discussion.

STRUCTURE OF THE OVARY OF A FULLY LAYING HEN.

At this point it will be useful to have a description of the morphological and histological structure of a normal ovary

in a laying hen.   The purpose is two-fold, first a perusal of
the chapters on reproduction in a book by Bradley (1950) and,
more recently, one by Sturkie (1954), reveal a lack of detailed
descriptive anatomy of the ovary.   Second, it is considered
necessary to present standard figures and terminology to avoid
confusion in a description of cell types and their function.
With such a basis, it will be easier to give a precise account
of the changes in different cell groups occurring in various
physiological states in the growing and mature female.

(a) Macroscopic appearance.   The right ovary of the chicken
is present as a visible rudiment at the time of hatching
(Fig 27), but later decreases in size and persists throughout
life as an inconspicuous vestige.   Rarely a fully formed and
apparently functional right ovary may persist (Domm, 1927;
Crew 1931).   The normal functional ovary is situated on the
left side near the cephalic lobe of the kidney, close to the
median line and posterior to the lungs.   It is attached to the
dorsal body wall by the mesovarium, and fig 4 shows its
macroscopic appearance.   It consists of several large yolky
ova, within their thecal cell layers, and a large number of
smaller follicles ranging from 2 to 10 mm. in diameter all
attached by stalks to the main mass of the ovary, which consists
of the medulla and ground cortex regions.   On all the large
follicles a somewhat avascular streak, called the stigma, is
present and it is along this region that the thecal layers
rupture to release the ovum at ovulation (Fig 5).   In addition
to the different sized follicles, a number of post-ovulatory
follicles in various stages of regression are present, which in
the early stages, resemble empty beech-nut cases hanging on
the substratum (Fig 4).   Nalbandov & James (1949) made a study

of the vascular supply to the ovary and showed that it receives
its blood supply from the ovarian artery which usually arises
from the left reno-lumbar artery, but may arise directly from
the dorsal aorta.   The arterial system of large stalked
follicles is comparatively simple, but the venous system is
complex, and Nalbandov & James believe that this functioned
to retard circulation, and thus facilitated the deposit of
large quantities of material in the yolk of the developing ova.
Large follicles are furnished with three concentric and
intercommunicating layers of veins in the theca externa, and
according to Nalbandov & James minute capillaries are present
in the apparently avascular region of the stigma.   Minute
follicles, before becoming stalked, have no vascular systems
of their own, but receive their nutriment from the blood supply
of the highly vascular medullary region of the ovary.
According to Bradley (1950) there is a nerve supply to the ovary
which is derived from the abdominal and pelvic plexuses of the
sympathetic system and from the posterior continuation of the
sympathetic trunk.   However, beyond this statement no details
are given of the system.

(b) Substratum tissue of the ovary.   The main ovarian mass in
a functionally active ovary consists of two somewhat ill-defined
parts (i) an outer cortex consisting of minute follicles, stromal
cells and other ground connective tissue, and (ii) an inner
medulla.   In the latter blood vessels and lymphatics enter and
leave the ovary for the nourishment of the developing ova, and
a certain amount of modified muscle and fibrous connective tissue
is incorporated in the mesovarium.   Fig 6 is a diagrammatic
representation of a part of the ovarian mass, compiled as a result
of a thorough study of the components seen in a large number of

microscopical preparations.

(i) Cortical Components.

1.   Germinal epithelium.

The surface of the ovary is covered by an epithelium which consists of a single layer of flattened cells, the nuclei of which are fusiform in shape.   At places the flattened cells become cuboidal and columnar in form, with rounded vesicular nuclei.   Below the germinal epithelium Masson's trichrome stain reveals a delicate layer of connective tissue, the tunica albuginea, which separates the epithelium from the underlying tissues (Fig 6).

2.   Stroma.

The connective tissue framework of the cortex is dispersed with stroma cells, most of which are of the elongate, fibroblast type with fusiform, deeply basophilic nuclei. Scattered amongst the stroma cells are larger cells with slightly basophilic, vesicular nuclei (Figs. 6,7).   These are undifferentiated cells which are difficult to distinguish from some types of migratory medullary cells in haematoxylin and eosin stained sections.   Their possible function in the physiology of the ovary is discussed on pages 59,60 and 75.

3.   Ova.

Excluding the large stalked follicles and small follicles just visible to the naked eye, there are myriads of developing ova ranging from 30-400μ in diameter in the cortex. Each minute ovum consists of a uniformly granular cytoplasm (ovoplasm) in which the nucleus is eccentrically placed.   The nucleus (germinal vesicle) is covered by a well defined nuclear membrane, and the chromatin is in the diffuse diplotene stage, except in the smallest follicles where it is more differentiated

and may even show the formation of ill-defined chromosomes (Fig 8). In the germinal vesicle of the smallest ovum, one or two acidophile nucleoli (plasmosomes) occur which fragment and disappear as the follicle grows. The germinal vesicle of follicles above 250 μ in diameter shows entirely fragmented chromatin.

In all ova up to 200 μ in diameter, there is present on one side of the germinal vesicle a cluster of granules, the so-called yolk body of Balbiani, which consists of Golgi element, mitochondria and mitochondrial yolk (Brambell 1926). The yolk body is very prominent in osmic acid fixed ovaries (Fig 6). In ovary sections fixed in formalin and Carnoy-Lebrun fluid the yolk body, though faint, is clearly marked and represents the non-lipoidal elements of the mitochondria (Fig. 7). Close to the body, usually on either side of it, are a few large spheres of fat, the primordial yolk, which can be seen in frozen sections of the ovary. During the growth of the ovum, the yolk body of Balbiani fragments and spreads out uniformly in the ovoplasm. The products, together with material diffusing through the granulosa cell layer, eventually form a well-defined layer of fatty yolk at the periphery of the ovoplasm leaving a space between it and the membrana granulosa surrounding the ovum (Fig. 9). This space is occupied by ground cytoplasm full of peripheral mitochondria. As the follicles grow, the process of yolk formation is further advanced, and in paraffin sections the cytoplasm of the ovum is seen to be full of large vacuoles of various sizes, from which the yolk has been dissolved away, (Fig. 10); spheres of eosinophilic protein material are seen at some stages. Later in this thesis the various ovum components will be discussed in relation to their histochemical

behaviour.   During follicle growth, the germinal vesicle moves
to the centre and thence to one one side and there remains with
fragmented chromatin.

The ova are primary oocytes until shortly before ovulation
when they become secondary oocytes after having shed one polar
body (Olsen, 1942).

4.   Membrana granulosa.

Covering the oocytes from the earliest stages in
their growth is a single layer of follicle cells constituting the
membrana granulosa (Figs. 6,7).   They are never multi-layered
as in the mammal follicle, but during rapid ovum growth. they
assume a pseudo-statified arrangement.   The granulosa cells are
of cuboidal form in minute follicles, and assume a columnar shape
as the follicle grows.   The nuclei of granulosa cells are
spherical and occupy more than half the cell space.   Compared
with the germinal vesicle, the nuclei are more basophilic and
show chromatin loops adherent to the nuclear membrane;   a
plasmosome is occasionally visible.   The cytoplasms of the
granulosa cells are variably packed with lipoidal granules
which are the precursors of yolk passing from the thecal layers
to the ovum (Fig. 12).   Mitotic divisions are commonly seen in
the granulosa during rapid growth of the ovum (Fig. 11).
From the stage when the ovum is about 2 mm. in diameter, the
granulosa cells secrete a transversely striated non-cellular
vitelline membrane (McNally, 1943) to surround the ovum (Fig 10).

5.   Thecae of follicles.

The oocytes before forming follicles lie embedded in
the general ovarian stroma under the germinal epithelium.   In
the follicle stage of growth, they are surrounded externally to
the granulosa layer by a capsule of ovarian stroma cells

forming the theca, which is further differentiated into internal
and external layers. The theca interna is compact and with
the Massons's trichrome stain is shown to consist of an inner
fibrous capsule immediately surrounding the granulosa (which
resembles the basement membrane of testis tubules), and an outer
layer of spindle-shaped cells (Figs. 12, 13). Included amongst
the latter are a number of nests of large, clear cells which
have been called medullary "luteal" cells and these will be
discussed later. Each nest consists of a few cells, the
nuclei of which are vesicular and stand out prominently against
the clear cytoplasm (Fig. 13). A few blood capillaries are
present amongst the various elements of theca interna, but
surrounding the latter are a few layers of flattened
stroma cells forming the theca externa, which gradually merges
into the general stroma and is abundantly supplied with blood
vessels and capillaries (Fig. 13).

6. Atretic follicles.

Follicles undergoing atresia are a constant feature
of the ovary, and follicles of all sizes can be seen in various
stages of atresia. A careful examination of this phenomenon
reveals that follicles may become atretic in three different
ways. Firstly, the most common mode of atresia is exhibited
by follicles up to 500 μ diameter and occurs in immature ovaries
as well as in fully functional ovaries. It begins by a
proliferation of the granulosa cells which stain deeply, and
form many irregularly arranged layers around the shrinking
cytoplasm of the ovum (Fig. 14a). As the atresia advances,
the proliferating granulosa cells lose their cell walls, so
that there is no clear demarcation between granulosa and
ovoplasm (Fig. 14b), and then the migrating granulosa cells

completely fill the entire follicle (Fig. 14c). The stages of atresia so far described occur whilst the thecal layers are intact. Later stages occur rapidly and begin with fibroblasts and other cells of the theca interna drifting inwards, so that the follicle appears as a heterogeneous compact mass of cells derived from all the three sources. Finally, hyalinisation occurs, and the entire follicle is reduced to a small connective tissue scar. (Figs. 6,15).

The second type of atresia is not very common in a pullet ovary but occurs frequently in ovaries of older laying birds. It is characterised by the multiplication of the thecal and granulosal layers which do not migrate inwards, but form a ring round the ovoplasm. There is great hypertrophy and hyperplasia of medullary "luteal" cells, and the ovoplasm of the oocyte together with the hyperplastic granulosa cells undergo fatty degeneration (Fig. 16). The follicle collapses (Fig. 17) and in the final stage of this type of atresia the medullary "luteal" cells migrate into the ovarian stroma and the thecal cells undergo hyalinisation.

The third type of atresia is common in the ageing hen as well as young hen ovaries, and is frequently seen in macroscopic follicles above 1.5 mm. in diameter. At first, the granulosa remains inactive and the thecal layers undergo extensive hypertrophy to become fibrous and very compact. Within the fibrous mass of thecal elements, a large number of capillaries appear. Later, the granulosa proliferates and ultimately fills the entire follicle (Fig. 18). There is no increase in medullary "luteal" cells due to the fibrous nature of the thecal layers, and the group of cells already present are pushed

outwards.   The ultimate scar left by such follicles is bigger
than that resulting from the two previous types of atresia.

  7.   Eosinophilic Cortical Cells.

          In the stroma of the ovary, but more especially in
the peripheral regions of the cortex, are large numbers of cells,
the cytoplasms of which are full of rounded, eosinophilic
granules.   These cells are of two types and are generally found
scattered sparsely throughout the stroma, but in some parts they
aggregate in large numbers.   One type has a large, vesicular
nucleus and the cytoplasm is packed with a variable number of
large granules.   It is not uncommon to see these cells
dividing (Figs. 19, 37).   The other type of cell is much smaller
in size, the nucleus is small, often bilobed, and the cytoplasm
is completely obscured by small, coarse eosinophilic granules
(Fig. 20).   These cells resemble oxyphilic granulocytes of the
blood.   Eosinophilic cortical cells attain their maximum
development in a functionally active ovary, but are absent in
ovaries of birds before 14-18 weeks of life.   These cells do
not disappear from the ovary after the active reproductive
stage, although they are somewhat reduced in old birds and in
inactive ovaries.   The significance of the cells will be
discussed later.

  8.   Vacuolar cells.

          In the cortex of a mature ovary, but more commonly
in older ovaries, are present irregular patches of cells, the
cytoplasm of which is full of fatty material.   In ordinary
histological preparations, this tissue is glassy in appearance
because the fatty material has been dissolved away.   The
cytoplasms of the cells constituting this tissue are irregular
in outline, variable in size and are either entirely empty or

show a number of vacuoles. The nuclei are degenerate, small and lie at one end of the disintegrating cell (Fig. 21). Such vacuolar cells are absent in young ovaries before ovulation and their morphological similarity to degenerating granulosa cells of post-ovulatory follicles suggests that at least a few patches represent the final stages in regression of post-ovulatory follicles. Some cells before fatty degeneration is advanced could be mistaken for secretory cells, but as will be shown later they lack any active chemical compounds associated with sex hormones.

9. Pigment.

In the cortex of the mature ovary are present irregular patches of orange-yellow coloured pigment. The distribution of the pigment is closely parallel with that of the eosinophilic cortical cells, and it is not infrequent to see the two intermingled in the same area(Fig. 22). At some places in the cortex the pigment granules are coalesced into irregularly shaped masses but more frequently they appear as rounded bodies. Occasionally, very small pigment granules are found sticking to the walls of vacuolar cells in sites of the ultimate degeneration of post-ovulatory follicles. The chemical nature of the pigment will be described later.

10. Vascular and Non-vascular Spaces.

The cortex of an adult ovary is not compact but spongy due to the presence of a large number of tissue spaces of irregular outline which permit the growth of follicles (Fig. 23). Vascular sinuses and smaller types of blood vessels ramify in the cortex to bring nutriment for the growing follicles.

(ii) Medullary Component.

The Medulla, or zona vasculosa, as the name implies is the central vascular part of the ovary. In the functional ovary

the blood vessels, sinuses and lymph spaces are well developed
for transporting materials for the elaboration and deposition
of the enormous quantities of yolk in the follicles. The
medulla is so greatly developed that its peripheral branches
encroach upon the cortex, and the clear demarcation between
cortex and medulla, which is characteristic of young ovaries,
is no longer discernible (Fig. 23, 24). Parts of the medulla
form the attachment of the ovary to the dorsal body wall, and
for this purpose fibroblastic, connective tissue cells and
modified muscle fibres exist in the mesovarium and penetrate
for some distance into the medulla. It is doubtful whether
the muscle is functional as such. In addition to the above
principal constituents of the ovary substratum there are a few
wandering phagocytic cells, lymphocytes and mast cells. The
latter stain metachromatically with toluidine blue.

(c)   Post-ovulatory Follicles.

At ovulation the ovarian follicle ruptures along the
relatively avascular stigma to release/ovum. As a result of
the sudden release of pressure, the discharged follicle shrinks,
its walls become considerably thickened and the cavity is greatly
reduced. A few scattered blood corpuscles remain in the cavity
due to varying amounts of blood vessel rupture at ovulation.
Unlike the mammalian ovary a corpus luteum is not formed after
ovulation, and it is best to call the ruptured avian follicle
a post-ovulatory follicle to avoid confusion with the functions
of the organ in the mammal. The post-ovulatory follicle in the
hen regresses quite rapidly until it is re-absorbed in the ovarian
mass and no longer visible to the naked eye. In an active laying
hen, four to six post-ovulatory follicles in various stages of
regression are generally present (Fig. 25). The walls of the

follicles are thick and consist mostly of connective tissue and fibroblasts excavated with the blood vessels, which appear dilated and congested as a result of shrinkage following ovulation (Fig. 26). The inner-most layers of cells lining the lumen of discharged follicles are the granulosa cells undergoing fatty degeneration. At places, the degenerating granulosa cells detach from the follicle wall, drift into the cavity, and there form an admixture with blood corpuscles. Outermost layers of the regressing post-ovulatory follicle wall represent the thin strip of ovarian cortex which covered the follicle during its growth period, and all or most of the cortical elements e.g. the flattened germinal epithelium, ovarian stroma cells, minute follicles and medullary "luteal" cells, are frequently present especially in the stalk region. Neither eosinophilic cells nor clear medullary "luteal" cells are found inside the follicle.

In the final stages of follicle regression the fatty degeneration of the granulosa is completed and the cavity assumes the appearance of vacuolar tissue described on p 32. The whole post-ovulatory follicle is finally re-absorbed, and t he vacuolated tissue incorporated in the cortex. Minute granules of pigment are present inside some of these cells, but the characteristic pigment of the cortex is absent.

(d) Large follicles (stalked & approaching maturity).

The most conspicuous part of an active ovary is the presence of several large follicles, ranging from 1.5 to 3.5 cm. in diameter (Fig 4). Large avian follicles, unlike ripe mammalian follicles, are stalked and during growth both stalk and follicle are covered with a thin layer of cortex. In the latter, may be found small satellite follicles (Fig 10). In addition to the large follicles, numerous follicles ranging from

3 mm. to 8 mm. in diameter are present on the surface of the ovary. They are generally creamy white in colour and filled with white yolk, but some show the beginning of the accumulation of yellow yolk (Fig. 4). Quite a fair proportion of the 3-8 mm. diameter follicles can appear in different stages of atresia especially towards the end of an egg laying season.

All large follicles are enclosed by thecal cell layers in which blood vessels are quite prominent, except in the stigma region where rupture takes place at ovulation. The thecal layers become very fibrous so that the medullary "luteal" cells, so prominent in thecae of smaller follicles, are inconspicuous. Granulosa cells form a single, pseudostratified layer covering the ovum, and capillaries penetrate the fibrous theca at many places to lie in close proximity with the granulosa cells (Fig. 10). In mammals during the growth of the follicle the granulosa cells proliferate, form many layers and then a cavity appears amongst them, which is filled with liquor folliculi containing oestrogenic hormone. The growth of the avain follicle is typified by the accumulation of yolk inside the ovum as a result of which the germinal vesicle comes to lie at one pole of the cell immediately below the vitelline membrane. Romanoff & Romanoff (1949. p 210) have described a fully formed unfertilized ovum as consisting of a central flask-shaped area of white yolk, called letebra, around which yellow yolk is laid in concentric layers separated by thin sheets of white yolk. Distally, the neck of the latebra expands out at the surface to form the nucleus of Pander immediately above which lies the germinal vesicle.

Contrary to the observations of Phillips & Warren (1937) and Kraus (1947) no muscle cells have been identified in the

thecal cell layers surrounding the follicles. According to these workers, muscle cells aid in expelling the ovum from the follicles at ovulation. From present observations, follicle rupture appeared to be heralded by a dilatation of blood vessels surrounding the stigma region and it is considered that further work on the vascular physiology concerned with ovulation, together with an investigation of the action of luteinising hormone is required to solve the problem of the mechanics of ovulation.

THE APPEARANCE OF THE OVARY IN DIFFERENT PHYSIOLOGICAL STATES.

After the preceding account of the functional ovary, the changes in the structure of ovaries of differently aged birds will now be described as seen macroscopically, and in haematoxylin and eosin stained sections. The significance of the cell changes mentioned will be further discussed when the histochemistry of the ovary is reported in the next section.

(a) Macroscopic. Fig. 27 shows the appearance of the ovary from the time of hatching to the time when the first egg is ovulated. The ovary during the inactive phase between laying periods is also shown. It can be seen that except for a slight decrease between 12 and 15 weeks there is a progressive increase in size towards sexual maturity. Bennett (1947) reported a similar phenomenon of a decrease in size of the ovary between 18 and 21 weeks in White Leghorn pullets although it continued to gain weight. The ovary of a day-old chick is a smooth, somewhat triangular structure with the adrenal closely adherent at the anterior end. The rudimentary right ovary is present at hatching and lies at the junction of the right iliac and post caval veins. After the 4th day it is reduced to a small linear structure, and is hardly visible in a week old chick. The left ovary on the other hand increases

in size, and by the end of the first week is about twice the
size of a day old chick ovary and is S-shaped in form.   From the
second week onwards, folds begin to appear on the ovarian surface,
the anterior end broadens and the posterior end elongates backwards.
Concurrently with these expansions and folding, the ovarian mass
increases.   By the 5th week, the ovary appears as a mass of
tightly packed leaves, and in surface view is irregularly concavo-
convex in shape.   The process of folding, thickening and growth
goes on gradually throughout the early period of sexual immaturity,
and except in size, the ovary at 21 weeks, i.e. during the period
just before first ovulation, is little different in outward
appearance from that at 5 weeks old.

Macroscopic follicles become visible to the naked eye on the
surface of the ovary between 20-24 weeks, and one or two enter
upon a rapid growth phase of development associated with the
accumulation of yellow yolk in the contained ovum.   Follicles
increase in size in a graded series until they reach 2.0-2.5 cms.
diameter when they ovulate.   If the largest follicle in succession
becomes atretic, as frequently happens, the next one takes its
place.   In a fully grown hen ovulation occurs when follicles
reach a diameter of about 3.5 cms.   The phenomenon of ovulation
of small diameter eggs by pullets is most likely a result of
selecting chickens for early maturing properties.   The egg
laying commences before the pullet attains maximum body size,
and physiologically, the production of gonadotrophins from the
pituitary has been induced prematurely.   Some support for this
view can be gained from the observation that the administration
of pregnant mare's serum (containing follicle stimulating
hormone) to pre-lay pullets will induce a rapid growth of small
diameter follicles which can be made to ovulate with luteinising

hormone (Nalbandov & Card, 1946). Comparatively small oviducts at the commencement of laying could also contribute to the laying of small pullet eggs as there is a low albumen content as compared to a full sized egg. The ovary when the first egg is ovulated is still immature in size and is frequently seen with only one or two large follicles and a post-ovulatory follicle (Fig. 27). The ovulation and oviposition of the second egg in the pullet's life may in this case be considerably delayed to enable other follicles to reach ovulable size. Frequently with pullets coming into lay, it has been observed that the first few eggs are laid with comparatively long intervals between them, and after a variable interval of intermittent laying, there is a sudden and simultaneous growth of many follicles, so that the ovary may show 10 to 15 large follicles with one or two undergoing atresia. It is not unusual for the ovary, soon after the attainment of this condition (Fig. 28), to ovulate two ova simultaneously and subsequently oviposit somewhat elongate, double-yolked eggs.

At the end of a laying period, the ovary is reduced to an inactive state and the ovarian surface is covered by follicles ranging from 3 to 10 mm. in diameter (Fig. 27).

(b) General Histology of Immature & Mature Ovaries

(1) 1 Day-old chick.

Microscopic examination of the ovary of a day-old chick reveals the ovarian tissue divided into two well-defined zones, the cortex and medulla. The medulla is surrounded by cortex except at the hilum region which is the seat of ovarian attachment.

The cortex which consists of germinal epthelium and proliferating cords of oogonia, is comparatively smaller in area than the medulla (Fig. 29).

The medulla consists of a large number of nests of medullary cells with slightly basophilic vesicular nuclei and very faintly stained cytoplasm. From now onwards in this thesis the cells which have previously been called medullary"luteal"cells are termed medullary cells since they originate in the medulla of the ovary during embryogenesis. The nests of medullary cells are embedded in a connective tissue stroma, and in the central parts of medulla many spaces appear between them. In the peripheral regions, immediately below the cortical cords of oogonia, the medullary and cortex stromal cells are closely packed and there are comparatively few small spaces (Fig. 30). Mitotic figures are commonly seen amongst medullary cells and they seem to multiply; groups break away and form new nests of cells. The term "medullary cords" has been given in the past to describe the arrangement of the nests of medullary cells along the edges of spaces in the medullary region. Besides the tissue spaces, which are sometimes filled with fluid, there are also present small blood vessels and sinuses.

The surface of the ovary is covered by a germinal epithelium consisting of cuboidal and columnar-shaped cells. Underneath are found cortical cell cords which are derived from germinal epithelium during embryogenesis (Lillie, 1952. p. 462-467). These cords are of various shapes enclosed in delicate fibrous envelopes, and consist of a large number of oogonia that are almost twice as big as the medullary cells (Fig.30). Nuclei of these cells are correspondingly large and most of them show the formation of chromatin loops typical of the early prophase stage of division.

Other stages of division are also seen. The nuclei of oogonia
are so large that the cytoplasm is obscured except for a small
acidophile area near the nuclus which is possibly a part of the
Golgi Zone. The whole cortical region of the ovary is clearly
demarcated from the medullary region by its lightness of staining
(Fig. 29).

(2). Changes during the first week. Great mitotic activity
is seen during the first week, both in the cortex and in the
medulla. The noticeable change in the cortex is a continued
growth of a few oogonia prior to giving rise to primary oocytes,
which when formed are conspicuous with large nuclei and
consolidated cytoplasms (Fig. 31). By the third day of life
some of the stroma cells become granulosa cells, and a few
minute follicles are formed towards the medulla (Fig. 34).
However, most of the cortical cells (oogonia) at this age are
still of the primitive type undergoing enlargement and
multiplication. In the medulla, intense multiplicative
activity is seen amongst the medullary cells which produces more
extensive "medullary cords". The latter fragment, and more
spaces are formed between them, which extends the space system
in the medulla (Fig. 32). Together with the process of enlargement
of "medullary cords", the cortical stroma cells multiply and the
medulla becomes a little more vascular. By the 5th or 6th day,
most of the primary oocytes are surrounded by granulosa cells to
form follicles and only at a few places one finds undifferentiated
oogonia (Fig. 33). As the follicles are formed, they migrate towards
the medulla and some of the stroma cells from the medulla grow in
between them (Fig. 34). At the end of the first week, medullary
cells continue to grow and divide, and many of them lie in a
subcortical position (Fig. 46).

(3) <u>Ovary from 2nd to 4th week.</u>   During the second week, the process of oocyte formation is almost completed and only occasional cortical cords of oogonia remain.   The growth of newly formed oocytes is rather slow and the diameter of the largest follicle measures 60 to 70 μ.   The cortex consists of tightly packed, minute follicles with few easily visible stromal cells.   It is covered externally by columnar-shaped cells of the germinal epithelium, and internally it is sharply demarcated from the central medulla region.   There is intense mitotic activity amongst potential granulosa cells, remaining oogonia and stromal cells in the cortex.   In the medulla the connective tissue spaces are more distended, and there is further increase in vascularity.

During the third week, some of the follicles enter upon a rapid growth phase, the diameter of the largest being 160 μ. The interspersed stromal cells exhibit continued multiplicative activity and the follicles become firmly embedded in them. A few binuclear follicles, and the formation of biovular or even triovular follicles begin to make their appearance.

More follicles enter upon rapid growth during the 4th week, resulting in the appearance of a larger number of follicles from 120 to 180 μ diameter.   The follicles, which had already attained 160 μ diameter by the third week, are only slightly increased in size during this period.   Polyovular follicles are commonly seen.   The stromal cells continue to divide and the cortex appears very cellular, compact and clearly demarcated from the medulla. For the first time , some of the follicles show the earliest stages of stresia revealed by the proliferation of the granulosa cells. (Fig. 14a).

(4).   <u>Ovary from 5th to 8th week.</u>   A large number of follicles continue to grow and some attain a diameter of 250-300 μ

by the 5th week.   The growth of follicles and hypertrophy
of stroma results in a slight thickening of the cortical
layer of the ovary.   The phenomenon of atresia is by now well
established and small follicles in different stages of this
process are commonly seen.   Throughout this period, the
medullary cells appear to start migrating in the cortical stroma
tissue, and show the development of a more granular cytoplasm.
In this state they are not easily distinguishable from the
general cellular elements of the stroma.

By the 6th week, some of the medullary cells revert to show
a progressively lighter staining reaction in the cytoplasm due
to the loss of some granular material (Table 4).   The cortex in
section no longer shows uniform thickness due to the great growth
and inward protrusion of some follicles, and folding of the ovarian
surface layers.   Some of the largest follicles are 350 -375 μ
in diameter.   As follicles protrude inwards from the cortex
they carry with them stromal cells which form the beginnings
of the thecal tissue.   Some of the migrating medullary cells
or large cortical stromal cells become incorporated into the
thecae.   At this age it is difficult to distinguish between
medullary cells and large cortical stroma cells, but generally
the former appear in groups of two or three and the latter are
dispersed throughout the cortex.   Polyovular and binuclear
follicles are more frequent in appearance.

By the 8th week, the processes of the previous weeks become
well established and some of the follicles attain a diameter of
550 μ (Fig. 14).

(5). Ovary from 9th to 16th week.   During this period there
are no major changes in the appearance of the ovary apart from
a general size increase of all component structures (Fig. 27).

The process of growth as it affects the individual follicles is slow, the diameter of the largest follicle at 14 weeks being 660 μ as compared to 550 μ at 8 weeks.

Atresia of small follicles becomes very widespread but the final stages of this phenonmenon, leading to the formation of scar tissue, are not seen in the 9 week old ovary.

The medulla of the 9-16 week ovaries remains narrow and consists of the usual vascular and non-vascular sinuses. Large numbers of medullary cells lie in a sub-cortical position, and migration into the cortex continues. In haematoxylin-eosin stained sections, the cytoplasm of some of these cells assumes a glassy clear appearance similar to their condition in the adult ovary (Fig 61).

Polyovular follicles, containing 2-5 ova, and binuclear follicles are commonly seen (Figs. 8, 35, 36). Polyovualr follicles containing 2-5 ova have been reported in mice by Fekete (1950) who considered that such follicles developed as a result of atypical differentiation of the germinal epithelium. He also considered that hereditary and hormonal factors played a part in their development since one genetic strain was found to contain more polyovular ova than others, and the germinal epithelium was reported to proliferate at oestrous. Similar observations have been made by Bacsich (1951) on foetal and early post-natal human ovaries. He reported that polyovular follicles succumbed first during early destruction of follicles so that by 6 months they were not found in human ovaries. In the chicken Brambell (1926) noted biovular follicles but regarded them as two oocytes situated in such close proximity that interfollicular cells were missing. Since then a number of workers including Domm (1937), Asmundson et al(1937) and

Taber (1948) have described the occurrence of polyovular follicles in the chicken ovary treated with gonadotrophins. From the present study of the normal chicken ovary it appears that polyovular follicles exist in immature ovaries and are a normal phenomenon. The occurrence of poly-nuclear follicles together with polyovular follicles, which have been observed, has not been reported previously in the chicken ovary.

At the age of 16 weeks, the oviduct is still undifferentiated and the comb is small, rarely exceeding 2.5 cms. in length and 1.0 cm. in height.

(6) <u>Ovary from 17th week to the time of first ovulation.</u> From 17th week to 20th week, the first signs of oviducal differentiation are evident and the comb starts on a rapid growth phase heralding the approaching maturity of the ovary. Follicles in the cortex continue to grow, the polyovular and binuclear follicles become less common in appearance, but atresia is very common. The medullary cells are not glassy-clear in appearance now, but show a granular cytoplasm indicating secretory activity.

A striking feature about the 17th week is the appearance of cells in the theca externa of follicles with cytoplasms which are full of large spherical-shaped eosinophilic granules. These cells appear in groups around the follicles, and are prominently distinguished from other stromal cells (Fig. 37). They are the mononuclear type of cortical eosinophilic cell described on p. 32. During ovarian growth these cells divide extensively and are also to be found throughout the peripheral regions of the cortex. The increase in their number is associated with the appearance of the other type of eosinophilic cell, which is somewhat smaller in size and is the blood leucocyte.

From about the 17th week until the 20th week the germinal

be seen (Fig. 38). This might indicate that as the ovary is
growing rapidly at this stage, the germinal epithelium is
undergoing compensatory hyperplasia. On the other hand, the
appearance of minute follicles below the germinal epithelium at
this time, coupled with the mitotic activity in the epithelium,
could suggest neo-formation of follicles as reported in some
mammals by Allen (1923), Morgan (1943), Latta & Pederson (1944)
and Barton (1945). Neo-formation of follicles in post-natal
life of mammals has, however, been vehemently denied by
Everett (1943), Bookhout(1945), Zuckerman (1952) and others.
The significance of the activity of the avian germinal epithelium
during this period must await further experimentation.
Just before the first ovulation the germinal epithelium again
                                      the
becomes inactive and its cells assume/flattened shape
characteristic of the adult ovary.

As mentioned earlier in this thesis, there is with pullets
                   the
a great variation in/age when the first egg is ovulated, depending
on the time of hatching and other factors. Hence a description
of the cellular changes in the ovary from 20 weeks onwards
is best followed by taking ovaries in different stages of
growth as they approach maturity irrespective of the age of
the donor pullet. This most critical period in the maturity
of the ovary is associated with a rapid rate of increase of
size. (Fig. 27).

During the stage bordering on maturity, the medulla becomes
distended owing to increased vascularity and the extension into
it of collagenous and muscular elements from the mesovarium.
In the cortex there is intense mitotic activity of the stromal
cells, and extensive development of blood vessels results

in a reduction in the thickness and compactness of the cortex
(Fig. 39).   Soon after the appearance of protruding macroscopic
follicles on the ovarian surface, the medulla and cortex are so
intermingled that a distinct cortex can no longer be recognised
except in regions occupied by small follicles.   The formation of
a theca interna around the largest follicle is now quite clearly
seen (Fig39).   Atresia of minute follicles, which is a normal
feature of ovarian growth, reaches its culmination before maturity
(time of first ovulation) and stages of scar tissue formation
are to be seen.   Hitherto atresia of follicles had been quite
common, but there had not been a complete breakdown to scar
tissue.

Bordering on the first ovulation the cytoplasm of most of the
medullary cells is slightly granular.   Only a few groups of
cells show glassy-clear cytoplasms and they form nests in the
thecae of follicles, together possibly with some large cortical
stroma cells (Fig. 13).   They increase in number and soon
after the first ovulation become more conspicuous in the thecae
of many follicles, which is the condition found in a fully
functional ovary.

Polyovular or binuclear follicles are no longer to be seen
in ovaries immediately before first ovulation or any time
afterwards.

(7). Ovary of hen in full laying condition.   The structure
of the fully functional ovary has already been dealt with on
pages   24 - 37.

It is not proposed at this stage to repeat all that has been
said, but just to call attention to a few further interesting
points.   Firstly, the growth of   very large follicles

results in a stretching of the cortex so that the demarcation between cortex and medulla is hardly discernible. Secondly patches of vacuolar cells, developed as a result of the final involution of post-ovulatory follicles, are a common appearance in the cortex. Thirdly, a good deal of scar tissue representing the final stages of follicle atresia is frequently seen. Fourthly, the occurrence of pigment in the cortex is a notable feature, and lastly, the eosinophil cells of the cortex attain their maximum development. In haematoxylin-eosin stained sections most of the medullary, or large cortical stroma, cells, in the thecae of the largest follicles have glassy-clear cytoplasms. (Fig. 13). In certain regions of the cortex notably in areas occupied by minute follicles, grouped medullary cells appear with a granular, secretory cytoplasm which is characteristic of a pre-ovulatory (18-24 weeks) ovary. It would appear, therefore, that the medullary cells incorporated in the thecae of large follicles are in a non-secretory condition.

(8). <u>Ovary during moult</u>. The first outward sign of the approach of moult in a hen is a period of irregular egg laying replacing the phenomenon of laying regular uniform clutches of eggs. The ovary shows only a few follicles in the ovulable size range (Compare Fig. 5 with Fig. 4). Egg laying eventually ceases, and the larger follicles start becoming atretic.

Occasionally, any type of large follicle in a fully functional ovary may be found undergoing atresia, exhibiting constrictions in the outer thecal layers. In such follicles there is extravasation of blood in the thecal layers and liquefaction of the yolk. This type of atresia is especially prevalent at the beginning of the hen's moulting period (Fig. 40) when most of the existing large follicles become atretic. Finally

the ovary reverts to an inactive state, when all the large follicles are reabsorbed and small pearly white follicles are scattered over its surface ; the follicles rarely exceed 8 mm. in diameter (Fig. 27). A large proportion of the small follicles also become atretic and appear as solid white or blackish pinheads on the surface of the ovary.

Microscopically, one sees widespread atresia of all sizes of follicles and there is a reduction in the number of both types of eosinophilic cortical cells. A most noticeable feature is the conspicuousness of the nests of medullary cells, and there appears to be an increase in the number of nests, as well as in the cells constituting each nest (Fig. 41). The cytoplasm of medullary cells is almost glassy clear, which as will be described later, signifies that the cells are not actively secreting steroid material at this time but are in a state of anabolism.

(9). Ovary of aged hen. It has been possible to study the ovaries of a number of ageing birds ranging from 3 to 11 years in age, some of which have been periodically active and others completely inactive. Macroscopically, there was hardly any observable difference in appearance to the corresponding phases of the pullet ovary. Microscopically, however, the aged ovary was characterised by an increase of phenomena associated with senile tissue. There was extensive hyalinisation of the cortical stroma, resulting in a preponderance of scar tissue. Enormous quantities of pigment were to be found in the cortex, and a remarkable reduction in the number of follicles was evident.

Patches of vacuolar cells, derived from post-ovulatory follicles and from the fatty degeneration of some of the cortex cells, were of widespread occurrence, but the eosinophilic

cortical cells were reduced in numbers. Compared with ovaries from moulting hens hypertrophy and hyperplasia of medullary cells was more advanced but the cytoplasms were similarly glassy-clear in appearance. This might indicate that the output of gonadotrophin from the pituitary is reduced during moult and old age, and is not sufficient to stimulate the expulsion of steroid from medullary cells. Atresia of follicles of the type mentioned for ageing ovaries on p. 31 is commonly seen, together with other types.

ASSOCIATION OF CELL CHANGES IN THE OVARY WITH PHYSIOLOGICAL FUNCTION AS REVEALED BY CYTOCHEMISTRY.

In the development of the chicken ovary, described in the previous two sections, an examination of haematoxylin and eosin stained sections has shown that three cellular structures in the anatomy of the organ periodically change their activities, or make an appearance at significant times in the different physiological states associated with egg-laying phenomena. It is now the intention to report and discuss results of examinations into the chemical changes within the cells which reveal their nature, and indicate whether or not they are important factors in the production of intraovarian hormones. Additional evidence is provided to support the hypothesis that studies of cell activity within an organ can provide useful information for the task of relating form to function in the physiological activity of an organ. Hitherto, this had not been investigated with the chicken ovary, and thus little had been done to ascertain the precise way in which components of the ovary functioned in the process of reproduction in the intact bird.

Observations will be reported later to show that techniques

of hormone administration combined with cytochemical examinations throw a good deal of light on the function of the ovary. But before performing work of this nature, it was necessary to have a picture of the normal cytochemical reactions of cells in the various phases of ovarian activity. There has been no systematic histochemical study of the different phases of reproduction in the bird ovary, although, as reported earlier, Marza & Marza (1935) studied the chemistry of the yolk of the hen's egg, and Marshall & Coombs (1952) studied the lipids in the ovary of wild birds.

The three cellular structures in the ovary which showed any activity at all, which indicated that they were related to effects produced by the ovary, and which were amenable to histochemical study were:-

(a)   Eosinophilic cortical cells, and pigment in the cortex.

(b)   The post-ovulatory follicle (POF).

(c)   Medullary cells and atretic follicles.

Table 3 gives the reactions of different cells of a functionally active ovary to the histochemical tests employed during the present work.

(a)   Eosinophilic Cortical cells and Pigment.

Apart from definite blood eosinophil leucocytes, cells with large eosinophilic spherical granules appear in the cortex of the ovary by the 16th-17th week in the growth of a pullet (Fig.42). The granules stain positively with Sudan black B, osmic acid (Fig. 43) and Baker's acid haematin. They react with the latter both before and after pyridine extraction indicating that apart from containing some lipid, the granules are mainly composed of proteinaceous material. Baker (1946)

and Cain (1947) showed that acid haematin positive material, which was negative after pyridine extraction, indicated the presence of phospholipid, and any material remaining positive after pyridine extraction was another type of lipid in strong combination with protein. The granules of eosinophilic cortical cells did not reveal any steroids, iron or mucopolysaccharides (Table 3), and it is therefore not likely that they play any part in the elaboration of hormones in the ovary. Taber (1951) noticed these cells in the ovaries of PMS-treated birds but she failed to establish any significance for their presence. It is an interesting observation to find aggregations of cells, with eosinophilic cytoplasmic granules, in the ovarian cortex and is worthy of some discussion. As has been previously mentioned a few of them are defintely eosinophilic leucocytes with bilobed nuclei (Fig.20) but the majority are larger, with vesicular nuclei and large spheroidal granules (Figs. 37, 43). Vast collections of eosinophil leucocytes have been observed in various organs of the animal body and Wislocki et al (1947) used the appendix of man as the source of this type of leucocyte for histochemical studies of the granules. In the appendix, it is most probable that the eosinophils are attracted by bacterial activity, for it is believed that they are concerned with detoxifying activities of the body. Reference to Table 3 establishes that in the ovary of the chicken the large eosinophilic cortical cells are not mast cells, since they exhibit negative metachromasia indicating the absence of acidic mucopolysaccharides which are usually associated with mast cell granules (Asboe-Hansen, 1954). After metachromatic staining a few definite mast cells are

revealed in the connective tissue of the ovary. As eosinophils appear at a time when active atresia of minute follicles occurs, and also throughout the time when the ovary is ovulating and regressing, it is feasible to suppose that the intra-organ disruption of tissue in some way stimulates the development of the cells in this site, for mitotic activity is commonly seen amongst them (Figs. 19,37). The cells resemble the myelocytes which give rise to the corresponding blood leucocytes and thus a conclusion which could be drawn from the present work is that the ovary is at times a site of haematopoetic activity with regard to the formation of eosinophils. It is well known that in Pisces, Amphibia and Reptiles, extra-medullary myeloid metaplasia of blood cells commonly occurs (Jordan, 1938) and that in Mammals it can occur under certain cir- cumstances (Maximow & Bloom, 1952, p.98).

Irregular patches of an orange-coloured pigment are another striking feature of the ovary and it was first described by Pearl & Boring (1918). They reported that it originated in the corpus luteum, which has subsequently been shown to be a misleading term for the interior of discharged and atretic follicles in the chicken ovary. In the present study pigment occurring as clusters of rounded bodies, which may or may not be coalesced, have been found in the cortical regions of the ovaries of mature and ageing hens. It was never found in immature pullets which suggested that it was a product of extensive egg ovulatory activity in the ovary, especially as it increased in amount with age.

The pigment was positive to tests for iron but not to any other chemical test (Table 3). A lipid, not phospholipid in

nature, was associated with the sites of pigment (Fig. 44)
but it is unlikely that it is an integral part of the pigment,
since after extraction of the ovary with fat solvents the lipid-
reactive areas disappeared leaving intact pigment masses.
The distribution of the pigment was sometimes closely associated
with aggregations of eosinophilic cortical cells and, at first,
it was thought that the pigment might originate from the
breakdown of these cells. However, histochemical tests revealed
that there was no trace of iron in the eosinophilic cells
but it was abundant in the pigment.   In view of Fell's (1925)
observation that the pigment was haemosiderin a test for the
differential diagnosis of this pigment (Lillie, 1954) was made
which confirmed Fell's results.   Some of the pigment is no
doubt derived from the aborting blood vessels of post-ovulatory
follicles, since in the vacuolar tissue of old ovaries, which
represents the penultimate site of the regression of POF,
aggregations of pigment are found.   A somewhat analagous
collection of haemosiderin pigment has been found in the uterus
of mice at sites where placentae had been implanted and
blood vessels were subsequently breaking down (Jones 1947).
Some of the iron deposits in the chicken ovary could be derived from the yolk
of atretic follicles since iron is demonstrable in the yolk of hen ova.

(b). Post-ovulatory follicles (POF).

        Presence of fat laden cells and pigment in the discharged
follicles of the hen led Pearl & Boring (1918) to homologize it
with the mammalian corpus luteum.   Fell (1924) however,
showed that the fat laden cells were degenerating granulosa
cells and hence could not be compared with mammalian corpus
luteum.   Davis (1942) failed to find any proliferation of

granulosa in /the/avian POF such as occurs to form a corpus luteum
in viviparous reptiles, and which helps in the retention of the
embryo in the uterus. He thus agreed with Fell that there is
lack of relationship between the mammalian corpus luteum and the
avian POF. Riddle & Schooley (1944) chemically extracted avian
POFs and could not find any progesterone, using the sensitive
McGinty test which detects as little as 0.25 to 1.0 μg. of
progesterone. It can thus be considered that /the/avian POF is
definitely not homologous with /the/mammalian corpus luteum.
In spite of these observations Rothchild & Fraps (1944)
suggested that the avian POF is not functionless but appears to
control the destiny of the ovum it once contained, when the
latter is in the oviduct. Since Rothchild & Fraps (1944)
did not make reference to any hormone being secreted and since
Riddle & Schooley (1944) could not detect any progesterone in
the structure, it was considered worthwhile to examine the POF
using histochemistry to find out whether there really was any
basis for the production of steroid hormone. The histological
structure of the POF, its regression and reabsorption to form
vacuolar tissue have already been described. Table 3 shows the
reaction, to various histochemical tests, of both recent and
regressed POFs, including the vacuolar tissue. The recent POF
which is the one that would influence its discharged ovum in the
oviduct, is negative to the entire range of histochemical tests,
except for the degenerating granulosa cells which are slightly
sudanophilic suggesting fatty degeneration. The walls of the
follicle are just connective tissue with congested blood vessels.
The connective tissue shows faint, diffuse birefringence due to
alignment of fibres. There is no metaplasia of granulosa cells

which occurs in the corpus luteum of mammals. The fully regressed POF, which constitutes the vacuolar tissue, gives only a purple reaction with Naphthoic acid hydrazide whereas the corpus luteum of mammals (dog and rat) gives an intense blue colour indicating steroids (Ashbel & Seligman, 1949). The post-ovulatory follicle of the chicken, therefore, does not contain steroid, nor does it contain phospholipids since with Baker's acid haematin test the interior gives mainly a greyish-blue reaction indicating other types of lipid associated with proteins. In paraffin sections, small granules of iron-positive pigment were noticed amongst the vacuolar cells, which are derived from the breakdown of extravasated blood cells in the walls of a POF. Since both histological and histochemical studies fail to reveal any sign of secretory activity during any phase of the involution of the POF, it is concluded that the thecal layers function to protect and nourish the ovum during its growth. Once ovulation occurs the POF regresses rapidly, and if it does hormonally influence the fate of its ovum, at least steroids are not elaborated for the purpose. The regression of the POF is rapid especially in the first few days, so that even in a regularly laying hen it is rare to find more than 5-6 POFs.

(c). Medullary Cells and Atretic Follicles.

From observations on the histological structure of the ovary from the time of hatching to the attainment of sexual maturity, and at various times in the subsequent activity of the ovary, it was clear that the medullary cells were continuously changing in their appearance and were most likely the cells concerned in the elaboration of hormones in the ovary.

Histochemical and optical methods to determine the sites of biosynthesis of steroid hormones have been applied to mammalian ovaries and adrenals by Dempsey & Bassett (1943),Claesson & Hillarp (1947ab),Mackay & Robinson (1947),Ashbel & Seligman (1949) , Barker (1951), Rennels (1951) and Seligman & Ashbel (1952). By applying similar methods to the chicken ovary it was found that the medullary cells are the seat of synthesis of carbonyl-lipid compounds, which, since present in the ovary, most likely indicate sites of androgen and, or, oestrogen activity. The Naphthoic acid hydrazide test has been used to indicate sites of steroid hormone metabolism. However, Karnovsky & Deane (1955) have shown that much of the carbonyl reaction can be due to aldehydes produced by autoxidation of very unsaturated fats in tissues. Thus it could be argued that the positive Naphthoic acid hydrazide reaction in the cells of the ovary merely reveals the presence of unsaturated lipids and not steroidal sex hormones. In support of this argument are the facts that (a) the Sudan black B lipid reaction is positive in the medullary cells in frozen sections of the ovary and (b) the F.S.A. reaction is positive under similar circumstances, but negative in Orth-fixed paraffin sections which indicates aldehyde groups liberated from complex lipids. However, the Sudan black B and F.S.A. reactions are positive in all the secretory and resting phases of the medullary cells thus revealing merely lipid material invariably associated with sites of steroid metabolism. Moreover, cholesterol and its esters, which are generally held to be precursors in the formation of sex hormones, are easily revealed by birefringence and a positive Schultz reaction in the storage-phase medullary cells of the ovary and

when the cells are actively secreting hormone the reactions become very faint or negative. There is a close correlation between secretory activities of the cells (as revealed by changes in granularity of cytoplasm and changing birefringent properties) and secondary sex character development (Table 4) which is good, if not conclusive, evidence that the medullary cells and, or, large cortical cells are sites of sex hormone synthesis and secretion. Unsaturated lipids associated with the sites of sex hormone production could form some of the metabolites for their synthesis.

Table 4 shows the reaction of medullary cells to histochemical and optical tests at different periods of the bird's life. Their activity in relation to comb and oviducal differentiation is given in the table since they are reliable indices of active hormone production. The medullary cells give a positive reaction to all the tests when the chick hatches (Fig. 45 shows the lipoidal cells in the medulla of a 1 day old ovary). In a week old chick the medullary cells are lying sub-cortical amongst the developing follicles, and they react positively to Sudan black and Naphthoic acid hydrazide tests (Figs. 46&47). By the second or third week of life, medullary cells show a weak reaction but they then progressively become positive again until by 12-16 weeks the reaction is most intense and is demonstrated by the birefringent areas in Fig. 48. The cells during this period are gradually elaborating and storing steroid material; the appearance of some Maltese crosses in the birefringent areas indicated the presence of cholesterol esters and phospholipids. During the period 18-20 weeks the growth of the comb and more especially the oviduct, is accelerated

and corresponds with a gradual loss (secretion) of histochemically-reactive material from the medullary cells (Fig. 49). At first ovulation a weak reaction is shown indicating rapid secretion of steroid material into the blood stream. Additional support for the above interpretations can be drawn from the results of experiments, which are later described, on the effect of the administration of PMS to 12 weeks old pullets. Normally at this age there is intense chemical reaction in the medullary cells indicating storage of steroid, but the gonadotrophin stimulation caused a precocious development of comb and oviduct and a corresponding weak reaction in the medullary cells typical of observations made on pre-laying pullets.

During the laying period the elaboration of hormone material is comparatively enormous so that some cells especially in the thecal regions of the follicles continue to show medium to intense reactions while others which are secreting show weak reaction (Fig. 50). There is intense reaction in the ovaries of moulting and ageing birds (Fig. 51) which corresponds to reduced hormone secretions, and is indicated by the withering of comb and reduced oviducal activity. Both oestrogens and androgens are purported to be secreted in the ovary of the bird, but it/has not been possible to differentiate, with any degree of certainty, two different cell structures responsible for the elaboration of the two sex hormones. However, certain observations can be discussed which throw light on the problem. It will be recalled that large cells with vesicular nuclei and weakly basophilic cytoplasms have been described as forming a part of the ovarian stroma. They are different from the fibroblastic type of stroma cell (Figs. 6,7) and in the active ovary are

sometimes sudanophilic and birefringent (Figs. 49, 50) forming a diffuse backgound in the cortex. Now, it has been shown that medullary cells migrate during ovarian growth from the medulla of the chick's ovary to the cortex and become intimately incorporated in the stroma. Owing to morphological and histochemical similarity of some secretory stages of the medullary cells with the larger type of cortical cells, it is difficult to single out for study their separate activities in the ovarian function. If one concedes the established hypothesis that the medulla of the ovary secretes androgens and the cortex oestrogens, a theoretical explanation can be offered that the medullary cells in the cortex, derived from medulla, elaborate the androgens, and the large cortical cells the oestrogens. Conclusive cytochemical evidence will have to await the development of tests to distinguish between oestrogens and androgens which are, chemically, two very similar compounds. Studies of secondary sex character changes are of no help in determining the source of the two hormones in the normal ovary since the comb and oviduct, which are good criteria of male and female sex hormone activity respectively, develop and regress simultaneously.

A last point to be discussed in connection with medullary cells and sex hormone production and secretion is the phenomenon of atresia. In all minute atretic follicles there are found aggregations of cells giving a positive reaction to histochemical tests for lipid, cholesterol and other steroids (Table 3 and Fig. 48). Since atresia of minute follicles occurs quite frequently a few weeks prior to first ovulation at a time when the oviduct commences its rapid growth phase, it would seem that there is some connection between the two observations. The

cells which appear in the thecae of follicles are either medullary cells or the larger cortical cells. For reasons stated above, it is not possible to differentiate the two, but they correspond to what have been called "luteal cells", "interstitial cells" or "medullary interstitial cells" in the past. It is suggested that the phenomenon of atresia of minute follicles serves to build up a reservoir of hormone precursors. This view would appear to be in agreement with that of Marshall & Coombs (1952) who in discussing the lipid changes in the ovaries of wild birds described a "lipoidal atresia" of small follicles functioning to produce a cholesterol reserve and contributing to the formation of "interstitial cells" just prior to the breeding season.

The only other site in the ovary which reacts positively to histochemical tests used in the present study, is the yolk of ova. In minute follicles the primordial yolk is situated around the Balbiani body and in larger follicles it forms a peripheral layer of granules. It gives intense reaction to all the cytochemical tests except birefrigence in polarised light when the activity is low. Allen et al (1924) and Marlow & Richert (1940) chemically extracted follicles of the chicken ovary and detected oestrogens, especially in small follicles. Kopec & Greenwood (1929) reported that an oestrogenic action of the yolk from fully formed eggs could be demonstrated by the development of female plumage after the injection of the yolk into ovariectomised hens. From the present work it is not considered that the presence of steroid material in yolk plays any significant part in the hormonal control of ovarian activity in the bird since the parts mentioned above are intensely reactive at all times in the development of the ovary, and the

yolk is a constant feature of follicles from the earliest stages in their development. The fact that the abovementioned workers detected oestrogen in follicles could be accounted for by a diffusion of steroidal material into the ova, via the granulosa cells, at times when medullary or large cortical stroma cells are actively synthesising and secreting hormone.

OVARIECTOMY IN RELATION TO ANDROGEN PRODUCTION.

Ovariectomies were performed in the present work to find out whether it would be possible to see, in the induced growth of the right gonad, cells which could be compared with the particular type in the chicken ovary which secrete androgens. The right gonad in the normal laying hen is a small and almost indistinguishable remnant lying on the post-caval vein beside the right adrenal gland (Fig. 3). Goodale (1916) reported the regeneration of the right gonad following ovariectomy in the pullet. Subsequently the extensive researches of Benoit (1923, 1924) and Domm (1924, 1927, 1929 a) and Zawadowsky (1926, 1927) showed conclusively that the rudimentary right gonad hypertrophied following ovariectomy and the poulard developed the plumage and head furnishings characteristic of the cock. The fact that the hypertrophied right gonad produced an androgenic factor causing comb growth was established by these workers from the observation that surgical removal of the right gonad resulted in the head furnishings reverting to the pale shrunken condition as seen in a capon. In the present work, to study the endocrine influence of the hypertrophied right gonad and more especially the cellular basis of hormone secretions, 25 young pullets ranging from 6-10 weeks in age were ovariectomized. A few birds died during surgical operation and in a few cases the left ovary regenerated

due to incomplete removal of the organs. However, out of the 25 young pullets, it was eventually possible to get 18 complete poulards showing male plumage and head furnishings. These birds were killed at varying intervals of time after ovariectomy for histological and histochemical study of the developing right gonad.

Domm (1927) showed that ovariectomy in the pullet could result in varying responses. The hypertrophied right gonad could develop either into a testis-like growth, an ovotestis or a pure ovarian structure. He explained this variation on the basis of the embryological composition of the right gonad at the time of ovariectomy. If the right gonad consisted of medullary elements only, as is frequently the case at the time of ovariectomy, the resultant right gonad would develop into a testis-like organ. If the right rudiment in embryogenesis developed, in addition, a cortex region which persisted until the time of operation, the hypertrophied right gonad could develop into an ovary or ovotestis, depending on the extent of cortical tissue present when it was induced to develop.

In the present work two birds developed ovotestes, but no typical right ovary structures were ever encountered. Birds with ovotestes retained their female plumage except for a brief, initial, transient, gonadless period when male plumage began to develop. The ovotestes had a core of sterile tubules with a few peripheral patches of follicles which were small and did not exceed 700 μ in diameter. Occasionally some could be seen undergoing atresia, with groups of glassy-clear medullary or large cortical stromal cells surrounding them (Fig. 52). The ovotestis structure described here differs in some respects from that reported by Domm (1927). He found that the organ

consisted of an anterior testis portion and a posterior ovary
portion with pedunculated follicles sometimes reaching 1.5 cm.
diameter. Greenwood (1925) described an ovotestis similar in
structure to that found in the present work.

A typical testicular right gonad is well developed 4 to
6 months after ovariectomy and is easily seen as a lobulated
organ lying along the right iliac and post-caval veins (Fig.53).
Goodale (1916) described the structure perfectly when he
mentioned it as resembling, macroscopically, a small testis
divided into lobes, but resembling neither testis nor ovary
in histological structure. He called it early nephrogenic tissue
with the Wolffian body and ducts undergoing compensatory hypertrophy.
There is remarkable uniformity in the descriptive accounts of
the hypertrophied right gonad given by subsequent workers (
Benoit 1923, 1924; Greenwood 1925; Domm 1927; Gray 1930).
Domm (1927) described the right gonad as a testis-like organ
lacking spermatogenesis in the tubules. But later, observations
of Benoit (1924) and Zawadowsky (1926, 1927), reporting
spermatogenesis in the hypertrophied right gonad, led Domm
(1928, 1929 b) to re-examine the problem. He concluded that,
since Benoit's (1924) and Zawadowsky's (1926, 1927) pullets
were operated on when under 6 weeks old and his were done between
3-6 months, the age at the time of operation was a significant
factor in determining whether spermatogenesis would occur.
However, in a series of further experiments performed on birds
less than 30 days old, Domm (1929 b,1930) was able to induce the
formation of varying degrees of spermatogenesis and this was
explained as being due to the inclusion in the right gonad of
primordial germ cells, which ordinarily disappear from the

right side a month after female chicks hatch (Brode, 1928).

was
Spermatogenesis, whenever it occurred ../ atypical and the

spermatozoa abnormal; artificial insemination into a pullet

of sperms from the right gonad did not result in any fertile

eggs (Domm, 1930).

In the present work it was not intended to stimulate

spermatogenesis, but just to study any active hormone-secretory

tissue from the right testis-like gonad which exerted

masculinizing effects. The responsible cell groups could then

be studied and compared with cells of the normal ovary. All

birds were therefore ovariectomized when more than 5 weeks old

and only those right gonads were studied which, from outward

appearances of the poulards, were exerting androgenic effects.

a
The right testis-like gonad when formed was/ lobulated

structure lying on the right iliac-post caval vein junction.

Its ventral free surface was convex in shape and the dorsal ,

attached surface considerably flattened. The outline and

size varied from bird to bird, and with the stage of development,

but more frequently the anterior end was broad and round while

the posterior end tapered into a vas deferens extending to a

varying length over the right kidney. The gonad was covered

by a thin coelomic epithelium underneath which was a layer

fibrous connective
of vascular/tissue corresponding to the tunica albuginea of

the testis. At 4 - 6 months of development connective

tissue strands from the tunica albuginea penetrate

inwardly into the gonad, dividing it into lobes.

Each lobe consists of dense connective tissue stroma in which are found cords of cells and sterile tubules (Fig. 54) either closely packed or widely distributed. More often they are closely packed in one part of a lobe and widely distributed in another. In certain regions of the gonad the tubules are small or solid, and in others, they are more developed in size. The largest tubules resemble seminiferous tubules of immature cockerels and consist of a single layer of cells standing on a thin basement membrane. The cells have vesicular nuclei placed basally close to the membrane, and their distal ends extend into the lumen of the tubule and almost completely obscure the cavity. In some of the tubules, but more commonly in the smaller type, the cells show mitotic activity and some of them slough off into the lumina of a few of the tubules (the cells do not appear to be primordial germ cells). Inter-tubular tissue is scanty, consisting of blood elements and connective tissue. More often, the central parts of lobes in the right gonad are occupied by differently shaped cords of cells firmly embedded in abundant fibrous connective tissue.

Lying underneath the connective tissue capsule of a part of the right gonad is a group of tubules lined by cuboidal epithelium, representing the hypertrophied Wolffian body which forms the epididymis of the right gonad (Fig. 55). The tubules which are embedded in fibrous tissue, lead into the straight Wolffian duct (vas deferens) which has already been described. Frequently the lumina of the tubules are filled with eosinophilic colloidal secretion.

The foregoing account of the structure of the right gonad agrees in all essential points with the descriptions given by

previous workers. None of the workers except Gray (1930), was concerned with the cellular basis of hormone secretions. He studied the hypertrophied right testis-like gonad at different stages of its growth and suggested that the cell cords and tubules, which he called medullary cords, were the source of hormone secretion. He argued, that the connective tissue, blood elements and lymph nodes could not be the source of androgens and since interstitial Leydig cells were absent, the medullary cords and tubules were the only elements which could be secretory. Gray made a significant observation that in the earlier stages of its growth, the right gonad was full of medullary cells some of which were fat laden. Later, the fat laden cells transformed, lost their fatty, vacuolar appearance and acquired granular cytoplasm. The cells increased in number and were thought to differentiate into cords or tubules. From Gray's diagrams, it would appear that the fat laden medullary cells are identical with those which are in the medulla of a day-old chick ovary, and which are also present in the rudimentary right gonad at the time of hatching. According to Gray, the well known phenomenon of the reversion to the female feathering in the poulards after about $1\frac{1}{2}$ years of its existence is due to a secondary proliferation of cell cords from the germinal epithelium. The secondary cords, though indistinguishable from the primary when incorporated in the gonad, were equivalent to the cortex of the ovary and secreted oestrogens for the ultimate henny plumage of the poulard.

In the present work, in addition to an ordinary histological study of the right gonad, a number of histochemical tests for steroids were applied to determine the cellular source of

secretions. The cords of cells in the immature gonad were faintly positive to Schultz's test for cholesterol but gave a stronger reaction than that in the tubules which later differentiated from the solid cell cords. The same staining pattern was true for Naphthoic acid hydrazide reaction. Under the polarising microscope, one could detect small faint birefringent areas scattered throughout the tubules when early formed. When frozen sections of the gonad were stained with Phosphine 3R and irradiated with UV light, a faint, diffuse, greenish fluorescence was visible which was difficult to localise to any particular cell type. Sudan black B staining showed that there was scattered lipoidal material throughout the entire gonad structure both in the immature state and later when tubules were differentiated. The epididymal tubules and the fibrous connective tissue stroma were negative, but there was a little lipoid in tissue immediately surrounding the tubules.

All the above histochemical reactions indicate that the cords of cells in the right gonad are the only possible sites of hormone production. The tubules which are derived from these cords as development proceeds are slightly less reactive, and the connective tissue and hypertrophied Wolffian body are entirely negative. All the reactions showed that there was no abundant storage of steroids in the gonad at any time, and indicated that cells were in an active secretory phase, corresponding with the rapid masculinisation process which occurs after ovariectomy. The observations recorded are in accord with the accepted views that, histochemically, positive material is less likely to be revealed during the height of secretory activity (Claesson & Hillarp 1947 ab),

Claesson et al. (1949) since secretory products are quickly dispersed. The distinction between actively secreting cells and those which never elaborate secretions is made obvious by examining them in all physiological conditions of an organ. The latter never reveal any reactive material, but the former reveal it in abundance when in the storage phase, and only sparsely in the active phase.

Assay of Hormone Substances from the Right Gonad of the Poulard.

In the assay no significant results were obtained either from painting day-old chick combs with testis or right gonad extracts. The chicks receiving known dosage of testosterone propionate, however, showed growth of combs. 45 ml. blood from poulards when extracted failed to reveal the presence of any active sex hormone. From these results, it is concluded that with the quantities of tissue used, insufficient hormone was obtained to survive the extraction procedure, and to produce growth response when subsequently used for assay.

EFFECTS OF HORMONES ON CELLULAR STRUCTURE OF THE OVARY.

(a) Gonadotrophin administration.

For the present work, it seemed desirable to re-examine the effects of gonadotrophin on the cellular changes in the young ovaries, using histochemical as well as ordinary microanatomical methods in order to gain further evidence for the activities of particular cells in the physiological functions of the ovary. Follicle stimulating hormone (FSH) is known to cause growth of follicles in the ovaries of adult hens, and such follicles can be induced to ovulate by the administration of luteal hormone (LH) (Nalbandov & Card, 1946). Previously it was found that high doses of FSH caused cessation of egg-laying after a few days due to the production of too many ovulable follicles

for the endogenous LH to have any action on them. (Bates, et al 1935). In pullets less than 120 days old, there is general agreement that precocious growth and ovulation of follicles cannot be induced by FSH and LH stimulation. Only a few conflicting reports exist, however, on the effects of such stimulation on the cells of immature ovaries. Nalbandov & Card (1946) were unable to observe any effect of FSH on the growth of minute follicles before the chick was 120 days old, although slight weight increases were noticed in the ovaries and oviducts. The increases were attributed to medullary hypertrophy but no constituents of the medulla were examined. With FSH therapy Taber (1946) failed to observe any growth in the cortex of the ovary of young chicks less than a week old, but reported extensive medullary growth. She stated that there was inhibition of follicle growth in chicks less than four weeks old, but in those of 12 weeks, there was no inhibition. The growth rate of the medulla was stated to decrease in birds of 4 weeks onwards, until in 8 week old pullets there was little noticeable medullary growth. Polyovular follicles were observed in treated birds at all ages. Oviduct hypertrophy was noticeable in treated birds older than two weeks, and in three week olds the oviducts of treated birds showed growth resembling that of preovulatory condition. The works of Domm & Van Dyke (1932), Domm (1934) and Asmundson et al (1937) somewhat contradicted the above findings. They reported that gonadotrophins not only caused increase in ovarian weight but also the growth of follicles in some young chicks. Asmundson et al (1937) showed that if treated before 3 weeks there was stimulation in the growth of interstitial cells only in the cortex, but in older birds (6 weeks) both

interstitial cells and follicles exhibited increased growth.
It was not stated clearly how the interstitial cells were typed.
Prolonged P M S administration resulted in the formation of
mis-shapen follicles which were pushed out of the ovary due to
extreme cellular development and crowding in the cortex.
Domm & Van Dyke (1932) and Domm (1934) used on 3-8 week old
birds a gonadotrophic substance from sheep pituitaries
(Hebin), and reported increase in follicular and interfollicular
tissue after two weeks' treatment. In younger chicks Domm
(1937) could not detect follicular growth and the ovarian
weight increase after Hebin treatment was explained as being entirely
due to medullary growth.

As there was an appearance of premature oviduct and comb
growth with gonadotrophin treatment it was stated that oestrogenic
and androgenic substances were being produced in the ovary.
Variable results reported as to the time and degree of stimulation
of various structures were no doubt due in part to the different
hormones used, and other variable experimental conditions, such
as the dosage of hormone, length of treatment, the age and breed
of birds for experiments. In looking for the source of hormone
secretions in P.M.S.-treated pullets one might be inclined to look
for the induced development of a right gonad, since it is known
that after sinistral ovariectomy in pullets, the right gonad
develops and exerts an androgenic effect on comb growth.
However, Domm (1933) showed that after gonadotrophic treatment
the masculinizing effect on the head furnishing was due to
androgen secretion from the ovary itself as there was no growth
of the right gonad. Further, he found that Hebin failed to
cause comb enlargement and acceleration in the growth/of right
the
gonad of sinistrally ovariectomised pullets in the same time

72.

that effects are made visible in normal treated young pullets. Precocious comb growth after Hebin injections in normal pullets was thought by Domm to be due to observed hypertrophy of medullary tissue in the ovary, but he did not mention either any cellular changes or offer any explanation for the simultaneous oviducal growth which is normally dependent on oestrogens. Taber (1948, 1951) later showed that nests of vacuolar, lipoidal cells (medullary cells) when caused to hypertrophy by gonadotrophic stimulation developed granular cytoplasm. Cessation of PMS injections resulted in the reappearance of vacuolar, lipoidal cytoplasms and correspondingly the comb regressed in size. From these observations Taber concluded that the stimulated medullary cells were responsible for androgen secretion. She was aware of the oestrogen production in PMS-stimulated ovaries of young pullets since the oviducts increased in size, but she did not attempt to explain its source.

Together with comb and oviduct growth general histological observations in the present work show that, compared with the normal pullets of two weeks, treatment with gonadotrophin caused a general size increase in the ovary but the hypertrophy of the medullary region was proportional to that of the cortex (Figs. 56,57). This is contrary to the views of Domm (1937) and Taber (1948, 1951) who reported great increase of the medullary component. A noticeable change in the cortex of a 2 week old pullet after gonadotrophin treatment was the inhibition of follicle growth (Figs. 58,59). There was widespread collapse of existing follicles, especially of very minute ones, and thus, the cortex showed fewer follicles compared with the control pullet. There was increased mitotic activity in the interfollicular tissue of the cortex contributing to growth.

If this interfollicular tissue is identical with that termed "interstitial" by Asmundson et al (1937) then observations in the present work agree, in that there is an increase in such tissue which might eventually cause mechanical extrusion of follicles. After gonadotrophin treatment a large proportion of medullary cells migrated into the cortex and developed very granular cytoplasms indicating secretory activity. In the normal ovary of the same age, most of these cells had only slightly granular cytoplasms. Thus the precocious comb and oviduct growth noticed in treated birds paralleled the change in the state of medullary cells.

In 13 week old pullets, when normally most of the medullary cells appear in the glassy-clear, inactive state, PMS treatment resulted in the transformation of these cells into the active secretory phase shown by the formation of granular cytoplasm (Figs. 60,61). This change was most striking and corresponded with stimulated comb and oviduct growth. The follicular growth still appeared to be inhibited by treatment at this stage, but the inter-follicular portion of the cortex was vastly increased and a general size increase of the ovary was again induced. The diameter of the largest follicles from treated and control ovaries both ranged from $500-600\mu$ and the medullary growth was in proportion to the growth of the cortex.

In a group of pullets, 19 weeks old, when rapid comb growth and oviduct differentiation occur normally, PMS treatment resulted in a great increase in the size of the ovary (Fig. 62, c.f. Fig.27) but no macroscopic follicles were visibly made to develop on the ovarian surface. Extensive oviduct and comb growth was also induced. Normally, in birds of this age, the ovary remains small and the medullary cells are in a granular

(secretory) phase. After PMS treatment the medullary cells still appeared in the granular phase and there was a noticeable increase in the size of follicles (Figs.63, 39). It appears that the response of minute follicles at this age and not earlier, points to the existence in the ovary of a phenomenon of gradual development of sensitivities of component structures to hormone stimulation. The one uniform result induced by PMS in pullets of all ages, was the formation of a large number of polyovular follicles; no binuclear follicles were ever encountered.

Histochemical observations support the findings of a secretory activity displayed by medullary cells. The ovaries of 2-week old PMS-treated pullets showed that sudanophilic material was present inside the surviving follicles as black spheres of primordial yolk. Collapsing follicles also showed a striking sudanophilia but it was difficult to localise it to any particular cell type. The medullary cells, which were mainly subcortical in position, were faintly positive to Sudan black and faintly birefringent. No fluorescence was observed in any part of the ovary nor was any Schultz-positive material indicated. Compared with a medium to intense reaction in normal 2-week old chicks (Table 4.), the Naphthoic acid hydrazide reaction was only faintly positive in the medullary cells, but a more positive reaction appeared in the yolk of intact and collapsing follicles. In older treated birds (13 weeks and 19 weeks) all the histochemical reactions cited above followed the same pattern. However, the yolk inside the larger follicles, and the contents of the blood vessels fluoresced in ultra-violet light, the significance of which was not clear. The above results indicated that induced comb growth and oviducal differentiation of PMS-treated birds closely corresponded with the loss of histochemically reactive material

from the ovaries, showing that the hormonally active principles
in medullary cells were mobilised to participate in the changes
noticed. . Correlated with the loss of chemically reactive
material was the striking histological change in the medullary
cells of PMS-treated pullets, namely the transformation from
the glassy-clear (storage phase) to the granular (active secretory
phase) cytoplasm. These results are in accord with those
reported by Claesson & Hillarp (1947ab) Claesson et al (1949)
and Rennels (1951), who showed mobilization of histochemically
reactive sterol from the ovaries of rabbits, guinea pigs and
rats after gonadotrophic stimulation.

Experiments with gonadotrophins again show that the medullary
cells play a very important role in hormone secretions in the
bird ovary. Domm (1937) was of the opinion that a hypertrophied
medulla after PMS-treatment was responsible for comb growth, but
present observations did not show any disproportionate growth in
the medulla. Thus it was necessary to analyse the cell components
of the ovary for causative agents. It has already been shown
that in the normal ovary, during growth, the medullary cells of
the immature chick multiply and later migrate into the cortex.
It is a forced migration owing to the increasing development of
vascular channels in the medulla of the ovary. Embryologically,
the cells originate in the medulla but due to the migration,
they become intimately incorporated in the stroma of the cortex.
Sections stained with haematoxylin and eosin show that granular-
phase medullary cells, i.e. when secreting, can hardly be
distinguished from certain large cortical stromal cells.
Androgens and oestrogens are chemically not very dissimilar and
the histochemical tests devised for the one also reveal the other.
So, until such time as cytochemical tests for differentiating
the two compounds become available, one is justified only to

make the observation that the secretions of medullary cells, together possibly with other cells of the cortex, are responsible for comb and oviducal growth in the chicken.

Oestrogen administration.

It is well known that oestrogens, when administered to intact females in sufficient quantity, cause ovarian atrophy; the effect being produced indirectly by inhibiting the gonadotrophic activity of the pituitary (Burrows, 1945 p. 260). The arrested development of the gonad is not permanent, since, if oestrogen treatment is discontinued, normal conditions are restored. Taber (1951), in an attempt to suppress ovarian function, treated immature female chicks, when a few days old, with oestrogens. The hormone caused a retardation in the rate of comb growth and the phenomenon was correlated with the appearance of a larger quantity of osmiophilic and basophilic material in the medullary cells. Thus, exogenous oestrogens inhibited the secretion of androgens from the cells which reverted to an inactive accumulating phase. From these observations, Taber concluded that medullary cells in the medulla of the ovary secreted androgen.

In the present work, it was intended to inhibit pituitary gonadotrophic activity to attempt to reveal cellular changes in the ovary correlated with comb regression. Pullets were treated with oestrogens at different ages, but it was not possible to induce comb growth regression in any age group of birds. There was only one uniform result produced by oestrogen treatment in immature birds, and that was hypertrophy of the oviduct by direct action of the hormone. In laying pullets the already grown oviducts were naturally unaffected by oestrogen, but oviposition ceased after the 2nd or 3rd injection and all the large follicles became atretic. After treatment, in one

5 year old non-laying hen, the oviduct at autopsy was well differentiated and a shelled egg was recovered from the uterus. This could be considered as an unusual, isolated instance of beneficial stimulation by oestrogens. However, it was not possible to repeat the rejuvenation of other older hens.

Histological examination of the ovaries of oestrogen-treated pullets and hens did not reveal any significant structural difference from untreated ovaries, except that mitotic divisions seemed to have increased in the germinal epithelium of immature birds. These observations are in accord with the view that the administration of sex hormones has no outstanding direct effect on the tissue of organs which elaborate the particular hormones. Under the conditions of the experiments in the present study, the histological changes in the ovary and the comb regression reported by Taber (1951) for chicks of a few days old could not be confirmed in pullets ranging from 1-18 weeks old. The observations were fully in accord with unchanged structure in the medullary cells.

ADRENALECTOMY IN RELATION TO SECONDARY SEX CHARACTERS AND THE STRUCTURE OF OVARY.

Since the adrenal glands of animals are embryologically intimately connected with the gonads, and both sets of organs produce steroid hormones, it was considered worthwhile to study the role of the adrenal in determining the functioning of the reproductive processes. Extensive researches dealing with the effect of adrenalectomy on the gonads and pituitary gland of mammals have been reviewed by Parkes (1945) and Zuckerman (1953). However, there have been comparatively few similar studies on the fowl. Parkin (1931) examined a few constituents of the blood of adrenalectomized fowls and reported an increase in uric acid content and a decrease in blood sugar as a result of adrenal

insufficiency. He stated that unless the operated birds were given adrenocortical extracts, death occured from 38-146 hours after the removal of the second gland. Parkes & Selye (1936) reported adrenalectomy in ducks and chickens to be rapidly fatal unless substitution therapy was resorted to. Sub-total adrenalectomy of 8 weeks old male chickens, according to the latter authors, resulted in a decrease in the size of testes and a temporary disturbance of their endocrine activity. The saddle feathers of a Sebright male showed a few millimetres (equal to a week's growth) of capon-like feathers which after one week reverted to the normal henny plumage of the Sebright cock. Bulbring (1937) showed adrenalectomy in drakes to be fatal within 8-10 hours depending on the time of year when the operations were performed. Herrick & Torstveit (1938) studied the effect of adrenalectomy on 5-6 month old cockerels and reported that life could be maintained up to 82 days by the administration of adreno-cortical extract and salt water. In such birds, the comb started regressing within 2 days of the operation and the testes became smaller in size. Histologically the epithelium of seminiferous tubules appeared to break down and obscure the lumina. From their observations, Herrick & Torstveit concluded that the adrenal glands played an important part in maintaining the testes of the chicken. Their results were subsequently confirmed by Hewitt (1947) who observed a decrease in testis size even after unilateral adrenalectomy. He also showed that as a result of bilateral adrenalectomy of ovariectomized pullets the right gonad failed to develop and in intact pullets unilateral or bilateral adrenalectomy resulted in an inhibition of the growth of the ovary.

However, there are no reports in the literature on the

effects of adrenalectomy on the structure and histology of the ovary in the female fowl. For the present work, 25 pullets were adrenalectomized to study the cellular aspect of adrenal-ovarian relationship in the chicken with a view to further examining cells most likely to be concerned in secreting hormones. Tables 5 & 6 give the ages, comb measurements, nature of operation and other details of the birds studied. A few pullets are not included in the tables since they died during the operations, owing to haemorrhage or failure to emerge from anaesthesia. The latter phenomenon occured more commonly during the second operation when the birds possessed only one adrenal gland, and thus it is quite feasible that such deaths were due to a lowered resistance to the anaesthetic which is known to occur through adrenal insufficiency (Zuckerman, 1953). At post-mortem examinations no other causes of death could be found. The birds were divided into four main groups:

(1) Birds 1-6.   Unilaterally adrenalectomized; either gland with, or without ovary (Table 5.).

(2) Birds 7&8.   Bilaterally adrenalectomized and ovariectomized. Maintained on DOCA (Table 5).

(3) Birds 9-20.   Bilaterally adrenalectomized without post-operative therapy (Table 6).

(4) Birds 21-25. Bilaterally adrenalectomized; maintained on DOCA and salt water (Table 6).

In the first group, contrary to Hewitt's observation on cocks, it was found that unilateral adrenalectomy in the pullet did not interfere with ovarian development. Birds 1 and 2 both developed normally and laid eggs, commencing at the normal maturity age for the flock. In those pullets (3-6) in which the ovary was removed together with the adrenal (Table 5), male plumage developed and the comb enlarged as in the case of

poulards . At autopsy, a right gonad was found in all the unilaterally adrenalectomized-ovariectomized birds except No. 6 which had a small **regenerated** left ovary. In the latter case, it appeared that regeneration of the ovary had started late after the operation, for the comb was very large and thick at the base, and the plumage was of male type for some time.

In the bilaterally adrenalectomized-ovariectomized group (Table 5), many birds died during, or soon after, the second operation (removal of second adrenal gland and ovary) in spite of operating on slightly older chickens which was found efficacious when dealing with birds in the third group. The two bilaterally adrenalectomized-ovariectomized birds that survived were given 2 mg. of DOCA, 48 and 24 hours before the second operation and afterwards daily for a week. The dose was then gradually reduced and given at longer intervals. Both birds developed male plumage and head furnishings and at autopsy showed the growth of small right gonads. Hewitt (1947) did not mention effects on plumage or head furnishings but reported that the right gonad did not develop in ovariectomized bilaterally adrenalectomized birds. However, since he kept his birds only for two months after the last operation, it is possible that there was not enough time for the right gonad to develop, as in the present study the right gonad was found to be small even at six months after the second operation. This shows that the rate of development of the right gonad is retarded if both adrenal glands are removed together with the ovary. A further explanation for the discrepancy between the results of Hewitt and those of the author could be in the fact that the former ovariectomized his birds when they were 16 weeks old. In the present work it was found that even in 16 week

old birds with intact adrenals, there was not as rapid a development of the right gonad after ovariectomy as in similarly operated young birds, i.e. 6 to 8 weeks old. At this point, it should also be mentioned that it is possible for the rudimentary right gonad, which lies very close to the adrenal gland (Fig. 3.) to suffer damage during right adrenalectomy, thus resulting in failure to hypertrophy subsequently.

In the third group detailed in Table 6, no post-operative treatment was given after bilateral adrenalectomy except salt in the drinking water. Birds 9-15 died within a day or two after the second operation and changes were not apparent in the ovarian structure. Birds 16-20 which were completely adrenalectomized at 12-18 weeks old survived better than birds 9-15 which were 6-10 weeks old at the second operation. This phenomenon is similar to the findings of Sisson & March (1935) who reported that older rats survived bilateral adrenalectomy better than younger animals. It is known that the ovary can produce a substance which prolongs the life of the adrenalectomized animals (Emery & Schwabe 1936), and thus the uniformly fatal result of bilateral adrenalectomy-ovariectomy with out substitution therapy in 6-10 week old chickens, referred to in the second group above, may be due to lack of such ovarian secretions. The deaths of birds 9-15 after bilateral adrenalectomy could be due to the inability of the ovaries to secrete the survival factor during the early stages of their development, since it was found that birds 19-20 which were operated on at 12-18 weeks survived reasonably well. The external effects produced by bilateral adrenalectomy were a slight decrease in comb size and in those surviving 20 days or more,/general weakness and apathy for food. There was no change in the new growing

feathers. At death, the ovaries were found to be not much different in outward appearance from normal birds of the same age, but they were much smaller in size.

Microscopically the ovary showed degenerative changes in its various components. Follicular growth ceased and the differentiation of the theca into interna and externa regions was ill-defined; instead, the follicles were surrounded by the adjoining stromal cells. Blood capillaries, so characteristic of the normal thecal layers, were no longer prominent with the result that the follicles appeared to be surrounded by a compact and somewhat avascular cellular envelope (Fig. 64). Medullary cells were not conspicious, and the medulla region of the ovary showed a most striking change in the extensive development of variously shaped spaces (Figs. 65, 64).

The above changes were noticeable in all the birds which survived from 2 to 10 days after the removal of the second adrenal gland. In the two birds which survived more than 20 days, ovarian atrophy was most striking. In these cases, the space invasion of medulla extended well into the cortex resulting in the marked reduction in the thickness of the compact cellular layers surrounding the follicles (Fig.66). Apart from these perifollicular cells, all the other cortical cells seemed to disappear. The phenomenon of the normal type of atresia, though present, was not very common but instead a peculiar type of follicular degeneration could be seen in a few follicles (Fig. 67). The cytoplasm and the germinal vesicle of such follicles liquefied and at the same time the granulosa and the ill-defined thecal layer collapsed. Ultimately, the disorganized thecal and granulosal cells together with the collapsed follicle were eliminated by fat infiltration.

In the fourth group (table 6) pullets were completely adrenalectomized when 12-15 week old. They were twice injected with 2 mg. DOCA, at 48 hours and 24 hours before the second operation, and then the same amount was given daily for a week or ten days after the operation. Thereafter, the dose was reduced to 1 mg. twice a week, and finally it was given only once a week or fortnight. With this treatment two birds (21,24) died within 40 days and three (22, 23, 25) survived for 6-8 months. In all these birds, except no. 24 which lived for only 21 days, comb enlargement was noted as against the decrease of comb size seen in the third group. The plumage remained the normal female type but none of the birds laid eggs. The ovaries were immature and showed a few fluid-filled follicles on the surface. Histologically, they resembled the ovaries from birds which did not receive DOCA (Fig. 66). There was some oviduct growth and differentiation into regions, but compared with a laying pullet of the same age it was much smaller in size. Oviducal differentiation and comb growth as noticed in the present case may be due to either indigenous ovarian secretions, or the action of DOCA, or a product of a complex inter-conversion of steroids in the bird body. An androgenic action of DOCA on the comb of capons, and an oestrogenic action on the oviduct have been discussed in a review by Parkes (1945). Adrenocortical hormones are known to be converted into androgens by liver and other tissues (Sayers 1950) and general interconversion of steroids in the body has been widely reported (Samuels 1949, Hechter et al 1950 and Hechter et al 1951).

In conclusion, one may summarize that it is possible to maintain adrenalectomized pullets for a long period on DOCA and salt water, but in such birds, the ovary remains small and immature, and cannot produce enough oestrogens for

full development of the oviduct. The ovary itself becomes degenerate, the thecal cell layers are absent and there is extensive development of spaces in the medulla.

Summary and Conclusions.

Histological and cytochemical methods have been used to study changing cell activities in the chicken ovary during different artificial and natural physiological states of reproduction. A continuous series of observations have been made on the ovary from the time of hatching, through puberty, the laying season and moulting to old age. Several interesting phenomena have been revealed.

(1) Atresia of minute follicles is evident in the ovary commencing at the 4th week of life, and reaches maximal activity in the period bordering on sexual maturity. The Schultz test and birefringent optical properties reveal at times an abundance of cholesterol and its esters in cells of these atretic follicles, which indicates that the phenomenon serves to build up a store of sex hormone precursors prior to functional reproductive activity.

(2) Polyovular and binuclear follicles are present in the ovary during the growing period prior to sexual maturity. It is a normal occurrence and such follicles are destined for destruction so that it is rare to find them in a fully functional ovary. Double yolked eggs frequently laid by pullets are not due to the ovulation of biovular follicles but to a simultaneous ovulation of two follicles at the flush period of the commencement of laying.

(3) It has been shown that medullary cells react positively to the Naphthoic acid hydrazide and other tests for ketosteroids, which suggests that they are the source of hormone secretion in

the ovary. Further evidence to support this hypothesis was gained from observations that the cells changed in their reactions during different reproductive phases of the ovary, which corresponded to simultaneous changes in the development of secondary sex characters of the bird. In the Brown Leghorn the development of these characters is a reliable criterion of sex hormone activity.

Investigations show that, chemically, it is at present impossible to distinguish two different cell types responsible for the production of androgen and oestrogen hormones in the ovary, although certain salient observations bearing on the problem have been made. Medullary cells possess clear cytoplasms when in the medulla of the ovary at the time of hatching. They multiply and migrate into the cortex after the first week, and later some are incorporated in the thecae of small developing follicles whilst others become intimately mixed in the stroma of the ovarian cortex. The latter at times are difficult to distinguish from certain large types of cells in the cortex of the ovary.

Apart from these two cell types no other cells or tissue structures in the ovary showed any indication of being concerned in hormone production. There appeared to be three possibilities for the sites of sex hormone synthesis and secretion. Firstly, that the medullary cells produce androgens, secondly the large cortical cells elaborate oestrogens and thirdly the two cell types act synergistically to produce chemical mediators, which have androgenic and oestrogenic effects on target organs according to the differential development of responsiveness in the latter. On present evidence it is not considered that there is sufficient proof to make a distinction between these possibilities.

(4) Experiments of administering gonadotrophic hormones to differently aged birds showed that comb and oviduct growth

could be induced to develop together with simultaneous changes in the cytoplasm of medullary or large cortical cells. From histological and histochemical observations it was considered that additional evidence was provided to show that these cells are intimately concerned in the elaboration of the sex hormones in the ovary. Contrary to previous work, no disproportionate growth of the medulla in the immature ovaries was noticed to account for the androgenic effects on secondary sex characters following gonadotrophin administration.

(5) Studies on the hypertrophied right gonad of the poulard, which is purported to secrete the androgens responsible for comb growth and male plumage, showed that cords of cells (which had previously been shown to develop from infantile medullary cells by Gray, 1930) were the only histochemically reactive elements in the right gonad from which steroids could be produced. It was not possible to extract chemically any androgenic material from the right gonad but this failure was most likely due to either the unsuitability of the extraction procedure for small quantities of tissue, or that the right gonad, being in an active secretory phase, did not contain enough hormone for detection by bio-assay.

(6) Since adrenal glands are intimately connected with the gonads in embryogenesis, and are known sources of steroid hormones, the role of the adrenal gland in reproductive phenomena of the female chicken was studied. It was shown that (a) unilateral adrenalectomy did not interfere with the normal development and subsequent ovulatory activity of the ovary ; (b) Indiscriminate bilateral adrenalectomy without substitution therapy proved fatal, but by carefully selecting the age for the operations and keeping the birds subsequently in a warm room, it was possible to keep them alive from 1-4 weeks without any post-operative

treatment. In such birds lack of adrenals had no effect on plumage but there was some inhibition of comb growth. Ovarian growth was also inhibited and there was extensive development of tissue spaces in the cortex, followed later by follicular degeneration; (c) It was possible to maintain bilaterally adrenalectomised pullets for long periods on DOCA and salt water, but in such birds the ovaries remained small and immature and ovulation never occurred. Compared with the bilaterally adrenalectomised birds in (b) there was some observed comb growth which was most likely due to either a direct action of DOCA or the conversion of the latter in the body to androgenic substances; (d) Without the administration of DOCA and salt water it was impossible under any circumstance to keep bilaterally adrenalectomised-ovariectomised birds alive even for a day. However, with such post-operative treatment two birds were maintained alive for 8 - 9 months, and it was observed that there was a development of the right gonad with associated comb growth and male plumage. The right gonad developed slowly compared to poulards with intact adrenals. From the above observations it is clear that the adrenal gland is essential for the normal maintainance and development of the ovary.

(7) Large eosinophilic cortical cells appeared in the thecae of growing follicles by the 17th week of life. They possessed vesicular nuclei and large, spherical, eosinophilic granules in the cytoplasm. These cells divide mitotically and give rise to smaller types of eosinophilic cortical cells with bilobed nuclei. Both types of eosinophilic cells attained maximum development in a fully functional ovary and were never absent from the ovary at any subsequent age although they were somewhat reduced in numbers. The large mononuclear type of

eosinophilic cortical cell morphologically resembled the eosinophil myelocytes in the blood-forming organs. Since mitotic divisions were frequently seen amongst them it is highly probable that the immature ovary is a site of granulocytopoiesis. Support for this conclusion can be gained from the known occurrence of extramedullary granulocytopoesis in the gonads of lower vertebrates. The production of the smaller type of eosinophilic cortical cell, the eosinophil leucocyte, in the ovarian cortex is most likely connected with the intra-organ tissue damage which occurs in the bird ovary due to frequent ovulations.

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Table 3

NO.	TEST	EOSINOPHILIC CORTICAL CELLS	ERYTHROCYTES	MEDULLARY CELLS CLEAR PHASE	MEDULLARY CELLS GRANULAR PHASE	PIGMENT	POST OVULATORY FOLLICLE RECENT	POST OVULATORY FOLLICLE REGRESSED (VACUOLAR CELLS)	ATRETIC FOLLICLES	PERIPHERAL & PRIMORDIAL YOLK	NOTES
1.	BAKER'S ACID HAEMATIN TEST (PHOSPHOLIPIDS)	+	+	+	−	−	−	*GREYISH	*GREY..BLUE	+	*INDICATES MATERIAL OTHER THAN PHOSPHOLIPID.
	(II) AFTER PYRIDINE EXTRACTION	+	−	−	−	−	−	−	−	−	
2.	OTHER LIPIDS, CHOLESTEROL & STEROIDS.										*DEGENERATING GRANULOSA LAYER IS SLIGHTLY POSITIVE
	i SUDAN BLACK B	+	+	+	FAINT	+	*−	+	+	+	**SOME SCATTERED BIREFRINGENCE DUE TO C.T. FIBRES
	ii SCHULTZ TEST	−	−	+	−	−	−	−	+	+	
	iii BIREFRINGENCE TEST	−	−	+	−	−	** −	−	+	−	
	iv FLUORESCENCE	−	FAINT FLUORESCENCE OCCASIONALLY IN BLOOD VESSELS & SINUSES	+	−	−	−	−	+	+	
	v NAPHTHOIC ACID HYDRAZIDE REACTION.	−	−	+	−	−	−	PURPLE	+	+	
	vi FSA	−	FAINT REACTION IN BLOOD VESSEL LUMEN & WALLS.	+	+	−	−	−	−	FAINT REACTION.	
3.	PEROXIDASE REACTION.	−	*+	−	−	−	−	−	−	−	*WEAK REACTION REVEALS HAEM & HAEMATIN COMPOUND
4.	TEST FOR IRON.	−	+	−	−	+	−	*−	−	**+	*VERY FAINT REACTION POSSIBLY DUE TO DEGENERATING ERYTHROCYTES **LARGE YOLK SPHERES
5.	METACHROMASIA	−	−	−	−	−	−	−	−	−	OCCASIONAL POSITIVE MAST CELLS ARE SEEN SCATTERED IN THE CONNECTIVE TISSUE OF THE OVARY

TESTS 1 & 2 ON FROZEN SECTIONS.
TESTS 3-5 ON PARAFFIN SECTIONS.

Table. 3.

Table 4

STAINING REACTION OF MEDULLARY CELL CYTOPLASM

AGE	COMB (LENGTH X HEIGHT. CMS.)	OVIDUCT (LENGTH. CMS.)	PARAFFIN SECTIONS HAEMATOXYLIN & EOSIN	FROZEN SECTIONS SCHULTZ TEST	SUDAN BLACK B	BIREFRINGENCE	FLUORESCENCE	NAPHTHOIC ACID HYDRAZIDE REACTION
I DAY	0·7 X 0·15	UNDIFFERENTIATED	CLEAR	M	I	M	M	M
I WEEK	0·8 X 0·15	– DO –	CLEAR	M	I	M	M	I
2 WEEKS	0·9 X 0·2	– DO –	SLIGHTLY GRANULAR	M	I	W	W	M
3 WEEKS	1·1 X 0·3	– DO –	GRANULAR	W	M	M	−	W
5 WEEKS	1·6 X 0·7	– DO –	BEGINING TO CLEAR	W	I	M	W	W
8 WEEKS	2·4 X 0·9	– DO –	CLEAR	I	I	I	M	M
16 WEEKS	2·8 X 1·4	– DO –	GLASSY CLEAR	I	I	I	I	I
18–20 WEEKS	3·7 X 3·0	BEGINING OF GROWTH 36·0	GRANULAR	W	M	M	M	M
AT FIRST OVULATION	4·0 X 3·9	ALMOST FULLY GROWN 46·2	A FEW GLASSY CLEAR, REST SHOW ALL STAGES OF GRANULATION	W	M	W	−	W
LAYING HEN	4·2 X 4·5	FULLY GROWN 59·6	MORE GLASSY CLEAR CELLS THAN AT FIRST OVULATION.	M	I	M	M	M
MOULTING HEN	WITHERED & DRY 3·2 X 2·4	REGRESSED 29·4	MORE CELLS GLASSY CLEAR & ONLY SOME GRANULAR.	I	I	I	I	I
OLD BIRD	WITHERED 3·5 X 2·8	REGRESSED 26·5	MOST CELLS CLEAR BUT SOME GRANULAR.	I	I	I	M	I

I – INTENSE REACTION
M – MEDIUM REACTION
W – WEAK REACTION

Table. 4.

NO.	PULLET	AGE (IN WEEKS) AT OPERATION		NATURE OF OPERATION		COMB (LENGTH × HEIGHT IN CMS)		POST-OPERATIVE TREATMENT	AGE AT AUTOPSY (MONTHS)	EXTERNAL APPEARANCE AT AUTOPSY	REMARKS
		FIRST	SECOND	FIRST	SECOND	AT LAST OPERATION	AT DEATH				
1	C7734	6	—	L. ADRENALECTOMY	NONE	2.0 X 0.8	4.2 X 4.5	NONE	10	COMPLETE FEMALE PLUMAGE	LAYING
2	C7806	6	—	R. ADRENALECTOMY	– DO.–	1.7 X 0.7	4.2 X 3.9	– DO.–	12	– DO.–	– DO.–
3	C7814	6	—	L. ADRENALECTOMY + OVARIECTOMY	– DO.–	1.5 X 0.6	4.5 X 4.5	– DO.–	10	MALE PLUMAGE	RT. GONAD DEVELOPED BUT NO OVARIAN REGENERATION
4	C7793	7	—	– DO.–	– DO.–	2.0 X 0.7	4.1 X 4.0	– DO.–	6	MALE PLUMAGE MIXED WITH SOME FEMALE FEATHERS	– DO.–
5	C7780	7	7½	R. ADRENALECTOMY	OVARIECTOMY	1.7 X 0.7	3.2 X 2.8	– DO.–	4	– DO.–	V. SMALL RT. GONAD
6	C8020	6	7	– DO.–	– DO.–	1.9 X 0.9	4.6 X 4.8	– DO.–	11	FEMALE PLUMAGE WITH A FEW MALE FEATHERS	REGENERATED LT. OVARY
7	C8058	6	8	L. ADRENALECTOMY + OVARIECTOMY	R. ADRENALECTOMY	2.0 X 0.8	4.3 X 4.5	DOCA & SALT WATER	8	MALE PLUMAGE WITH ONLY A FEW FEMALE FEATHERS	SMALL RT. GONAD
8	D746	6	9	– DO.–	– DO.–	2.1 X 0.8	4.5 X 4.4	– DO.–	9	– DO.–	– DO.–

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resembled the interstitial cells of the mammalian ovary. Nests of clear cells in the cortex of the ovary, which appeared to be synonymous with Van Durme's medullary cells, were earlier termed intertitial

NO.	PULLET	AGE (IN WEEKS) AT OPERATION		NATURE OF OPERATION		COMB (LENGTH × HEIGHT IN CMS)		POST-OPERATIVE TREATMENT	SURVIVAL TIME	EXTERNAL APPEARANCE AT AUTOPSY	REMARKS
		FIRST	SECOND	FIRST	SECOND	AT LAST OPERATION	AT DEATH				
9	C8836	5	6	R. ADRENALECTOMY	L. ADRENALECTOMY	2.0 x 1.2	—	NONE	16 HRS	—	—
10	NWB	6	7	– DO.–	– DO.–	2.2 x 0.8	—	– DO.–	1 DAY	—	—
11	C8599	6	8	– DO.–	– DO.–	2.7 x 1.7	—	– DO.–	1 DAY	—	—
12	C8493	7	9½	– DO.–	– DO.–	2.2 x 1.1	—	– DO.–	1 DAY	—	—
13	C8269	8	9	– DO.–	– DO.–	2.1 x 0.8	—	– DO.–	2 DAYS	—	—
14	C8810	5	10	– DO.–	– DO.–	2.1 x 1.0	—	– DO.–	18 HRS	—	—
15	C9395	10	10½	– DO.–	– DO.–	1.9 x 0.9	—	– DO.–	1 DAY	—	—
16	C8263	8	12	– DO.–	– DO.–	2.0 x 0.9	2.0 x 0.9	– DO.–	10 DAYS	—	—
17	C9479	8	12	– DO.–	– DO.–	2.0 x 1.2	2.0 x 1.2	– DO.–	6 DAYS	—	—
18	C9476	7	12	– DO.–	– DO.–	2.1 x 1.1	2.1 x 1.1	– DO.–	5 DAYS	—	—
19	C8816	11	14	– DO.–	– DO.–	2.3 x 1.2	2.3 x 0.9	– DO.–	27 DAYS	SMALL & WITHERED COMB. ♀ PLUMAGE.	OVARY IMMATURE.
20	C8187	16	18½	L. ADRENALECTOMY	R. ADRENALECTOMY	2.3 x 1.3	2.3 x 1.0	– DO.–	22 DAYS	– DO.–	– DO.–
21	D249	9	15	– DO.–	– DO.–	2.3 x 1.2	2.6 x 1.4	DOCA & SALT WATER	38 DAYS	NORMAL ♀ PLUMAGE. A LITTLE COMB GROWTH.	– DO.–
22	D746	9	14	– DO.–	– DO.–	2.0 x 1.0	3.4 x 3.1	– DO.–	145 DAYS	NORMAL ♀ PLUMAGE. COMB GROWN.	OVARY IMMATURE, OVIDUCT DIFFERENTIATED.
23	M2238	6	13	– DO.–	– DO.–	2.2 x 1.0	3.0 x 2.9	– DO.–	130 DAYS	– DO.–	OVARY IMMATURE.
24	C9380	9	13	– DO.–	– DO.–	2.0 x 1.5	2.0 x 1.5	– DO.–	21 DAYS	PLUMAGE NORMAL. NO COMB GROWTH.	– DO.–
25	9485	8	12	– DO.–	– DO.–	2.0 x 0.9	3.2 x 2.5	– DO.–	144 DAYS	COMB INCREASED. PLUMAGE NORMAL.	OVARY IMMATURE WITH 3 FLUID FILLED FOLLICLES. OVIDUCT DIFFERENTIATED.

INDEX TO ILLUSTRATIONS

All tissue sections stained with haematoxylin and eosin, unless otherwise stated.

AL - Atretic follicle (large).
AM - Atretic follicle (microscopic).
BB - Balbiani body.
BF - Biovular follicle.
BV - Blood vessels.
Cap - Capillaries.
CC - Compaction of cells.
CX - Cortex.
E - Epididymis.
EL - Large eosinophilic cortical cell.
F - Follicles.
G - Membrana granulosa.
H - Hilum.
L - Large cortical stroma cells.
Li - Lipoidal granules.
M - Medulla.
MC - Medullary cell. (clear).
MG - Medullary cell (granular).
MT - Mitotic division.
Oc - Primary oocytes.
Oo - Oogonia.
Ov - Ovary.
P - Pigment.
POF - Post-ovulatory follicle.
PY - Peripheral yolk.
RG - Right gonad rudiment.
RT - Right gonad.
S - Stigma.
SF - Small fibroblastic stroma cell.
ST - Tissue spaces.
TB - Extravasated thecal blood.
TF - Fibrous layer of theca interna.
TL - Right gonad tubules.
TT - Thecae (combined interna and externa).
V - Yolk vacuoles.
VM - Vitelline membrane.

Fig. 1.

Fig. 2.

ADRENAL GLAND   RUDIMENTARY RIGHT GONAD

A

RIGHT LUNG

KIDNEY

B   C   LIVER

POSTERIOR VENA CAVA
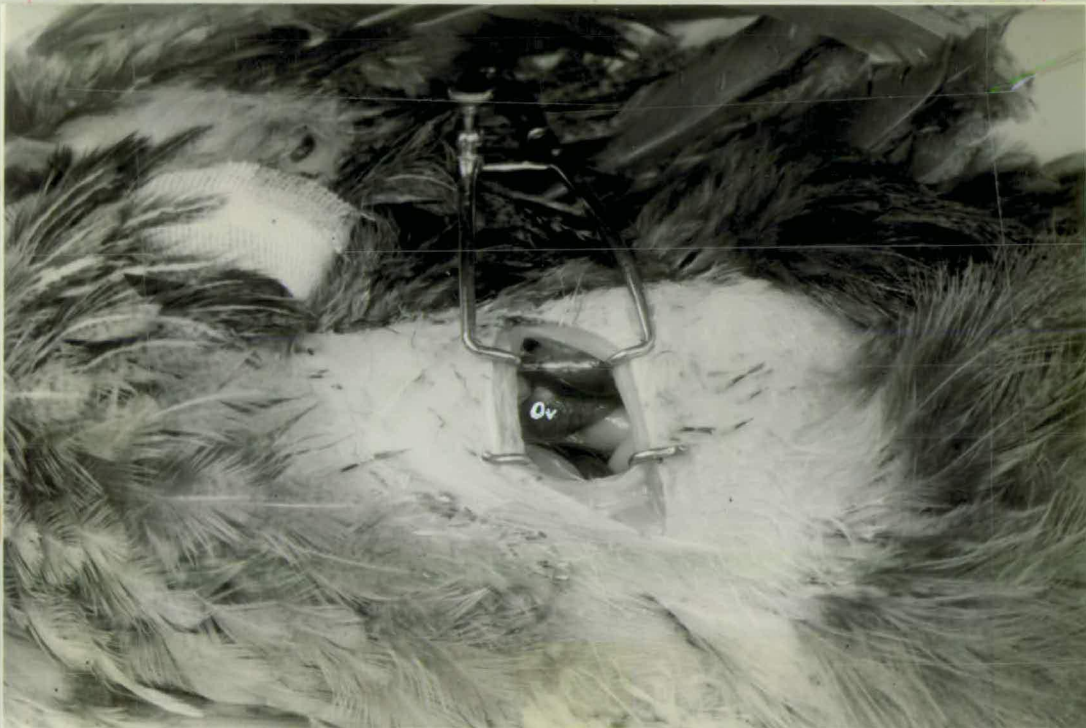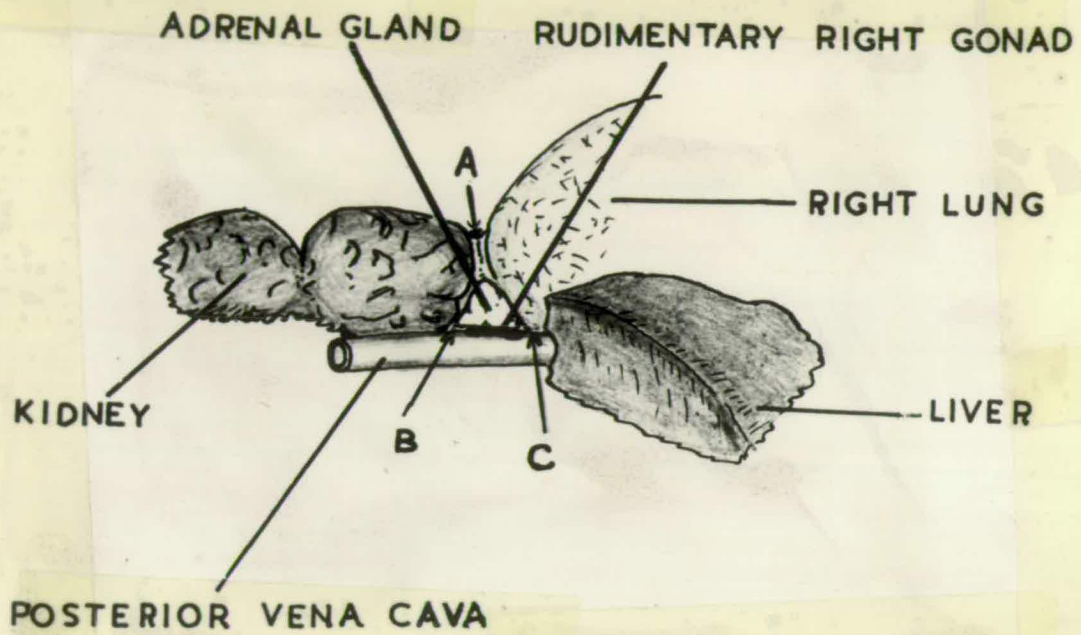
Fig. 3.
Right adrenal in relation to other viscera.
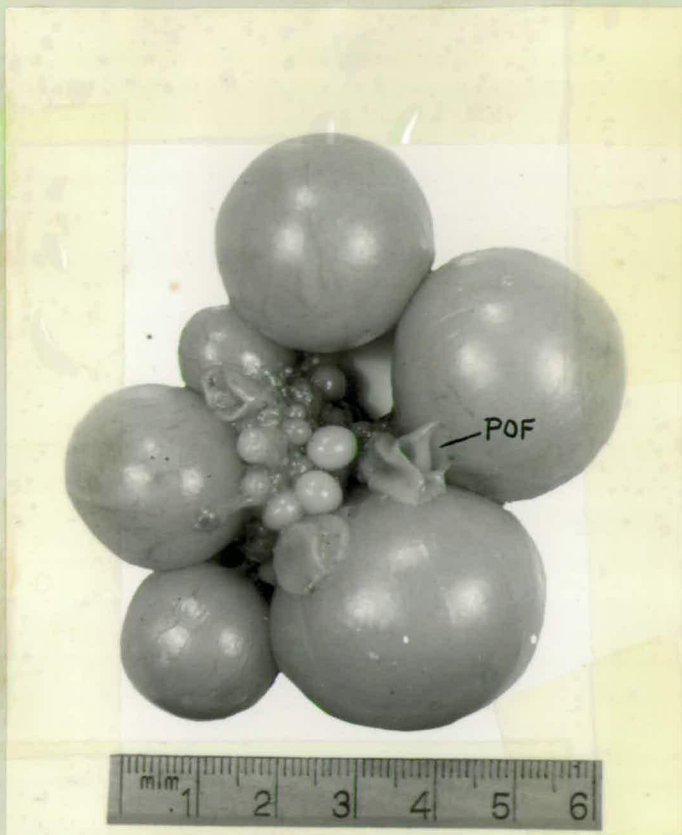
Fig. 4.
Fully functional ovary showing 3 POFs and ova.

Fig. 5.
Ovary preceeding the moult. Note fewer large ova compared with Fig. 4.

YOLK VACUOLES

LARGE
CORTICAL CELLS

FIBROBLASTIC
STROMA CELLS

MEMBRANA GRANULOSA

VITELLINE MEMBRANE

THECA INTERNA

THECA EXTERNA

EOSINOPHILIC
CORTICAL
CELLS.

PIGMENT

GERMINAL EPITHELIUM

TUNICA ALBUGINEA

BALBIANI BODY

MEDULLARY CELL NEST (IN THECA)

GERMINAL VESICLE

NUCLEOLUS

STAGES IN THE
ATRESIA OF
FOLLICLE.

VACUOLAR TISSUE

SCAR TISSUE

LYMPH SINUS

BLOOD SINUS

CORTEX.

MEDULLA.

ATRETIC FOLLICLE.
(LARGE)

Fig. 6.

Diagram of a section through part of a functional ovary.
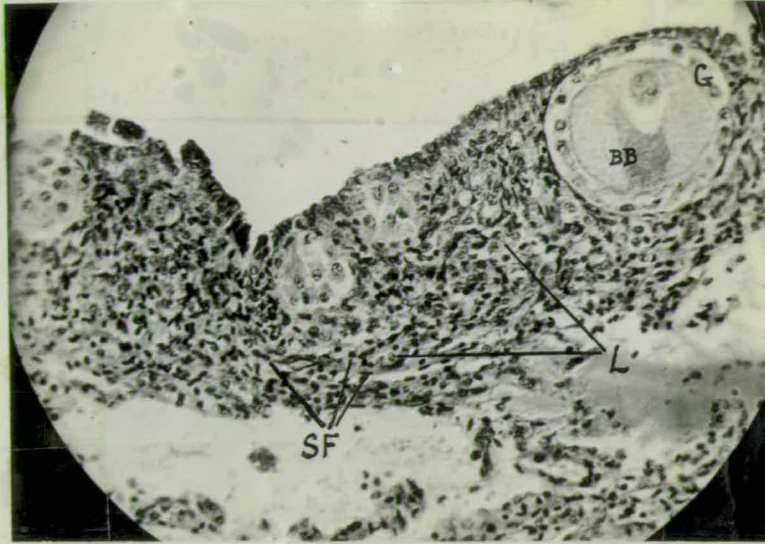
Fig. 7.   X 250.
Cortex of the ovary showing two types of cortical stroma cells.
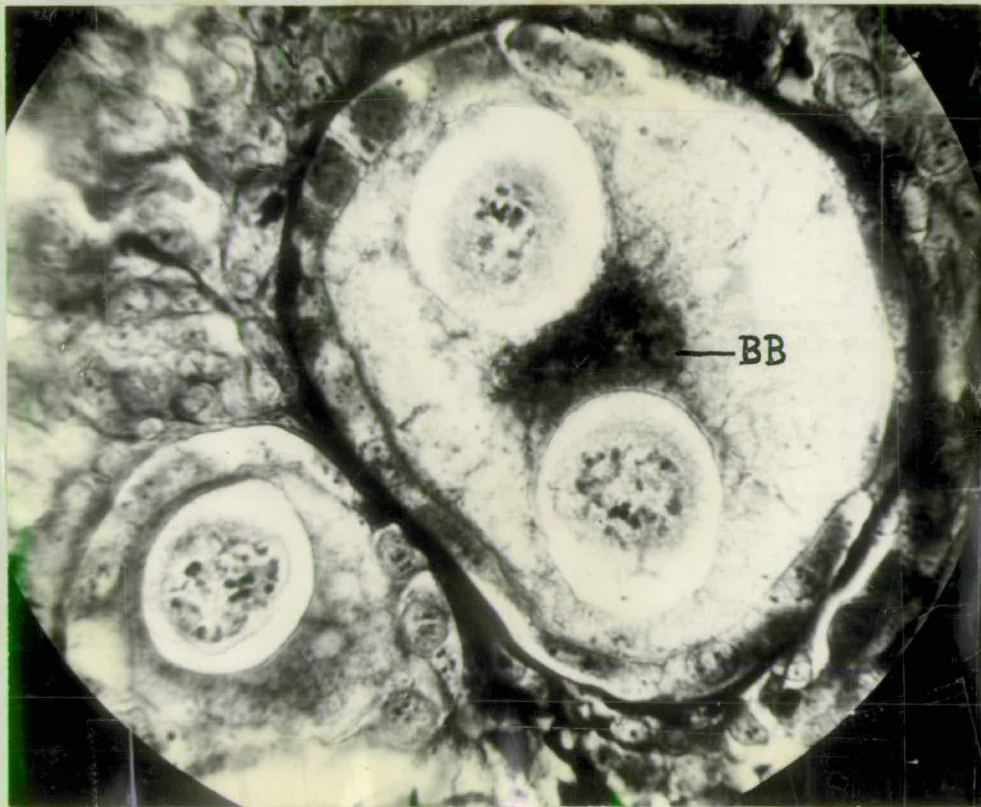
Fig. 8.   X 1500.
Osmic acid & Iron haematoxylin.
Diffuse chromosomes , and binuclear follicle showing osmiophilic Balbiani body.
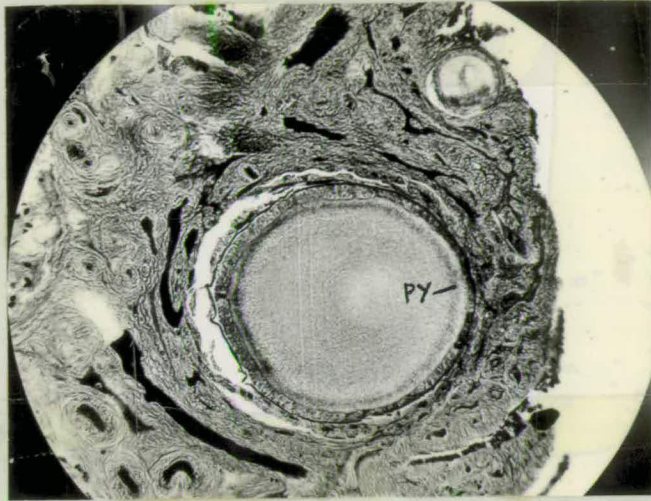
Fig. 9.   X 75.
Frozen section.   Sudan black B.
Note the peripheral, sudanophilic yolk in the ovum.
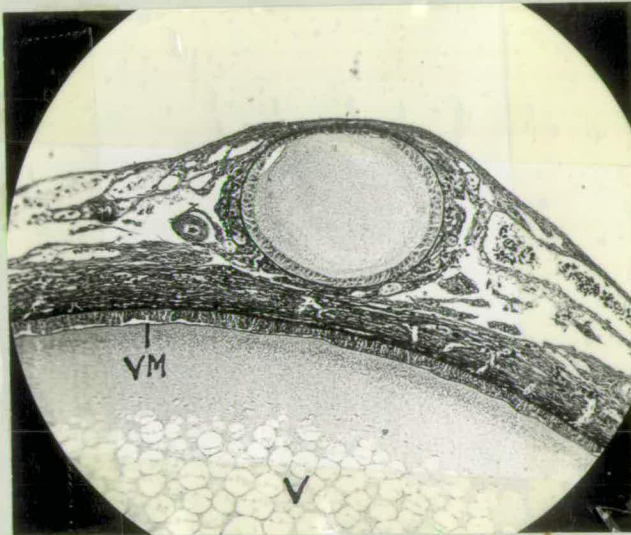
Fig. 10. X 80.
Note a small satellite follicle in the theca externa of a
large follicle. Yolk vacuoles and the vitelline membrane
are also seen in the large follicle.
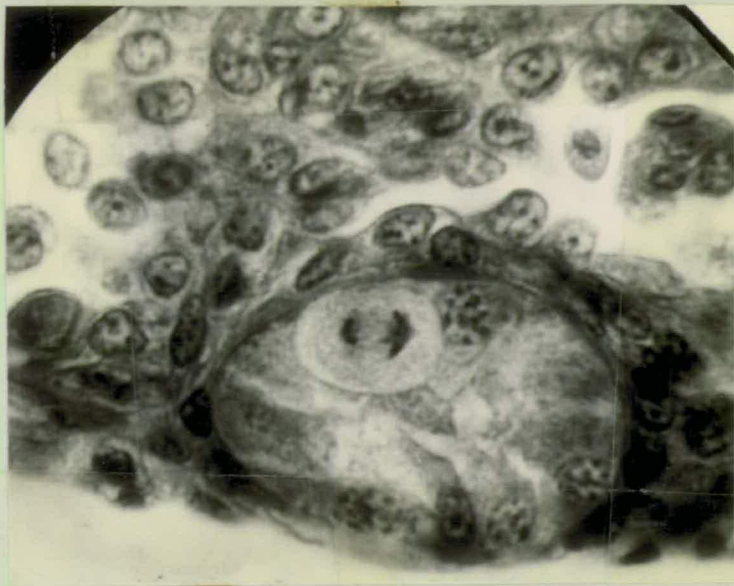
Fig. 11. X 1300.
Anaphase in the granulosa cells of a young follicle.
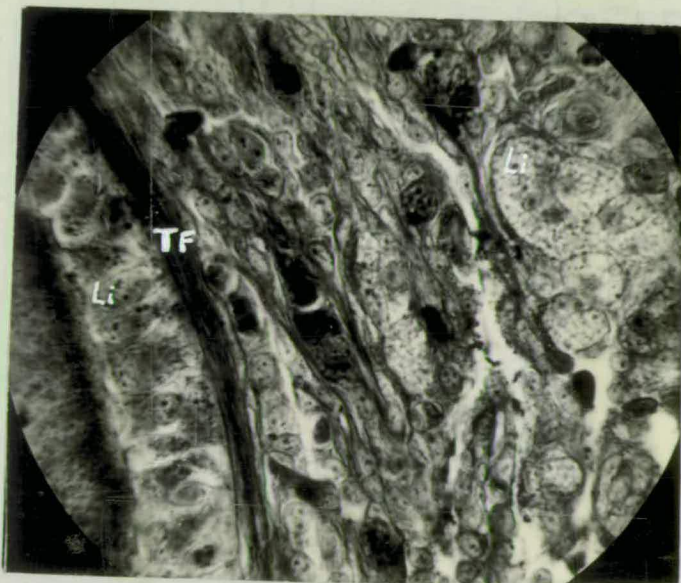
Fig. 12. X 1000.
Osmic acid & Iron haematoxylin.
Note fibres of the theca interna adjacent to the granulosa
cells, and lipoidal granules in the cytoplasm of medullary
and granulosa cells.
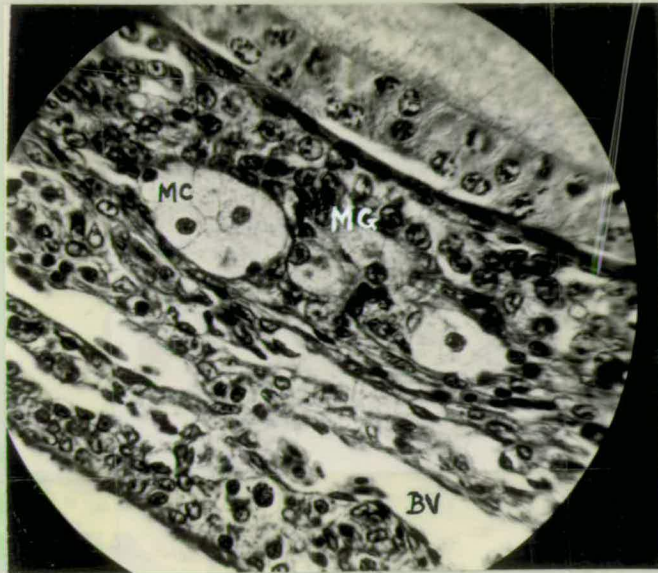
Fig. 13. X 600.
Masson's trichrome stain.
Note nests of medullary cells with clear and granular
cytoplasms in the theca interna of follicle.

Fig. 14. X 30.
Ovary of 14 weeks old pullet showing three stages of atresia
(a,b,c). Note also the migration of medullary cells towards
the thecae of follicles, and the larger follicles protruding
into the medulla.
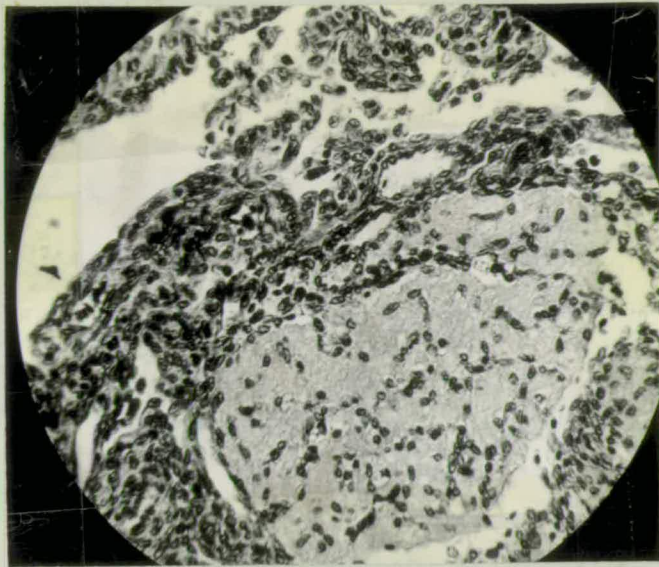
Fig. 15. X 400.
Scar of atresia.

Fig. 16. X 100.
Atretic follicle showing hypertrophy and hyperplasia of
medullary cells, and fatty degeneration of granulosa.
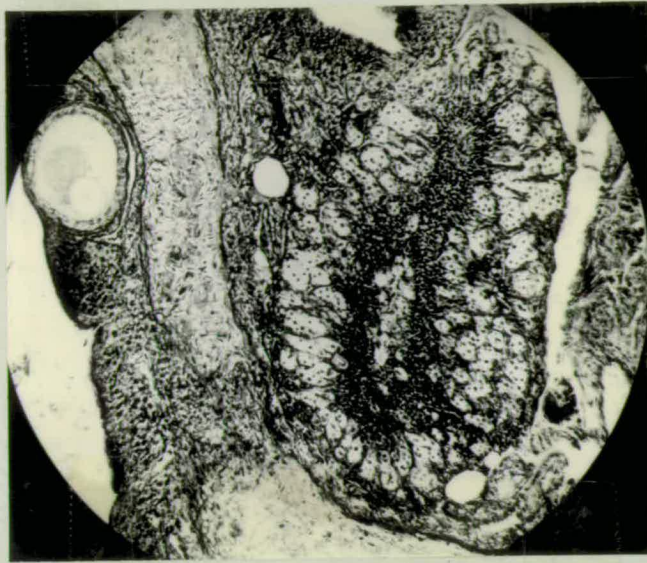
Fig. 17.   X 100.
Collapse of an atretic follicle of the type shown in Fig. 16.
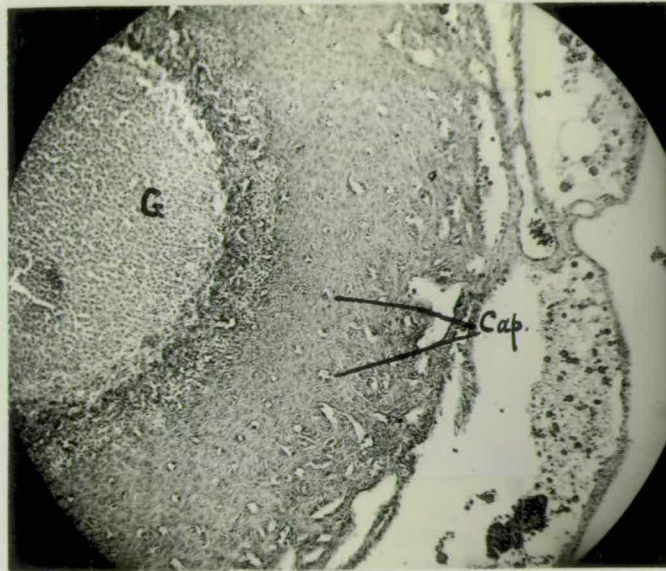
Fig.18.   X 15.
Atresia of a large follicle showing capillaries in the
hypertrophied thecae, and the proliferation of granulosa.
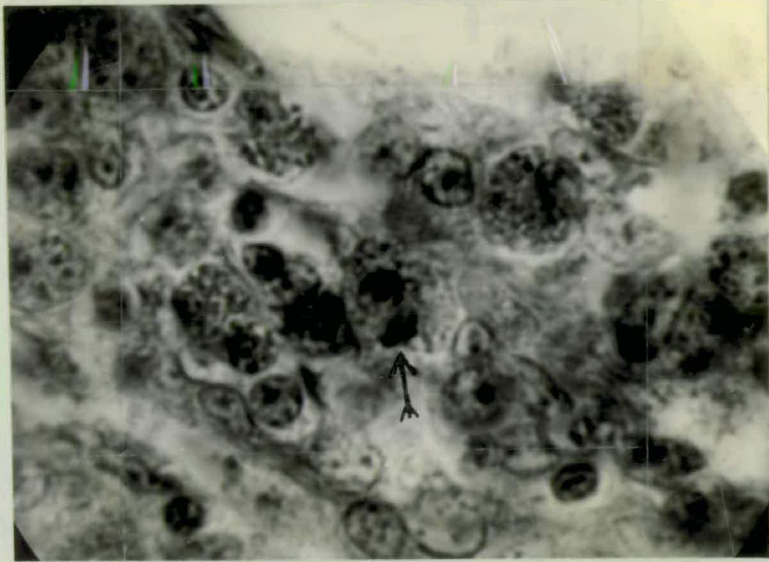
Fig. 19. X 1500.
Metaphase of division in large eosinophilic cortical cell.
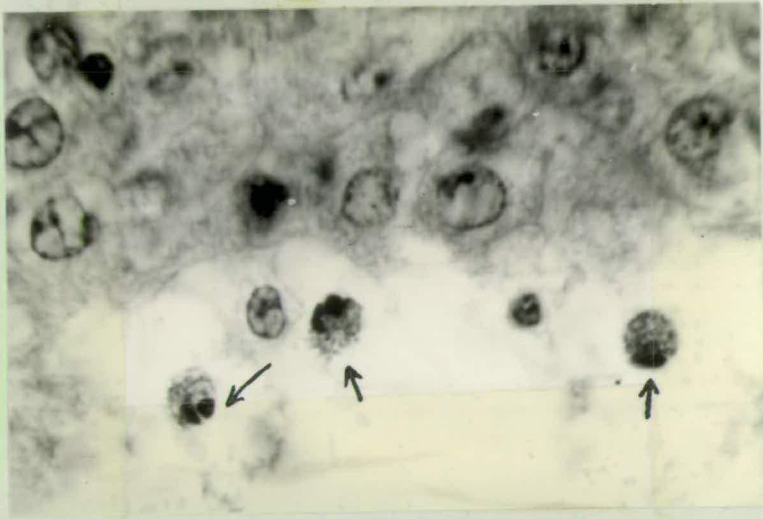
Fig. 20. X 1500.
Eosinophilic leucocytes with bilobed nuclei.
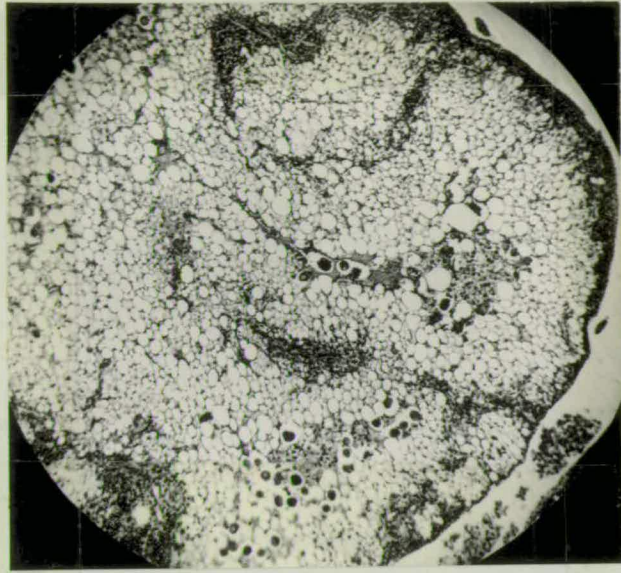
Fig. 21. X 20.
Vacuolar tissue in the cortex of ovary.
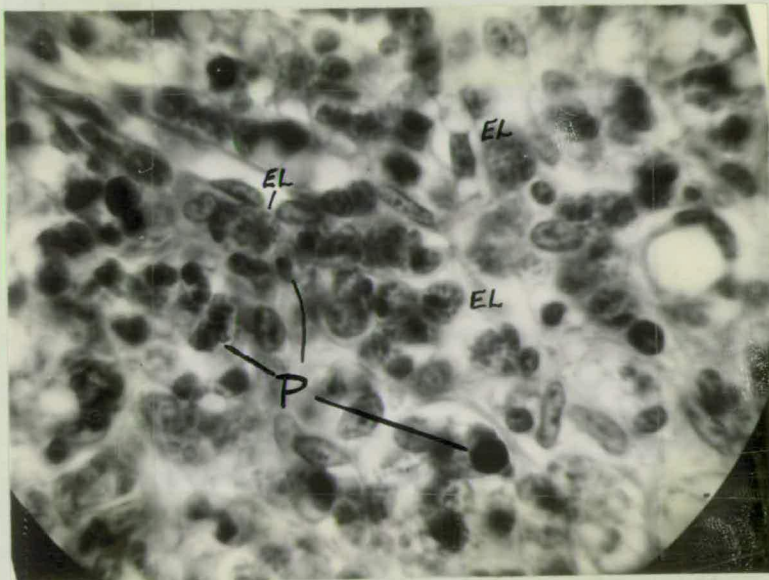
Fig. 22. X 1000.
Pigment and large eosinophils in the cortex of ovary.
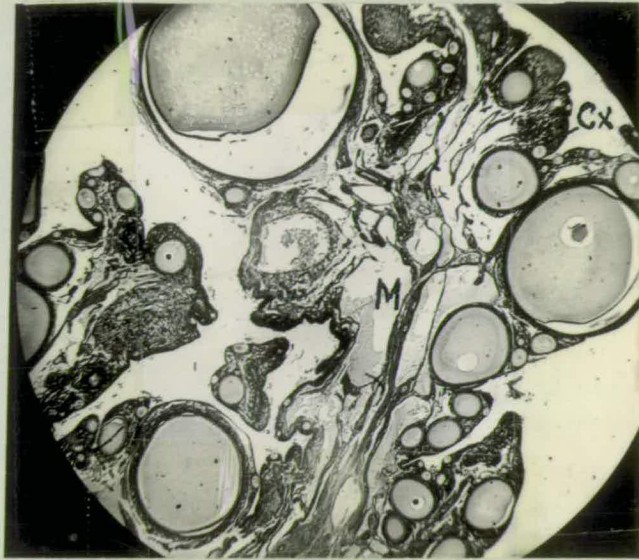
Fig. 23. X 10.
Functional ovary showing blood and lymph sinuses in the
medulla, and ill-defined cortex.

Fig. 24. X 20.
8 weeks old ovary showing compact cortex clearly demarcated
from the medulla.
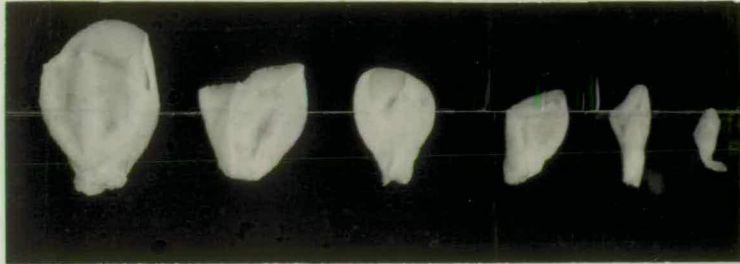
Fig. 25.
Stages in the regression of POF.
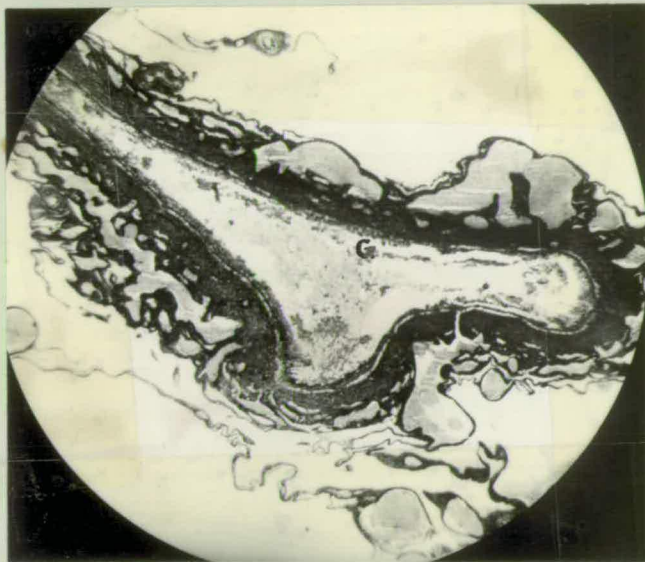
Fig. 26. X 10.
Masson's trichrome stain.
Section through the middle of a POF showing the distended
blood spaces in thecal walls, and degenerating granulosa.
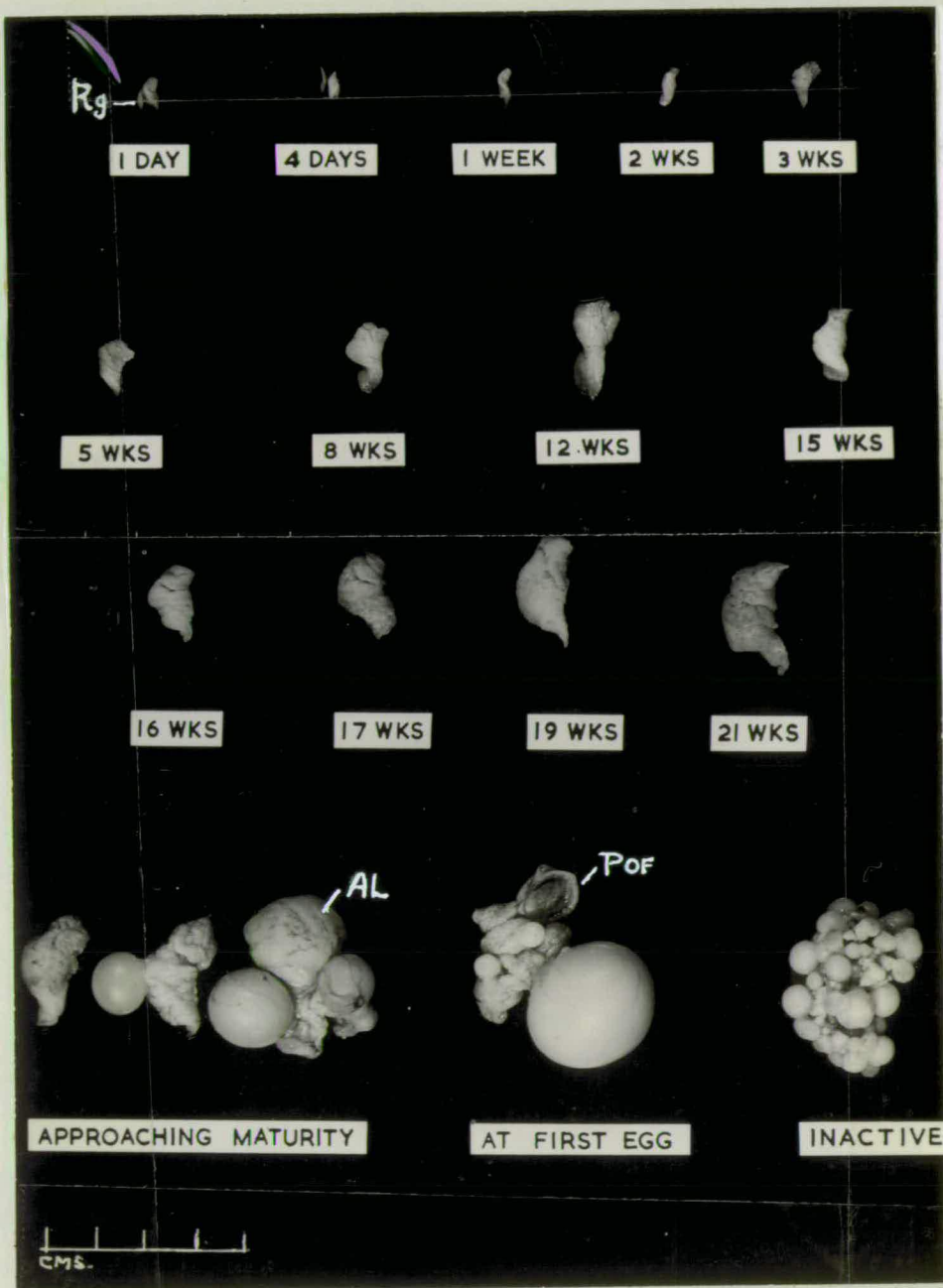
Fig. 27.
Stages in the growth of the ovary. Note the small
rudimentary right gonad up to 4 days, and the large
atretic follicle in the pre-lay stage of the ovary.

Fig. 28.
Ovary of a pullet in early stages of laying. Note the large
number of maturing follicles and two large atretics.

Fig. 29. x 15.
Ovary of a day old chick showing cortex and medulla clearly
demarcated. Note the hilum of the ovary.
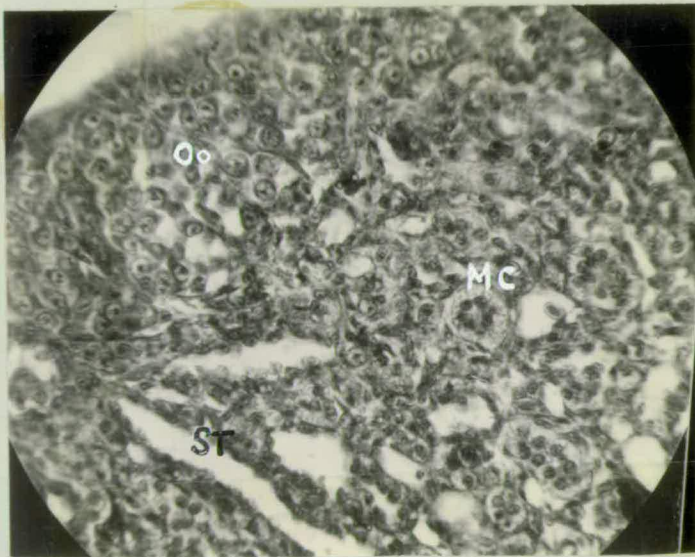
Fig. 30. X 330.
Ovary of a day old chick showing cortical cords of oogonia,
medullary cells and a few spaces.
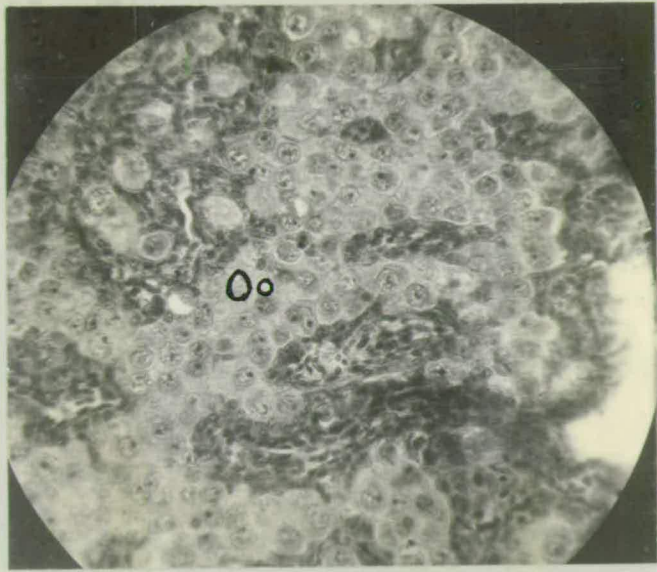
Fig. 31. X 330.
Ovary of a 4 day old chick showing enlargement of oogonia
in the cortical cords prior to oocyte formation.
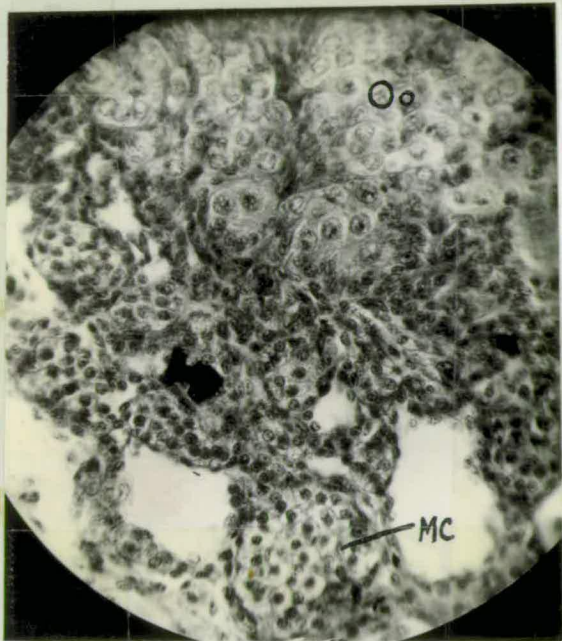
Fig. 32. X 330.
Ovary of a 4day old chick showing increased spaces, and
hypertrophy of medullary cells.
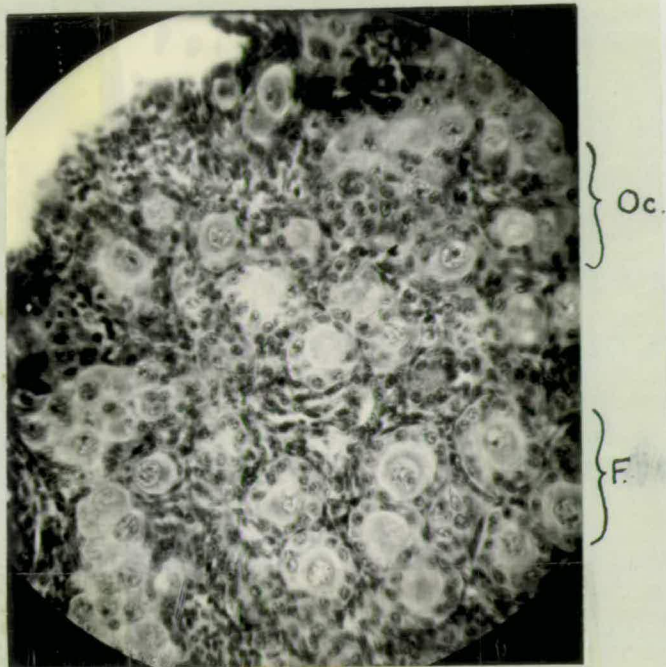
Fig. 33. X 330.
Ovary of a 6day old chick showing the begining of primary
oocyte formation from cortical cords.Note minute follicles.
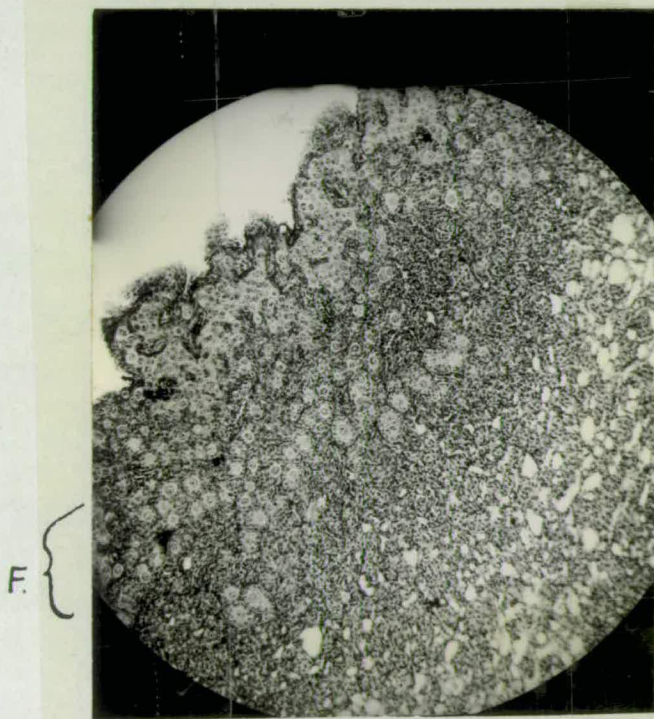
Fig. 34. X 70.
Ovary of a week old chick showing the inward migration of
follicles, and breaking up of cortical cords.Compare fig. 29.
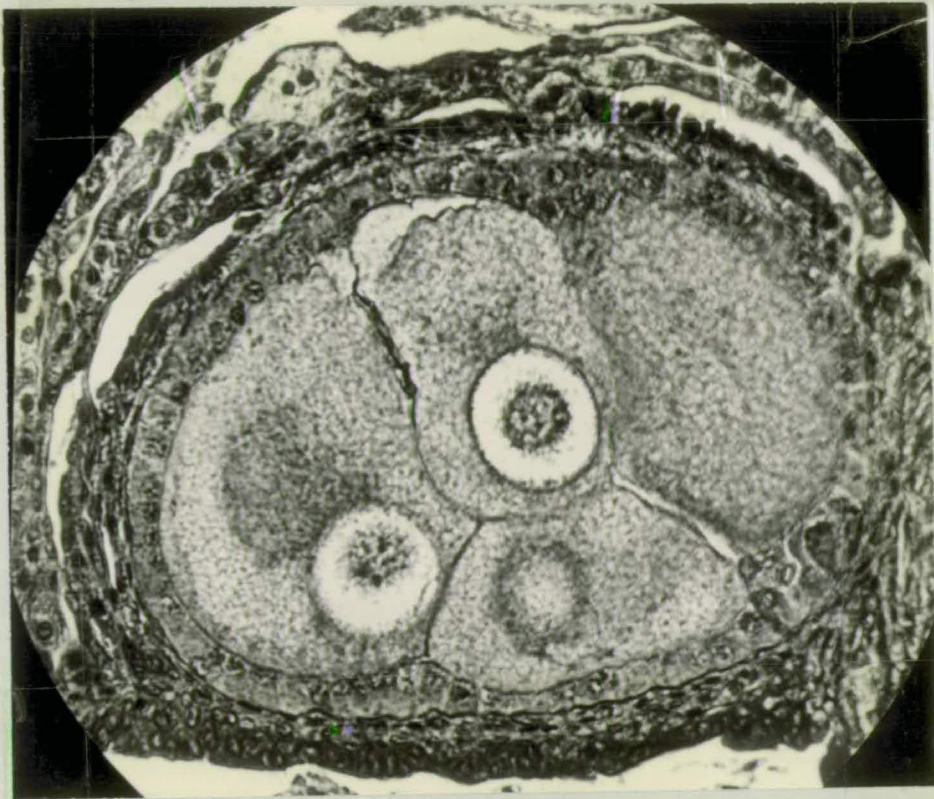
Fig. 35. X 600.
Masson's trichrome stain.
A polyovular follicle.
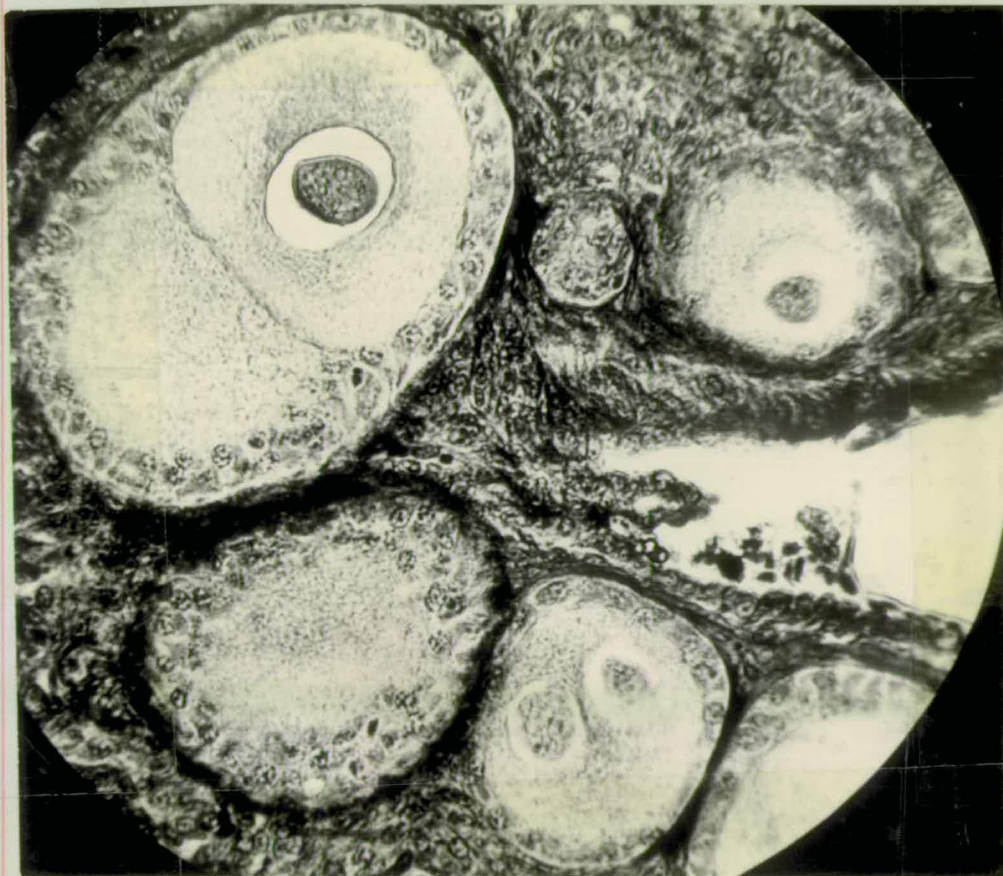
Fig.36. X 800.
Masson's trichrome stain.
A polyovular and a binuclear follicle in 12 week old ovary.
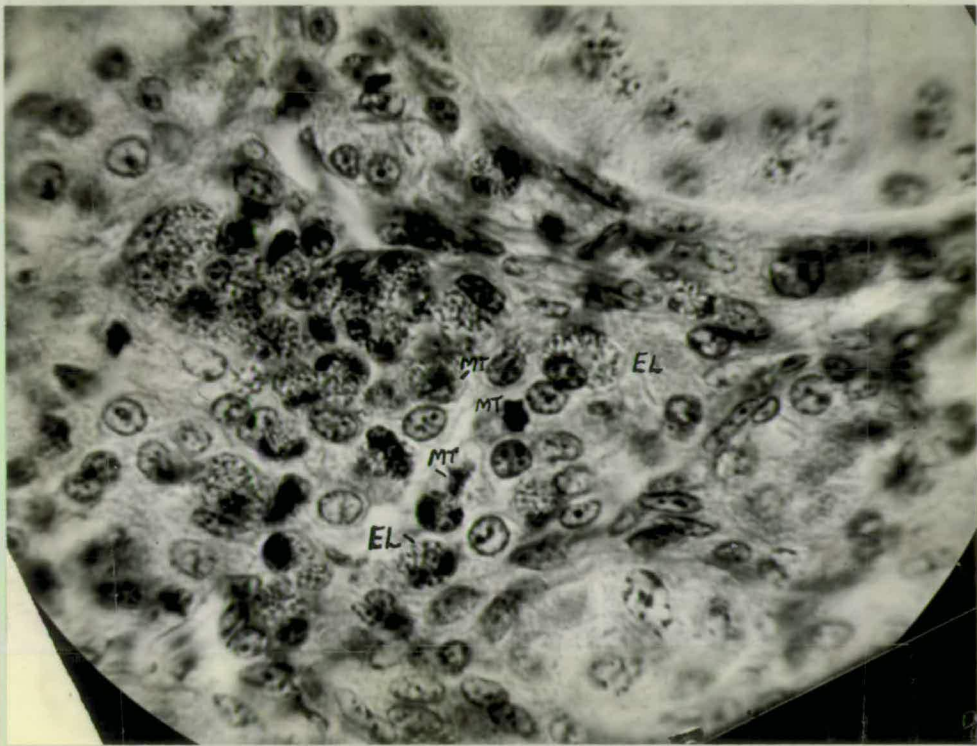
Fig. 37. X 1000.
Ovary of a 17week old pullet showing a group of large
eosinophilic cortical cells. Note mitotic divisions in some
of these cells.
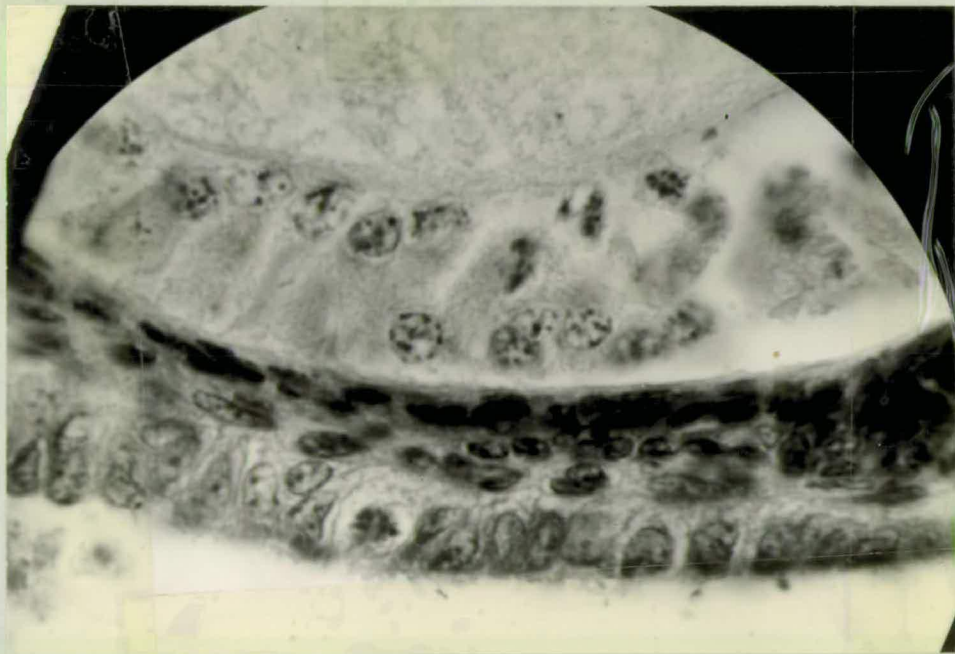
Fig. 38. X 1500.
Mitosis in the germinal epithelium of an 18 week old pullet.

Fig. 39. X 10.
Ovary of a 19 week old pullet showing blood sinuses and spaces
in the medulla, and fully formed thecae of follicles. Cortex
not compact.

Fig. 40.
Ovary at the begining of moult showing atresia of all large
follicles. Note extravasated thecal blood.

Fig. 41. X 80.
Masson's trichrome stain.
Ovary of a moulting hen showing the hypertrophy of medullary
cells both in atretic and intact follicles.
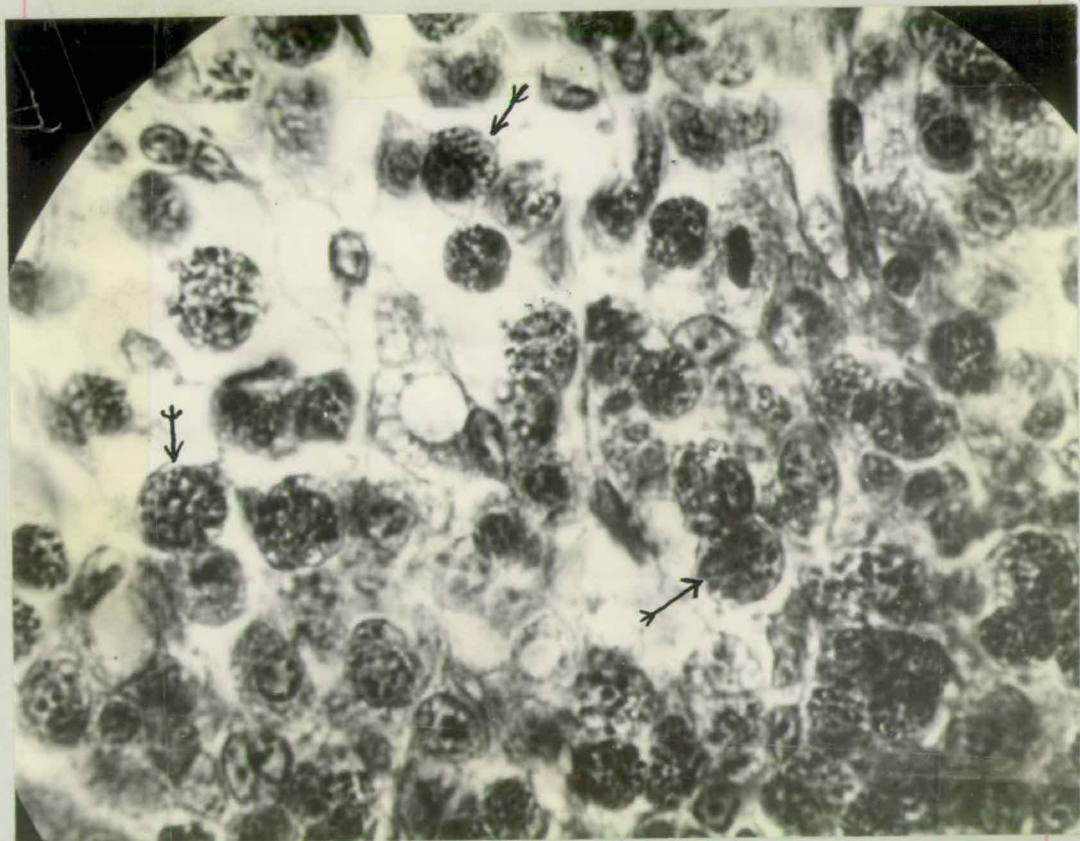
Fig. 42. X 1500.
Group of large eosinophilic cortical cells in the ovarian
cortex.
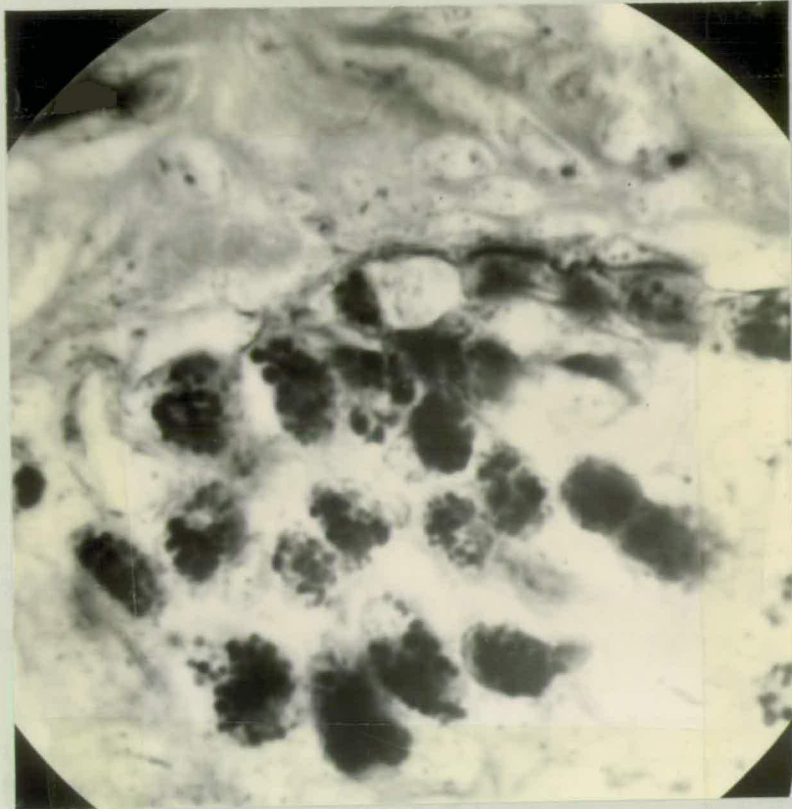
Fig. 43.   X 1500.
Osmic acid & Iron haematoxylin.
Osmiophilic granules in large eosinophilic cortical cells.
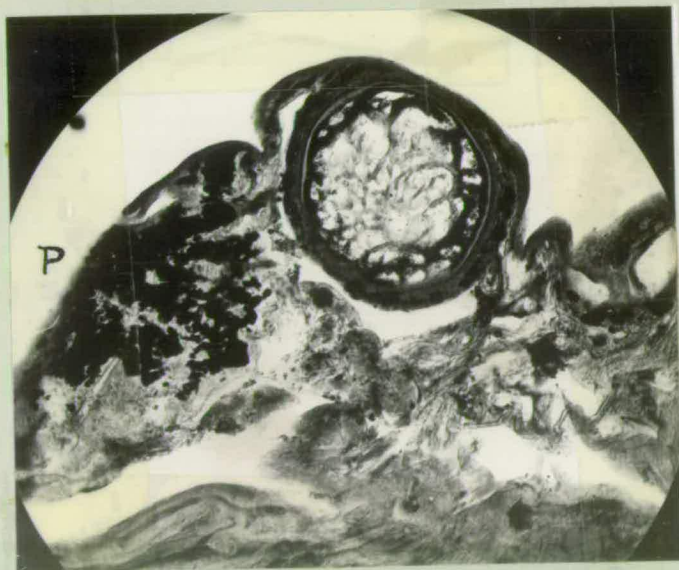
Fig. 44. X 75.
Frozen section. Sudan black B.
Sudanophilic pigment in the ovarian cortex.
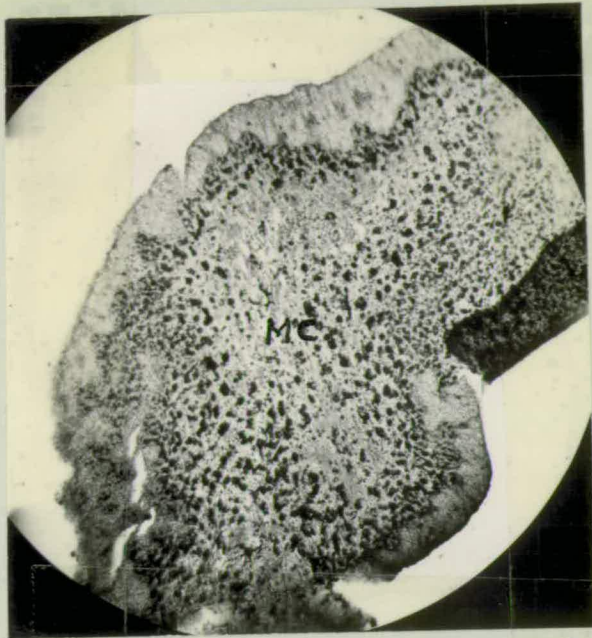
Fig. 45. X 30.
Frozen section. Sudan black B.
Ovary of a day old chick showing sudanophilic medullary cells.

Fig. 46. X 120.
Frozen section. Sudan black B.
Ovary of a week old chick showing sudanophilic medullary
cells , most of which are sub-cortical in position.
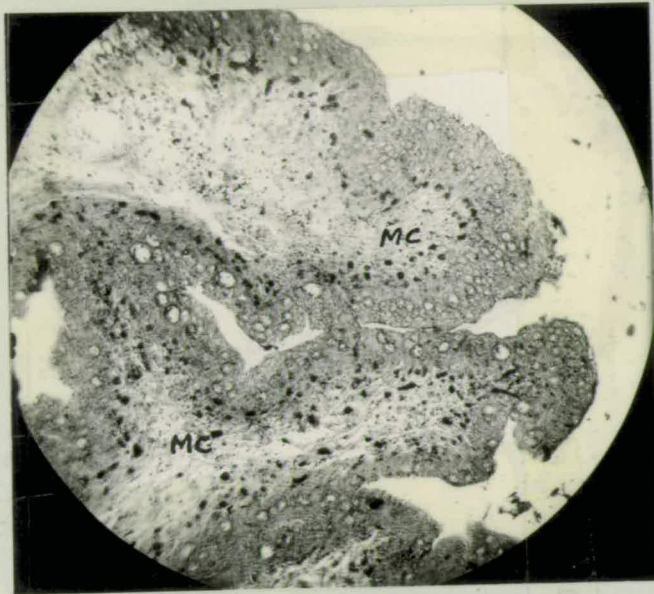
Fig. 47. X 18.
Frozen section. Naphthoic acid hydrazide.
Ovary of a week old chick showing positive medullary cells.
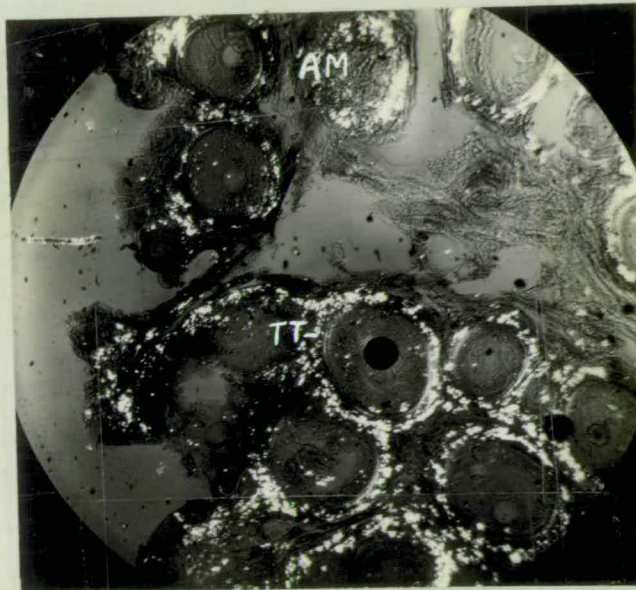
Fig. 48. X 75.
Frozen section. Polarised light.
14 week old ovary showing intense birefringence in the thecal
layers and atretic follicles. Note also scattered birefringence
in the cortex.
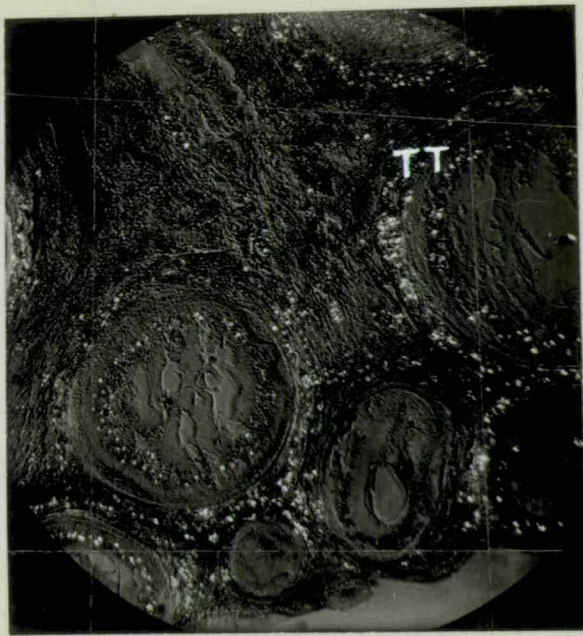
Fig. 49. X 60.
Frozen section. Polarised light.
Ovary of 21 week old pullet showing faint birefringence in thecae.
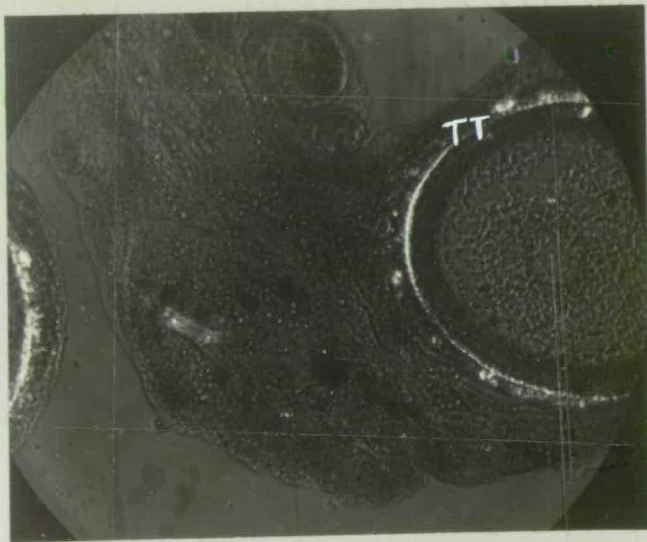
Fig. 50. X 60.
Frozen section. Polarised light.
Fully functional ovary showing reduced birefringence in thecal cells.
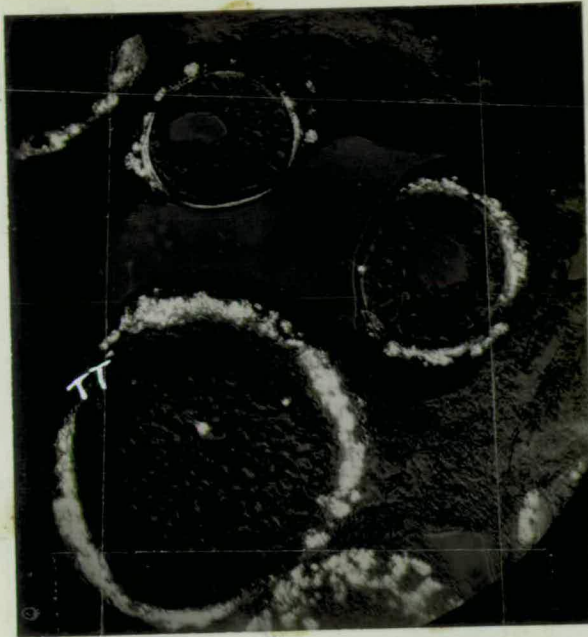
Fig. 51. X 60.
Frozen section. Polarised light.
Ovary of a moulting hen showing intense birefringence in
the thecal layers.
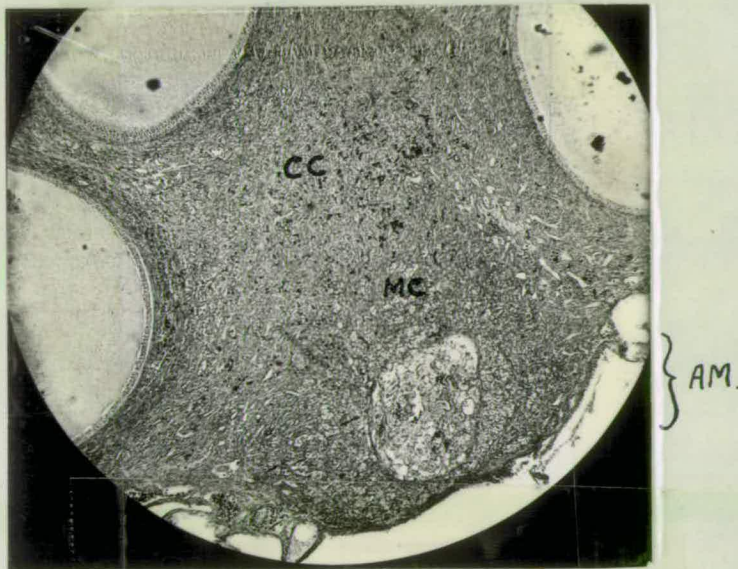
Fig. 52. X 50.
Ovotestis showing three follicles and another undergoing atresia.
Note a central core of compact cells.

Fig. 53.
Dissected poulard to show the right gonad in situ.
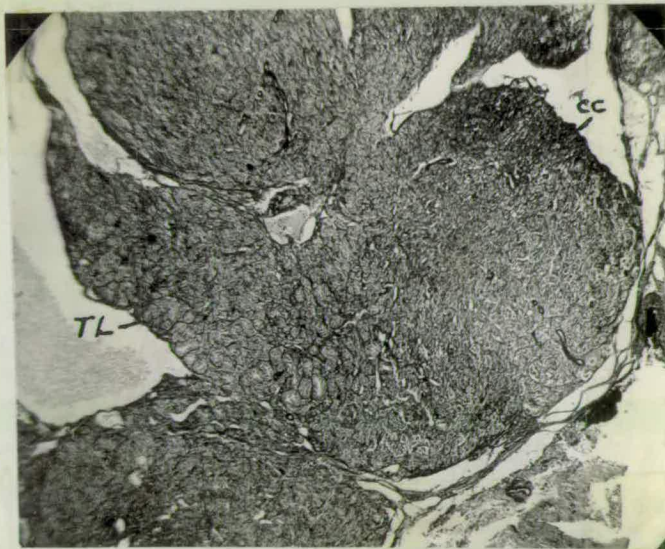
Fig. 54. X 25.
Right gonad showing solid cords of cells and tubule formation.

Fig. 55. X 25.
Right gonad showing hypertrophied epididymal region.
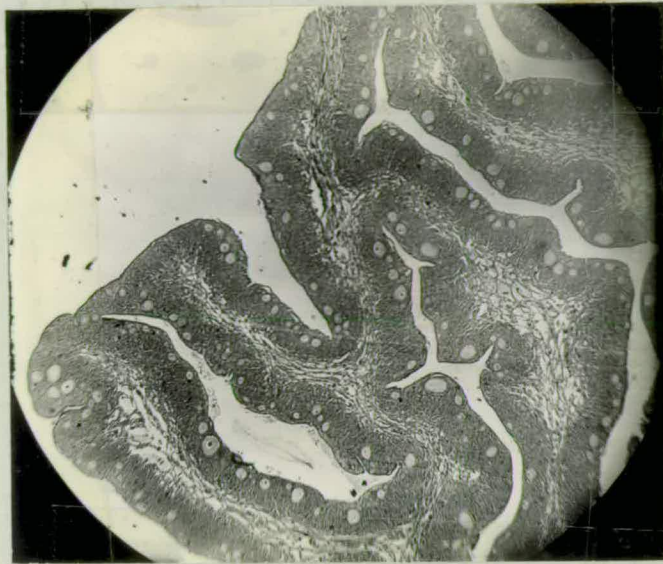
Fig. 56. X 15.
Ovary of a two week old chick treated with gonadotrophin showing
inhibition of follicle development.

Fig. 57. X 15.
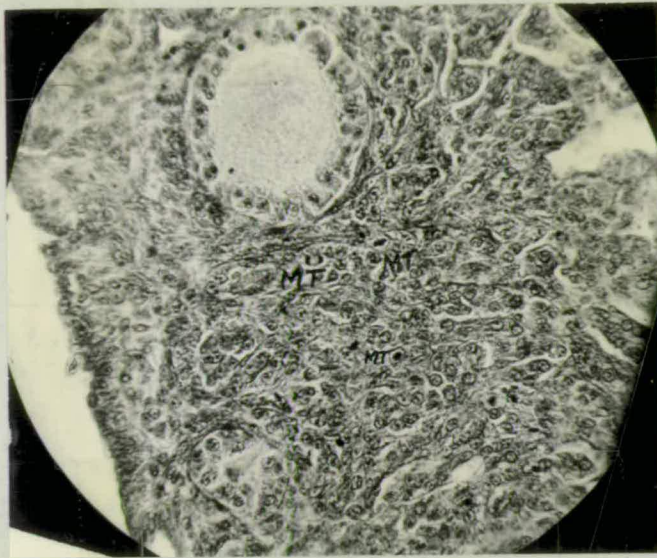Ovary of a 2 week old chick showing extensive follicle development.

Fig. 58. X 320.
Ovary of a 2 week old chick treated with gonadotrophin showing
few follicles, but abundant inter-follicular tissue. Mitoses
can be seen in the latter.

Fig. 59. X 320.
Ovary of a normal 2 week old chick showing the development of
many follicles.

Fig. 60. X 180.
Ovary of a 13 week old pullet treated with gonadotrophin
showing the medullary cells in the granular phase.

Fig. 61. X 180.
Ovary of a normal 13 week old pullet showing medullary cells
in a state of synthesis (clear cytoplasm).

Fig. 62.
External appearance of 19 week old ovary treated with gonadotrophin.
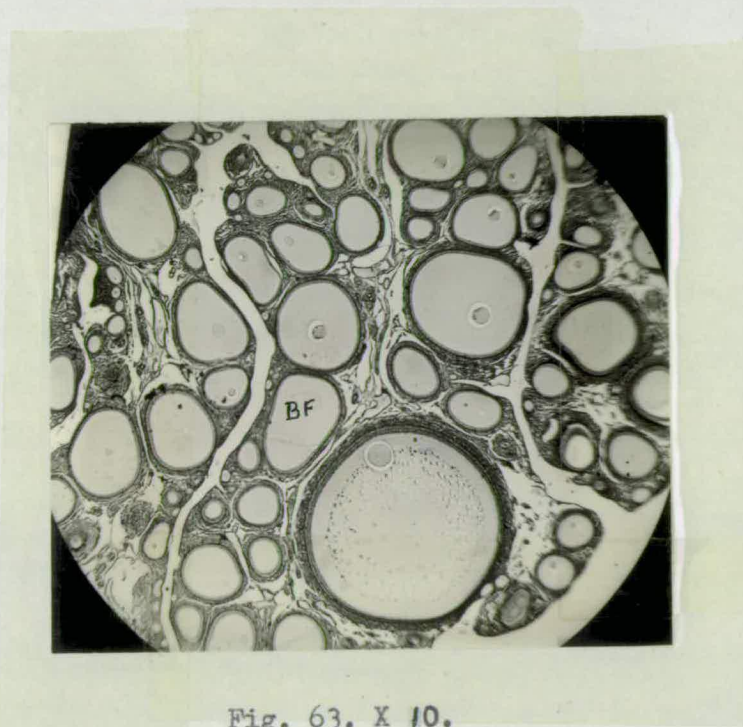Compare Fig. 27.

Fig. 63. X 10.
Ovary of 19 week old pullet showing increased follicle size.
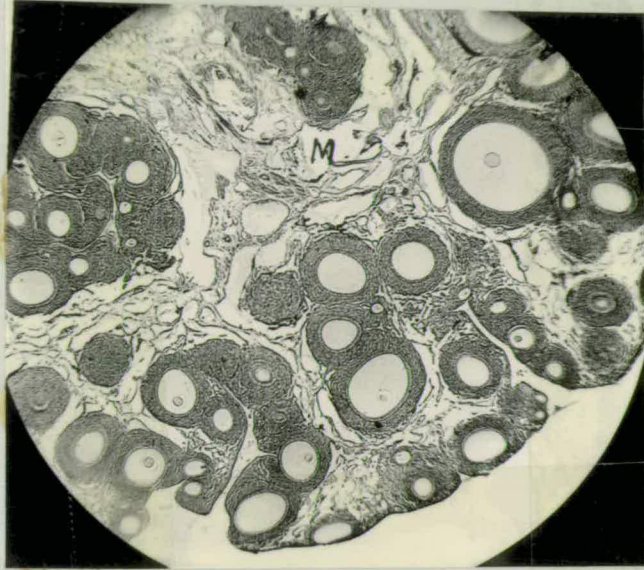Note a biovular follicle. Compare Fig. 39.

Fig. 64. X 30.

Ovary of a 13 week old bilaterally adrenalectomised pullet
showing extensive development of medulla, and compact avascular
thecae.

Fig. 65. X 20.

Ovary of a 13 week old pullet to show normal development of
medulla and thecae. Compare Fig. 64.

Fig. 66. X 20.
Ovary of an 18 week old pullet, 4 weeks after bilateral
adrenalectomy. Note the extensive spaces in the medulla
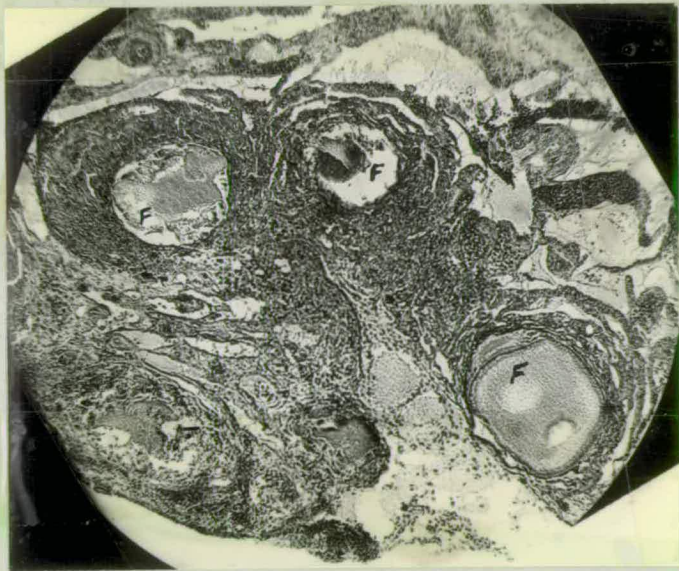and the reduction of thecal layers.

Fig. 67. X 75.
Part of the ovary in Fig. 66 showing degeneration of follicles.