Title of the paper: STUDY ON THE OVARY, OVIDUCT AND UTERUS OF THE EWE.

By: ROBERT HADEK, Dr.Med.Vet., Vienna.

From: The Department of Veterinary Histology and Embryology of The University of Glasgow Veterinary School.

Submitted as Thesis for the Degree of Ph.D. in the Faculty of Medicine.

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

Contents

Vol. I.

Introduction

The purpose of this work has been to study the histological and histochemical aspects of the reproductive tract of the ewe which have not been adequately studied to date and to re-examine certain aspects of published work when results seemed to have been inconclusive or not adequately substantiated.

Literature

Haller and Kuehlemann (1754) and Bonett (1884 & 1888) described the macroscopic appearance of sheep ovaries of the German breed, Kupfer (1928), Quinlan and Mare (1931) and Quinlan et al. (1939) of the South African Merino sheep during different phases of the sexual cycle and pregnancy. Kelley (1937) described the macroscopic appearance of the Australian Merino sheep ovary during heat and after ovulation, Allen and McKenzie (1931) the features of the ovaries of different American breeds, Grant (1934) the gross anatomy of the ovaries of the Scottish Blackface, and finally Robinson (1950 & 1951) the features of the artificially produced corpora lutea in the English breeds. Although these authors have described the macroscopic appearance of the ovaries during an-oestrus and different phases of the sexual cycle, only Bonett (1884) has compared the weight of ovaries obtained from ewes of different ages and Robinson has discussed the influence of the freshly ruptured follicle on the ovarian

weight. Therefore it was decided to repeat in this work the studies of Bonett (1884) and Robinson (1950) on a larger scale to see whether these statements apply equally well when both problems are considered simultaneously.

Although - strangely enough - a comprehensive paper on the sheep ovary was not published yet Lesbouyries (1948) in his book described the occasional presence of the remnants of the rete ovarii in the sheep ovary. Voss (1950, 1951 & 1952) has enumerated in a series of papers the differences in the thickness of the individual connective tissue fibres between a highly domesticated and a primitive German sheep breed. Stieve (1934) gave an account of the sheep Graafian follicle immediately preceding ovulation. The first paper on the ruptured follicle of the ewe was published by Paterson (1840) who thought that the corpus luteum was only an organised blood clot. Marshall (1901 & 1903) contradicted him and from him originates the description of the organised corpus luteum as we know it today. Warbitton (1934) subsequently expanded Marshall's (1903) views and in addition to the connective tissue and lutein cells described the presence of an embryonic cell type.

Lately Harrison (1946 & 1948) has described fairly extensively the histology of the ovary of the horse and that of the goat and the signs of oogenesis in these animals.

In addition to these papers mentioned above the works of previous authors on the ovary of mammals was reviewed and

and an attempt was made to determine to what extent they were applicable to the sheep; expanded the observation of Bonett (1884 & 1888) and Robinson (1951) on ovarian weights at different phases of the sexual cycle; attempted to relate the cyclical variations of follicular development, number of follicles, formation of corpora lutea and its age; confirmed Lesbouyries' statement on the rete ovarii and finally undertook the first systematic histochemical examination of the sheep ovary.

The histology of the sheep oviduct has been described by Andersen (1928), Casida & McKenzie (1932) and McKenzie & Terril (1937). However since their description did not wholly agree with the features on occasional examination of sheep oviducts, the study of the oviduct histology was repeated and histochemical tests were performed.

Papers on the histology of the sheep uterus were published by Robin (1858), Bonett (1880 & 1882) and Kolster (1902). All three noted an alteration in the behaviour of the uterine epithelium during different phases of the cycle and described a "menstruation" as taking place during heat. Further they described the glandless prominent areas of the uterine mucosa - caruncles - where placentation takes place and the intercaruncular areas which contain the uterine glands. Marshall (1903) repeated their work and doubted whether the description of "menstruation" was rightly used in connection with the sheep. Assheton (1906) presented the first clear cut picture of the uterine mucosa and its behaviour during

different phases of the sexual cycle and an-oestrus and dispelled the idea of "uterine menstruation" in the ewe. The work of these early workers were followed by the papers of Haklik (1926) on the evolution and involution of the uterine mucosa in the sheep during oestrus cycle and the problem has been re-examined several times by Casida and McKenzie (1932), McKenzie et al. (1933), Cole and Miller (1935) and McKenzie and Terril (1937). The uterine pigment which has been referred to by almost all of the previous investigators was the exclusive subject of the papers of Kazzander (1890) and Grant (1933). They both described the presence of melanin pigment cells but Grant (1933) in addition noted a round type of cell which he did not investigate but noted to be laden occasionally with pigment.

The histochemical aspect of the work of these earlier authors was confined to establishing the presence of fat and organic iron in the uterus and Bonett (1888) has described the variation in amount of fat in certain phases of the sexual cycle.

After reviewing the foregoing it was decided to confirm the uterine studies to the histochemistry of the endometrium, to an examination of certain "round" cells whose presence was only mentioned by Grant (1933) in his paper on uterine pigment, and to a study of the alterations in the glands with especial reference to changes taking place during the oestrus cycle.

In addition to these the study of the caruncular circulation was also contemplated to establish whether there

is any change taking place in caruncular circulation during the different phases of the cycle.

Material and Methods

The material on which this study is based can be divided into two groups. (In addition to these recorded observations I had the opportunity of seeing the genital tracts of many thousand ewes in all phases of the cycle.) Group 1 includes the genitalia of 120 ewes obtained from non-pregnant sheep of all ages which were used for gross anatomical observations and measurements; group 2 includes the genitalia of some 64 non-pregnant animals on which the histological and histochemical part of this report is based. The material of group 1 and of 54 animals of group 2 was obtained from animals slaughtered in Glasgow Corporation Abattoir. The remaining 10 animals in group 2 were experimental ewes kept in the Veterinary Hospital (Chart 1).

Anatomical observations and measurements. The genital tracts of the 120 ewes in group 1 were obtained from freshly slaughtered sheep a few minutes after death.

Before the carcase was opened the (1) breed, (2) age, (3) condition and (4) apparent stage of oestrus (mucous outflow or redness of vulva), were noted. When the carcase was opened the genital organs were immediately removed and carried to the laboratory for examination and measurement.

In the laboratory the sexual cycle (5) of the animal as determined by ovarian examination was recorded. The length of

the cervix (6), of the uterine body (7) and uterine horn (8) and that of the oviduct (9) were measured.

The data of the ovaries included the length (10), depth (11), width (12) and the weight of the organ (13) and the number of follicles and corpora lutea (14).

The external diameter of the oviduct (15), the external diameter of the uterine body cranial to the cervix (16), the external diameter of the horn cranial to the body (17) and the thickness of the uterine horn wall (18) (intercaruncular area) cranial to the medial ligament were measured. Weight of the uterus was recorded after above measurements were taken (19) (Chart 2).

Histological and histochemical technique. Of the 64 genital tracts used for histological and histochemical study, 54 were obtained from freshly slaughtered ewes from the Glasgow Abattoir and 10 from the sheep kept at the Veterinary Hospital. The genital tract of the latter was obtained by laparotomy.

5 mm. portions were obtained from all of the tissues in the genital tract. In some cases however the ovaries and oviducts were not cut into 5 mm. pieces but were fixed in toto for serial sections. From the oviducts as a rule separate pieces were obtained from the ampulla and from the isthmus. As far as possible the uterine and oviduct material took form as a transverse section of the entire uterine horn; in cases in which this proved to be impracticable because of the large size of the organ portions of transverse sections were obtained.

Each piece of this uterine tissue contained in addition to the caruncular (non-glandular) area a glandular part as well. In a number of cases 10 mm. blocks of the uterus were fixed for serial sections. All specimens were fixed within 10 minutes of the death of the animal or, in the case of operation, immediately after their recovery.

Of all the fixatives, Regauds (R) formol-saline (F-S) and Bouin (B) have served mainly for morphological purposes.

The following fixatives were used for histochemical purposes. Zenker-formol (Z-F) for ribo- and desoxyribonucleic acid reactions (Feulgen and Rossenbeck (1924), Brachet (1940)). Corrosive formol (C-F) as recommended by Roberts and Jarrett (1953) for the preservation of polysaccharide complexes. In addition the following were used: Alcohol (Alc.) and Dubosq-Brazil's fluid (D-B) on Dr. Steedman's (1952) advice. Acetone (Ac.) or Alc. and Ac. mixture were used for the fixation of the alkaline and acid phosphatases (glycero) (Gomori, 1952). The majority of specimens were embedded in paraffin but from a considerable number of blocks frozen sections were cut.

Paraffin sections were cut at 6μ and frozen sections at 30 - 50μ.

As a routine at least one section of each block was stained with haematoxylin and eosin for morphological purposes. Further sections were stained by Masson's trichrome stain, Gordon and Sweet and Laidlaw's Ag. stain for reticulum and Elastic van Gieson stain for the presence of elastic and collagen fibres.

In addition the following staining methods were employed on suitably fixed sections -

Inorganic substances -

Inorganic iron: The Prussian blue test and Turnbull's blue for Ferrous Iron (Glick, 1949).

Masked Iron (Gomori, 1952): Prussian blue test, Humphrey Dinitroresorcinol test for iron and Thomas and Lavolley Hydroxyquinoline test for iron (Glick, 1949).

Organic substances -

Polysaccharides: Periodic acid Schiff (henceforward abbreviated to P.A.S.) staining (McManus, 1946 & 1948; Hotchkiss, 1948a & b) was performed on corrosive-formol and alcohol and D-B fixed sections according to the method of Roberts and Jarrett (1953). Only occasional sections were counterstained with haematoxylin.

If staining with P.A.S. was positive, sections were treated with (a) diastase (saliva) at 37°C and (b) hyaluronidase (Benger "Hyalase") 1000 units per 100 cc. of buffered water for 2 hrs. at 37°C (Hale, 1946).

If controls (a) and (b) were negative (that is to say if the material was stained), then further sections were stained with (c) Southgate's mucicarmine (Alcohol and Formalin

fixed specimens), (d) Toluidin blue (Corrosive formol fixed), (e) Celestin blue (C-F and F-S fixation) and (f) Sudan black (C-F and F-S fixation). Further sections were stained in solutions of (g) Methylene blue buffered at pH 3.4, 5 and 7 for 24 hrs. (Roberts & Jarrett (1953)).

Nucleic acids: Desoxy-ribo (thymo) nucleic acid: For the staining of desoxy-ribo nucleic acid Feulgen's method (Feulgen and Rossenbeck, 1924) was used (Zenker-formol and Corrosive fixed blocks). To eliminate "pseudo" reactions the control of Oster-Mulinos (1944) was performed. No counterstaining was used.

For staining Ribo (yeast) nucleic acid: The method of Unna-Pappenheim (Brachet, 1940) was used on sections fixed either with Corrosive-formol or Zenker's fluid. For controlling the validity of the reaction Fisher's (1953) mineral acid method was used.

Lipids: Formalin fixed frozen sections were stained with Sudan 3 and Sudan 4 (Sharlach R) and paraffin embedded sections with Sudan black B.

Pigments: (i) Phenolic pigments: the bleaching in dilute acids and alkalis; the acidified permanganate test and the Ag. test was performed according to Gomori (1952).

Enzymes -

Alkaline phosphatase: Gomori's (1939) method was used. The sections were incubated for 1 and 2 hours respectively, at 37°C. To eliminate error due to preformed Ca control sections were incubated in a buffer of pH 4.5 for 30 min. at 37°C to remove preformed Ca.

Acid phosphatase: Wolf-Kabat's (1943) modification of Gomori's method was used. Sections were incubated 24 and 72 hours at 37°C.

Microscopical measurements: all measurements were made on Bouin-fixed specimens.

For the study of caruncular circulation the "Modified" Pickworth staining method (Bacsich and Wyburn, 1940) was used.

The breeding season and the sexual cycle in the ewe.

The domestic sheep in Scotland is seasonally polyoestric. The breeding season starts in autumn but the exact time of its beginning is subject to breed, geographical and thermal influences. The breeding season in some of the lowland sheep is considerably longer than that of the highland breeds. The majority of sheep from which material was obtained were blackfaced highland sheep and their sexual season begins in late October. The average length of the cycle is 16.5 days (Grant, 1934). Oestrus or heat averages 20 - 24 hrs.; met-oestrus, 2 days; di-oestrus 9.- 11 days; and finally, if the sheep is not successfully mated, pro-oestrus, 2 - 3 days.

The genital tract of the sheep shows cyclical alterations during the breeding season when it is accompanied by heat. According to Grant (1934) and McKenzie & Terril (1937), quiescent cycles might take place after the last and before the first cycle of the season when ovulation occurs without heat.

The Ovary

Gross Anatomy

The ovary of the sheep is a horse-bean shaped organ of grey-white colour and varies in length from 2 - 3 cms., in depth (continuation of the hilus) from 1 - 2 cms., and in thickness from half to 1 cm. There is a hilus (attached border) on one side, where it is attached to the ovarian ligament (Fig. 1). The fimbriated end of the oviduct hangs over the ovary and in those specimens which were killed during oestrus and met-oestrus it has covered the whole organ. The weight varied from 0.5 to 4.5 g. (mean 0.25 - 2.5) (Chart 3) and it was found that the factors within the same breed which influence it were age and the stage of the sexual cycle. When the weight of ovaries from ewes of different ages were compared in the resting state (an-oestrus), it was found that the lightest were those from the young sheep, 0.50 g. (1 year old), and that it gradually increased with age, the heaviest being found in animals of 5 - 6 years of age.

With regard to the alteration in ovarian weight during

the breeding season, it was lightest before the beginning of the season and it became progressively heavier with each cycle after the formation of each corpus luteum. The heaviest ovary, 4.680 gm. was obtained from a $2\frac{1}{2}$ year old ewe at the end of the breeding season. It showed two fairly recently formed and two older corpora lutea.

Histology

The ovary of the sheep is covered by the germinal epithelium. The germinal epithelium is a single layer of cuboidal cells. The cells were small during an- and di-oestrus and greatly increased in size during pro-oestrus and oestrus. In the same periods a number of cell divisions were also visible (Figs. 2 & 3). Beneath the germinal epithelium in specimens which were stained by reticulum stains and P.A.S. it is possible to recognise a distinct though thin basement membrane. Since P.A.S. stains some of the connective tissue fibres of the tunica albuginea as well it is on occasions difficult to distinguish it clearly but as figs. 4 & 5 show it has been established beyond doubt that it is a true basement membrane. Beneath it one finds a tunica albuginea which is comparatively thick in the ewe but which is missing from the sites of old corpora lutea. The follicles rupture at any point on the ovarian surface with the exception of the attached border.

Beneath the tunica albuginea the substance of the ovary can be divided into two layers, the thin and somewhat loose

cortex which contains fewer blood vessels, where the developing follicles are situated and which is missing at the hilus; and the denser medulla which contains more blood vessels (Fig. 1). Smooth muscle fibres from the ovarian round ligament enter the ovary at the hilus and end abruptly. The ovary has a framework of reticular fibres (Fig. 6). The individual ova and follicles are situated within a reticular framework (Fig. 7). Primordial and secondary follicles are recovered in the cortex and with their development into tertiary they gradually sink into the medulla. The connective tissue between the primary follicles and egg groups was rather scanty.

The medulla of the sheep ovary is formed of dense connective tissue with many large blood vessels. It is evenly covered by the cortex at all aspects of the ovary, with the exception of the hilus (attached border) where the medulla - as in other animals - is directly continuous with the ovarian ligament.

Masson's trichrome stain revealed a scarcity of collagen fibres in the cortex and elastic fibres were absent except in the walls of the blood vessels. Among the usual connective tissue elements found in the ovary a particular cell type was seen which was characteristic of the ovary. This cell was only somewhat bigger than a fibroblast but apparently it had no processes, its cytoplasm was scanty and it had a big vesicular nucleus. The nucleus stained only lightly with haematoxylin and the cytoplasm was eosinophilic. This type of connective

tissue cell was only encountered in the ovary and was interspersed by fibroblasts, macrophages and the usual cellular elements which occur in the connective tissue.

In the medulla of the ovary one very often encounters the vestiges of the rete ovarii. They are small duct-like structures usually circular in transverse section and their lumen is lined by a single layer of columnar or cuboidal cells. The cells have large, centrally placed nuclei. Occasionally the lumen is filled with some slightly P.A.S. positive material (Figs. 8 & 9).

Oogenesis and follicular development

Large numbers of sections from a number of ovaries which were obtained in an-oestrum, or in different phases of the sexual cycle, were examined with a view to comparing the number of follicles present in different development stages. While no attempt was made to determine the exact number of follicles present, it was evident that during an-oestrus the number of follicles was very markedly reduced (Fig. 10). With regard to the developmental stage of follicles it was found that during an-oestrus only a low percentage of the total were primary follicles and the remainder secondary and tertiary, contrary to the usual findings in oestric or pro-oestric animals. The proportion of follicles appeared to be the same throughout an-oestrus regardless of the time when the ovaries were obtained.

In ovaries which were obtained during pro-oestrus, the

greater proportion of follicles were primary but the secondary and tertiary follicles were increased in number as compared with an-oestric animals (Fig. 11). The greater number of follicles was seen in ovaries obtained during the period of oestrus. The number of primary follicles decreased during met-oestrus and the di-oestrus ovary presented a picture similar to the one seen in an-oestrus ovaries.

The smallest oocytes which measured 20 - 22μ were recovered from the outermost layer of the cortex. They were enclosed in completely flat follicular cells to form a primary follicle (Fig. 12). These cells were long and narrow with an elongated nucleus and a very faintly stained cytoplasm. Once the oocyte reached a certain size the flat follicular cells were apparently transformed into granulosa cells. This involved an alteration in the shape of the cells, the cells becoming square to cuboidal and this change was first noted as a rule at the two opposite poles of the oocyte where two two granulosa cells appeared (Fig. 12). The smallest oocyte in which they were present measured 28.5μ. Gradually the ovary became surrounded by a row of granulosa cells which have grown always further from the two cells which appeared first. In the ovary of the sheep first one complete layer of cells have surrounded the oocyte and only after this layer was complete was there a beginning of the formation of a second cell layer. The second cell layer started the same way as the first one with two cells appearing at two opposite poles outside the first layer. The follicle,

after the completion of the second layer of granulosa cells, measured approximately 66.5μ in diameter. The earliest appearance of the zona pellucida was seen in a follicle of this size. It was an extremely thin structure.

The first theca interna cells appeared when the secondary follicle had a diameter of 60μ or so. They were flattened cells and created the impression of a connective tissue cell.

On investigating a large number of ovaries in different phases of pro-oestrus and early met-oestrus, it was found that follicular development in the sheep takes place during pro-oestrus, oestrus and early met-oestrus while during other phases of the cycle the ovarian activity, at least follicular development, seemed quiescent. When the follicle reached the size of 80μ the granulosa cells were no longer compact but presented a "sponge" (web)-like appearance. The individual cells by means of their processes were in contact with one another, i.e. forming a syncytium (Fig. 13). The ovum was in the middle of the follicle and the follicle was still at the periphery of the cortex, just beneath the tunica albuginea. The meshes of the granulosa layer began to show the presence of liquor folliculi when the follicle reached the diameter of 160μ or so. At this stage the granulosa was 43μ thick and the theca interna 37.5μ . Follicles of the size of 150 - 300μ were in a deeper layer of the cortical zone than those of smaller size and the theca interna acquired a thecal

cone which pointed towards the ovarian periphery.

From that (160μ) size onwards the growth of the theca interna was more rapid relative to that of the granulosa. When the follicle reached approximately 500 - 700μ the theca interna and granulosa were equal in thickness measuring 100 - 130μ (Chart 4).

At different phases of follicular development the theca interna at one aspect of the follicle becomes thicker than at other aspects and since this thickening assumes the shape of a cone it is referred to as thecal cone. In small follicles up to 300μ a marked thecal cone points towards the periphery of the ovary (Fig. 15a & b). It is absent in follicles of 400 - 500μ which have migrated deeper into the ovary (Fig. 15c). It can be seen again in follicles of 600 - 800μ when it points towards the ovarian medulla (Fig. 15d). Once the follicle has apparently reached the limit of its inner migration in the ovary a gradual change takes place in the position of the thecal cone which then again points towards the ovarian periphery (Fig. 14 and 15e). Preceding the follicular rupture the thecal cone points towards the nearest point of ovarian surface (Fig. 15f).

In several cases, however, there was no possibility of inward follicular migration. As a result all sorts of follicular forms come into being; the most unusual being the ellipsoid forms, but pyramid-like or irregularly shaped follicles were also frequently encountered.

The cells of the theca interna in the small follicles

are flat cells with a dark oval nuclei and eosinophil cytoplasm. The cells are concentrically arranged around the basement membrane. It was usual to find in large follicles one layer of very flat theca interna cells with small nuclei which lay next to the basement membrane. External to them some larger cells were found. They were also flattened but were considerably bigger than the previously mentioned ones. They had round nuclei and fairly well stained eosinophil cytoplasm. During oestrus the cells were somewhat polygonal but never lost their concentrical arrangement (Figs. 16 & 17). The cells of the thecal cone were not as flat as the other theca interna cells and their nuclei are vesicular.

In the small follicles some capillaries are found which were arranged circularly. In bigger follicles, in addition to the above described capillaries, small sinus-like blood vessels were seen. Two "wreath"-like (Harrison, 1948) rings of blood sinuses and capillaries could be detected in the theca. One ring near to the basement membrane consisting of sinuses and capillaries which surround the basement membrane in an almost complete circle, and an outer circle of blood vessels which was on the periphery of the theca interna (Figs. 18 & 19). In the terminal stages of follicular development the theca interna increased in thickness, through oedema the cells became looser than was hitherto the case, and between the cell layers large spaces appeared which in paraffin sections were apparently devoid of any material (Fig. 17).

A "festooning" of the basement membrane between the theca interna and granulosa was occasionally found. This "festooning" was achieved by minute projections of the theca interna, which protruded into the follicle proper (basement membrane and granulosa) and pushed in the basement membrane and the granulosa layer. Many of these projections contained capillaries (Fig. 20).

The growth of the follicle and ovum

In a large number of cases the diameter of the follicle and ovum was measured together with the thickness of the theca interna and granulosa. This material (Chart 4) was treated statistically to establish the connection between (i) follicular growth and growth of the ovum on the one hand follicular growth and that of the (ii) theca interna on the other, and (iii) the granulosa.

(i) Comparison of follicular and oocyte growth shows that in the early stages of development the growth of the oocyte is much more rapid than that of the follicle as a whole. In the primordial follicle of 26μ the oocyte already measures 22μ. But when the follicle reaches the size of approximately 300μ the growth rate of the oocyte is abruptly reduced and thereafter the follicular growth is much more rapid than that of the oocyte. While in the remaining period of growth the follicle reaches the size of 5,000 - 10,000 μ the ovum reaches the maximum size of 145μ only. If these measurements are reproduced graphically - the average size of the ova plotted against the actual follicular

size - then a steeply rising almost vertical line appears which represents the quick growth period of the ovum after which the growth abruptly flattens out into an almost horizontal line which corresponds with the slow growth period. From all available data the formula for the regression line for the whole growth period -

$$Y = 85.4117 + 20.1933 (\log_e F - 5.8605)$$

was obtained where Y is the size of the ovum and F is the size of the follicle (Graph 1). Further to prove the nature of the growth a log. chart (Graph 2) was obtained. It is seen from this graph the growth line is continuous.

The thickness of the theca interna (ii) has shown an increase in a straight line when the regression line was -

$$Y = 78.16 + 39.13 (X - 6.24)$$ (Graph 3).

When data referring to the thickness of the granulosa (iii) was treated in a similar way the result was not conclusive, although it is suggestive of a parabola which it was expected to describe (Graph 4).

Multinuclear ova, polyovular follicles and accessory oocytes.

Large numbers of polyovular follicles and multinuclear ova were recovered in the sheep's ovary. These structures were only found in the ovaries of animals killed during pro-oestrus, oestrus and met-oestrus.

The number of multinuclear ova was low at the beginning of pro-oestrus, showed a gradual increase during pro-oestrus and was highest in animals which were slaughtered at oestrus. They

were found degenerating in animals which were slaughtered during met-oestrus and early di-oestrus. All oocytes with more than one nucleus were in the early stages of development and none was found within a secondary follicle. While the most frequent of multinuclear oocytes were those which had two nuclei, a large number had three, and occasionally even four or five nuclei. Multinuclear ova as a rule were larger than oocytes with single nuclei (Figs. 21 and 22).

Hartmann (1926) has classified the polyovular follicles into the following three groups:

Type I. The ova are separated by intervening masses of granulosa cells,

Type II. The ova are in contact by broad surfaces, and

Type III. The ova are linearly arranged within the follicles.

In the sheep it was possible to find all three types of deviation from the normal. Type I, by which the oocytes are described to be separated by granulosa cells needs modification with regard to that of the sheep; namely the cells which separate the oocytes were often not granulosa cells but the flat follicular type of cells which one encounters surrounding the oocyte before the actual granulosa cell formation takes place (Fig. 23). Type II, the broad surface contact was quite usual in the ovary of the sheep and oocytes resembled a nest of eggs lying together. The most frequent number of oocytes in one group was five.

Type III, or linear arrangement of oocytes similarly occurred

very often and was encountered regularly in the sheep's ovary. The number of oocytes encountered in this way was again very high (Fig. 25). While accessory oocytes were not encountered regularly, there were a few found to be enclosed in the theca interna of a fairly large and well developed follicle.

Follicular degeneration and atresia

Formation and degeneration of oocytes was found to be a continuous process in the ovary of the sheep. There were always a number of degenerating follicles present during an-oestrus. In late met-oestrus or early di-oestrus an atretic or degenerative wave seemed to sweep over the ovary and most of the follicles and oocytes showed a degeneration in its wake.

The degeneration attacked first the smallest then gradually the bigger follicles. Accordingly it was possible to find met-oestric ovaries which hardly contained any healthy primordial and secondary follicles at all, only some tertiary follicles still perfectly healthy.

The degeneration took different forms in the different stages of development. In the primordial follicles the sign of degeneration was the disorganisation of the cytoplasm in the oocyte into a web-like structure leaving part of the cell completely devoid of any vitellus and the picnotic changes in the nucleus. In secondary follicles lymphocytes have invaded the granulosa at the beginning of atretic process; the ovum become disorganised and the zona pellucida loses its circular

structure. In the more highly developed follicles which already possessed a cumulus oophorus and antrum folliculi, the degeneration affected the whole follicle at once. Coming from without inward, the first changes were noted in the shape of the granulosa cells which appeared flattened and the whole granulosa layer became flatter including the cumulus oophorus. The follicular fluid accumulated into little globules and the ovum underwent the same changes as those in the secondary follicles. An invasion of lymphocytes started (Fig. 26) and the antrum gradually became occupied by web-like threads and fibroblasts (Fig. 26). When the degeneration was complete the follicle was filled with connective tissue (Fig. 27).

The rupture of the follicle

Actually no rupture of follicle was witnessed when doing this work but several ovaries were recovered during met-oestrus when it seemed that rupture of Graafian follicles was imminent.

From the histologist's point of view the changes which took place in the follicle during heat immediately preceding ovulation can be summarised as follows.

Some larger follicles were not subjected to the degenerative wave which swept over the ovary following the ovulation during the last met- and di-oestrus. During di-oestrus however these follicles were in a "resting period". The theca interna was thin and so was the granulosa. The granulosa cells were small and polyhydral nucleus and

cytoplasm stained basophil (Fig. 16).

During pro-oestrus the follicle was in a growing phase. The theca externa cells bordering the theca interna became larger, the theca interna became wider than was during di-oestrus and the granulosa cells underwent marked changes. The basal layer of granulosa cells next to the basement membrane became cylindrical with a slightly acidophil cytoplasm, the one layer above it cuboidal and the cells in the layers above it remained polyhydral (Fig. 17).

During oestrus immediately preceding the rupture of the follicle at a point where the theca exerted the highest pressure on the tunica albuginea, the albuginea gradually disappeared and the theca externa and interna seemed to coalesce. Within a short period this layer became thin and the follicle bulged out (Fig. 28). This protruding follicular wall was thin and glassy and minute strongly injected thecal blood vessels were visible. It is customary to refer to this protruding follicular wall as glassy membrane or macula pellucida.

Within the follicle connection between the granulosa cells became loosened and in the cumulus oophorus spaces appeared among the granulosa cells which were filled with liquor folliculi (Fig. 29). Within a short period of the impending rupture of the follicle a minute opaque spot - area opaca - appeared in the middle of the macula.

The corpus luteum

Very fresh corpus luteum (i.e. within a few hours after

the follicular rupture) appeared either as a slightly bleeding small hole or slit of alternating size or as a little blood clot.

On naked eye examination of the ovary the one day old corpus luteum appeared as a small, bright red spot, slightly beneath the ovarian surface and the above mentioned opening was scarcely visible. In the two days old corpus luteum a gradual protrusion of the corpus luteum has taken place and in the three days old corpus luteum the opening was a circular elevation just above the ovarian surface. During the ensuing six or seven days the colour of the corpus luteum changed from red to pink and the growth in size continued. The corpus luteum was covered by epithelium on the 7th - 9th day after the follicular rupture. Thereafter the protrusion gradually decreased and the colour of the corpus luteum changed to yellow. On the 16th day when a new Graafian follicle ruptured the colour of the one cycle old corpus luteum was whitish-yellow.

After the rupture of the fresh follicle, the one cycle old corpus luteum further decreased in size and it migrated gradually into the ovary.

It was found that the corpora lutea of different ewes were almost of the same size at identical developmental stages, disregarding the age of the animal or the size of the ovary. A hole in the centre of the corpus luteum often persisted throughout the first sixteen days of luteal development.

On histological examination two to three hours after ovulation the newly ruptured follicle was found to contain a

coagulum of blood and follicular liquid surrounded by cell debris and the remnants of the follicular wall. At the point of rupture there was an oval hole in the follicular wall through which part of a blood clot was seen to protrude from the follicular cavity. At the periphery of the follicular cavity the follicular wall, due to the cessation of the internal pressure, was raised into folds (Figs. 30, 31 & 32).

After the lapse of 6 - 7 hrs. the theca interna cells broke through the basement membrane at the top of the folds and began to grow towards the centre of the cavity. The granulosa cells, according to the vigour of growth, occupied a more or less extended area on the periphery. While the cells which originated from the theca interna showed very active division, those of the granulosa were not dividing. There was still blood visible within the follicular cavity.

From the crest of the folds vigorously growing connective tissue trabeculae advanced towards the centre of the cavity. The trabeculae were always accompanied by numerous blood vessels. In addition, among the lutein cells of granulosa origin, there were extensive areas filled with blood.

In the 12 - 16 hrs. old corpus luteum round cells of granulosa origin were larger than in the previously described one and there were numerous blood sinuses among these cells. Groups of granulosa lutein cells were surrounded by connective tissue trabeculae and in the axis of the trabeculae the connective tissue cells showed a more vigorous growth and better

development than was visible in the specimen which was 6 hrs. younger. The blood which was found earlier lying freely among the granulosa origin lutein cells was mainly surrounded by connective tissue (Fig. 33). In the centre of the organised corpus luteum however there was present a cavity filled with what appeared to be the remnants of liquor folliculi.

The 24 - 48 hr. old corpus luteum appeared to be better organised and the slit-like opening of the corpus luteum was filled out by luteal tissue (Fig. 34). One of the 48 hr. corpora lutea was partly herniated and has shown a well developed plication (Fig. 34). The number of sinusoids among the granulosa cells has decreased considerably. The connective tissue was well developed and carried a large number of blood vessels (Fig. 35). In addition to this, connective tissue islands were found at almost every part of the plicae in the plicated specimen. The nuclei of the granulosa cells have become more vesicular than in younger specimens. The cytoplasm of the cells were full with very small, faintly eosinophilic granules.

In the 3 - 7 day old corpus luteum the connective tissue showed a vigorous growth and by the 7th day it has surrounded every group of luteal cells. The luteal cell groups were formed of 3 - 4 cells. Connective tissue trabeculae were leaving the area which was the theca externa carrying blood vessels into the inner part of the corpus luteum. Even in specimens which were herniated the connective tissue covered the whole corpus luteum. The origin of the connective tissue, however, was not the tunica albuginea of the ovary, but the connective tissue of the corpus luteum.

There is a fair amount of reticular tissue throughout the organ, which seem to have originated from the connective tissue accompanying the blood vessels (Fig. 36).

The corpus luteum reaches its maximum development on or about the 10th day. At that stage in addition to the connective tissue elements the following cell types can be differentiated:

a) granulosa lutein cells: a large round cell (30 - 34μ in diameter) with a centrally placed large vesicular nucleus. The cells in the paraffin embedded specimens are almost completely devoid of any cytoplasm and if any was present it was faintly eosinophil and was usually adjacent to the nucleus (Fig. 38a).

b) thecal lutein cells: elongated polygonal shaped cells with a centrally placed ovoid comparatively small darkly stained nucleus. The cytoplasm of these cells was not vacuolated and was strongly eosinophil. The length of the cell varies from 20 - 26μ and its width from 12 - 16μ . (Fig. 38b).

c) thin elongated cells which resembled the small theca interna cells. Small centrally placed darkly stained nucleus found within the eosinophil cytoplasm. These cells are often found to contain two nuclei and measured 14 - 18μ in length (Fig. 38c).

The big and the small cells formed part of an organised pattern and almost every large cell was surrounded by the small cells and the whole structure was vascularised (Fig. 37). In some

cases part of the follicular cavity was still present and was filled with what appeared to be the remainder of the liquor. In the non-pregnant animals twelve days after ovulation, i.e. at the approach of the next pro-oestrus, the corpus luteum began to degenerate. Degeneration was a very gradual process and began with the gradual decrease in cell sizes and pyknosis of the nuclei. The large cells of granulosa origin were the first to suffer and in the trabeculae the connective tissue showed an increase in amount to the detriment of the theca interna cells (Fig. 39 & 40). The connective tissue trabeculae have gradually increased in size and the granulosa cells become smaller. The whole process of degeneration gathered momentum at the time when the new heat set in and it advanced rapidly after the rupture of the new follicle. The strongly developed connective tissue ultimately completely replaced the lutein cells and a migration of macrophages set in (Figs. 41 & 42). Due to the replacement of the lutein cells by connective tissue and the disappearance of the blood vessels the corpus luteum became smaller and was transformed into a corpus albicans which gradually migrated deeper into the ovary. By the end of the second cycle the original corpus luteum was a connective tissue structure well within the ovary (Fig.. 42). Its shape was spherical and the organ was devoid of blood vessels and reticular fibres and was composed entirely of collagen and elastic fibres and of very few fibroblasts. In the centre of the organ a large number of big macrophages were visible. The number of these cells decreased with each successive ovulation until finally it was only a small

connective tissue structure which remained of the follicle (Figs. 42 & 43).

Histochemical reactions in the ovary

The basement membrane beneath the germinal epithelium has shown a positive P.A.S. reaction. The reaction was not affected after treatment with diastase and hyalase or extraction by methanol-chloroform mixture.

The contents of the rete ovarii tubules was found to give slight reaction with P.A.S. and a very weak reaction with Southgate's mucicarmine. The P.A.S. positive reaction did not disappear after methanol-chloroform, diastase or Hyalase. No staining was seen with Sudan black and Toluidin blue. With methylene blue reaction was obtained only at pH 7.0.

Histochemical reactions in the follicles and corpus luteum. Fat was only found in the theca interna cells of the large follicles. The fat was Sudan 3 and Sudan 4 (Sharlach R) positive.

In the developing follicles the liquor folliculi stained with mucicarmine and reacted faintly with P.A.S. P.A.S. reaction was also obtained in the zona pellucida and it was more pronounced than that of the liquid. A faint P.A.S. reaction was also obtained in the granulosa cells and preceding follicular rupture in the cells lying nearest to the basement membrane a number of P.A.S. positive granules appeared which stained more intensely than the rest of the cytoplasm (Fig. 47). This comparatively weak reaction of granulosa cells and liquor was enhanced after treatment with diastase and has disappeared after hyalase treatment.

On staining with Toluidin blue it was difficult to detect metachromasia in the earlier stages of follicular development except in the zona pellucida which was constantly metachromatic. Once however the follicle reached the diameter of 500μ the liquor folliculi and granulosa cells also showed metachromasia although the reaction was always strongest at places nearest the zona pellucida of the developing ovum. In further developed follicles (800 μ) and at certain stages of follicular development, the reaction disappeared temporarily.

The P.A.S. positive material was negative with Sudan black B which in paraffin sections stained the yolk granules only.

With Methylene blue at pH 3.0 the granulosa cells and zona pellucida reacted strongly and the theca interna gave a comparatively weak reaction. Similar reactions were obtained at pH 5.0, in addition the liquor folliculi was also positive.

In follicles of 500μ or larger the cells (cytoplasm) of the theca interna have shown a strongly positive alkaline (glycero) phosphatase reaction which was occasionally found in the theca externa and granulosa as well (Figs. 44 & 45).

Acid (glycero) phosphatases were found only in the nuclei of the granulosa cells in large follicles (800μ and over) (Fig. 46). Fibroblasts nuclei occasionally showed positive reaction as well.

In the degenerating follicles the liquor folliculi was strongly positive with Southgate's mucicarmine and P.A.S.

During degeneration the liquor folliculi formed droplets rather than a continuous mass and granulosa cells which were detached in the degenerating follicles were strongly metachromatic with Toluidin blue and stained strongly with Methylene blue even at pH 3.0. Moreover the reaction was not affected by treatment with hyalase.

A further change in the degenerating follicle was that the theca interna, granulosa and liquor folliculi now gave positive alkaline phosphatases (glycero) reaction.

At the beginning of corpus luteum formation the theca interna cells continued to show strongly positive alkaline phosphatase reaction which contrasts sharply with the negative granulosa (Fig.48) cells. At about the 10th day (after follicular rupture) the granulosa lutein cells began to exhibit positive alkaline phosphatases reaction which persist for a few days then begin to diminish and by the beginning of the following oestrus the reaction ceases.

In the few days old corpus luteum the frozen sections contained numerous fat globules which stained with Sudan 4. The number of the globules increased with the development of the corpus luteum. In frozen sections from corpora lutea at the maximum stages of their development the large cells contained a large amount of fat which stained deeply with Sudan 3 and Sharlach R. It was due to the amount of fat that the cytoplasm of the large cells was scanty when examined in paraffin embedded and haematoxylin-eosin stained specimens. If paraffin embedded

specimens were stained with Sudan black B in the smaller type cells the sudanophil elements were evenly distributed throughout the whole of the cells.

With the degeneration of the corpus luteum the lipid content of the cells gradually decreased and had completely disappeared from the one cycle old follicle. The macrophages which occupied the centre of the degenerated follicle remained however strongly sudanophil and P.A.S. positive (Figs. 43 & 49).

The Oviduct

Macroscopical Anatomy

The oviduct of the sheep is a partially pigmented narrow tube which, like that of other mammals, is enclosed within the folds of the uterine ligament. In its course it usually forms 5 - 6 loops which finally join the coiled end of the uterine horns. Its average length in the Scottish Blackface sheep was 16.3 cm. (Chart 6).

It was found that the length of the oviducts was influenced by the age of the animal and as a rule the older the animal was the longer its oviduct. The oviduct can be divided into three parts, viz. that of the infundibulum, ampulla and isthmus. One segment merges gradually with the other.

The infundibulum is the ovarian extremity of the oviduct. It is situated cranially to the ovary and is roughly in the triangular area formed by the ovary, the border of the broad ligament and ampullar part of the oviduct. It is conical and as a rule it measures 7.5 - 15 cm. in length and 0.2 - 0.3 mm.

in thickness. In oestrus it completely envelops the ovary. The free edge was fimbriated and the fimbrae continued into folds. These folds are 1 - 2 mm. in height and run centripetally towards the ovarian opening of the oviduct (abdominal ostium).

In the cranial part of the ovarian ligament one finds the ductuli aberrantes superiores which were thin white structures slightly raised above the oviduct surface and resembled small vessels injected with white material.

The ampulla is the second part of the oviduct, and connects the infundibulum to the isthmus. It was 5 - 7 cm. long and its diameter was 3.5 - 4.5 mm. as a rule. It formed 2 - 3 loops in its run. The thickness of the ampulla decreases from a maximum at the infundibular end to a minimum of about one-third to two-fifths at the isthmic end. The thickness of the wall shows also a considerable alteration, namely an increase from 0.2 mm. at the infundibular end to 0.5 mm. or 0.6 mm. at the point of joining the isthmus. Occasionally fine melanin pigment was found in its wall, never however in big quantities.

The isthmus was almost as long as the ampulla - 4-5 cm. - and forms 2 - 3 loops. The diameter of the isthmus is 1 - 2 mm. and the thickness of its wall is 0.75 mm. The wall is usually pigmented. On its mucous surface there are usually 4 low ridges which are connected by very fine structures to one another.

The tubo-uterine junction is very gradual in the sheep. At the distal end of the isthmus the uterine glands are already

appearing while the wall is still that of the oviduct. Then the uterine glands gradually increase in number while the folds decrease and the appearance of the first caruncles mark the boundary of the uterus.

The oviduct is supplied with the ovarian artery from the infundibular to the isthmic and from the isthmus caudally it is supplied with the uterine branch of the uterine artery. The ovarian artery is a branch of the uterine artery.

Histology

The wall of the oviduct is composed of three layers which are from without to inwards; serosa, muscularis and mucosa (Fig. 50).

The serosa is the peritoneum which on the dorsal and ventral aspect covers the oviduct. On the medial and lateral aspects one finds the lig. lata.

In the muscularis large numbers of smooth muscle fibres are surrounded by connective tissue. This layer is very thin in the infundibulum and ampulla and is thick in the isthmus.

Pigment granules are encountered in the muscularis connective tissue.

The mucosa is composed of an epithelium with a basement membrane and a very thin lamina propria. The loose connective tissue is raised into folds which give to sections, especially to those which originate from the ampulla, a most intricate appearance.

Detailed study was confined to the mucosa.

Cell types in the oviduct epithelium

In the oviduct of the sheep four cell types described by previous authors - the non-ciliated secretory, rod- and round-cells - have been observed. However only the first two types - non-ciliated secretory and ciliated non-secretory - were present at all phases of the cycle. The rod and round cells were found only during late met- and early di-oestrus. Variations in the proportion of secretory cells were noted at different levels of the oviduct. The secretory cells were most abundant in the infundibulum and ampulla though in the latter the number was rather less than in the former. There were comparatively few in the isthmus.

Ciliated and secretory cells are columnar cells of equal height, but the nuclei of the latter were placed nearer to the basement membrane than that of the former.

Both of these cells underwent a gradual increase of height during the sexual cycle, reaching their maximum height in oestrus and met-oestrus. Actual measurements were 19 - 24μ in di- and an-oestrus, 24 - 31μ in pro-oestrus and oestrus and 31 - 35μ during oestrus and met-oestrus (see Fig. 51). (Compare the height of cells on Figs. 52, 53, 54 and 57.)

The round cells as a rule were observed only during the di-oestrus and pro-oestrus period and lie between the bases of the other cells. They were considerably smaller than the columnar cells.

Rod cells appeared during met-oestrus and were found up to early di-oestrus. They are as high as the ciliated and

non-ciliated secretory cells and appeared to have been produced from the secretory cells by the extrusion of the nuclei. However, the change from secretory into rod cells was a gradual one. During late met- and early di-oestrum in some of the cells, the nuclei move nearer to the surface of the cell and create the impression of wandering out. These nuclei are elongated and thin and there is, as a rule, a constriction visible in the middle of their length. Once outside the cell they became round to oval and measured 2 - 5 μ in diameter. In the later phases of di-oestrus they were regularly recovered lying free in the lumen of the oviduct (Figs. 63 and 64). At this time the cells from which they originated appeared thin, rod-like and without nucleus. Their thickness is half of the ciliated cells and their cytoplasm is intensely stained with pyronin. As a rule they disappear before the beginning of pro-oestrus and none were ever found during oestrus.

Histochemical reactions

Lipids. Although lipid droplets were occasionally found scattered throughout the whole thickness of the oviduct wall, they were not found in the epithelial cells in any stage of the cycle.

P.A.S. + Material. In the sections stained with P.A.S., with the exception of the late di- and an-oestrus specimen, the secretory cells were easily recognised from their content of Schiff positive material. This was found to consist of minute granules which varied in colour from brilliant ruby to deep pink in different phases of the cycle.

The granules stained least intensely (pink) during early di-oestrus. The intensity of staining reached its maximum (brilliant ruby) during oestrus and met-oestrus.

In addition to the alteration of the staining intensity the granules also varied in position and amount. Thus during pro-oestrus the granules were found to be massed between the nucleus and the free surface. During oestrus and met-oestrus the granules within the cells could only be found in a thin line, just below the free surface, and at this time some Schiff + material appeared in the lumen of the oviduct as well. Towards the end of met-oestrus the Schiff + material disappeared from the cells and from the lumen of the oviduct. It became completely absent by the 4-5th day after cessation of heat (Figs. 55, 56, 57 and 58).

The P.A.S. + material was not affected by diastase or Hyalase and thereafter sections were stained with Sudan black which also proved negative. Further sections were then stained with Celestin blue and Toluidin blue. Metachromasia was observed with Celestin blue and with Toluidin blue.

Finally sections were stained with Southgate's mucicarmine and Methylene blue. Mucicarmine has stained all the P.A.S. + substance. Methylene blue staining was only positive at pH 7.0.

Phosphatases. Positive reaction was obtained in the epithelial cells with Gomori's method of alkaline (glycero) phosphatase reaction, during oestrus and met-oestrus. In the epithelial cells of the infundibulum and ampulla the material

appeared as a globular mass in the distal part of the secretory cells after 1-2 hours incubation (Figs. 59 and 61). In the isthmus on the other hand a positive alkaline phosphatase reaction was present in all cells, but it appeared to be localised to a narrow area immediately beneath the free surface (Figs. 60 and 62). Positive reactions were not obtained during di- and an-oestrus.

Only occasional nuclear reactions were obtained for acid phosphatase and they varied so much that no conclusions could be drawn.

Nucleic acids. Sections fixed in corrosive formol and Zenker's solution were stained with Feulgen's method for the presence of desoxyribonucleic acid and with Methyl green - Pyronin (Unna-Pappenheim) for the presence of ribo- and desoxy-ribonucleic acid. Pyronin suggested that there was no ribonucleic acid in the cytoplasm of the columnar ciliated and secretory cells during di- and an-oestrus. The ribonucleic acid content of these cells increased during pro-oestrus and reached its height during oestrus and met-oestrus.

Nucleated projections of the secretory cells. These projections can be divided according to their position in relation to the cell into two different groups. In one of these are included projections which are still in contact with the cells and form finger-like processes projecting into the lumen of the oviduct. The other group includes those which are lying free within the lumen of the oviduct (Figs. 63, 64 and 65).

Projection of both groups gives a positive Feulgen reaction. Those projections which are still in contact with the cells possess, in addition, a cytoplasmic covering which gives a faint reaction with Pyronin. This feature is absent from those which lie entirely free within the lumen of the oviduct.

The Uterus

Gross Anatomy. The uterus of the sheep is a pink coloured bicormate organ. Anatomically a uterine body and two tapering horns which coil at their end can be described. The horns without any outer landmark continue into the oviduct and the uterine body into the cervix. It is customary to divide the bicormate uteri, according to Seiferle (1936) into two sub-groups, namely (a) those by which the uterine body is undivided (uterus bicornis non subseptus - horse); and (b) those by which the uterine body is divided (uterus bicornis subseptus) (Fig. 67). The sheep with the other ruminants belong to this group of animals. As the continuation of the body at the anti-mesometrial border the two horns for the first 5 cms. of their length are kept together by a ligament.

The average length of the uterus was 20 cms. and the average weight was 150 gms. It was found that the age of the animal and the phase of sexual cycle influenced the weight and length of the organ (Chart 6); viz. uteri of younger sheep were lighter and those of older animals were heavier. Again uteri from animals of similar age were lighter during an-oestrus and pro-oestrus and were heavier during met- and early di-oestrus (Chart 6).

In the opened uterus and uterine horns macroscopically noticeable, very often pigmented round button-like prominences are visible. These prominences are called caruncles. Their size varies between very wide limits even within the same uterus. In every uterus the largest caruncles were found at the origin of the uterine horns from the body and the smallest at the cervical orifice. Four of the caruncles are in one horizontal line and there are four rows running vertically along the whole length of the uterine body and uterine horns (Fig. 66). The number of caruncles was approximately 100 in the whole uterus. They were often jet black in the Blackface sheep and the pattern of pigmentation and the hue of the black varied between wide limits. The caruncles were smaller in the young sheep and larger in the older ones. Further there were cyclical alterations taking place in the size of the caruncles. They were smaller at the end of di-oestrus and during pro-oestrus than during met-oestrus and oestrus.

The thickness of the uterine wall was from 3 - 5 mm. in the intercaruncular area and like the caruncle it showed alteration during the sexual cycle.

Endometrium - Histology. Two different regularly alternating areas can be identified in the endometrium of the ewe. The one is the caruncular area which is composed entirely of connective tissue and blood vessels and is devoid of glands and the other is the intercaruncular which contains the uterine glands. As has been described above the caruncular area was often found to be heavily pigmented.

The whole uterine mucosa rests on a reticular fibre network.

The uterine lumen is covered by an epithelium of columnar cells which continues without any apparent change into the glandular epithelium. The basement membrane of the epithelium is similarly continuous with that of the uterine glands. The glands are tubular, slightly branching coiled glands which reach down to the muscularis and usually invade part of the caruncular base.

The cells of the epithelium

In oestrus the uterus was lined by columnar epithelial cells of 27 - 31 μ in height. All of them had strongly basophilic basally placed nuclei and their cytoplasm was eosinophilic. At irregular intervals round cells with clear cytoplasm were seen on the basement membrane. During met-oestrus a growth of the epithelial cells took place and the epithelium became pseudo-stratified. The cells were tall columnar and measured 32 - 37 μ in height. In the mucosa there were enlarged tissue spaces due to oedema and the mucosa was much thicker than during any other phase of the cycle. At the same time a folding of the uterine mucosa was seen. The epithelium remained in its maximum developmental stage approximately until the 10th day after ovulation. During early di-oestrus a large number of lymphocytes could be found at irregular intervals in different places in the epithelium.

During the second half of di-oestrus an involution took

place in the uterine mucosa and the epithelial cells became lower and narrower. They measured 17 - 20μ in height and subsequently the epithelium was again simple columnar (Figs. 68 and 69). During an-oestrus or the resting period the uterine epithelial cells were very low and slender and measured between 15 - 18 μ in height.

The structure of the uterine glands

The uterine glands can be divided into two parts, namely (i) the opening or orifice of the glands and the deeper part (ii) which lies just above the muscularis and referred to as the body. The whole gland is lined by columnar to cuboidal epithelium similar to that of the uterine lumen and is surrounded by a basement membrane (Fig. 72 and 73).

During an-oestrus the glands were small and few and apparently non-branching. There was an increase in their number and growth was taking place during pro-oestrus and oestrus and secretion during met-oestrus and the first half of di-oestrus. Consequently, with reference to the alteration which took place in the sheep uterine glands during the sexual cycle, the following phases could be distinguished -

a) preparatory period - beginning 48 to 12 hrs. before heat and stretching to heat (pro-oestrus), characterized by an increase of glandular cell size;

b) proliferative period - extending from the onset of heat to ovulation, and is characterised by further increase in the glandular cell size and increased coiling of the glands;

c) secretory period covering the time of met-oestrus and early di-oestrus when secretory material leaves the cells;

d) quiescent period - late di-oestrus and lasting (approximately 6 days) until pro-oestrus or the period of an-oestrus.

The secretory cells during this period again became cuboidal or low columnar and were interspersed by narrow "rod" cells which were thin, columnar and devoid of any nucleus.

The structure of the caruncle

Reticular fibres were the main supporting elements of the caruncles but in addition to them collagen fibres were found (Fig. 70 and 71). Elastic fibres were only found around blood vessels. In addition to the commonly occurring connective tissue cells, big round (20μ diameter) cells with a round nucleus were encountered which were most numerous in the deeper layers of the mucosa and decreased in number towards the epithelium. The cells stained lighter red with eosin than the surrounding area and a number of dark black granules were occasionally seen in those which lay close to the muscularis. The nearer the cells were to the epithelium the more pigment granules they contained, (Figs. 80 and 81).

In addition to this occurrence of pigment, a large number of melanophores were found loaded with finer pigment granules than the ones encountered in the round cells. These cells, contrary to

the previous group, were most numerous beneath the epithelium and decreased in number towards the muscularis. The cells had a large oblong body and extended cytoplasmic processes which contained a large number of the fine pigment granules. A large vesicular nucleus was always almost in the centre of the cytoplasm. From a purely morphological point of view these cells were similar to the true pigment cells found in the lamina fusca. They had a varied number of processes and all of them contained minute black pigment granules.

Blood supply

Since placentation in the sheep is restricted to caruncular areas, and some of the earlier authors have described caruncular bleedings during oestrus, special attention was paid to the blood circulation within the caruncles during the different phases of the cycle.

During an-oestrus there were only very few capillaries visible in the caruncle and they were running diagonally (straight) to the surface (Fig. 87). During pro-oestrus (Fig. 82) an increase in the size of the blood vessels was noted and during oestrus (Fig. 83), met-oestrus (Fig. 84) and early di-oestrus (Fig. 85 and 86) a large number of additional blood vessels and capillaries appeared. In some animals an occasional small haemorrhage was noted to have taken place beneath the uterine epithelium. It was only visible in those animals whose caruncle was not pigmented (Fig. 86). In the later phase of di-oestrus the blood vessels underwent an involution, became smaller and decreased in number.

Histochemical reactions

Inorganic Iron. Inorganic iron in the form of minute granules was encountered in the glandular epithelium only. None was present during di-oestrus, an-oestrus or during pro-oestrus. However at the beginning of the proliferative period a number of small granules appeared and their number has considerably increased by the end of this period. The granules were only found in the distal part of the cells between the nucleus and the glandular surface. During the secretory period the number of granules has decreased within the cells and a number of them appeared within the lumen of the gland (Fig. 76 and 77).

Organic Iron. Organic iron was found associated with the erythrocytes in blood vessels and in some of the round cells.

P.A.S. Positive Material. In the endometrium of the ewe two types of P.A.S. reactive material were encountered. The (i) first one a weakly reacting P.A.S. positive material which was found during early di-oestrus in very small quantities in the uterine epithelial and glandular cells and somewhat later in the lumen of the uterus or that of the uterine glands (Fig. 75); and the (ii) second strongly reacting material in large quantities within the round cells which presence apparently was not associated with the sexual cycle.

(i) and (ii) remained P.A.S. positive after treatment with diastase, hyaluronidase and methanol-chloroform and has not shown any metachromasia with Toluidin or Celestin blue (Fig. 81). They

have shown only a faint staining in slight acid and a strong reaction in the neutral Methylene blue solution. Consequently it is assumed that the cytoplasm of the cells contain a type of mucoprotein.

Ribo- and Desoxyribonucleic acid. The ribonucleic acid content of the cells showed a cyclic alteration associated with the breeding season. The maximum amount of reacting material was to be found during the secretory phase and hardly any at all during the quiescent period. The rod cells whose occasional appearance is described during the quiescent period have never shown any ribo- or desoxyribonucleic acid reaction.

Fat. Neutral fat in the form of small globules which have stained equally well with Sudan 3 and Sharlach R was found in the glandular and epithelial cells during the proliferative phase. The globules were located between the nucleus and the free surface of the cells. During the secretory phase the globules were found in the lumen of the glands, in that of the uterus. In addition to this fat small globules were found in the round cells which apparently were not influenced by the sexual cycle.

Alkaline and acid phosphatase. Disregarding positive nuclear reactions which could be obtained in almost all phases of the cycle, positive alkaline phosphatase reactions were obtained in the cytoplasm of the epithelial and glandular cells during the secretory phase. The reaction followed the course of the glandular activity; thus the reactions were first obtained

from the orifice of the gland and only later that in the basalis. The reacting material completely disappeared during the quiescent period (Fig. 78 and 79).

Discussion

Ovaries. Although the number of ovaries which were weighed was comparatively low (120) it is still the highest number so far available for effective comparison. Chart 3 which shows the mean ovarian weights in different phases of the cycle showed that as a rule the ovaries tend to be the heaviest during di-oestrus and lightest in an-oestrus on the one hand and on the other that when an-oestrus ovaries are compared those from the oldest animals are the heaviest. These results confirm Bonnet's (1884) and Robinson's (1950) results.

Although Keller (1942a, b & 1943) and Höfliger (1948) have shown the presence of a collagen fibre ovarian skeleton in the cow, despite all the diligent search no such skeleton was found in the ovary of the sheep. The ovary was found to be supported by reticular fibres which surround the individual follicles.

The presence of a basement membrane in the ovarian epithelium has not been described before. Its existence was shown by the P.A.S. positive reaction (Gersh, 1947, Gersh & Catchpole, 1949) and the strength of this reaction was not affected by diastase or hyalase or the methanol-chloroform extraction, further it was stained by reticular stains.

Oogenesis at present is one of the most hotly debated problems of reproductive histology. While Desaive (1947 and

1948) has found that it is rhythmical in the rabbit and Höfliger (1948) assures us that it is also rhythmical in the cow, Green, Mandl and Zuckerman (1951) and Mandl and Zuckerman (1952b) have found it to be continuous in the primate and in the rat. Some of the authors either prefer not to take issues or accept both possibilities (Harrison, 1948). With regard to the result of the present work on the basis of the examined material it is impossible to arrive at a correct judgement. While on the one hand there is ample proof for it, that there is an increased number of primary follicles detectable during pro-oestrus and oestrus, the basic question whether there is any oogenesis in other phases of the cycle cannot be answered definitely. The most obvious solution was thought to be the observation of the number of follicles in ovaries at the beginning of an-oestrus and the comparison of this number with those found at the end of an-oestrus. It was thought that if there were no changes in the appearance of follicles during the resting phase this would be an indication for the cessation of oogenesis during an-oestrus. However there were quite a number of follicles detected during an-oestrus which were at the beginning of atresia. From this it is evident that the ovary, or at least some of them are not at a complete standstill during an-oestrus.

With regard to the origin of the newly formed oocytes Mandl and Zuckerman (1952a) conducted experiments in rats by which they established that despite the destruction of the germinal epithelium the formation of oocytes is a continuous

process. From this it appears that it was not the germinal epithelium which provided the ovary with the newly formed oocytes but some other structures. During no phase of the cycle was it possible to find any structure in the ovary of the sheep which could be described as Pflüger's tube. The absence of this germinal epithelial structure in addition to the presence of the basement membrane and the strongly developed tunica albuginea makes it difficult to assume that new formation of oocytes in the ewe is from the cells of the germinal epithelium. Indeed Cole and Miller (1935) expressed the view that whereas in the dog and cat - animals with scant or no tunica albuginea - the formation of new oocytes apparently originates from the germinal epithelium, in animals with strong tunica albuginea, the development takes place beneath the structure. On the basis of the present work this certainly seems to be the case in the sheep. The oocytes are surrounded by the follicular cells which are always flat.

A somewhat uncommon feature of the sheep ovary is the multinuclear ova and the polyovular follicle. These structures which according to Bacsich (1948, 1951) are to be regarded as developing under the influence of the same causative agent are described to occur in addition to the sheep, in the human embryo and neonatal (Bacsich, 1948, 1951), in the cow (Höfliger, 1948), in the goat (Harrison, 1948) and in the horse (Harrison, 1946). There are two schools of thought with regard to their origin, (a) those who assume a pathologic function of the "morphogenic process" (Dawson, 1951) or (b) those who assume hormonal activities.

While the majority of workers reject the idea of hormonal influence (Corey, 1928, Smith, Engle and Tyndale, 1934, Saunders and Cole, 1936, Collip, 1935, Clauberg, 1932, Zephiroff & Dobrovolskaya-Zavadskaya, 1940); Bullough (1946, 1944c), Harrison, (1946 and 1948) and Bacsich (1951) show that the presence of multinuclear ova and polyovular follicles bears a relation to the hormone secretion of the individual or mother.

In the sheep, like that of the goat (Harrison, 1948) and cow (Höfliger, 1948) the number of polyovular follicles increased from a very low beginning at early pro-oestrus to some 12% of the total, just after heat. However while in the goat only type I of polyovular follicles was recovered (Harrison, 1948), in the sheep all the three types as described by Hartman (1926) could be seen. It is assumed by earlier authors that the presence of polyovular follicles, like that of multinuclear ova is due to the influence of gonadotrophic (Bacsich, 1951) or oestrogenic (Harrison, 1948) hormones. On the basis of the present work while it seems reasonable to assume that a morphogenic process is at play - indeed what else could be - at the development of these pathological structures, it appears evident that this morphogenic process is released or induced by the hormonal influences. If it would be a purely morphogenic process, then the number of these pathologically developing structures ought to be higher and should not bear relation to the sexual cycle, and if it would be due to hormonal influences alone then again one ought to witness occasionally an almost completely pathological crop of oocytes produced during oestrus.

Harrison's (1948) investigation on the ovary of the goat further confirms this theory. He has shown namely that three days preceding heat 3% of all follicles are polynuclear and that this number increases up to 12% at the time of heat.

The presence of multinuclear ova and polyovular follicles as a rule can only be witnessed at the early stages of follicular development preceding the appearance of granulosa cells. None of them were encountered in the later stages of follicular development. Of the multinuclear oocytes some were found with small spoke-like processes which, according to Harrison (1948) are to be regarded as the cell membranes of the original oocytes.

The disappearance of the follicular cells which surround the early oocytes was described as degeneration in the goat (Harrison, 1948). In the ovary of the sheep however no degeneration of the follicular cells was witnessed and the impression was that the follicular cells gradually became transformed into granulosa cells. If Harrison's (1948) point of view would be accepted it would also be necessary to explain how these cells were produced by the adjacent connective tissue.

By students of early follicular development it is generally assumed that the spaces which appear among the granulosa cells in the developing follicle are caused by the pressure of the liquor folliculi. During this work however it was found that intercellular spaces in small follicles were visible before any liquor folliculi formation was seen to have taken place. The granulosa cells in these early follicles had a distinctly different

appearance from those which were seen in the smaller or larger follicles since they had processes with which they were in contact. Since it was not possible to show the presence of any substance in these spaces it is assumed that they were vacuoles.

The relation between the growth of the follicle and the growth of the ovum in the sheep's ovary was similar to that of the goat (Harrison, 1946) and sow (Parkes, 1931 and Green, 1951). The data are comparable to Brambell's measurements (1928) on the follicular growth in mice; Green, Mandl, Zuckerman's (1951) measurements on the follicular growth in the rat, and the comparative measurements of Parkes (1931) on sow, bovine, etc. Although the diagram presented in this paper in unison with all the previous results shows the two distinctive growth phases, following the advice of Robb (1954) it is presented as one continuous curve. Although this method of presentation is at a variance with all the published results of the previous authors who have always shown a separated "a" and "b" phase of growth, the division of the curve into two phases on the basis of my results would only be artificial. One would not rightly know which measurements to enumerate among phase "a" and which during phase "b" (Robb, 1954).

Since small follicles were much more numerous than large ones, like Harrison (1948) I had to estimate the regression line of a lower number of follicles than that of the smaller ones.

In general the development of the sheep's follicle is comparable to that of the cow (Hammond, 1927, Höfliger, 1948), the pig (Corner and Amsbough, 1917) and the goat (Harrison, 1948).

The zona pellucida appears in the follicle of 66.5μ diameter, it develops very gradually and is extremely slow in growth. In the mature follicle it shows a thickness of not more than 6 - 8μ .

The function and behaviour of the thecal cone was described at length by Strassmann (1941) who associated it with the follicular movement towards the ovarian surface. Harrison (1948) on the other hand questions such a simple function. He suggests the possibility that its formation might be due "to the better blood supply to the cortical pole of the follicle" and when the few "wreaths" of blood vessels surround the follicle it disappears. For the appearance of the thecal cone which points towards the medulla he gives the reason of arrested migration and for the reappearance of a new external one its eventual contribution to ovulation.

Harrison (1948) expressed the opinion that the follicle stimulating hormone acts on the theca interna and Westman (1934) and Harrison (1948) in the rabbit have noticed an enlargement in the theca interna upon administration of follicle stimulating hormones.

While working on this problem it was found that the theca interna increased in thickness during oestrus in the ewe when the follicle stimulating hormone activity is at its highest and similar changes were noted in the Geomys (Mossman, 1937), in the cow (Hammond, 1927), in the sheep (Warbitton, 1934), in the goat (Harrison, 1948), in the pig (Corner, 1919) and in the horse (Harrison, 1946).

With regard to the validity of Sckrochet's (1916) theory on the proteolytic enzyme production in the thecal cone in the ewe, this possibility certainly cannot be excluded. It might have been coupled during the last "actively growing phase" of the follicle with the follicular pressure and the disappearance of the tunica albuginea immediately preceding the rupture of the follicle. However no histochemical test was performed for proteolytic enzyme activity.

For the reason of the appearance of the "festooned" follicles no explanation can be given. It might be that it is a more efficient way to increase the amount of liquor folliculi through the dialysis of serum through the wall of the blood vessels if it is situated almost inside the follicle.

Although no special technique was used to investigate follicular circulation, with the help of the HE and Masson's trichrome staining, it was found that it is similar to that of the pig (Andersen, 1926), rat (Bassett, 1943) and goat (Harrison, 1948).

Although it was found in several cases that sinusoids were in close proximity with the basement membrane and even projecting into the follicle especially in the "festooned" follicles no blood vessel was ever found to pierce the basement membrane. In conformity with Corner's (1919, 1921) and Pincus and Enzmann's (1932) observation, the appearance of additional antra within the cumulus oophorus was found in follicles which were very close to rupture. These antra however were only filled with liquor folliculi

and not with blood as Corner (1919) describes it in the pig. Although this is contrary to Corner's (1919) view the appearance of additional antra in the cumulus oophorus immediately preceding the follicular rupture is described to occur in all animals in which ova is shed without a corona radiata, e.g. in the goat (Wester, 1921), in the pig (Corner, 1917 & 1919), and in the rabbit (Pincus and Enzmann (1932).

Stieve's (1934) account of the rupturing follicle in the sheep ovary was found to correspond to those observed when working on this thesis. He did not mention the formation of additional antra within the cumulus which is an invariable precursor of ovulation on the one hand and on the other he altogether omitted to describe the disappearance of the tunica albuginea and the thinning of the theca externa prior to ovulation.

It is a remarkable fact that no sheep ovum was found however near ovulation which would have shown division or the presence of a polar body.

The size of the freshly ruptured follicle is described by Kelley (1937) as 0.5 cm. and according to him the liquor's escape was responsible for at least 30% reduction in the luteal dimension when compared to the size of the Graafian follicle (see Zuckermann, Green, Fisher, Harrison on sizes). In fact it was found that the size of the newly ruptured follicle and the forming corpus luteum can alternate to a great extent depending upon the size of the follicle before rupture and whether bleeding has taken place. It is thought that at least in 30% of cases no bleeding takes

place and all the more remarkable is the fact that the corpora lutea at their maximal developmental stage look almost identical. The remarkably uniform size of the corpora lutea and the remaining liquor in the middle has been described by Kupfer (1928), Quinlan and Mare (1931), Casida and McKenzie (1932), Grant (1934), Cole and Miller (1934), Kelley (1937) and Grant (1934) which were found to be correct.

While the corpus luteum of ~~the human~~ man and that of the cow is yellow, it was found to be pink in the sheep and only gradually changed its colour - due to degeneration to yellow and later to white. Marshall (1903) has shown that the corpus luteum is formed from the theca interna and granulosa cells and not from the blood clot as described by Paterson (1840). Warbitton (1934) in the sheep adds to them the appearance of "embryonic luteal cells". According to this investigation the "embryonic luteal cells" described by her are the smallest theca interna cells. It is customary to describe three phases in the life of the corpus luteum, namely that of development, highest activity and degeneration. In the sheep the highest activity was reached about the 10th day after ovulation.

Ancel and Bouin (1909) statement with reference to the absence of true interstitial glands in self-ovulatory animals are proved to be correct with reference to the goat (Harrison, 1948) and now with reference to the sheep.

It was found in the sheep that atresia could happen at any stage of the follicular development. The main differences between

a healthy and ~~that of~~ an atretic follicle ~~were already~~ was described by Marshall (1903) and more recently by Sturgis (1949); and the finding described in this work confirms their description.

The period which is most favourable for conditions causing follicular atresia, is reported to be the second half of pregnancy (Hartman, 1926, Harrison, 1948, Marshall, 1903). While working on my thesis large numbers of degenerating follicles were always found during met-oestrus and di-oestrus. This fact is confirmed by Harrison (1948) although it is contrary to the results of Green, Mandl and Zuckermann (1951) in primate ovary. The fact that degeneration takes place at a period of the sexual cycle when in the non-selfovulatory animals the interstitial cells are fully functioning might serve as a further proof in favour of the theory. On the other hand if it is accepted - what is generally assumed - that the formation of oocytes and the maturing of follicles is under the influence of the follicular stimulating hormone of the pituitary, then it can be presumed that the wave of degeneration is caused by the discontinuity of the follicular hormone or eventually by the secretion of the luteinizing hormone. This theory however does not offer any explanation why some of the large follicles are exempt from the degeneration while others are affected. While doing this work it was thought that the amount of oestrogenic hormone in the liquor folliculi acts as a protective agent against the degenerative wave. However since there are follicles even of the largest size - which should contain a high amount of oestrogen - and are affected by the degenerative wave, while many of the smaller follicles -

which should contain less - escape, this theory is clearly not tenable and at present no explanation can be offered for this particular follicular behaviour.

In many of the text books it is customary to refer to the "Call-Exner bodies" which are apparently the places where the formation of liquor folliculi begins. Since none of the modern authors (Harrison, (1946 & 1948); Höfliger (1948); Green, Mandl & Zuckermann (1952)) with the exception of that of Lesbuiyer (1948) refer to them it is assumed that in the developing follicles they are really not bodies at all, merely holes between the spongy threads of the granulosa cells in the developing secondary follicles which become filled with liquor folliculi. In atrophic follicles however it was possible to find spherical bodies formed by the liquor folliculi which could eventually also be described as "Call-Exner bodies".

Histochemical reactions in the ovary

The histochemical reactions associated with the theca interna were the presence of neutral fat and alkaline (glycero) phosphatase. Similar histochemical reactions were described to occur in the theca interna in the ovary of the sow (Corner, 1917, 1919, 1940, 1944; Rossmann, 1942).

There were two further structures in which fat was found, namely in the cells of the corpus luteum and in the granules of the macrophages in the corpora albicans.

The presence of alkaline phosphatase in addition to the cells of the theca interna was confined to the lutein cells of

theca interna origin in the corpus luteum and to the liquor folliculi and theca in the degenerating follicles. The granules in the basal layer of granulosa cells stained well with P.A.S., gave only faint reaction with Toluidin blue and stained well with Methylene blue at pH 3. They have however disappeared after treatment with hyaluronidase. Consequently the granules were assumed to be hyaluronic acid.

The liquor folliculi has reacted with P.A.S. and has shown very weak metachromasia with Toluidin blue. Further it has stained well in Methylene blue at pH 3.0. The staining reaction has disappeared after hyalase treatment and consequently it was assumed that the follicular liquid contains a mucopolysaccharide which was or mostly was hyaluronic acid. At present it is not wholly understood why hyaluronic acid should give metachromatic reactions (Pearse, 1952) if it is not only because of the presence of an additional mucopolysaccharide. According to the works of Allen and Doisy (1924) and Allen (1926) the oestrogenic hormone is a mucopolysaccharide and consequently the presence of the reaction is assumed to have taken place because of the presence of the hormone within the follicle.

The reactions obtained with the degenerating follicle were stronger P.A.S., strong Methylene blue reaction at pH 3.0 and somewhat stronger Toluidin blue reaction than in the healthy follicles. If one accepts the presence of oestrogenic hormone in the follicle as the reason for mucopolysaccharide reaction, then the theory of Mey (1936), Seiferle (1936) and Höfliger (1948),

according to which the degenerating follicles of the ungulates act as interstitial cell gland, seem to be partly proved. The presence of lipids in the corpora of different mammals is an old established fact. The hormone progesterone is expected to be contained in the lipids. With regard to their chemical structures, that in the corpus luteum of the bovine was shown to be a carotid by Amman (1936), Höfliger (1948) that of the Rhesus monkey luteolipid by Rossman (1942) and attempts were made to establish the nature of that present in the corpus luteum of the pig by Yamauchi (1925) and Lang (1925). With regard to that of the goat it is known that the structure is not "luteolipid" (Rossman, 1942). No further study was undertaken to establish the correct nature of the fat in the sheep's corpus luteum. The function of the enzyme phosphatase is, as far as we know, the synthesis of fat (Bourne, 1941). In the case of the theca interna and corpus luteum it would serve to synthesize the fat present in these structures.

The nature of the granules in the big macrophages presented a rather difficult problem. Since the granules were very strongly staining with P.A.S. but were only weakly metachromatic and reacted with Methylene blue at pH 3 and contained neutral fat, cannot be described as mast cells in the customary sense of the word or globular leucocytes (Kirkman, 1950). On the other hand however the presence of the granules within the cells suggests that the cells are phagocytes.

Oviduct

It was found that the length of the oviduct increased with

the age of the sheep and there was about 4 cms. difference between the 1 year old and 5 year old ewe.

Andersen (1926) has already described that the oviduct of the sheep without any change continues into the uterus and this work has confirmed it.

The alteration in the number of P.A.S. positive granules in the secretory cells during the phases of the cycle and their complete absence during an-oestrus and di-oestrus suggests that their production is under hormonal influence. Furthermore, since during certain phases of the cycle (oestrus and met-oestrus) they are to be recovered outside the secretory cells, within the lumen of the oviduct, and at the same time a considerable decrease (in oestrus) or an almost complete disappearance (during met-oestrus) of this number is noticeable within the cell, they could only be interpreted as secretory granules, which are secreted and thus leave the cell during oestrus and met-oestrus.

Corner (1932) refers to a paper which deals with the recovery of "Leucofuchsin positive material" within the mammalian oviduct. However, neither the careful reading of the reference cited by him nor the scrutiny of previous papers written on the subject has enabled me to find the work to which he referred. Accordingly, to my knowledge this is the first occasion on which a systematic study has been undertaken with regard to the variation of Schiff positive granules throughout the various phases of the sexual cycle.

With regard to the nature of these granules it will be noticed that although they are P.A.S. positive they are neither

glycogen nor hyaluronic acid since they are not removed by treatment with ptyalin (diastase) nor with hyaluronidase. This is at variance with the findings of McAllister (1915), Jacovlev (1927), Iwata (1929), Jöel (1939a) who claim to have recovered glycogen in the human oviduct. In this respect the secretory process in the oviduct of the ewe shows a definite deviation from that of the human. Further, the secretory granules were negative to the Sudan stains, therefore the secretion was not glycolipid. Metachromasia was obtained with Toluidin blue and Celestin blue and it was therefore assumed that the granules were composed of sulphated mucopolysaccharide. They gave positive Southgate mucicarmine, Alcian blue 8 GS stainings as well.

Of the previous authors on this subject Lillie (1945) has described "small hyaline globules lying on the surface of the ciliated epithelium" of the fimbriated end of rabbit and guinea pig oviducts. They were, according to his description, Schiff positive and were lying between the ciliated cells but he did not think that he had done enough work to define them as mucins.

Phosphatase reactions were demonstrated during oestrus and met-oestrus and parallel to that of the P.A.S. staining have shown an increase and decrease in the amount of reacting material. This is in agreement with the observations of Leblond (1950) who observed it in the rat, Moog and Wenger (1952) in the hamster and Bourne (1943) in the chicken.

Other substances which have been located in the columnar

cells of the oviduct during the course of this work were ribonucleic acid. Similarly to phosphatase and mucoprotein the ribonucleic acid content has increased during oestrus and met-oestrus although it was never completely absent during other phases of the cycle. The rise in the ribonucleic acid content in the cells is known to be related to secretory activity in this way it has corresponded to the presence of phosphatase and the secretory globules during oestrus and met-oestrus.

The fat which was found with Sudan B and Sudan 3 (Sharlach R), in the oviduct of the ewes was always extra-cellular and was brought on the section by the knife from the mesovarium.

The finger-like processes which projected from the surface of the secretory cells into the lumen of the oviduct during different stages of the oestrus cycle contain the nucleus of the cells and soon become detached when the cell body appears rod-like. The appearance of these processes coincides with the cessation of secretion by the epithelial and thereafter the secretory cells are undergoing a form of degeneration. The rod-like remnants entirely disappear by the onset of the next cycle.

The nucleated appearance of the projections from the secretory cells does not support the opinion expressed by Casida and McKenzie (1932), McKenzie et al. (1933) and McKenzie and Terril (1937), that they represent a holocrine secretion, and this view is strengthened by the fact that these structures only appeared at met-oestrus and became detached in di-oestrus, that is to say at a time when secretion is at its minimum.

No cell count was made, but there was no evidence of gross alteration in the proportion of ciliated to secretory cells.

No variation in height was found between secretory and ciliated cells in any phase of the cycle. There is a variation of height of the epithelium during the cycle, but these variations affect both secretory and ciliated cells equally.

The Uterus

The histology of the sheep uterus has already been described by Assheton (1906), Marshall (1903), Cole and Miller (1935), Casida and McKenzie (1932), McKenzie et al. (1934), McKenzie and Terril (1937). The purpose of my investigation was to establish by means of histochemical tests the nature of uterine secretion.

The cells in the uterine glands are all similar thus differing from those in the human uterus (Aykroid and Gatenby (1940), Gatenby and Aykroid (1939 & 1941), Bartelmez (1933) and Bartelmez and Bensley (1933)).

Very weak P.A.S. positive reacting material was found in the distal part of the cells during met- and early di-oestrus and in the glandular lumen only during early di-oestrus. Since this substance did not react with anything else it is thought to be a mucoprotein.

Inorganic iron was found in alternating amount during the sexual cycle. It was always in the distal part of the secretory cells nearest to the glandular lumen and it was always in the form of minute granules. The number of the granules was highest during oestrus, met-oestrus and early di-oestrus, they were

completely absent during the later phase of di-oestrus and pro-oestrus. To my knowledge no one has ever recorded before the regular appearance of inorganic iron in secretory glands in the uterus or otherwise. It is thought that it might have a function in the early embryo and might provide the necessary iron component for the forming blood corpuscles.

The folding of the uterine mucosa and the alteration in the epithelium itself have already been described by Assheton (1906), Casida and McKenzie (1932), Cole and Miller (1935), McKenzie and Terril (1937) and Robinson (1952). Further the majority of the above-mentioned authors have similarly observed an immigration into the epithelium of leucocytes in the later phase of di-oestrus. Although a remarkable increase in the caruncular circulation was shown to take place during di-oestrus, there was no menstruation in the true sense (Markee, 1940) taking place. Although Marshall (1903) has already assumed that there is a difference between the human menstruation and the haemorrhage in the uterine epithelium of the ewe, the real understanding of the differences came only after Markee's (1940) paper. Uterine haemorrhage was occasionally found while working on this thesis but was always beneath the epithelium thus never resembling menstruation.

The presence of fat with the Sudan stains was shown in the uterine and glandular epithelium during met- and early di-oestric period.

The presence of fat with the Sudan 3 and Sudan 4 (Sharlach

R) stains was shown in the uterine and glandular epithelium only during the met- and di-oestric period.

The presence of alkaline phosphatase in the surface epithelial cells was highest during oestrus and early met-oestrus and in the glandular epithelial surface it appeared gradually like the presence of inorganic iron.

The presence of fat, mucoprotein and inorganic iron in the uterine mucosa are regarded as precursors of the constituents of uterine milk and are in agreement with Amoroso's (1952) statement on that subject.

The pigment granules in the round cells which were staining with P.A.S. and fat and occasionally contained haemosiderin had a number of different origins.

In some of the cells there were black melanin granules which they have absorbed after their liberation from the melanoblasts. Some of the macrophages contained iron which was haemosiderin but the major part of pigment granules which these cells contain were bigger than those of the melanin cells and since these granules have shown a P.A.S. positive reaction and stained with fat they were assumed to be "Abnützung's pigment" (pigment of wear and tear). These cells cannot be described as mast cells or globule leucocytes (Kirkman, 1950) since they did not show metachromasia with Toluidin blue, although according to Brusa (1950), Compton (1952), the presence of heparin granules and metachromasia is not a necessity in mast cells. However because of its regular appearance and identical shape it is

assumed that these cells represent a somewhat specified form of macrophages than those usually occurring in the connective tissue.

Resume (Summary)

1. The comparative weighing of sheep ovaries during an-oestrus has shown that older animals have correspondingly heavier ovaries.

2. The median weight of both ovaries were equal in all ages compared (1 - 4 yr.).

3. The uteri and the ovaries have shown cyclical alteration of weight which followed that of the oestrus cycle. Both were heaviest during di-oestrus and lightest during pro-oestrus.

4. The presence of a basement membrane has been established under the germinal epithelium.

5. Developing follicles were most numerous during pro-oestrus and oestrus, while there was a large number of atretic follicles present during met-oestrus and di-oestrus.

6. During an-oestrus oogenesis, follicular development and follicular atresia were either at a standstill or were at a very low level since the ovary created the impression of complete rest.

7. There was an indication that oogenesis, follicular development and follicular atrophy in the sheep, like that of the goat, are not continuous but periodically recurring processes associated with the oestrus cycle.

8. The presence of multinuclear ova and polyovular follicles has been established in the sheep ovary and were only found during pro-oestrus and oestrus.

9. The thecal cone like that of the goat was found to exhibit changes in the sheep which were similar to that of the former.

10. The follicular growth of the oocyte was found to resemble that of other animals. The two phases of growth (e.g. quick and slow) were graphically reproduced as one continuous line.

The formula for the regression line was-

$$Y = 85.4117 + 20.1933 (\log_e F-5.8608)$$

11. No blood vessel was found to penetrate the glassy membrane.

12. Additional cavities which appeared in the cumulus oophorus before follicular rupture were filled with liquor folliculi.

13. The corpus luteum in non-pregnant animals reached its maximum development approximately on the 10th day after ovulation.

14. The two cycle old corpus luteum - corpus albicans - contained only some connective tissue and a group of macrophages.

15. Of the histochemical reaction in the ovary of the sheep the presence of (i) fat has been established in the theca interna and the lutein cells of the corpus luteum, (ii) cytoplasmic alkaline phosphatase (glycero) was present in the theca interna, to a lesser extent in the theca externa and in those cells which were of theca interna origin, (iii) in the granulosa cells and liquor folliculi the presence of hyaluronic acid was demonstrated.

16. In the oviduct mucosa the secretory and ciliated cells were found to be regular features whereas the rod and round cells appeared only periodically.

17. During the end of met-oestrus and di-oestrus a degeneration has taken place in the secretory cells, their nuclei became extruded and the cells became transformed into rod cells.

18. With histochemical stainings secretory granules were seen

in the secretory cells during pro-oestrus, they increased in amount and appeared in the lumen during oestrus and decreased during met-oestrus. From the histochemical reactions of the granules their structure was concluded to be acid mucopolysaccharide.

19. In the uterus of the sheep with histochemical stainings cytoplasmic alkaline phosphatase (glycero) sudanophil fat and a very low amount of mucoprotein was found in the epithelial and glandular cells. The strength of the reaction followed the sexual cycle, e.g. it was completely absent during pro-, an- and late di-oestrus, was very weak during oestrus, improved during met-oestrus and was strongest during the end of met-oestrus and early di-oestrus.

20. In the glandular cells in addition to the previous substances inorganic iron was present which showed an alteration in amount comparable to that of the mucoprotein.

21. The "round pigment cells" (Grant, 1933) in the uterine mucosa have been identified as wandering macrophages which seem to start from the uterine muscularis and show an increasing content of tissue debris as they approach the uterine epithelium.

22. Changes in the caruncular blood vessels were observed during the oestrus cycle. The number of blood capillaries showed an increase during oestrus, reached its maximum during met- and early di-oestrus and was at its lowest during late di-oestrus, an-oestrus and pro-oestrus.

References Cited

Allen, E. (1926). The ovarian follicular hormone; a study of variation in pig, cow and human ovaries. Proc.Soc.Exp.Biol. & Med. 23; 383.

_____ & Doisy, E.A. (1924). The induction of a sexually mature condition in immature females by injection of ovarian follicular hormone. Amer.J.Physiol. 69, 577.

_____ & McKenzie, F.F. (1931). Oestrus periods, ovarian changes and tubal ova in the sheep. Anat.Rec. 48, Abs.

Ammann, K. (1936). Histologie des Schweine-Eierstockes unter besonderer Berücksichtigung des Ovarialzyklus. Diss. Zürich.

Amoroso, E.C. (1952). In Marshall's Physiology of Reproduction, 3rd Ed. London.

Ancel, P. & Bouin, P. (1909). Sur le homologies et signification des glandes a secretion interne de l'ovarie. C.R.Soc.Biol., Paris, 61, 497.

Andersen, D.H. (1926). Lymphatics and blood vessels of the ovary of the sow. Contr.Embryol.Carneg.Instn. 17, 107.

_____ (1928). Comparative anatomy of the tubo-uterine junction. Am.J.Anat. 42, 255.

Ascheim, S. (1911). Uber den Glycogengehalt der Uterusschleimhaut. Zbl.Gynek. 36, 100.

Assheton, R. (1906). The morphology of the ungulate placenta, particularly the development of that organ in sheep and notes upon the placenta of elephant and hyrax. Philos. Trans. B. 198, 143.

Aykroyd, C.E. & Gatenby, G.B. (1940). ' Cytology of Human Uterine Glands in Gravid and Non-Gravid Phases. Quart.J.micr.Sci. 82, 542.

Bacsich, P. (1948). Multinuclear ova and multinuclear follicles in the young human ovary and their probable endocrinological significance. J.Endocrinol. 6, i.

_____ (1951). Some observations on near term human foetal ovaries. Ibid, 7, xiv.

_____ & Wyburn, G.M. (1940). Cyclic variations in the vascular architecture of the uterus of guinea pig. Trans.Roy.Soc.Edinb. 60, 79.

Bartelmez, G.W. (1933). The Menstruating mucous membrane of the human uterus. Contr.Embryol.Carneg.Instn. 24, 46.

____ & Bensley, C.M. (1933). Human uterine gland cells. Spec. Cytol.3, New York.

Bassett, D.L. (1943). The changes in the vascular pattern of the albino rat during the oestrus cycle. Amer.J.Anat. 73, 251.

Bonnet, R. (1880). Über melanose der Uterusschleimhaut bei Schafen. Deutsche Zeitschr. f. Tiermed. 5, 64.

____ (1882). Über melanose der Uterusschleimhaut bei brünstigen und kurze Zeit trächtigen Schafen. Ibid. 7, 107.

____ (1884). Beiträge zur Embryologie der Wiederkauer. Arch. f. Anat. & Physiol. 170, 231.

____ (1888). Demonstration der Praparate und Zeichnungen zur Entwicklugsgeschichte des Schafes. Anat. Anz. 3.

Bourne, G. (1941). Cytology and Cell Physiology. Oxford.

____ (1943). The distribution of alkaline phosphatase in various tissues. Quart.J.exp.Physiol. 32, 1.

Brachet, J. (1940). La detection histochimique des acides pentosenucleiques. C.R.Soc.Biol. 133, 88.

Brambell, F.W.R. (1928). The development and morphology of the gonads of the mouse. III. The growth of follicles. Proc.Roy.Soc. B, 103, 258.

Brusa, A. (1950). Experimental contributions to the knowledge of the mastocytes in rodents' uteri. Biol.Latina, 2, 758.

Bullough, W.S. (1944a). Oogenesis and its relation to the oestrus cycle in the adult mouse. J. Endocrinol. 3, 141.

____ (1944b). The method of growth of the follicle and corpus luteum in the mouse ovary. Ibid. 3, 150.

____ (1944c). The effects of oestrone on the ovary of the mouse. Ibid. 3, 235.

____ (1946). Mitotic activity in the adult female mouse Mus musculus L. A study of its relation to the oestrus cycle in normal and abnormal conditions. Philos. Trans. B. 231, 453.

Casida, L.E. & McKenzie, F.F. (1932). The oestrus cycle of the ewe: histology of the genital tract. Miss.Agric.Exp.Sta. Res.Bull. 170.

Clauberg, C. (1932). Zur Physiologie und Pathologie der Sexual Hormonen. Zbl. Gynäk. 56, 964.

Cole, H.H. & Miller, R.F. (1935). Changes in reproductive organs of ewe. Am.J.Anat. 57, 39.

Collip, J.B. (1935). Interrelationship among urinary, pituitary and placental gonadotrophic factors. J.Am.Med.Assoc. 104, 556.

Compton, A.S. (1952). A cytochemical and cytological study of the connective tissue mast cells. Amer.J.Anat. 91, 301.

Corey, E.L. (1928). Effect of prenatal and postnatal injections of the pituitary gland in the white rat. Anat.Rec. 41, 40.

Corner, G.W. (1917a). On the lipoidal nature of structures in the corpus luteum cells of swine. Anat.Rec. 11.

____ (1917b). Variations in the amount of phosphalides in the corpus luteum during pregnancy. J.Biol.Chem. 29, 141.

____ (1919). On the orig. of the corp. lut. of the sow from both granulosa and theca interna. Am.J.Anat. 46, 117.

____ (1921). Cyclic changes in the ovaries and uterus of the sow and their relation to the mechanisms of implantation. Contr.Embryol. Carneg. Instn. 13, 117.

____ (1932). Cytology of the ovum, ovary and fallopian tube. Special Cytology, Caudry, 2nd Ed., New York.

____ (1940). Accessory corpora lutea in the ovary of the monkey Macaca rhesus. Anales Fac. de med. de Montevideo, 25, 553.

____ (1942). The fate of the corpora lutea and the nature of the corpora aberrantia in the rhesus monkey. Contr.Embryol.Carneg.Instn. 30, 85.

____ (1944). Alkaline phosphatase in the ovarian follicles and corpora lutea. Sci. 100, 270.

____ & Amsbaugh, A.E. (1917). Oestrus and ovulation in swine. Anat.Rec. 12, 287.

Dawson, A.B. (1951). Histogenetic interrelationships of oocytes and follicle cells. A possible explanation of the mode of origin of certain polyovular follicles in the immature rat. Ibid. 110, 181.

Desaive, P. (1940). Contribution radio-biologique a l'etude de l'ovarie. Arch.Biol.Paris, 51, 587.

____ (1947). Contribution a l'etude du mecanisme de l'evolution et de l'involution folliculaires dans l'ovarie de lapin adulte. Ibid. 58, 331.

Desaive, P. (1948). Contribution a l'etude du mecanisme de l'evolution et de l'involution folliculaires dans l'ovarie de lapin adulte (Deuxieme partie). Ibid. 59, 31.

Feulgen, R. & Rossenbeck, H. (1924). Mikroskopisch - chemischr Nachweis einer Noucleinsäure vom Typus der Thymonucleinsäure und die darauf beruhende elektive. Färbung vom Zellkernen in mikroskopischen Preäparaten. Z. physiol. Chem. 135, 203.

Fisher, E.R. (1953). Destruction of cytoplasmic basophilia with mineral acids. Stain Techn. 28; 9.

Gatenby, J.B. & Aykroyd, O.E. (1939). Cytology of the human uterine gland cell. Nature, Lond. 144.

____ (1941). Note on the cytology of the human uterine glands in the gravid phase. Proc.Roy.I. Acad. B. 46, 97.

Gersh, I. (1948). Histochemical studies of basement membrane. Fed.Proc. 7, 270.

____ & Catchpole, H.Z. (1949). The organization of the ground substance and basement membrane and its significance in tissue injury, disease and growth. Am.J.Anat. 85, 457.

Glick, D. (1949). Techniques of histo- and cyto-chemistry. Interscience, Publishers Ltd., London.

Gomori, G. (1939). Microtechnical demonstration of phosphatase in tissue sections. Proc.Soc.Exptl.Biol. & Med. 42, 23.

____ (1952). Microscopic Histochemistry, Chicago.

Grant, R. (1933). The pigmentation of the uterine mucosa. Brit.Vet.J. 89, 271.

____ (1934). Studies on the physiology of reproduction in the ewe. 1. Symptoms, periodicity and duration of oestrus; 2, Changes in vagina and cervix; 3. Gross changes in the ovaries. Trans.Roy. Soc.Edinb. 58, 1.

Green, S.H. Mandl, A.M. & Zuckerman, S. (1951a). The proportion of ovarian follicles in different stages of development in rats and monkeys. J.Anat.Lond., 85, 325.

____ (1951b). The number of oocytes in the mature rhesus monkey (Macaca Mulatta). J.Endocrinol. 7, 81.

Green, T.A. (1951). The development and growth of the ovarian follicle of the sow. Anat.Rec. 109, 38

Haklik, T. (1926). Das Endometrium des Schafes mit besonderer Rucksicht auf die Evolution. Kozlemenyek az osszehasonlito elet-es kortan körebol. 20, 1.

Hale, C.W. (1946). Histochemical demonstration of acid polysaccharides in animal tissues. Nature, Lond., 157, 802.

Haller & Kuhlemann (1754). Observationes quaedam cina negotium generationis. Lipsiae.

Hammond, J. (1927). The Physiology of Reproduction in the Cow. Cambridge.

Harrison, R.T. (1946). The early development of the corpus luteum in the mare. J.Anat.Lond., 80, 167.

____ (1948). The changes occurring in the ovary of the goat during oestrus cycle and early pregnancy. Ibid. 82, 21.

Hartman, C.G. (1926). Polynuclear ova and polyovular follicles in the opossum and other mammals, with special reference to the problem of fecundity. Amer.J.Anat. 37, 1.

Höfliger, H. (1948). Das Ovar des Rindes. Acta Anat., Suppl.5, Basle.

Hotchkiss, R.D. (1948a). A microchemical reaction resulting in the staining of polysaccharide structures in fixed tissue preparations. Arch.Biochem. 16, 131.

____ (1948b). Histological and histochemical use of periodic acid. Stain Techn. 23, 99.

Iwata, M. (1929). Beiträge zur Morphology der Menschlichen Tube. Mschr. d. Geburtsh. 81, 283.

Jakovlev, J. (1927). Uber Glycogen in den Eileitern der Frau. Jow. Aknsh. i. Zhensk. Boliez. 38, 24.

Joel, K. (1939a). The glycogen content of the Fallopian tubes during the menstrual cycle and during pregnancy. J.Obst. & Gynek. Brit.Emp. 46, 721.

____ (1939b). The lipoid content of the Fallopian tubes during the menstrual cycle and during pregnancy. Ibid. 46, 731.

Kazzander, J. (1890). Über die Pigmentation der Uterinschleimhauf des Schafes. Arch.f.mikr.Anat. 36, 507.

Keller, L. (1942a, b, 1945). cited after Höfliger. The papers are not available in Britain and cannot be borrowed from the Continent.

Kelley, R.B. (1937). Studies in fertility of sheep. Comm.Austr.Counc. for Sci. & Ind.Res.Bull.No. 112.

Kirkman, H. (1950). A comparative morphological and cytological study of globule leucocytes (Schollenleukocyten) of the urinary tract and of possibly related cells. Am.J.Anat. 81, 9.

Kolewe, H. (1913). Anat. & Hist. Untersuck der Gebarmutter und des weibl. Begattungsorgane der Ziege. Diss. Berlin, 1913.

Kolster, R. (1903). Weitere Beiträge zur Kenntniss der Embryotrophe bei Indeciduaten. Anat. Hefte 20, 231.

Kupfer, M. (1928). The sexual cycle of female domesticated mammals. 13th & 14th Report of the Dir. of Vet. Educ. & Res. Dept. of Agric. Pretoria, 1209.

Lang, L. (1935). Der Brunstzyklus des Rindes nach Untersuchungen am Ovarium unter besonderer Berücksichtigung der in ihm auftretenden Lipoide. Zeitsch. f. Konst. Lehre, 10, 79.

Leblond, C.P. (1950). Distribution of periodic acid-reactive carbohydrates in the adult rat. Am.J.Anat. 86, 1.

Lesbouyries, G. (1949). Reproduction des Mammiferes Domestiques. Paris.

Lillie, R.D. (1945). Studies on the histochemistry of normal and pathological mucins in man and in laboratory animals. J. of Tech. Meth. & Bull. of Int. Assoc.Med.Mus. 25, 1.

McAllister (1915). referred to in Ascheim's paper.

McKenzie, F.F., Allen, E., Guthrie, M.T., Warbitton, V., Terrill, C.E., Casida, L.E., Nahm, L.T., Kennedy, T.W. (1933). Reproduction in the ewe - a preliminary report. J. of Anim.Prod. 1933, 278.

McKenzie, F.F. & Terrill, C.E. (1937). Estrus, ovulation and related phenomena in the ewe. Missouri Agric.Experim. Stat.Res.Bul.264.

McManus, J.F.A. (1946). The demonstration of certain fatty substances in paraffin sections. J.Path. & Bact. 58, 93.

____ (1948). Histological and histochemical uses of periodic acid. Stain Techn. 23, 99.

Mandl, A.M. & Zuckerman, S. (1951). Functional changes in the uterine vessels. J.Endocr. 7, xxxv.

____ (1952a). The growth of the oocyte and follicle in the adult rat. Ibid. 8, 126.

____ (1952b). Cyclical changes in the number of medium and large follicles in the adult rat ovary. Ibid. 8, 341.

Markee, J.E. (1940). Menstruation in intraocular endometrial transplantation in the Rhesus monkey. Contrib.Embryol. Carnegie Instn. 29, 51.

Marshall, F.H.A. (1901). Preliminary communication on the oestrus cycle and formation of the corpus luteum in sheep. Proc.Roy. Soc.Lond.B. 68, 47.

____ (1903). The oestrus cycle and formation of corpus luteum in the sheep. Phil.Trans.B. 196, 47.

Mey, R. (1926). Untersuchungen über das Vorkommen einer interstitiellen Eierstockdrüse beim Rind im intra-und extrauterinen Leben. Arch.f.Gynäkol. 128, 177.

Moog, F. & Wenger, E.L. (1952). The occurrence of a neutral mucopolysaccharides at sites of high alkaline phosphatase reactivity. Am.J.Anat. 90, 339.

Mossman, H.W. (1937). The thecal gland and its relation to the reproductive cycle; a study of the cyclic changes in the ovary of the pocket gopher, Geomys bursarius (Shaw). Ibid. 61, 289.

Oster, K.A. & Mulinos, M.G. (1944). Tissue aldehyde and their reaction with amines. J.Pharmacol.Exptl.Therap. 80, 132.

Parkes, A.S. (1931). The reproductive processes in certain mammals. II. The size of the Graafian follicle at ovulation. Proc.Roy.Soc. B. 105, 185.

Paterson, R. (1840). The corpus luteum. Edinb.Med. & Surg.J. 54, 390.

Pearse, E. (1952). Modern methods in histochemistry. J.Clin.Med. 7, 1.

Pincus, G. & Enzmann, E.V. (1932). Fertilisation in the rabbit. Jour.exp.Biol. 9, 403.

Quinlan, J. & Mare, G.S. (1931). The physiological changes in the ovary of the merino sheep in S. Africa and their practical application in breeding. 17th Rep. of the Dir. of Vet. Serv. etc. 663.

Quinlan, J., Claassens, C.C., Bonsma, H.C. & Rose, P.E. (1939). Observations on the gestation period of Ronderib-Afrikander sheep. Onderstepoort Jour. of Vet. Sci. 12, 251.

Robb, R.C. (1954). Personal communication.

Roberts, G.B.S. & Jarrett, W.F.H. (1953). Histochemical identification of higher polysaccharides. In print.

Robin, C. (1858). Sur quelques points de l'anatomie et de la physiologie de la muquese et de l'epithelium uterine pendant la grossesse. Journ. de l'Anat. e de la Phys de l'homme et des animaux. Paris, 1858.

Robinson, T.J. (1950 & 1951). Control of fertility in sheep, Pts. I & II. J. Agric. Sci. 40, 275 & 41, 6.

Rossman, I. (1942). On the lipin and pigment in the corpus luteum of the Rhesus monkey. Contr.Embryol. Carnegie Instn. 30, 65.

Saunders, F.J. & Cole, H.H. (1936). Age and the qualitative ovarian response of immature rat to mare gonadotrophic hormone. Proc.Soc.Exp.Biol. N.Y. 33, 504.

Sckochet, S.S. (1916). A suggestion as to the process of ovulation and ovarian cyst formation. An.Rec. 10, 447.

Seiferle, E. (1936). Cited after Ellenberger-Baum: Handbuch der Vergleichende Anatomie der Haüstiere. 18th Ed. Jena, 1943.

____ (1936). Die sog. interstitillen Zellen des Eierstockes und ihre Bezichungen zur Stroma und Ovarialzyklus im besonderen beim Schwein. Ztschr. f. Zellforsch. 25, 421.

Smith, P.E., Engle, E.T. & Tyndall, H.H. (1934). Different ovarian responses after injection of follicle stimulating and pregnancy urine in very young female rats. Proc.Soc.Exp.Biol. N.Y. 31, 744.

Steedman, H.F. (1952). Personal communication.

Stieve, J. (1934). Die beziehungen zwischen Follikelsprung und Brunst beim Hausschaf. Z. mikrosk.-anat. Forschg. 36, 481.

Strassmann, E.O. (1941). The thecal cone and its tropism towards the ovarian surface, a typical feature of growing human and mammalian follicles. Amer.J.Obstet.Gynaec. 41, 363.

Sturgis, S.H. (1949). Rate and significance of atresia of the ovarian follicle of the Rhesus monkey. Contrib.Embryol.Carneg. Instn. 33, 60.

Voss, G. (1950). Messend-zoologische Untersuchungen an Ovarien verschiedener Schafrassen. Klatt-Festschrift, Zool. Anz. Suppl.15.

____ (1951). Studien über den Feinbau der Ovarien verschiedener Schafrassen. Diss. Kiel.

____ (1952). Analytical investigations on the ovaries of sheep in relation to breed. Zool.Jb. Anat. 72, 438.

Warbitton, V. (1934). The cytology of the corpora lutea in the ewe. J. Morphol. 56, 181.

Wester, J. (1921). Eierstock und ei befruchtung und unfruchtbarkeit bei den haustieren. Berlin.

Wolf, A., Kabat, E.A. & Newman, W. (1943). Histochemical studies of tissue enzymes. III. Am.J.Path., 19, 423.

Yamauchi, M. (1925). Untersuchungen uber den Follikelapparat der ovarien bei Mensch und Rind mit besonderer Berücksichtigung der in ihm auftretenden Lipoide. Zeitschr. f. Konst. Lehre. 10, 28.

Zephiroff, P. & Dobrovolskaja-Zavadskaja N. (1940). Sur une substance antisexuelle isolee de l'urine d'une fillette de quatre ans. C.R.Soc.Biol., Paris, 133, 405.

Contents

Vol. II.

Chart 1.

Period of Cycle	Yr.	For macroscopical description (Group 1)	For micro-anatomical studies (Group 2)		Total	
		From Abattoir	From Abattoir	From Garscube	Macroscopical studies	Micro-anatomical studies
An-oestrus	1	2	-	-		
	2	8	4	-	26	11
	3	6	6	-		
	4	10	1	-		
Pro-oestrus	1	-	-	-		
	2	4	4	-	22	14
	3	6	4	-		
	4	12	4	2		
Oestrus	1	-	-	-		
	2	10	2	-	21	12
	3	3	5	-		
	4	8	5	-		
Met-oestrus	1	-	-	-		
	2	4	1	-	24	15
	3	10	1	-		
	4	10	3	8		
Di-oestrus	1	3	-	-		
	2	4	5	-	27	16
	3	8	5	-		
	4	12	4	-		
TOTAL		120	54	10	120	64

The number, origin and sexual phase of genitalia used for this study.

Chart 2.

Serial No.

Breed: Age: Condition:

Ext. sign of heat:

Phase of sexual cycle:

Length of genital tract:

	(I)	(II)	(III)
Right ovary -	length:	width:	Remarks:
	depth:	weight:	
Left ovary -	length:	width:	Remarks:
	depth:	weight:	
Oviduct -	length:	Diam. amp.:	isthmus:
Uterus -	length:		thickness of wall:
	body width:		weight:
	horn diam.:		

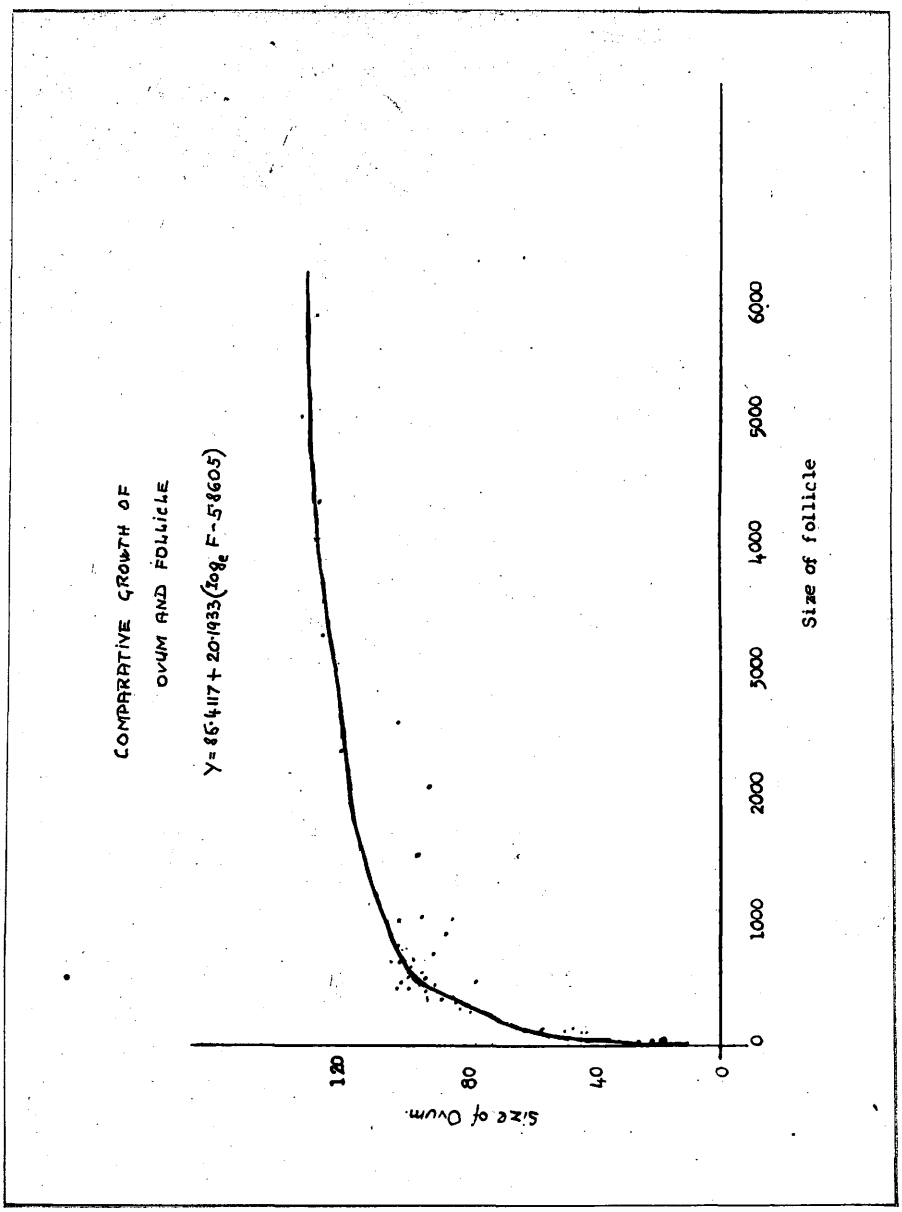
Specimen sheet.

Ovary	OESTRUS				MET-OESTRUS				DI-OESTRUS				AN-OESTRUS				PRO-OESTRUS			
	1 Yr.	2 Yr.	3 Yr.	4 Yr. & over	1 Yr.	2 Yr.	3 Yr.	4 Yr. & over	1 Yr.	2 Yr.	3 Yr.	4 Yr. & over	1 Yr.	2 Yr.	3 Yr.	4 Yr. & over	1 Yr.	2 Yr.	3 Yr.	4 Yr. & over
0.75 g.	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-
1.00 g.	-	-	-	-	-	-	-	-	1	-	-	-	2	6	-	-	-	-	-	-
1.25 g.	-	-	-	-	-	2	-	-	-	-	-	-	-	2	2	-	-	2	-	-
1.50 g.	-	2	-	-	-	1	3	2	-	-	-	-	-	-	4	2	-	-	2	2
1.75 g.	-	2	1	-	-	1	4	4	-	2	2	2	-	-	2	4	-	2	3	5
2.00 g.	-	-	2	4	-	-	2	2	-	2	4	8	-	-	-	4	-	-	1	3
2.25 g.	-	6	-	2	-	-	-	2	-	-	2	2	-	-	-	-	-	-	-	-
2.50 g.	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

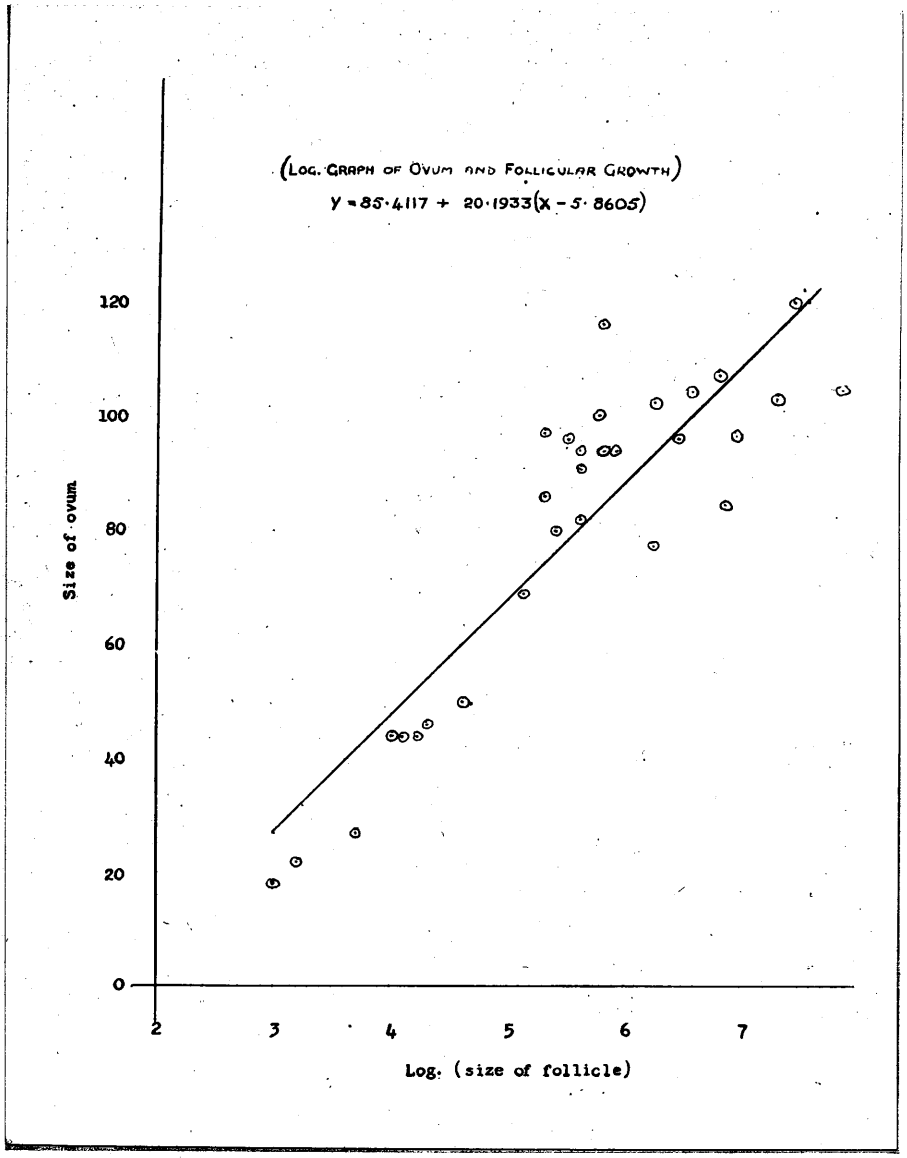
Chart 3.

The mean ovarian weight in different age groups and different phases of the cycle.

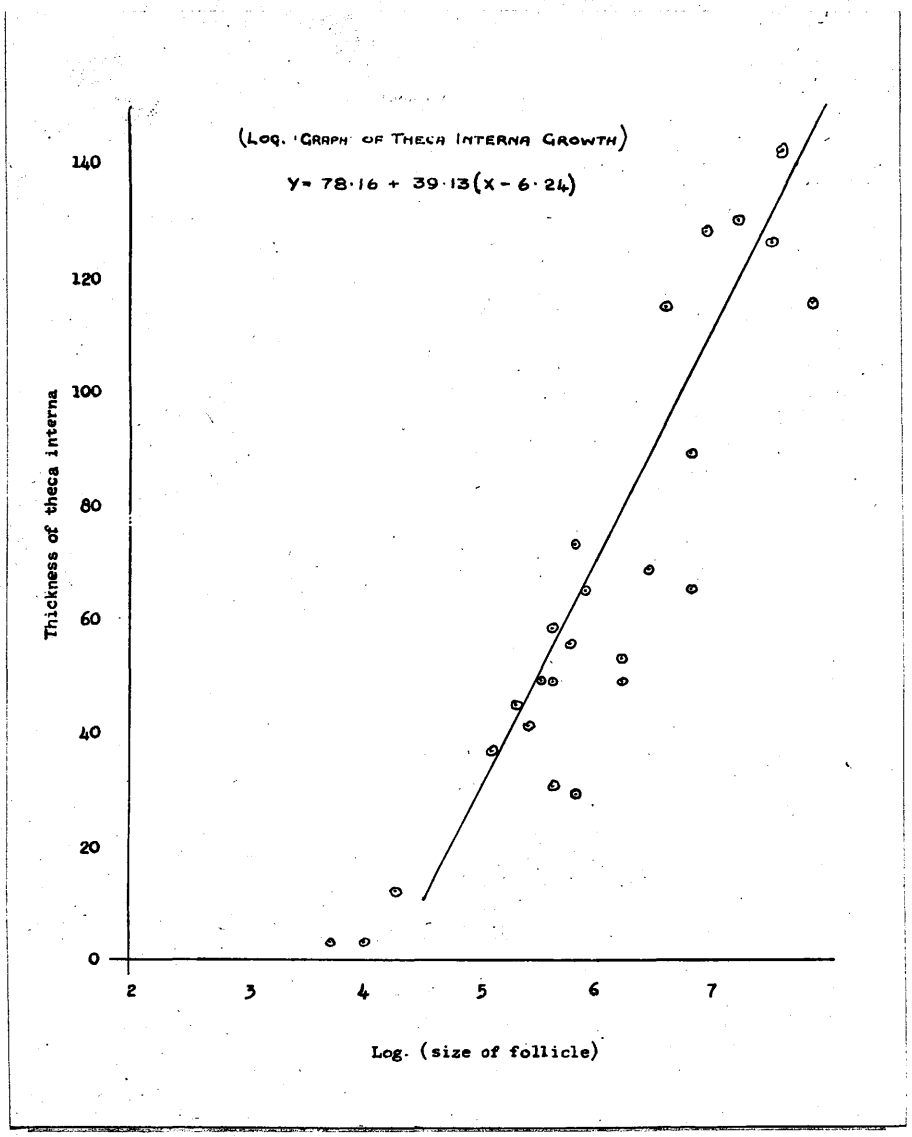
Graph 1.


COMPARATIVE GROWTH OF
OVUM AND FOLLICLE
Y = 85·4117 + 20·1933(log_e F - 5·8605)
Size of Ovum
120
80
40
0
0
1000
2000
3000
4000
5000
6000
Size of follicle

Graph 2.


(Log. Graph of Ovum and Follicular Growth)
y = 85·4117 + 20·1933(x - 5·8605)
Size of ovum
120
100
80
60
40
20
0
2
3
4
5
6
7
Log. (size of follicle)

Graph 3.


(Log. Graph of Theca Interna Growth)
Y= 78·16 + 39·13(X - 6·24)
Thickness of theca interna
0
20
40
60
80
100
120
140
2
3
4
5
6
7
Log. (size of follicle)

Graph 7

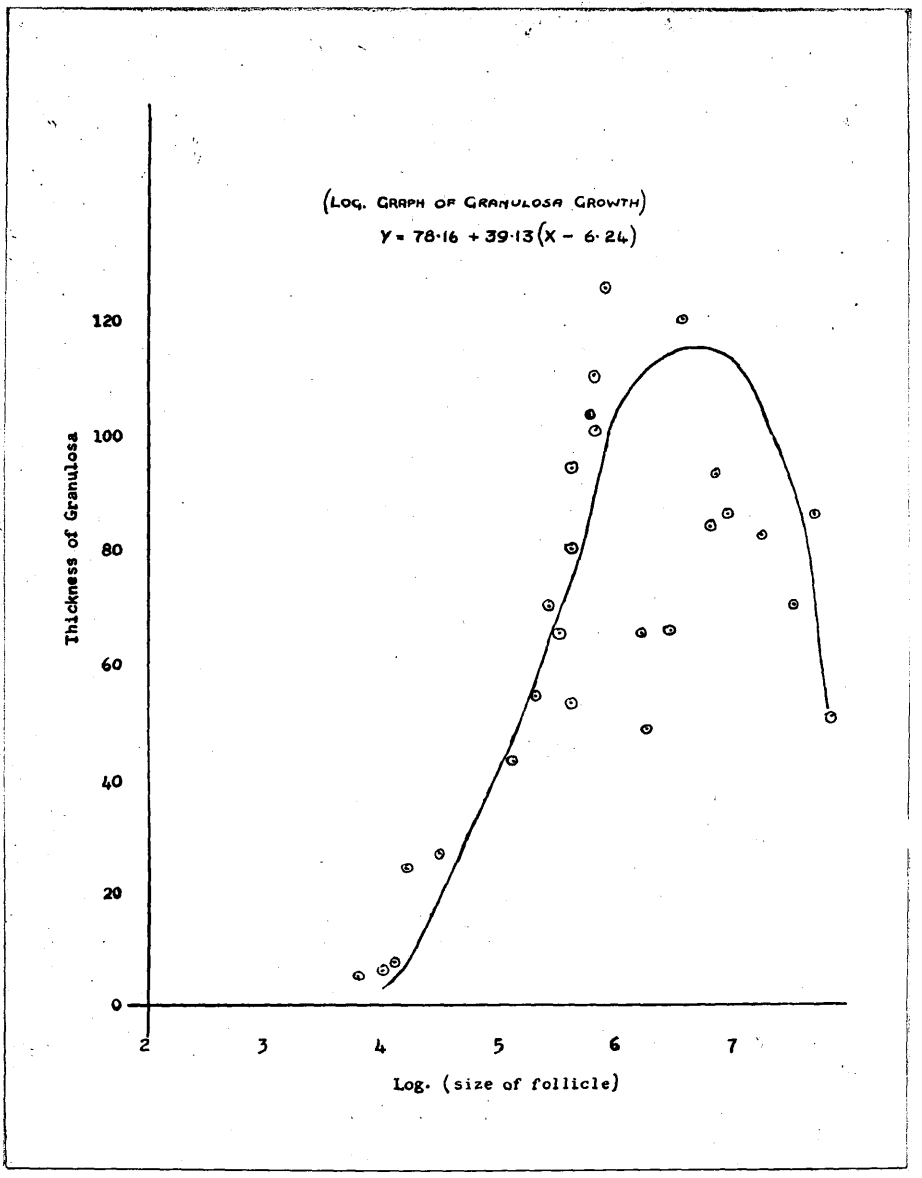

(Log. Graph of Granulosa Growth)
Y = 78·16 + 39·13 (X - 6·24)
Thickness of Granulosa
120
100
80
60
40
20
0
2
3
4
5
6
7
Log. (size of follicle)

Chart 4.

Diameter of Follicle	Size of Ovum	Thickness of Granulosa	Thickness of Theca interna
μ	μ	μ	μ
20	17.8	- not present	-
25	21.5	- not present	-
39.6	27	5.5	3
55.2	44	6.1	3
59	44	7.3	not measured
66.5	44	- not measured	-
73	46	24	12.5
101	50	26.6	22(?)
163	69	43	37
197	86	54	45
199	97	- not measured	-
218	80	71	41
241	96	65	49
259	82	81	49
273	94	53	32
275	91	94	58
316	100	103	55
326	116	101	29
332	92	110	73
362	94	126	64
479	77	65	49
514	102	48	53
635	96	65	68
718	104	120	115
930	107	83.7	65
934	86	93	89
1042	96	86	130
1528	103	82	131
1750	120	70	63
2130	90	86	143
2577	105	51	115
3610	144	96	222
4450	137	58	27
5927	146	32	172

Comparative measurements at different follicular developmental stages.
(Each number is the average of 3 measurements)

Chart 6.

	Age of Animals			
	1 yr.	2 yr.	3 yr.	4 yr.
Av. length of oviduct - cms.	15.3	15.9	16.5	17.3
Av. length of uterus - cms.	7	15	17	20
Av. size of caruncles dur. an-oestrus - mm.	2	4	-	5
Av. size of caruncles dur. pro-oestrus and early di-oestrus - mm.	-	7	-	9

Comparative measurements of the length of oviducts and uteri.

Plate 1.

Fig.1.

Ovary, H.E.x40;

Fig.2.

Ovary, Germinal epithelium during Di-oestrus, H.E.x20;

Plate 2.

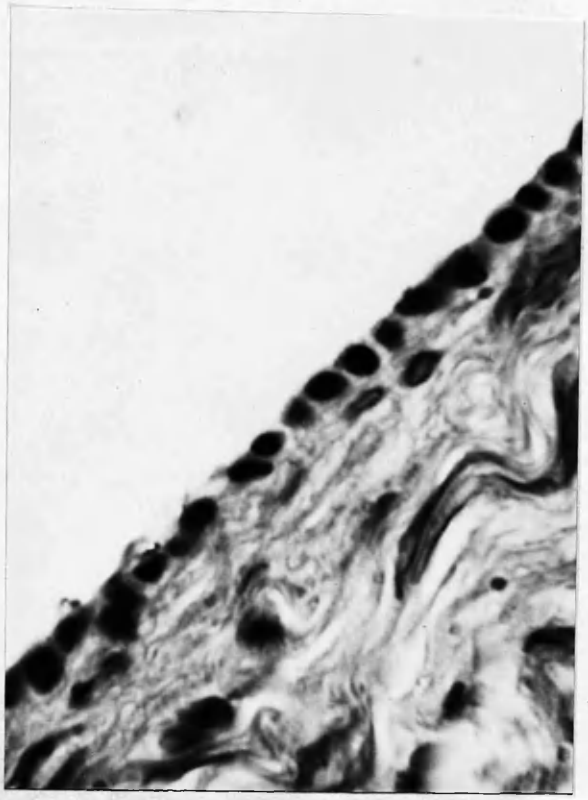


Fig.3.

Ovary, Germinal epithelium during Pro-oestrus,H.E.x1400;

Fig.4.

Ovary, Basement membrane, Gordon-Sweetx1200;

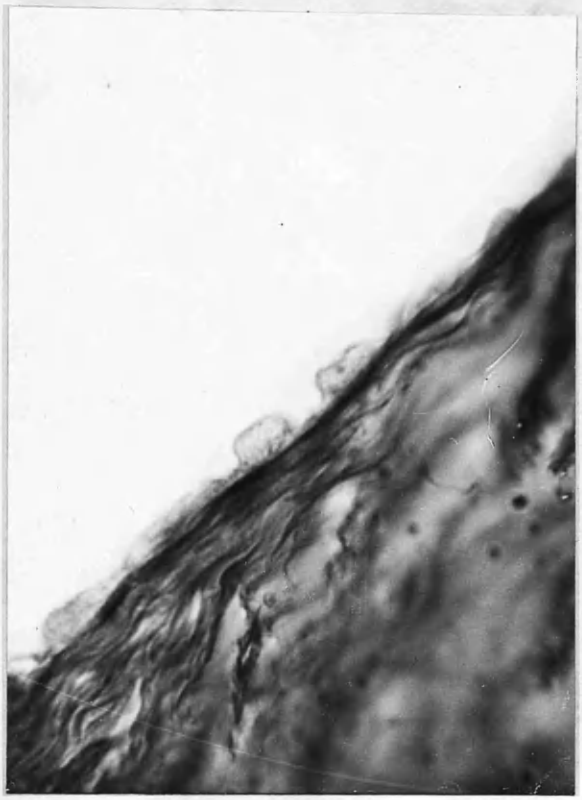


Fig.5.

Ovary, Basement membrane,
P.A.S. x1200;

Fig.6.

Reticular fibers
of Cortex,
Gordon-Sweet x42;

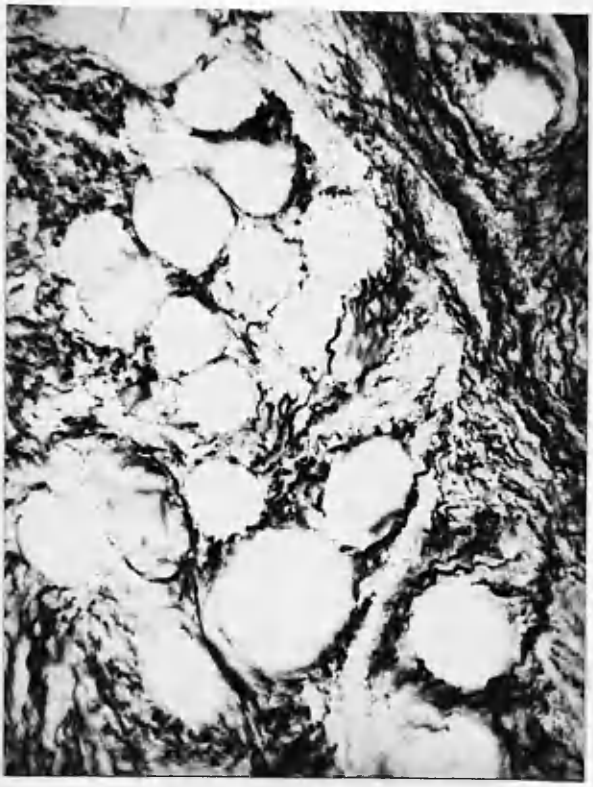


Fig.7.

Ovary,Reticular fibers of Cortex, Gordon-Sweetx500;

Fig.8.

Retee ovarii, l.s.x500;

Plate 5.

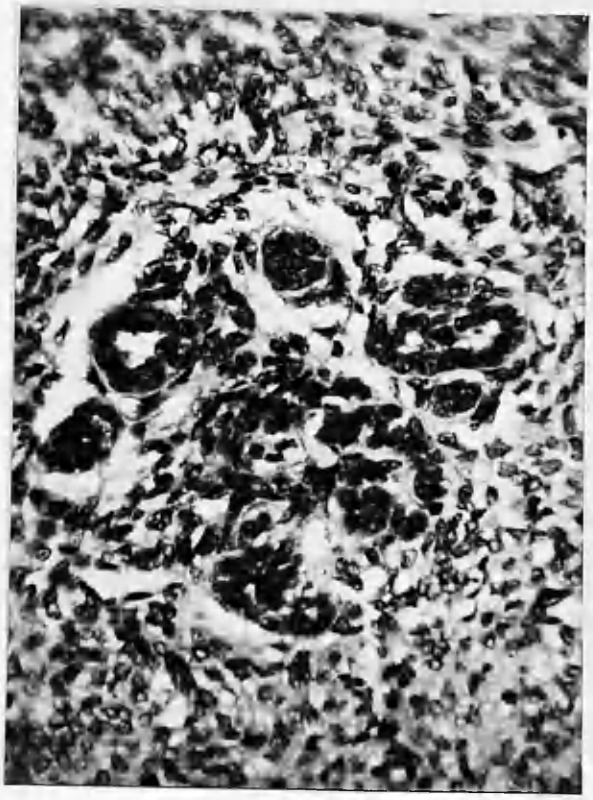


Fig. 9

Ovary, Rete ovarii,
H & E x 400.

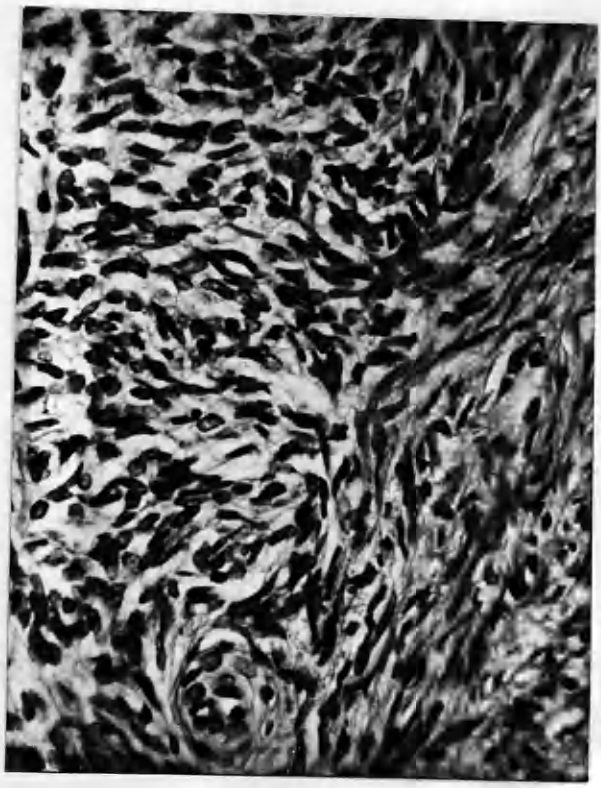


Fig. 10

Ovary, Cortical area
devoid of follicles,
H & E x 700.

Plate 6.

Fig. 11.

Ovary, Cortical area with follicles, H & E x 700.

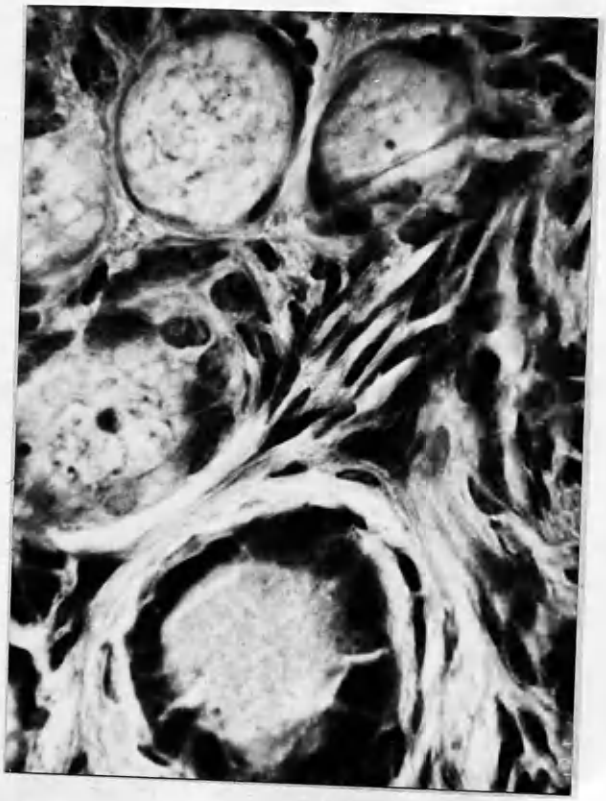


Fig. 12.

Ovary, primordial and developing secondary follicles, H & E x 1150.

Plate 7.

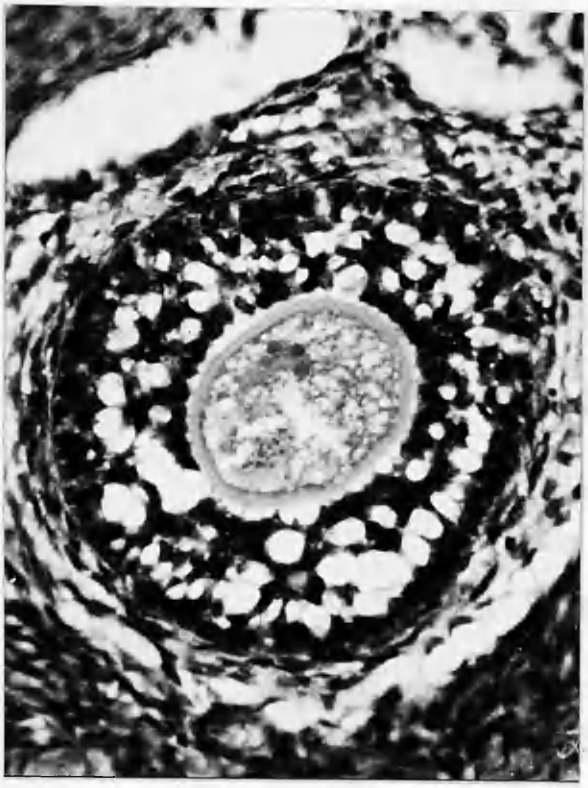


Fig. 13

Ovary, Developing granulosa cells in secondary follicle, H & E x 400.

Fig. 14

Ovary, Thecal cone of small tertiary follicle, H & E x 42.

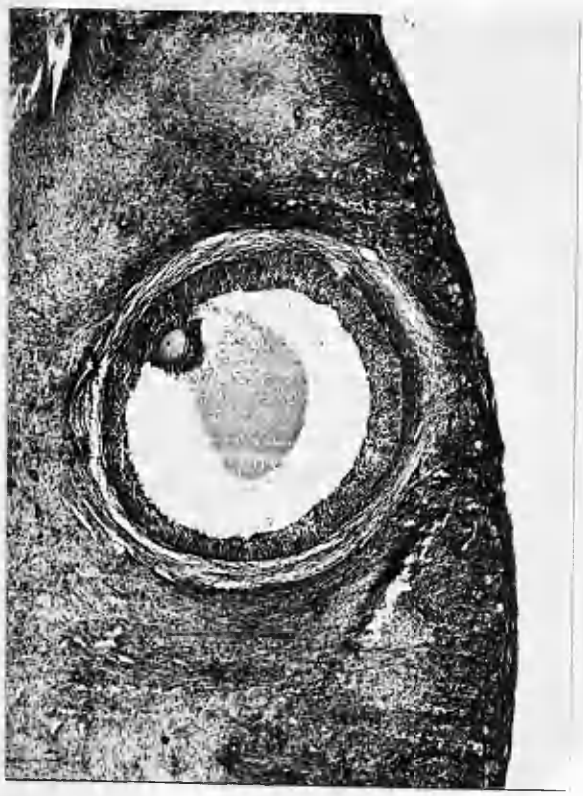
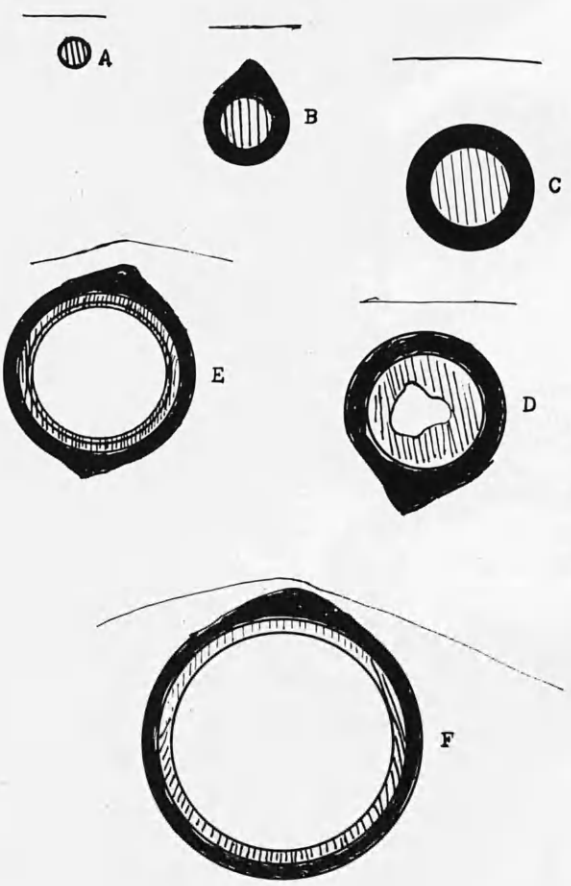


Plate 8.



DRAWING OF THECAL CONES IN DIFFERENT POSITIONS AT VARIOUS STAGES OF FOLLICULAR DEVELOPEMENT

Fig. 15

Ovary, Drawings of thecal cone in developing follicles (after Harrison).

Plate 9.

Fig. 16

Ovary, Granulosa and thecas of large tertiary follicle in "resting" stage, Mass. x 400.

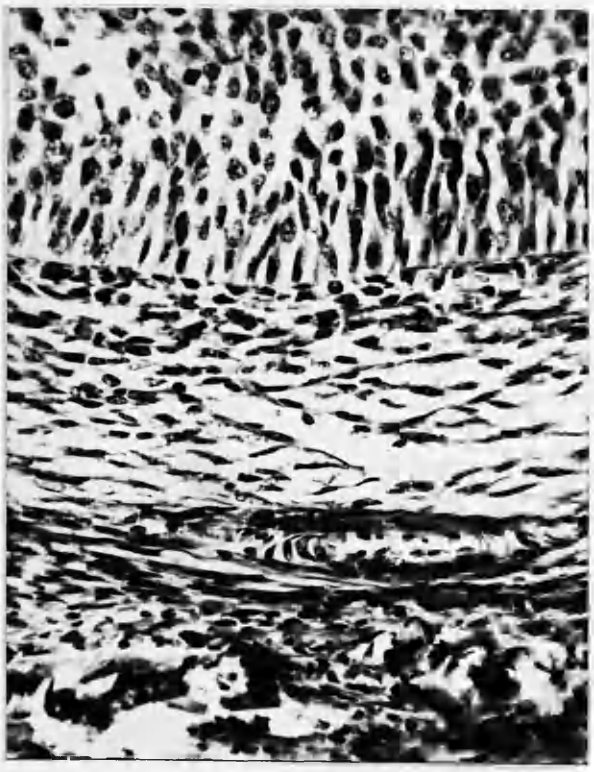


Fig. 17

Ovary, Granulosa and thecas of large tertiary follicle in "actively growing" phase, Mass. x 400.

Plate 10.

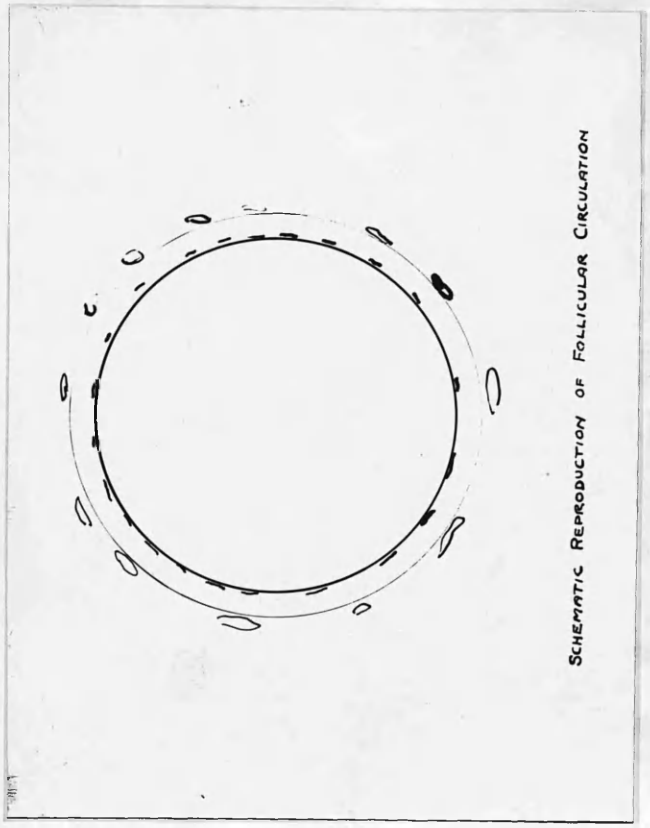


Fig. 18.

Ovary, Drawing of follicular circulation (2 wreaths)

Fig. 19.

Ovary, Drawing of follicular circulation (2 wreaths)

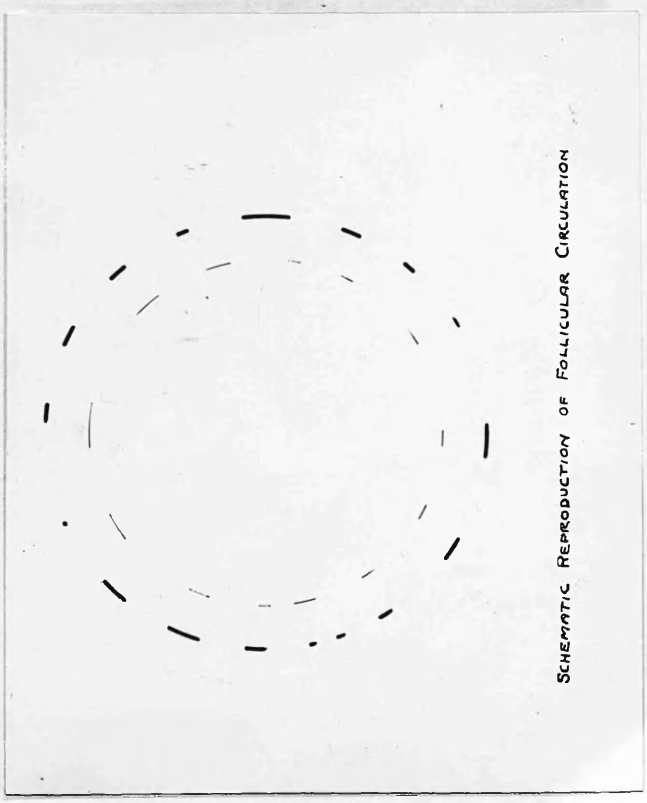


Plate 11.

Fig. 20.

Ovary, "Festooned" follicle, H & E x 400.

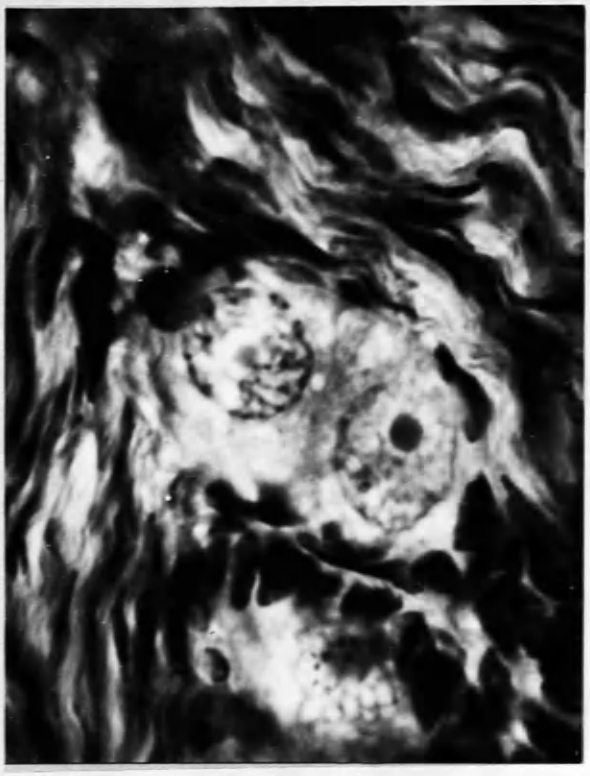


Fig. 21.

Ovary, Multinuclear ovum with 2 nuclei, Mass. x 1150.

Plate 12.

Fig. 22.

Ovary, Multinuclear ovum with 3 nuclei, Mass. x 1150.

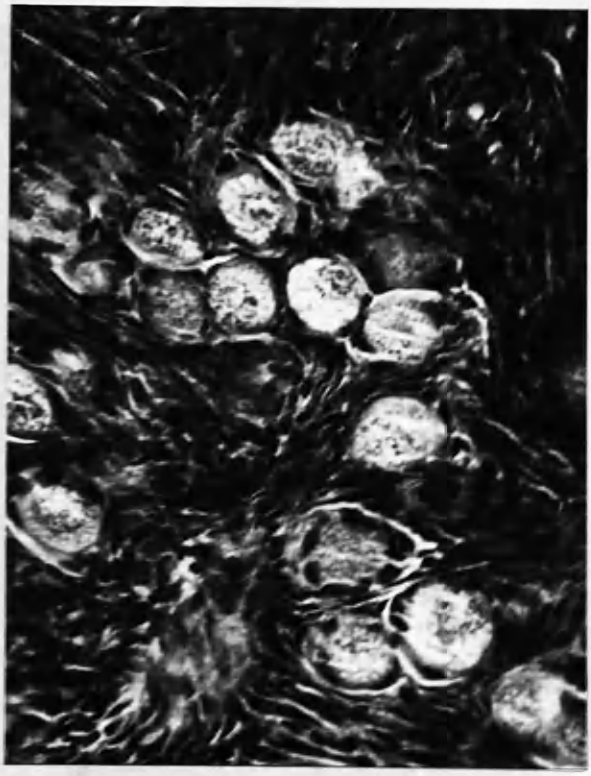


Fig. 23

Ovary, Polyovular follicle, Type I. Mass. x 475.

Plate 13.

Fig. 24
Ovary,
Polyovular follicle,
Type II, Mass. x 475

Fig. 25

Ovary, Polyovular
follicle, Type III,
Mass. x 475.

Plate 14.

Fig. 26

Ovary, Follicular atrophy - early stage, H & E x 95.

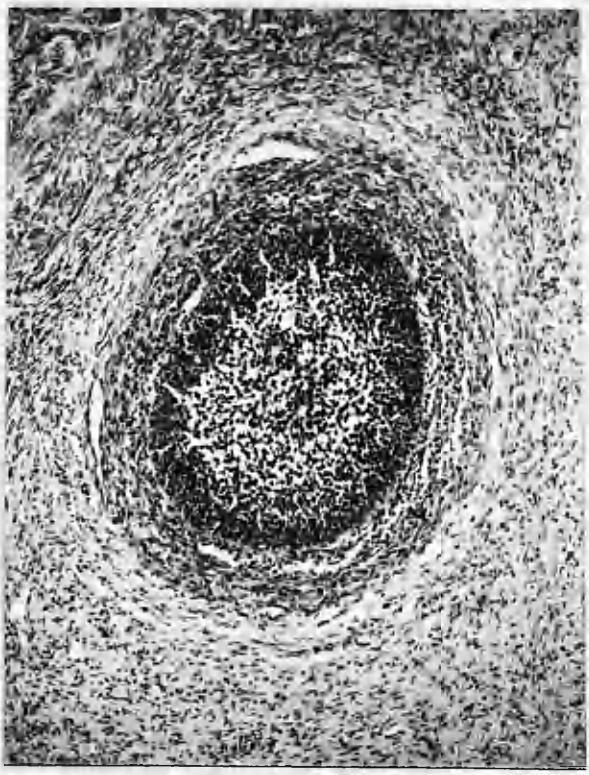


Fig. 27

Ovary, Follicular atrophy - late stage, H & E x 110.

Plate 15.

Fig. 28

Ovary, Graafian follicle preceding rupture, H & E x 14.

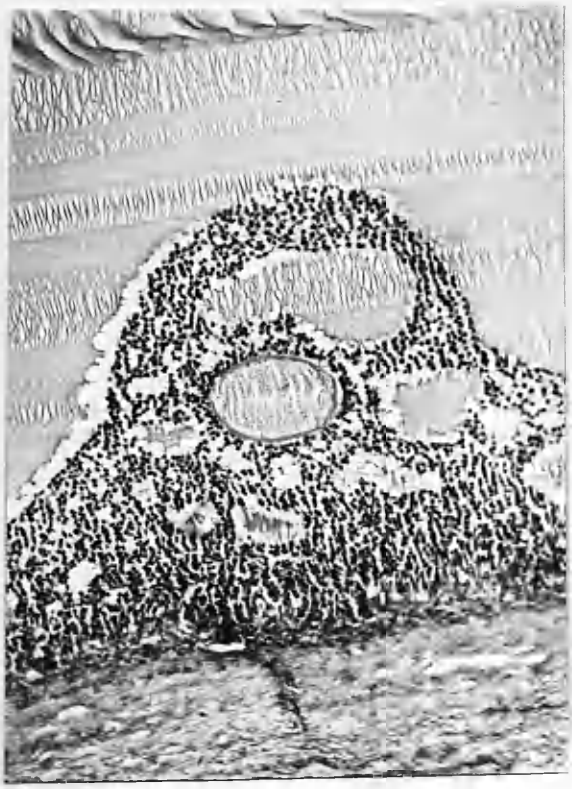


Fig. 29

Ovary, Cumulus oophorus from Fig. 26, H & E x 100.

Plate 16.

Fig. 30

Ovary, recently ruptured follicle (approx. 3 hrs. after follicular rupture), H & E x 30.

Fig. 31

Ovary, recently ruptured follicle (approx. 6 hrs. after follicular rupture) H & E x 20.

Plate 17.

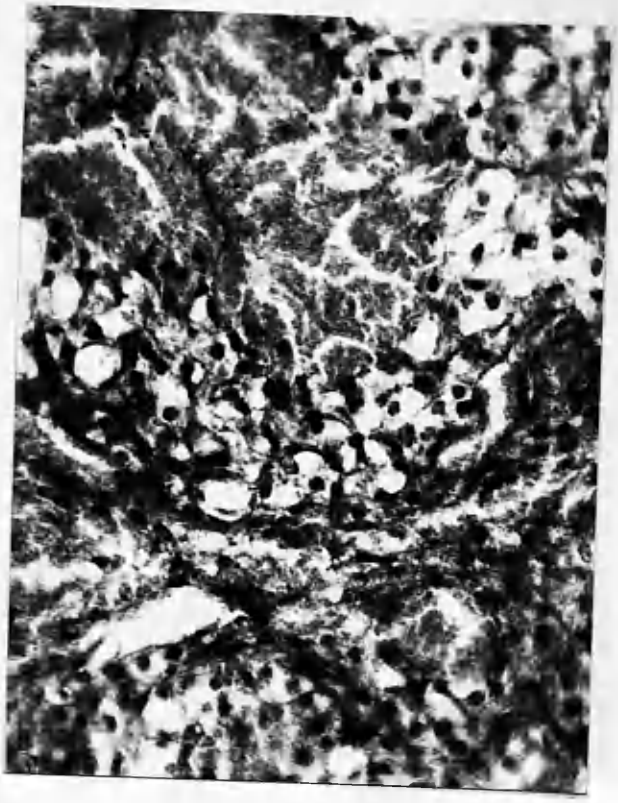


Fig. 32

Ovary, Granulosa and theca interna cells in 6 hr. old corpus luteum, H & E x 400.

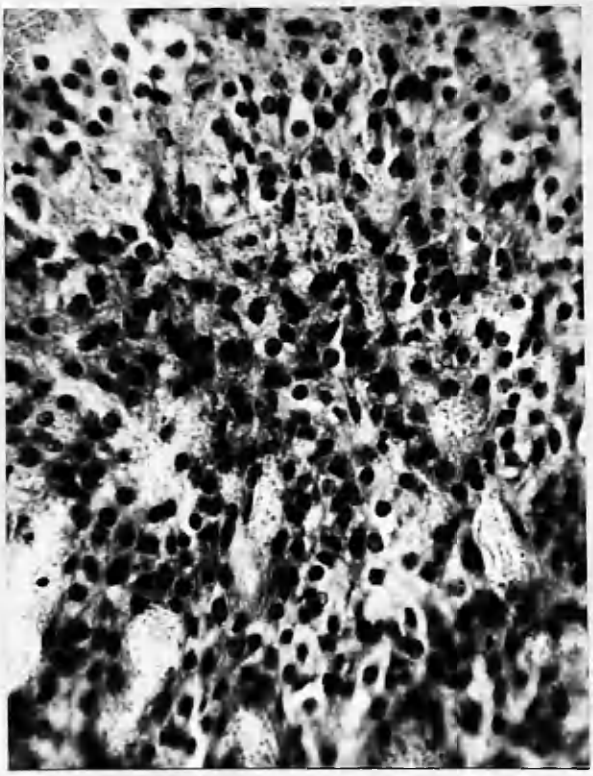


Fig. 33

Ovary, theca interna and connective tissue in 16 hr. old corpus luteum, H & E x 400.

Plate 18.

Fig. 34

Ovary, 24 hr. old corpus luteum, slight plication, H & E x 30.

Fig. 35

Ovary, 24 hr. old corpus luteum, Mass. x 400.

Plate 19.

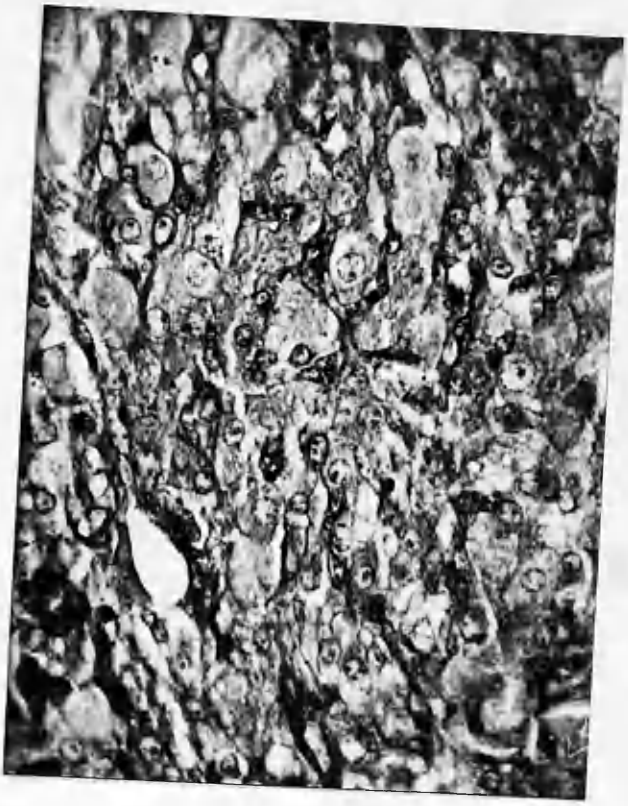


Fig. 36

Ovary, 5 day old corpus luteum, Mass. x 400.

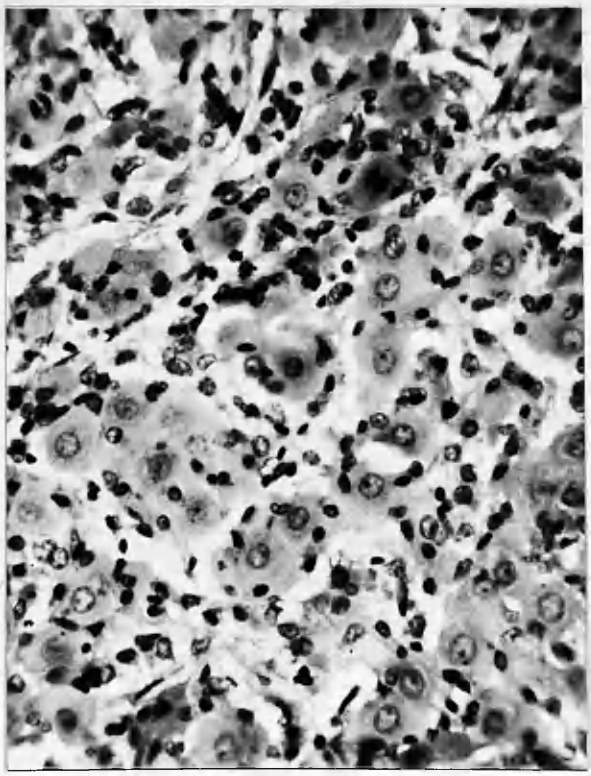


Fig. 37

Ovary, 10 day old corpus luteum, H & E x 400.

Plate 20.

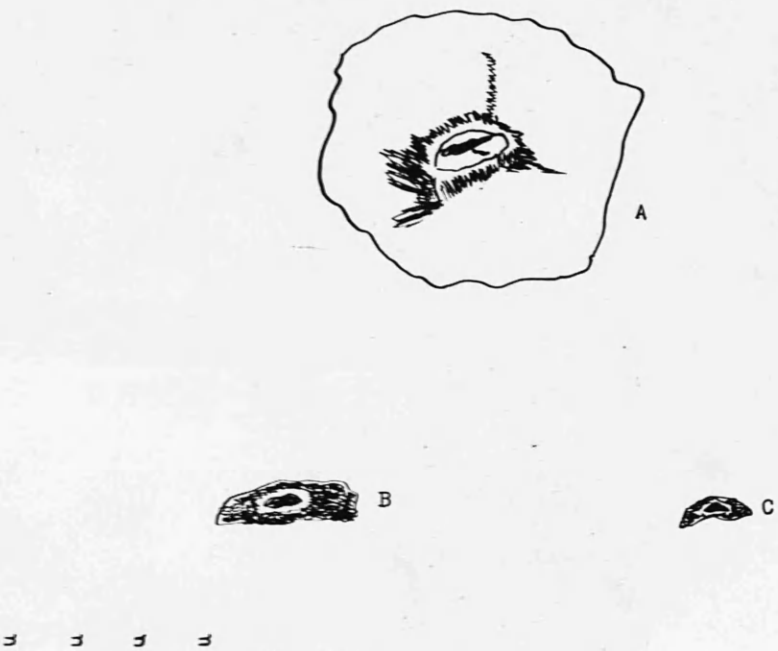


Fig. 35.
DRAWING OF CORPUS LUTEUM CELLS

Fig. 38

Ovary, drawing of lutein cells in
10 day old corpus luteum.

Plate 21.

Fig. 39

Ovary, 1 cycle old corpus luteum, H & E x 90.

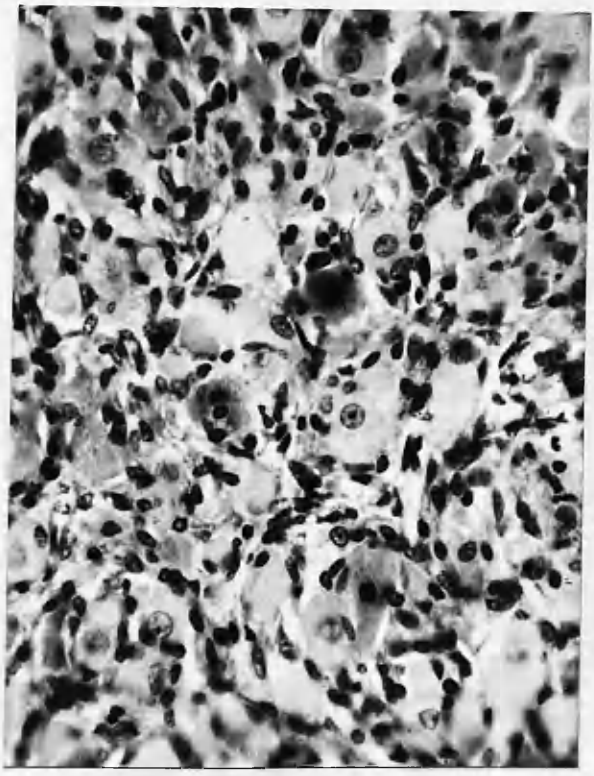


Fig. 40

Ovary, 1 cycle old corpus luteum, H & E x 400.

Plate 22.

Fig. 41

Ovary, degenerating corpus luteum after oestrus, Mass. x 400.

Fig. 42

Ovary, 2 cycles old corpus luteum, (Corpus albicans) Mass. x 400.

Plate 23.

Fig. 43

Ovary, 3 cycles old corpus luteum/corpus albicans/Mass. x 400.

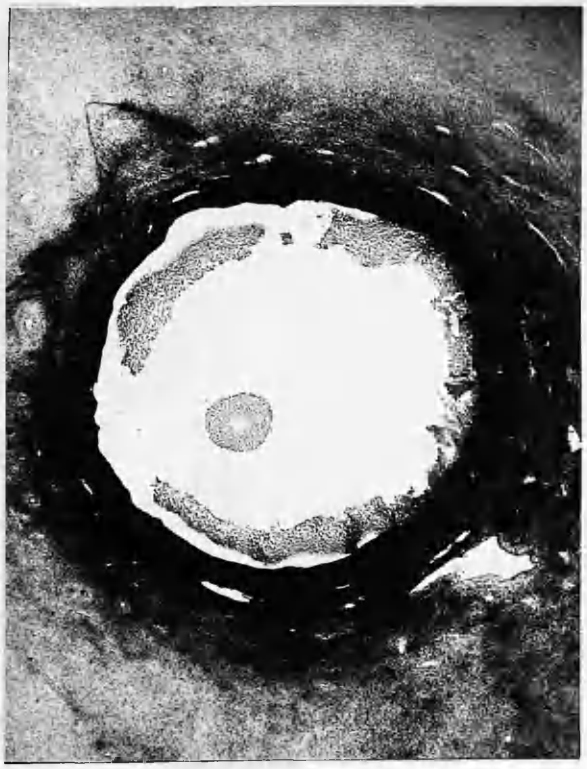


Fig. 44

Alkaline phosphatase (glycero) reaction in Graafian follicle x 18.

Plate 24.

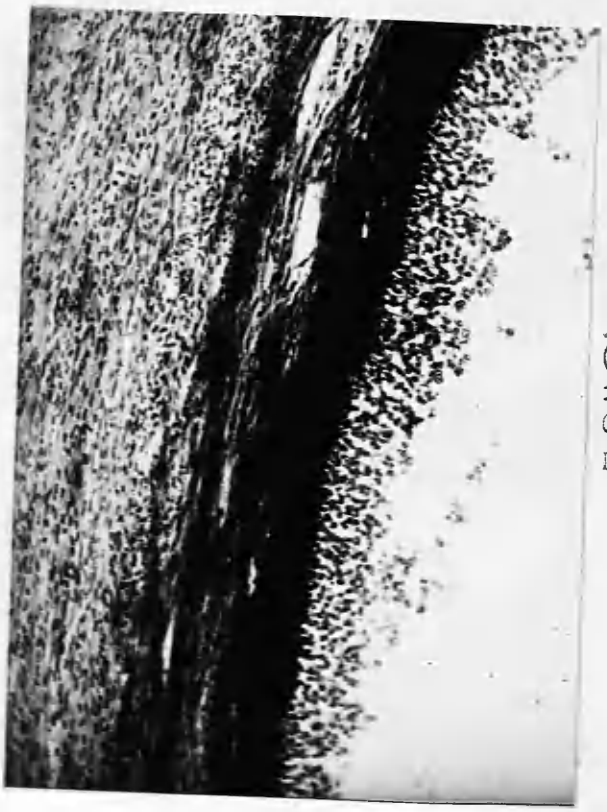


Fig. 45

Alkaline phosphatase (glycero) reaction in follicular wall of Graafian follicle preceding rupture x 120.

Fig. 46

Acid phosphatase (glycero) reaction of Follicular wall x 110.

Plate 25.

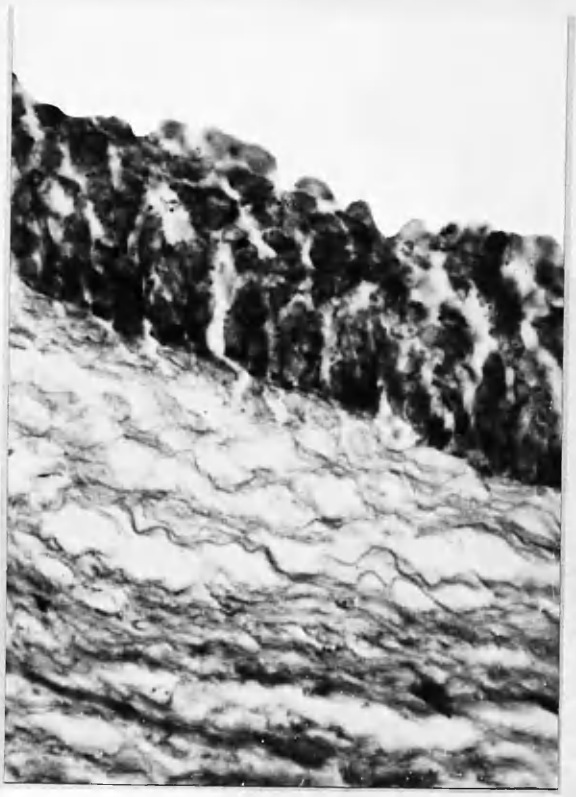


Fig. 47

Ovary, PAS reaction in granulosa cells of Graafian follicle preceding rupture x1400.

Fig. 48

Ovary, Alkaline phosphatase (glycero) reaction in theca interna cells of newly ruptured follicle x 200.

Plate 26.

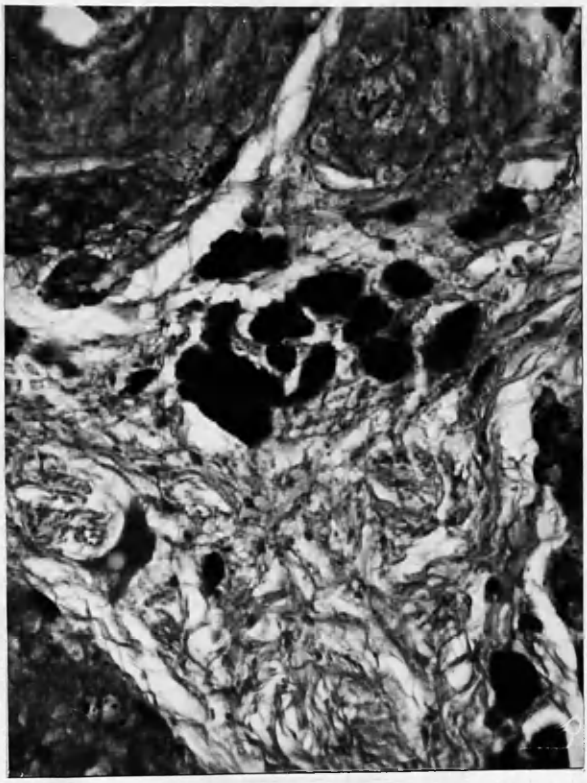


Fig. 49

Ovary, P.A.S. reaction in
corpus albicans
x 400.

Plate 27.

Fig. 50

Oviduct, T.S. H & E
x 60.

Plate 28.

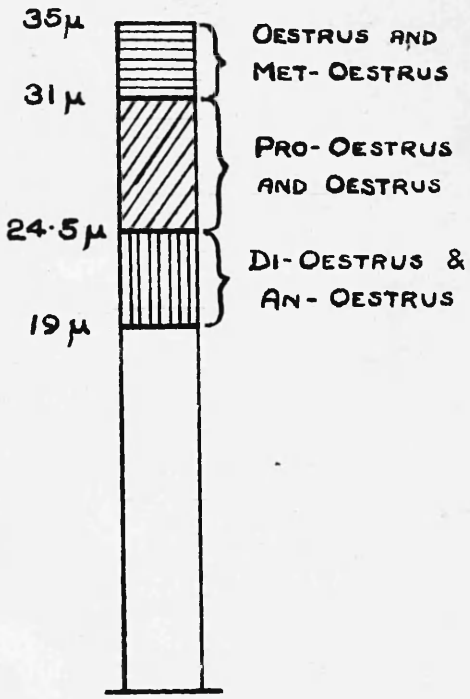


Fig. 51

Oviduct, Diagram of alterations in oviduct cell sizes.

Fig. 52

Oviduct, an-oestrus, H & E x 400.

Plate 29.

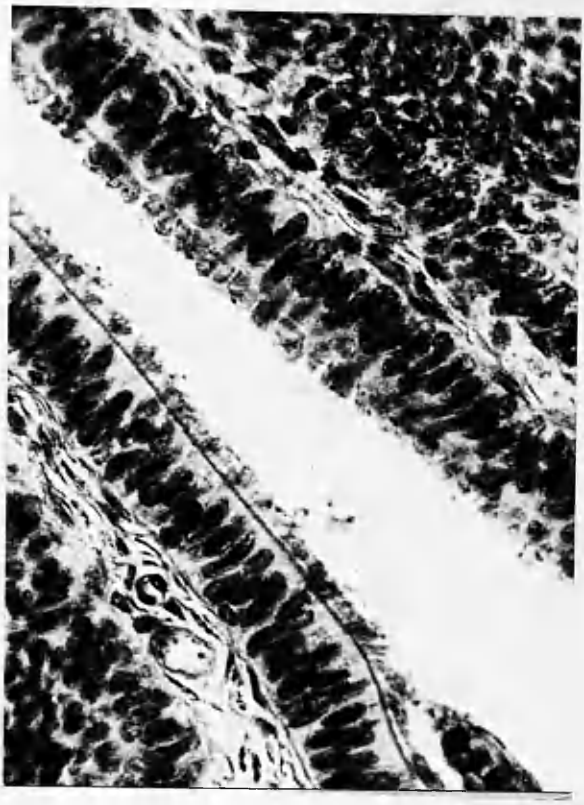


Fig. 53

Oviduct, pro-oestrus,
Mass. x 400.

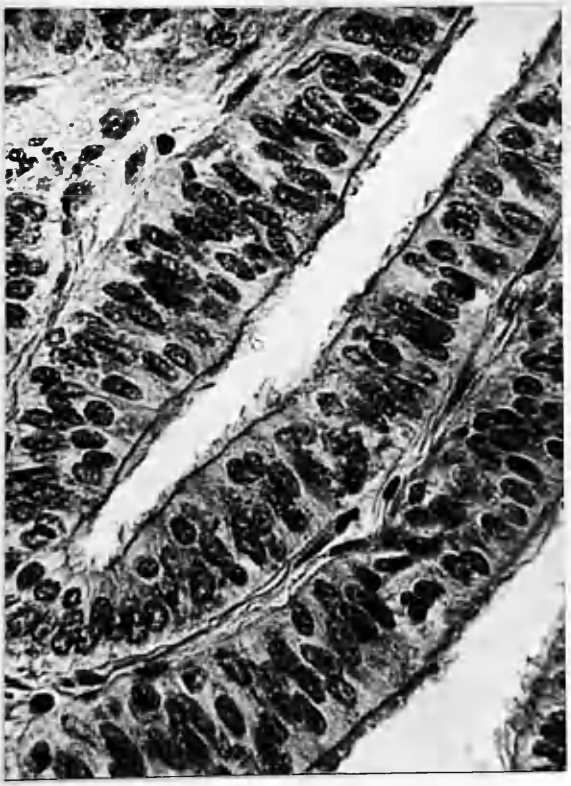


Fig. 54

Oviduct, oestrus
H & E x 450.

Plate 30.

Fig. 55

Oviduct, oestrus,
P.A.S. x 400.

Fig. 56

Oviduct, oestrus,
P.A.S. x 1200.

Plate 31.

Fig. 57

Oviduct, met-oestrus,
P.A.S. x 400.

Fig. 58

Oviduct, met-oestrus,
P.A.S. x 1200.

Plate 32.

Fig. 59

Oviduct, infundibulum, met-oestrus, alkaline phosphatase (glycero) x 200.

Fig. 60

Oviduct, isthmus, met-oestrus, alkaline phosphatase (glycero) x 200.

Plate 33.

Fig. 61

Oviduct, infundibulum, met-oestrus, alkaline phosphatase (glycero) x 400.

Fig. 62

Oviduct, isthmus, met-oestrus, alkaline phosphatase (glycero) x 400.

Plate 34.

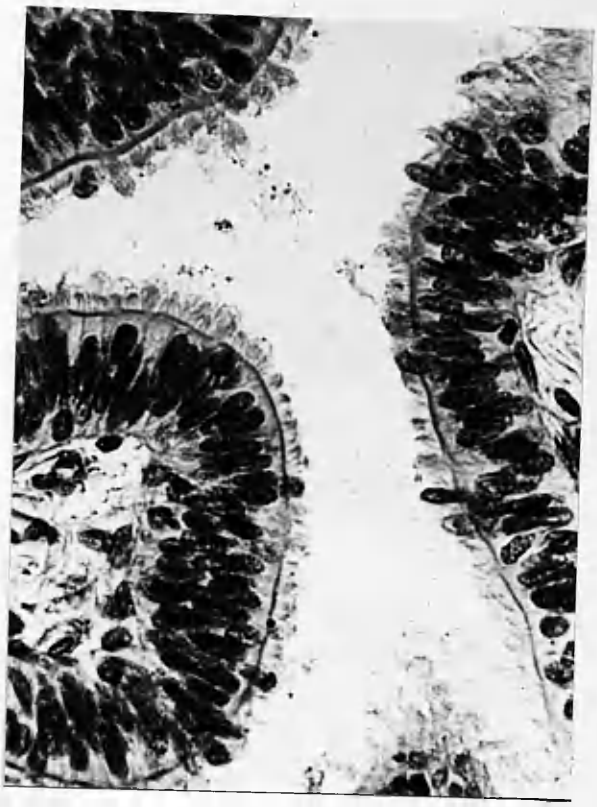


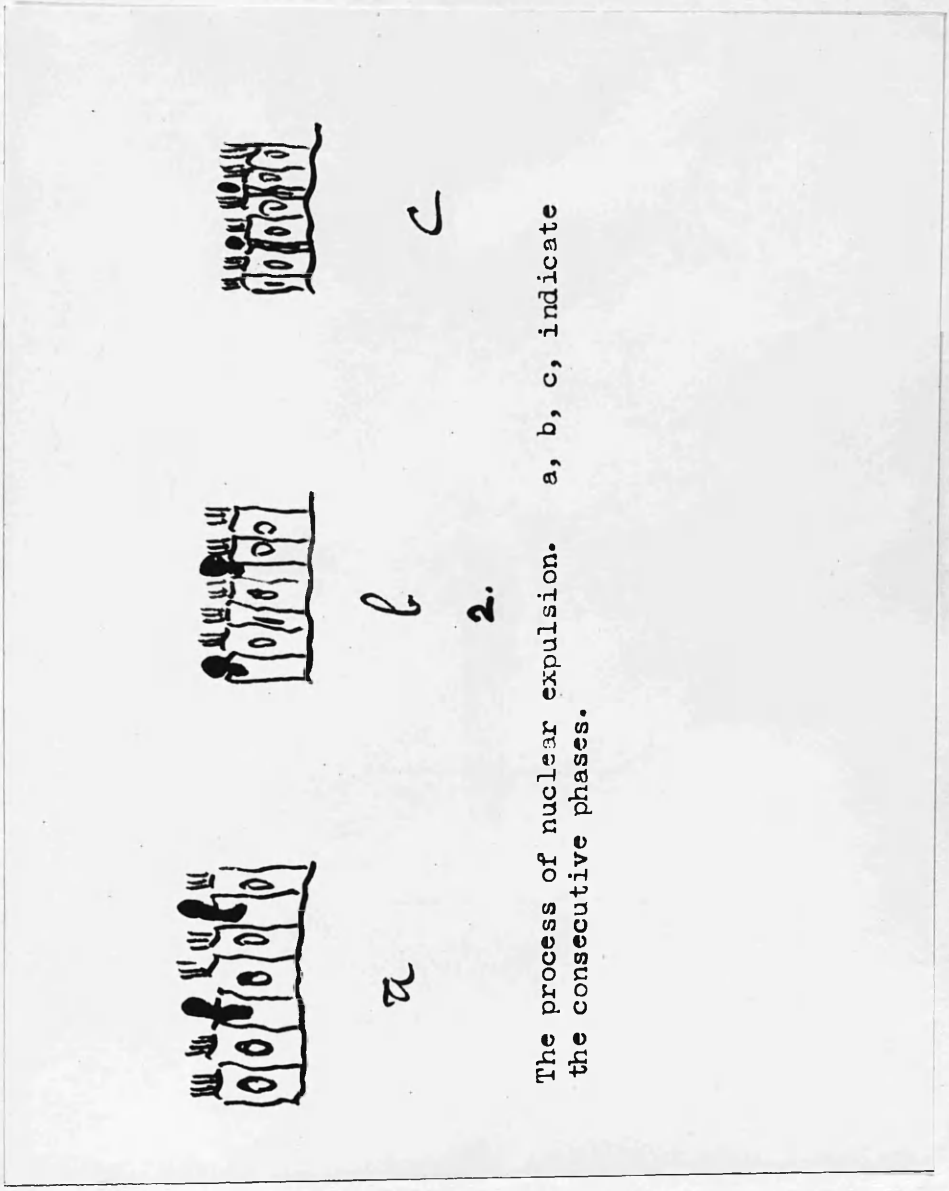
Fig. 63

Oviduct, di-oestrus, H & E x 400.

Fig. 64

Oviduct, late di-oestrus, H & E x 400.

Plate 35.



The process of nuclear expulsion. a, b, c, indicate the consecutive phases.

Fig. 65

Oviduct, diagram of nuclear expulsion.

Plate 36.

Fig. 66

Uterus, T-S, H & E x 10.

Plate 37.

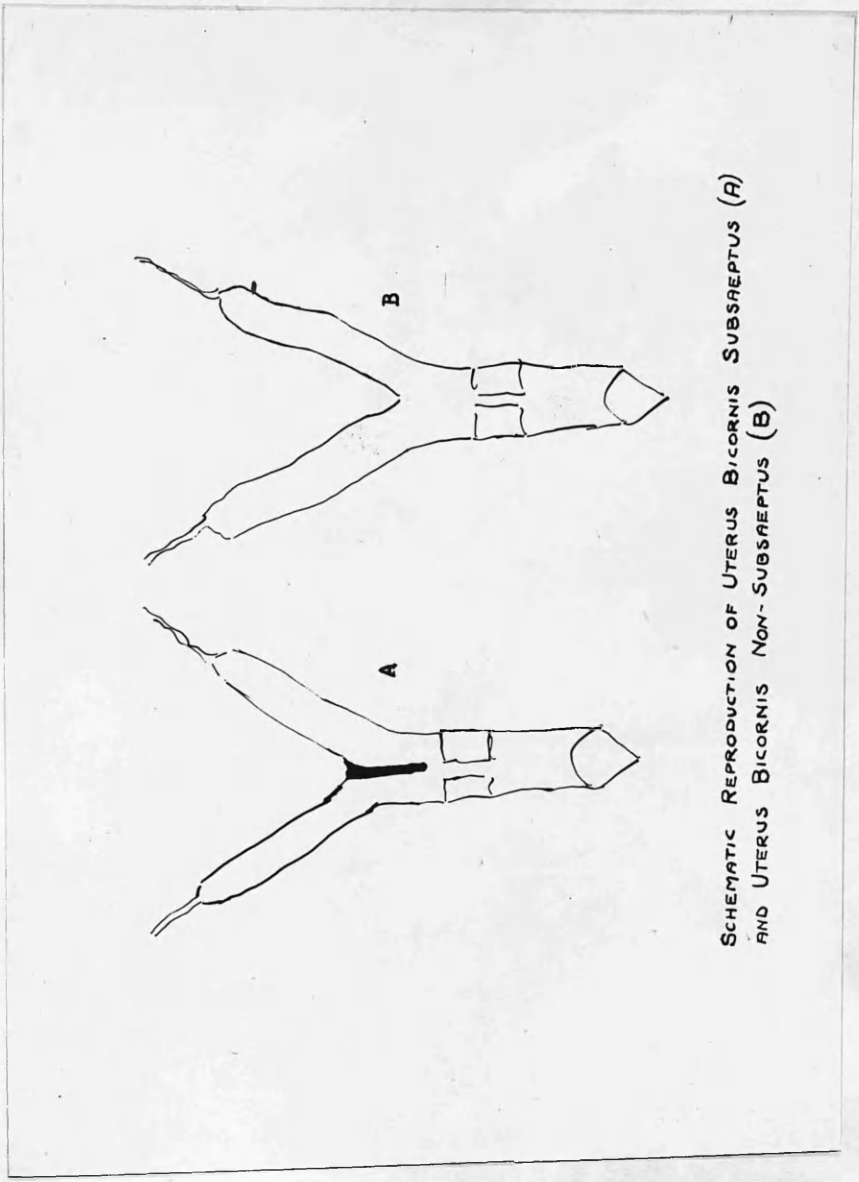


Fig. 67

Uterus, diagram of uterus bicornis,
non-subseptus and subseptus.

Plate 38.

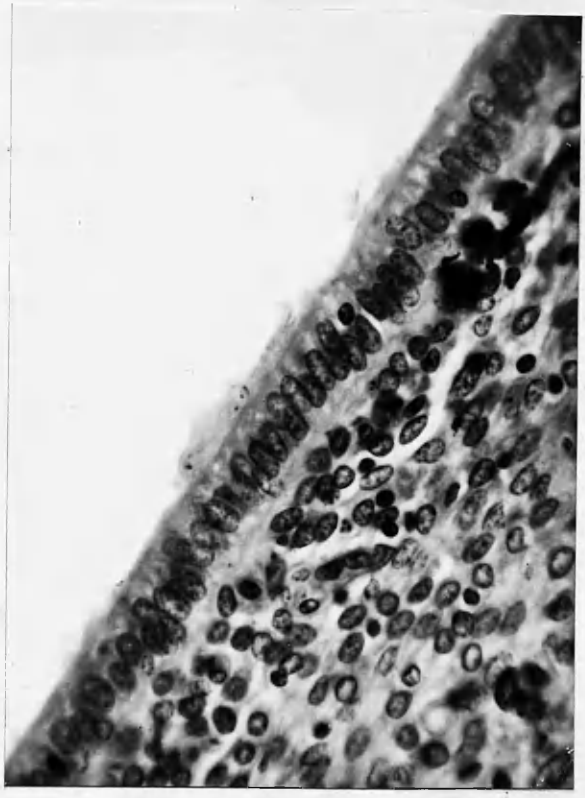


Fig. 68

Uterus, early pro-oestrus, epithelium, H & E x 400.

Fig. 69

Uterus, early di-oestrus, epithelium, H & E x 400.

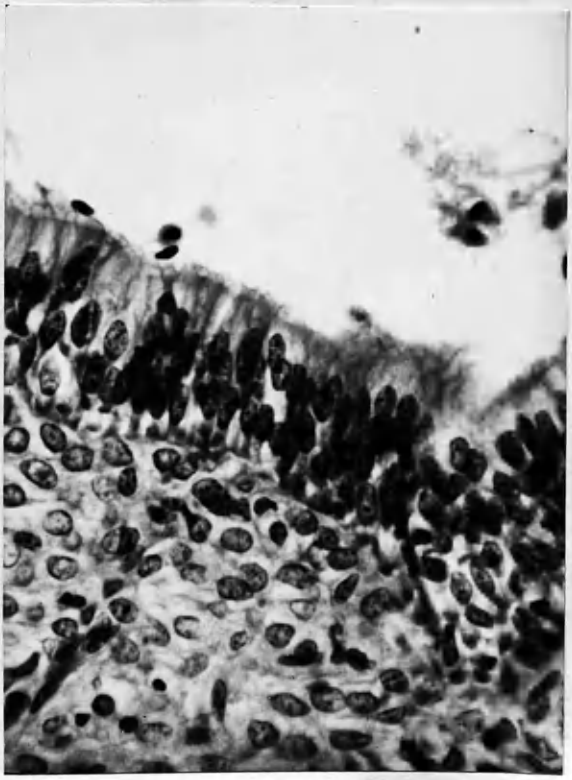


Plate 39.

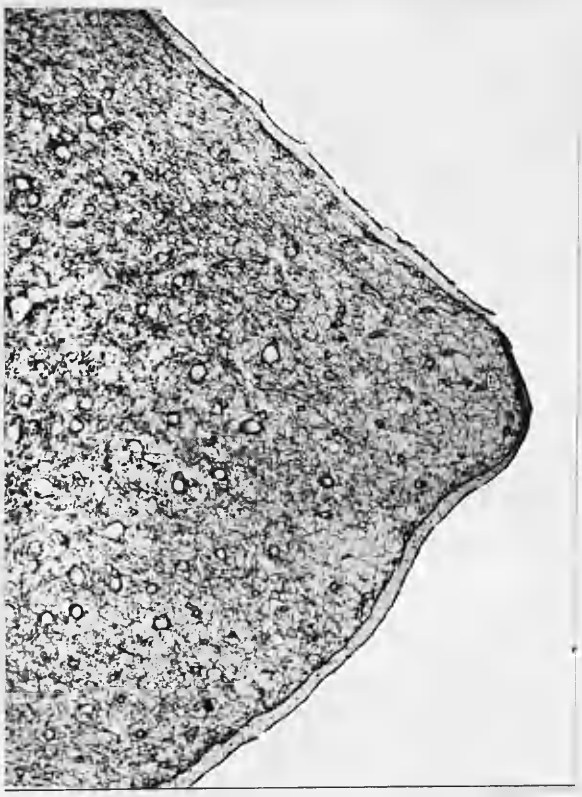


Fig. 70

Uterus, caruncle,
Gordon-Sweet x 150.

Fig. 71

Uterus, caruncle,
Gordon-Sweet x 1140.

Plate 40.

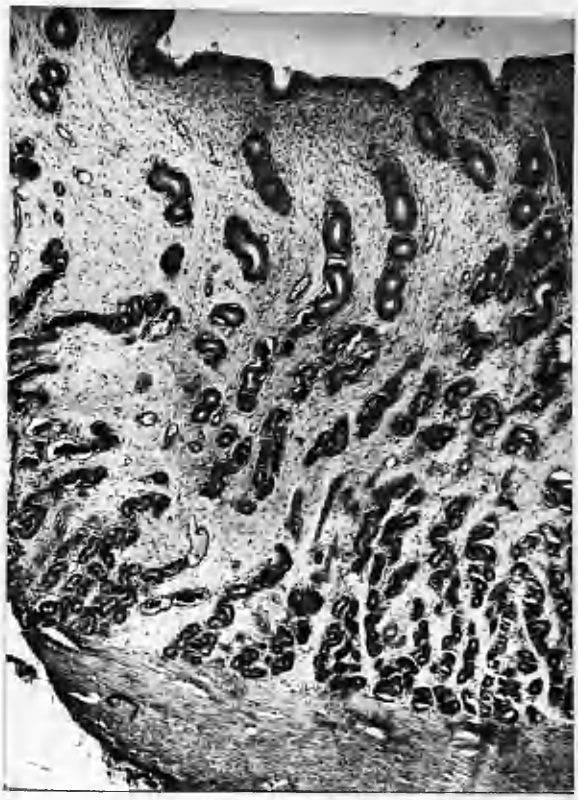


Fig. 72

Uterus, early di-oestrus, intercaruncular area, x 140.

Fig. 73

Uterus, early di-oestrus, intercaruncular area, x 280.

Plate 41.

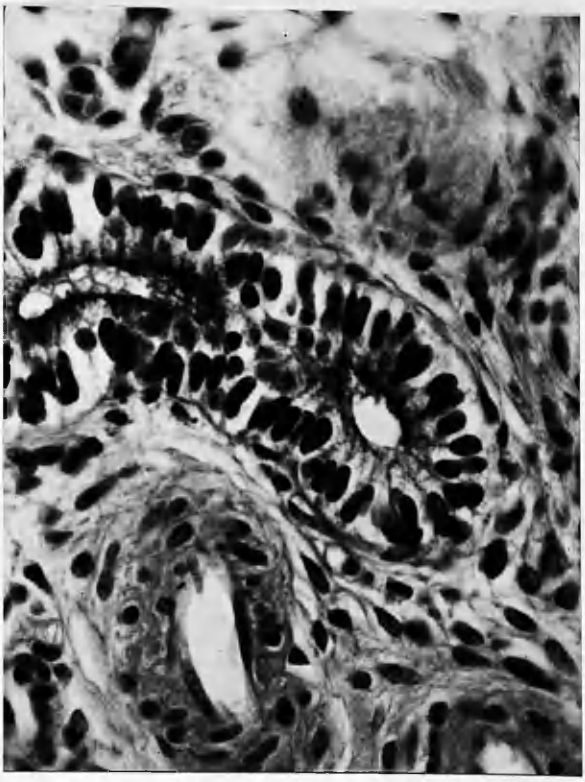


Fig. 74

Uterus, early pro-oestrus, gland, Mass. x 380,

Fig. 75

Uterus, early di-oestrus, gland, P.A.S. x 400.

Plate 42.

Fig. 76

Uterus, oestrus, gland, inorg. Fe x 400.

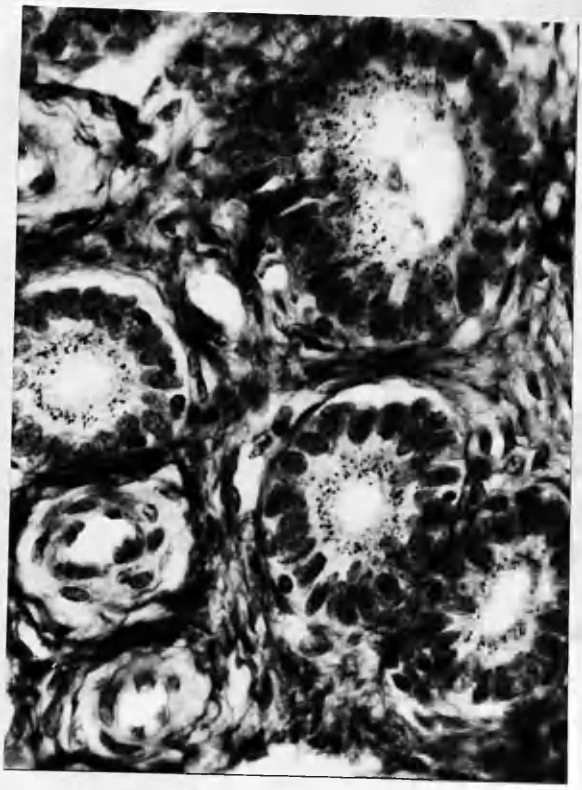


Fig. 77

Uterus, early di-oestrus, gland, inorg. Fe x 400.

Plate 43.

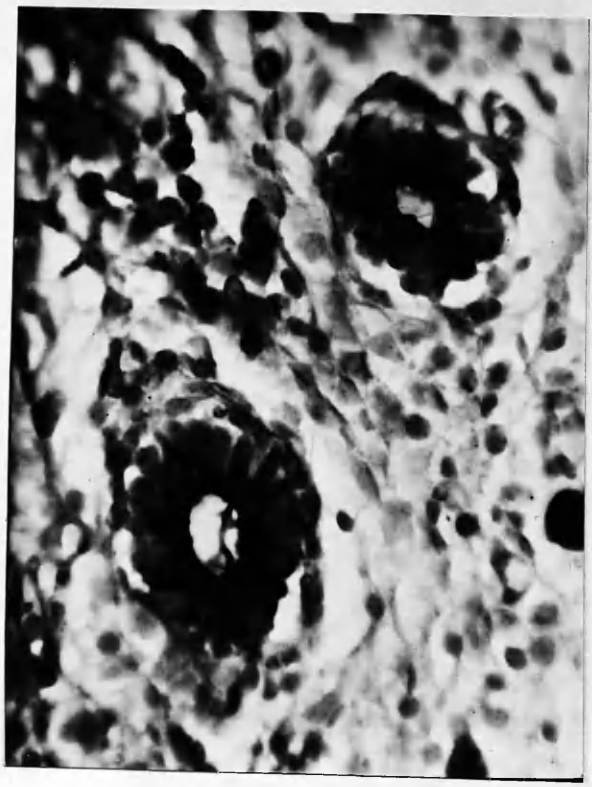


Fig. 78

Uterus, met-oestrus, gland, alkaline phosphatase (glycero) x 400.

Fig. 79

Uterus, early di-oestrus, alkaline phosphatase (glycero) x 400.

Plate 44.

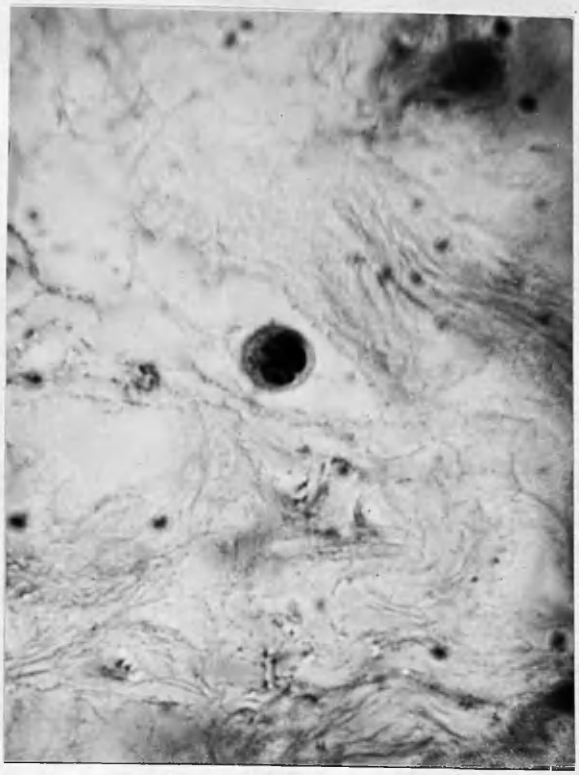


Fig. 80

Uterus, uterine macrophage near muscularis H & E x 400.

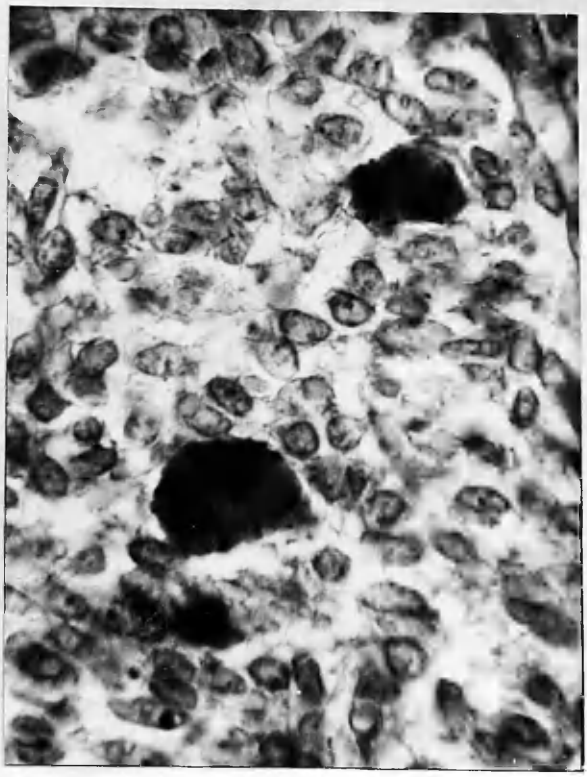


Fig. 81

Uterus, uterine macrophage beneath uterine epithelium, P.A.S. x 1200.

Plate 45.

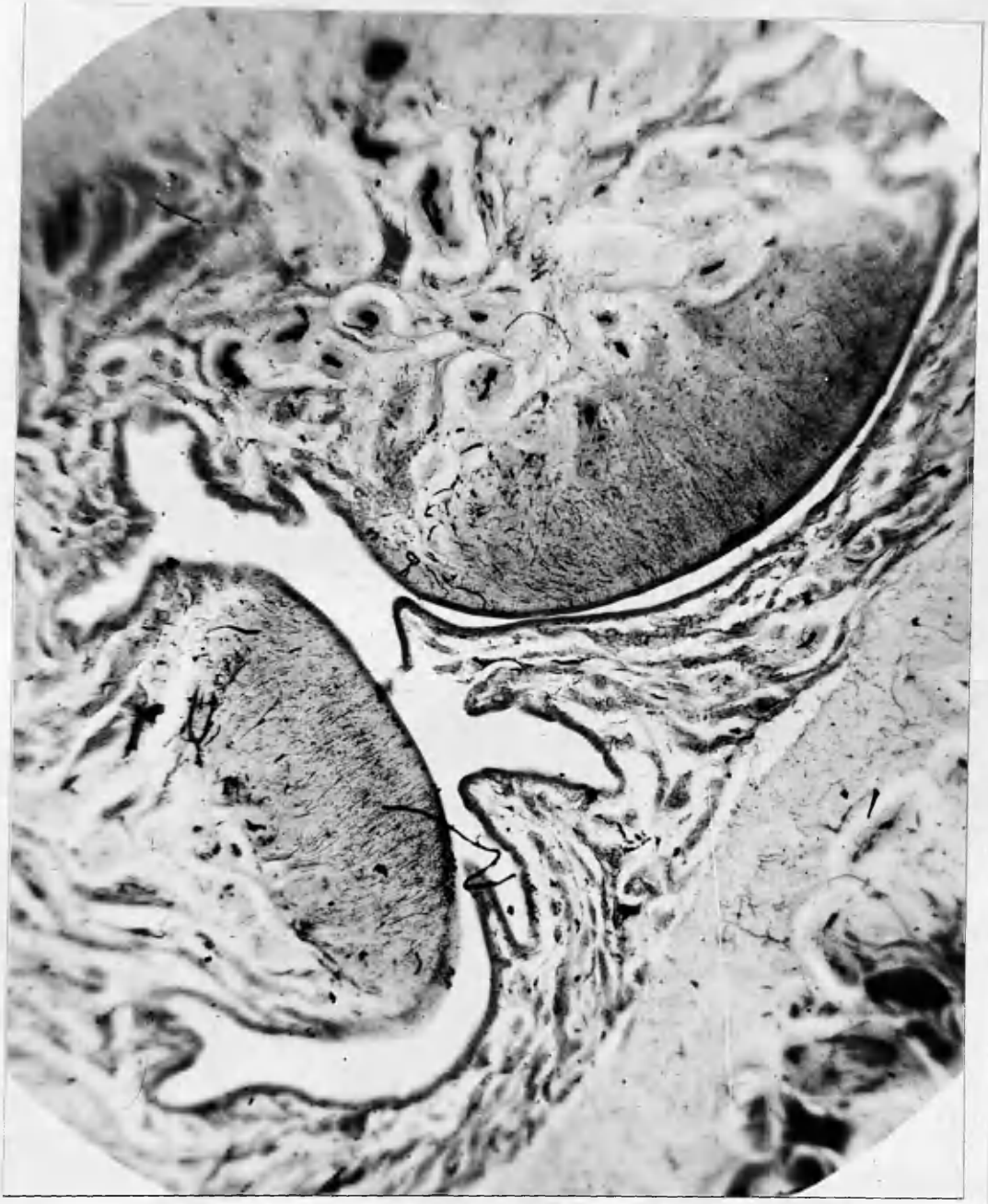


Fig. 82

Uterus, pro-oestrus caruncular circulation, Mod. Pickworth x 100.

Plate 46.

Fig 83

Uterus, oestrus, caruncular circulation, Mod. Pickworth x 100.

Plate 47.

Fig. 84

Uterus, met-oestrus, caruncular circulation, Mod. Pickworth

x 100.

Plate 48.

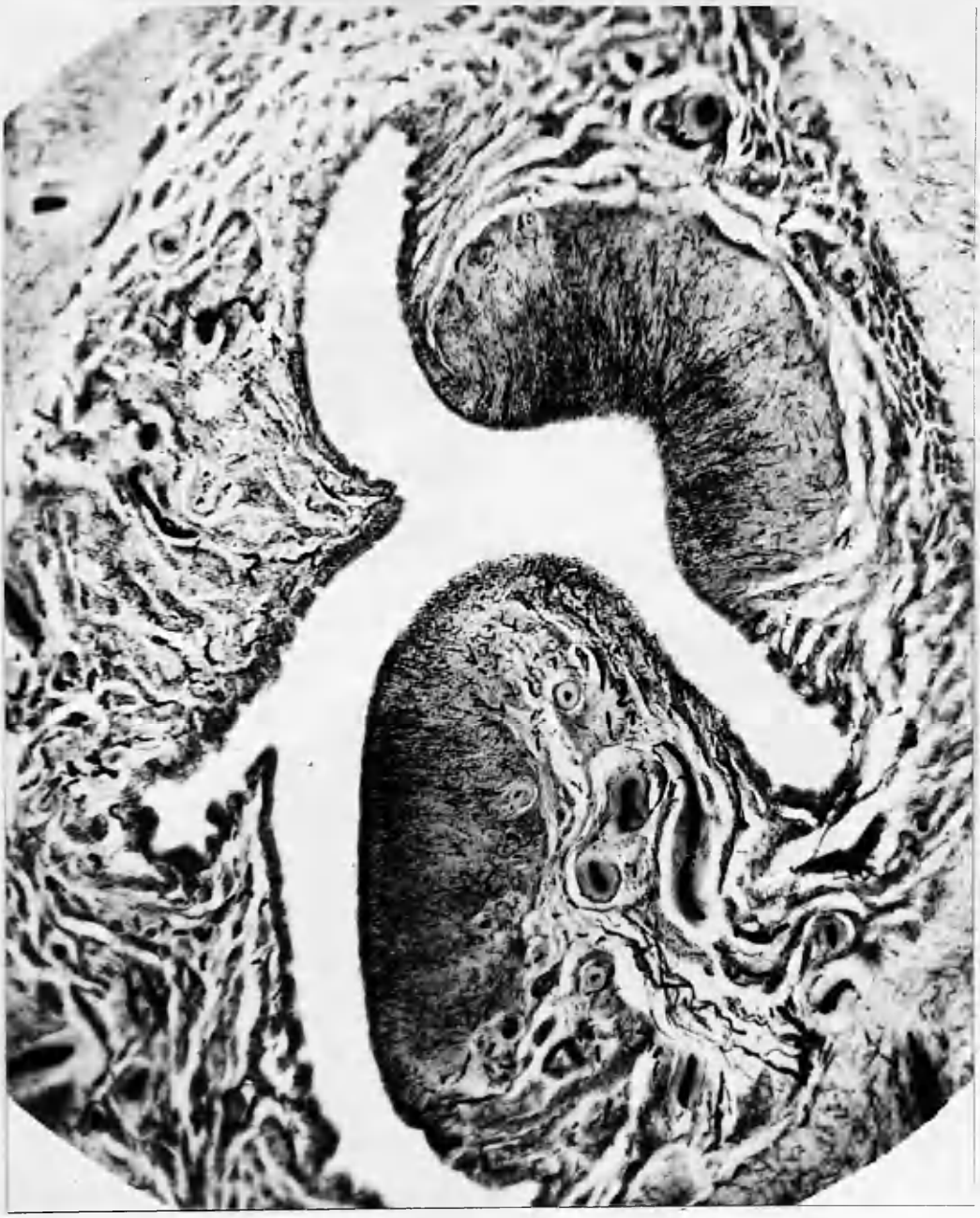


Fig. 85

Uterus, di-oestrus, caruncular circulation, Mod. Pickworth x 100.

Plate 49.

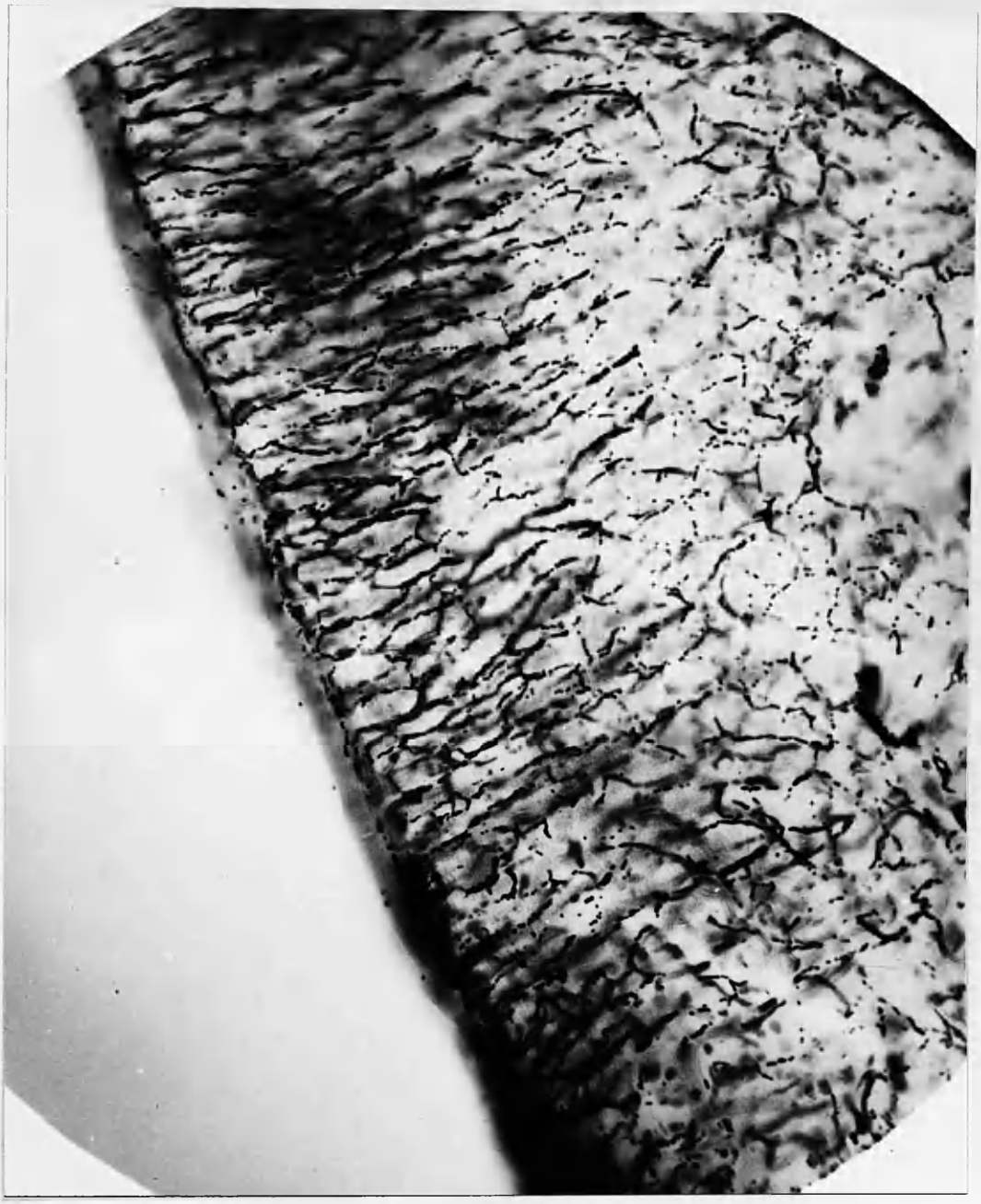


Fig. 86

Uterus, Di-oestrus, caruncular
circulation, Mod. Pickworth
x 240.

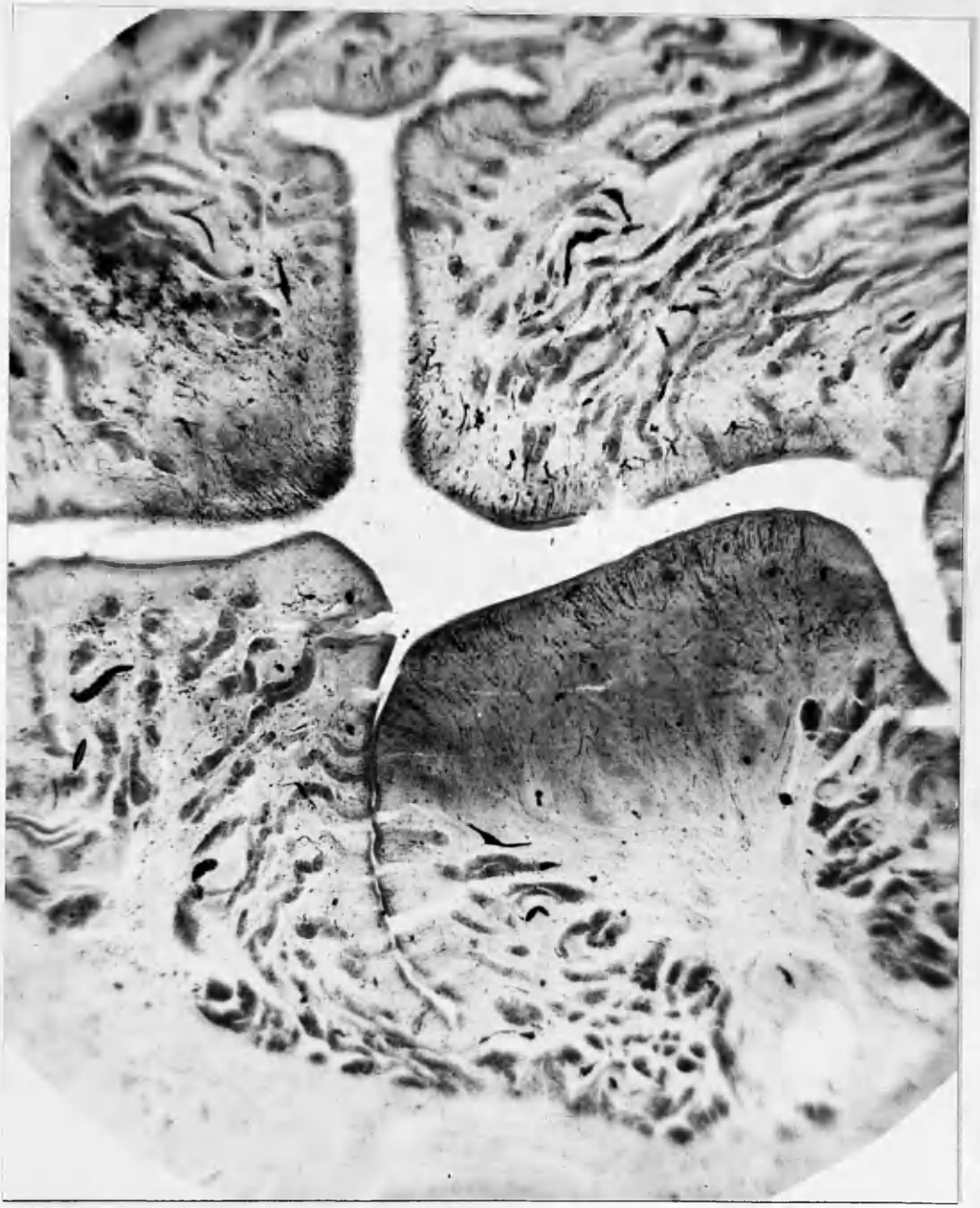


Fig. 87

Uterus, an-oestrus, caruncular
circulation, Mod. Pickworth
x 100.