PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Contributions to the Physiology of Mammalian Reproduction. Part I. The Oestrous Cycle in the Dog. Part II. The Ovary as an Organ of Internal Secretion

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V. *Contributions to the Physiology of Mammalian Reproduction.* Part I.—*The Œstrous Cycle in the Dog.* Part II.—*The Ovary as an Organ of Internal Secretion.*

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[Plates 7 and 8.]

Part I.—The Œstrous Cycle in the Dog.

Contents.

1.—*Introduction.*

The present paper, which is divided into two parts, deals mainly with the œstrous cycle in the Bitch and with the factors controlling its periodicity.

Before describing the changes which the generative organs periodically undergo and the causes which assist in producing these changes, it seemed important to give a brief comparative account of what is known regarding the œstrous cycle and its frequency of recurrence in the order of mammals to which the Bitch belongs. This we do in the present part (Part I).

Within the limits of the order in question there appears to be hardly less variation in the capacity for breeding possessed by its members than is shown by those of the entire mammalian class. The section of this paper dealing with this subject contains various observations on the breeding habits of carnivores which have not hitherto been recorded. It is followed by a description of the histological changes in the

uterus of the Bitch throughout the cycle, and these are compared with the similar changes undergone by the uterus in the Ferret, the Sheep, the Monkey, and the human female.

The next section of this paper deals with ovulation and the periods when it occurs, and this is followed by an account of the bearing of the facts recorded on the various theories which have been put forward regarding the relation between ovulation and menstruation.

We then, in the second part, adduce evidence in support of the view that the proœstrum and œstrus are brought about by an ovarian secretion, and give an account of our own experiments bearing on this subject. Lastly, we describe a series of experiments carried out to test the doctrine that the corpus luteum exercises an influence on the attachment of the embryo and the formation of the placenta.

The work was commenced in the autumn of 1903, and has since been continued with short intervals of interruption. A few observations which are published here were made at an earlier period. The investigation was started in collaboration with Professor Swale Vincent, but owing to the departure of the latter for Manitoba in the summer of last year, this arrangement had to be discontinued. We wish to thank Professor Vincent for permission to publish the results obtained during the time of his collaboration. We would like also to record our sense of obligation to Mr. Walter Heape, of Trinity College, Cambridge, who has done so much to further the subject under discussion; and to express our indebtedness to Professor Schäfer for the valuable advice and assistance which he has given throughout the progress of our work.

The expenses of the investigation have been defrayed by grants made by the Government Grant Committee of the Royal Society, the Moray Fund Committee of the University of Edinburgh, and the Carnegie Trust for the Universities of Scotland, to the members of all of which we tender our thanks.

2.—*The Œstrous Cycle in Carnivores.*

In describing the periods into which the œstrous cycle is divided, we have made use of the terminology originally proposed by Heape (1900) and subsequently adopted by one of us (Marshall, 1903, 1904) in giving an account of the cycle in the Sheep and in the Ferret.

The Bitch, with which the present paper principally deals, is monœstrous, that is to say, the sexual season contains a single non-recurrent œstrous cycle. Consequently there is no diœstrous interval, the entire cycle being divided simply into four periods as follows:—(1) Anœstrum, or the period of rest, (2) Proœstrum, or the period when the genital organs become congested and bleeding occurs, (3) Œstrus, or the period of desire, and (4) Metœstrum, or the period when the genital organs resume the quiescent condition, supposing that the animal does not become pregnant during œstrus.

The procestrum in the Bitch is characterised externally by the vulva being swollen, reddened, and moistened with mucus, and by the existence, usually but not invariably, of a discharge of blood from the vagina. The discharge of blood generally ceases at the commencement of œstrus, but in some cases a relatively slight sanguineo-mucous flow may continue throughout this period or even beyond it. Such has been noticed to be the case in a Dandie Dinmont terrier. It is stated by STONEHENGE (1887), however, that a bitch will rarely take the Dog until bleeding is over. The procestrum lasts for from about a week to 10 days, while œstrus may extend for about another week.

The average duration of the complete cycle in the Bitch is about six months, there being two "heat" periods annually, in typical cases in the spring and autumn. Though this is the general rule, individuals often show considerable variation. Thus Bitches belonging to the smaller breeds may come on heat regularly every four months. An experienced breeder of Irish Terriers informs us that with the Bitches of this breed the cycle frequently recurs after four months, but that every six months is the rule. On the other hand, with large Dogs the heat periods tend to occur at longer intervals (HEAPE, 1900). Mr. ROBERT LEADBETTER, of Hazlemere Park, Buckinghamshire, who has been very successful in breeding Great Danes, tells us that with the Bitches belonging to this breed the "heat" periods not infrequently recur at intervals of eight months. There can be no doubt that though in the case of certain Bitches generally belonging to the smaller varieties, the cycle may be repeated oftener than every six months, this is only when they fail to become pregnant, for more than two litters are seldom, if ever, produced within a year. On the other hand HEAPE states (1900) that the "sexual seasons of any Bitch may be interfered with to the extent even of complete cessation if she is systematically prevented from breeding." It should be noticed, however, that the periodicity of the season tends to become very irregular with advancing age, and this quite apart from whether the Bitch is prevented from becoming pregnant. The periodicity of the cycle depends to some extent upon physical condition, and also apparently upon climatic influences, for it is stated by RINK (1877) that in Danish Greenland the Bitches usually have but one sexual season in the year.

The period of gestation in the Bitch is usually from 59 to 63 days. With Dogs of smaller breeds there is said to be a tendency for this period to be slightly less than in the case of larger animals. The lactation period is variable in its duration, and may extend until the next "heat" period, for in one case under observation the mammary glands of a Bitch which showed all the usual signs of procestrum contained a considerable quantity of milk, and had evidently been very recently employed in suckling.

The wild Dog (*Canis azaræ*) of South America is said to breed only in winter, and therefore but once annually (RENGGER, 1830). The same is the case with the Wolf and the Fox (SPALLANZANI, 1784, MILLAIS, 1904) in their wild state, but in the Zoological Society's Gardens in London these animals, and also the Jackal, are said to

experience two sexual seasons annually like the Bitch (HEAPE, 1900). We are informed, however, that the Wolves in the Gardens at Dublin* only come "on heat" once a year if permitted to breed; otherwise they come "on heat" more frequently. It thus appears that the greater frequency of recurrence of the œstrous periods of Wolves in captivity as compared with those in the wild state is due in part to their not being permitted to breed. All these animals are said to be monœstrous.†

The female of the Cape Hunting Dog (*Lycaon pictus*) in the Gardens at Dublin for three years came "on heat" regularly in the middle of October and gave birth to pups at the beginning of January, the period of gestation being 80 days. Subsequently the Bitch came "on heat" in August, a litter being produced in November, or two months earlier than previously, so that two litters were born in one year.

The female of the domestic Cat normally breeds two or three times a year. SPALLANZANI (1784) states that the heat periods are in February, June, and October, but there can be no doubt, if only from our own observations, that individuals show much variation. HEAPE (1900) says that there may be as many as four sexual seasons annually, but this presumably is only when the animal is not permitted to breed. He says further that the domestic Cat when it becomes feral has apparently only one annual sexual season. We can find no record as to whether œstrus recurs during the same sexual season if the Cat is not allowed to copulate (*i.e.*, whether the Cat is polyœstrous), but our own observations show that there may be four "heat" periods in one season, the duration of the diœstrous cycle being about a fortnight.

The wild Cat according to HAMILTON (1896), experiences only one sexual season within a year, but according to a statement by MIVART (1881), there may be two. MILLAIS (1904), says that it is uncertain whether this species has one or two breeding seasons in a year. "The young are usually born in May, two to five in number . . . . The probability is that wild Cats breed only once in the twelvemonth, but I have seen young animals killed in Scotland in October which certainly could not have been more than 40 days old. The mother must have dropped them as late as the end of August or the beginning of September."

Mr. ALFRED HENEAGE COCKS in a letter to Mr. MILLAIS says: "I have received wild Cats barely full grown at such times as would lead me to suppose that they were born rather later than May, but hardly so late as September; the latest of these which I noted on receipt was probably barely six months old on February 15. It was therefore probably born about the latter part of August, but may not have been born until September. In captivity I have never observed a female wild Cat come in season during the summer . . . . Many years, when owing to the death of the young or the fact that the pair had not bred together in the spring, I have kept male and female together all summer, but they have shown no inclination to breed."‡

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* For the information published in this paper regarding the breeding habits of the animals in the Royal Zoological Society's Gardens at Phœnix Park, Dublin, we are indebted to Professor D. J. CUNNINGHAM and Dr. R. F. SCHARFF.

† The period of gestation in the Wolf is 63 days (Dublin Zoo); that of the Fox is said to be two months, or about the same time (MILLAIS, 1904).

‡ In a more recent letter Mr. COCKS informs us that the old female in his possession came in season and took the male last autumn (1904), after the death of the kittens which were born earlier in the same year. The Cat, however, did not become pregnant in consequence of the autumn copulation. The most usual

Mr. COCKS refers to the fact that a female in captivity was seen to copulate on one occasion on February 17 and also on February 21, so that the œstrous period in the wild Cat must last for at least five days.* In the domestic Cat we have observed the period to be about the same time.

The larger species of the Felidæ in their wild state probably agree in having a single annual breeding season. In captivity some members of the group at any rate come "on heat" more frequently. The Lionesses in the Dublin Zoological Gardens, a place famous for the success in Lion breeding achieved there, experience two œstrous periods annually, even if allowed to become pregnant. If the copulation is unsuccessful, or if not permitted to breed, they are said to come "on heat" three or four times within a year, a frequency at least as great as that shown by the domestic Cat. Mr. ROBERT LEADBETTER, who has bred several litters of Lion cubs at Hazlemere Park, Buckinghamshire, informs us that Lionesses do not as a rule begin to come "on heat" until they are three years old, and that if not successfully served at the first "heat" period they may experience a recurrence of the cycle every three weeks until they become pregnant. It thus appears that in captivity at any rate the Lioness may be polyœstrous, in this respect apparently resembling the Cat under domestication. The female Puma in the Dublin Gardens is stated to come "on heat" only once a year if breeding, or twice annually if there is no gestation.

The duration of the œstrus in the Lioness is at least a week (LEADBETTER MS.).

The period of gestation in the Lioness is from 15 (Dublin Zoological Gardens) to 16 (LEADBETTER MS.) weeks. That of the Tigress is 22 weeks (LEADBETTER MS.), while the Puma carries its young for 15 weeks (Dublin Zoological Gardens).

Most species of Bears, both in their natural state and in the Zoological Society's Gardens in London are monœstrous and have a single annual breeding season. The Grizzly Bear, however, according to SOMERSET, bears young only once in two years (HEAPE, 1900). The Bears in the Zoological Gardens at Dublin, on the other hand, may experience more than one annual sexual season if they fail to become pregnant, but only one if pregnancy occurs, the period of gestation being seven months. HEAPE (1900) says that with the Bears in the Gardens in London the period of œstrus may continue for as long as two or three months continuously, but this, as he remarks, is an unnatural condition, copulation not resulting in pregnancy.

month for wild kittens is May, but the range of date in Mr. COCKS' collection has been from April 26 to July 22. According to the same authority the period of gestation in the wild Cat is normally 68 days, or a week longer than in the domestic Cat.

* "The male wild Cat," Mr. COCKS informs us "(like the Stag), has a 'rutting season,' and calls loudly, almost all day and night, making far more noise than the female." This information is of interest, inasmuch as the males of most carnivores, although they undoubtedly show signs of increased sexual activity at some times more than at others, are not known to have anything of the nature of a regularly recurrent rutting season. Nothing of the kind is known in the Dog, nor so far as we are aware in the males of the domestic Cat or the Ferret, all of which seem to be capable of copulation at any time of the year. On the other hand the males of Seals appear to have a rutting season at the same time as the sexual season of the female.

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The Ferret is monœstrous and may have one, two or three sexual seasons annually, but when there is more than one the seasons do not recur at regular intervals rhythmically but are concentrated in the spring and summer, the autumn and winter being almost invariably occupied by a prolonged anœstrum (MARSHALL, 1900). This tendency towards a concentration of sexual seasons during a particular part of the year may be regarded as an approach to a polyœstrous condition, for if the "heat" periods were to recur at still shorter intervals they might quite well be considered as belonging to one sexual season. But so far as we are aware the Ferret does not experience œstrus more than twice annually if permitted to breed.

The Polecat, of which the Ferret is a domesticated variety, is also monœstrous and breeds but once a year. Mr. COCKS has kindly sent us the following information regarding Polecats in captivity: "I have bred 25 litters, of which—

16	per cent.	were born between	May 23 and end,
48	,,	,,	June 1 and 15,
28	,,	,,	June 16 and end,
8	,,	,,	July 1 and 7."

Mr. COCKS thinks that the number of litters born subsequently to the middle of June in the wild state is small. The sexual season of the Polecat is therefore fairly restricted and regular.*

Probably the Stoat, and perhaps also the Weasel, are monœstrous in their wild state (BELL, 1874), but these animals do not appear to breed in captivity. Mr. COCKS (1900), has succeeded in getting the Pine Marten to breed in captivity, the period of œstrus probably lasting for a fortnight.†

Mr. COCKS, who has kept these animals for over 30 years, tells us that he has no doubt that they only breed once a year.

The Otter in its wild state breeds only in the winter, according to BELL (1874), but in captivity the female comes in season very regularly once a month all the year round in the absence of the male (COCKS, 1881). Mr. COCKS' subsequent experience, concerning which he has been good enough to give us information, confirms the fact that there is no anœstrous period, about which there previously appeared to be some doubt. In the wild state, according to COCKS, "it may be asserted with some confidence that while the young are born during any month of the year, yet that more litters are born between October and February, both inclusive, than during the remaining seven months." Mr. COCKS adds that he has no doubt that the Otter only breeds once in about a twelvemonth.‡ SOUTHWELL (1888), states his belief that the Otter in the wild state, as a general rule only breeds in winter, but that it occasionally breeds also at other times.

The various species of Seals are very regular in their breeding habits and are almost certainly monœstrous, having one litter of pups annually.§ In the case of the Harp Seal the œstrous cycle appears to have attained to an almost perfect rhythmic regularity, for from Greenland to the north-east of Newfoundland where this species occurs in abundance, the pups are stated to be born each year between March 8 and March 10. But HINDEMAN says that at Jan Mayen the pups are not born until about

* The gestation period in the Polecat is about 40 days, or about the same as that of the Ferret (COCKS, 1891, HARTING, 1891).

† The Pine Marten's gestation period is about 14 weeks (COCKS, 1900).

‡ The Otter's gestation period is about 61 days (COCKS, 1881).

§ The period of gestation in the common Seal is nine months, the rutting season being September (MILLAIS, 1904).

March 23 or 24 (MILLAIS, 1904). TURNER'S Notes (1875), on the breeding of Seals also point to the conclusion that these animals are monœstrous.

The Walrus affords an example of a mammal which produces young only once in three years. Parturition takes place in May or June, the sexual season being about the same time of the year. The period of gestation is about a year, and the nursing or lactation period two years (MILLAIS, 1904).

From a consideration of the instances given above it is evident that the species of the order Carnivora show a very wide range of variation in their sexual and reproductive capacity. That the recurrence of the œstrous cycle may be concurrent with the seasons and may take place with a rhythmic regularity is well shown in certain members of the group, while it is no less evident that the periods when heat occurs are largely influenced by environmental conditions. Thus it is mentioned by BISCHOFF (1863) that the breeding season of the Fox is affected by the nature of the country where it lives, and MILLAIS (1904) refers to the fact that whereas the cubs of this animal in most parts of Great Britain are not born until the end of March or beginning of April, in the South of England they are frequently produced as early as January.

The influences of domestication and captivity upon the periodicity of œstrus are even more marked than those of climate, and the more frequent recurrence of the cycle in the Ferret, the domestic Cat, and certain of the larger Carnivora in Zoological Gardens must be ascribed to this cause. The periodicity of the œstrus in certain of those animals in the Gardens at Dublin is said to be markedly affected by accommodation, heating, and feeding, and can to a certain extent at any rate be regulated. This is comparable to what occurs among other mammals, such as Mares or Sheep (HEAPE, 1900, MARSHALL, 1903). Taking all these facts into consideration and remembering that the monœstrous condition, so far as is known, occurs most commonly among animals in a state of nature, it may, perhaps, be inferred that this is the more primitive condition, polyœstrum being secondarily acquired not merely within the limits of the order Carnivora, but in the Mammalia as a whole.*

3.—*The Histology of the Uterus during the Cycle.*

For the purpose of describing the changes through which the non-pregnant uterus of the Bitch passes, we divide the œstrous cycle into four periods as follows:—

(1) Period of rest	Anœstrum.
(2) Period of growth and congestion	Procœstrum.
(3) Period of destruction	
(4) Period of recuperation	Œstrus. / Metœstrum.

* There can be no doubt that there are numerous instances of animals in which the wild representatives of the species or genus are monœstrous, while the domesticated forms are polyœstrous, the polyœstrum being apparently independently acquired in each case, *e.g.*, the Sheep, Sow, Cat; the greater frequency of the sexual season in the Ferret as compared with that of the Polecat may also be cited. It would seem more

This scheme of division is identical with that adopted by MILNES MARSHALL (1893), HEAPE (1894, 1897), and others in describing the menstrual changes in the Primates.* (*Cf.* also LEOPOLD, 1877.)

(1) *Period of Rest.*—The normal non-gravid uterus of the Bitch has been described by TURNER (1876) and other writers on the zonary placenta, to whose works the reader is referred. A short account of the uterus before the first "brunst," as well as of that of the newly-born pup, is given also by BONNET (1902) in a recent memoir on the embryonic development of the Dog.†

The mucosa is bounded at the surface by a layer of epithelium composed of cubical or columnar cells. This epithelial layer is continuous with that which bounds the glands, which is composed similarly of a single layer of cells. The sub-epithelial mucosa or stroma is a connective tissue containing fusiform cells showing clearly marked nucleoli. Small blood-vessels are contained in the stroma, which is of fairly uniform character throughout. Leucocytes do not appear to occur in the mucosa outside of the vessels. Pigment is not present at this stage, at least ordinarily. On the outside of the stroma are the normally arranged muscle layers containing large blood-vessels.

(2) *Period of Growth and Congestion.*—The mucosa at this period is slightly thickened, at the same time becoming more compact, but we find no growth to several times the original thickness of the layer; such as has been described by RETTERER (1892), who has contributed a short account of the Bitch's uterus without, however, giving any figures. The growth is accompanied by enlargement and congestion of the capillaries, which at the same time become much more numerous.

There is no apparent change in the histology of the epithelium or of the uterine glands.

It has, however, in some cases been noted that previous to the external signs of "heat," there is a discharge of mucus containing a considerable number of polymorph leucocytes from the vaginal aperture. A similar discharge has also sometimes been found after external bleeding, when it has been shown to arise very largely from the uterus, although a few squamous epithelial cells, presumably from the wall of the vagina, are contained in it.‡

probable, therefore, that this greater frequency in the periodicity of œstrus is a secondary condition than that it is of the nature of a reversion to a primitive ancestral condition. But the solution of the problem must depend somewhat on the view taken regarding the legitimacy of the biological conception of reversion.

* The terms anœstrum, etc., are HEAPE'S (1900), as previously mentioned.

† A very brief account of the Bitch's uterus during the heat period is also given by BONNET (1902), as well as by RETTERER (1892).

‡ In the case of one Bitch in our possession this discharge was considerable and the leucocytes were extremely numerous. The discharge continued for at least a week, but may have lasted longer. Consequently we considered that this animal was suffering from "leucorrhœa" (*cf.* the case of the female Monkey described by HEAPE (1897), which it appeared closely to resemble). But this Bitch came "on heat" about three weeks after we first noticed the discharge, the external bleeding being apparently normal.

It is evident that the changes which take place during the growth period are essentially similar to those which occur during the growth period in the Sheep (Marshall, 1903), the Ferret (Marshall, 1904), and the Monkey (Heape, 1894, 1897), but there does not appear to be any serous infiltration through the stroma like that described in the human female at this period (*cf.* Lipes, 1904). Fig. 1 (Pl. 7) represents part of a transverse section of a uterus of this period, showing the congested blood-vessels.

(3) *Period of Destruction.*—At the commencement of this period the walls of the congested vessels break down and red corpuscles and leucocytes become extravasated through the stroma. Some of the vessels, however, may be observed at this stage to be still intact. This is seen in fig. 2 (Pl. 7). The extravasated blood tends at first to collect immediately subjacent to the epithelium lining the lumen of the uterus, as shown in figs. 2 and 3 (Pl. 7). This collection of blood beneath the surface epithelium evidently corresponds to what Gebhard (1895) describes in the menstruating human female as the sub-epithelial hæmatoma. The blood is not markedly aggregated in "lacunæ," as is described in the case of Monkeys by Heape (1894, 1897), but occasionally small aggregations are found immediately under the lining epithelium. As a rule, the extravasated blood is diffused throughout the stroma in the area under description.

During the second stage of the period of destruction blood finds its way into the cavity of the uterus and thence it passes to the vagina, when external bleeding is to be observed. This is accompanied by an increase in the mucous secretion, comparable to what has been described in the human female (Lipes, 1904). It would seem probable that the appearance of the cells marked *sec* in fig. 2 is associated with this secretory activity.

Authorities are not agreed regarding the extent to which denudation of the uterine mucosa takes place in the human female, some stating that this is considerable, while according to others even the surface epithelium is retained intact. According to Lipes (1904) the denudation is variable, depending upon the rapidity and degree of the hæmorrhage. In Monkeys (*Semnopithecus entellus* and *Macacus rhesus*) the whole of the surface epithelium and the superficial stroma are destroyed. Sutton (1880), however, states that no denudation takes place in *Macacus rhesus*. Retterer (1892), who has written a short account of the processes occurring during heat in the Bitch, says that the epithelium is nowhere removed except at the places where the blood escapes into the cavity of the uterus. In the Sheep denudation of the epithelium is probably very rare, while its normal occurrence in the Ferret must be regarded as doubtful (Marshall, 1903, 1904). In the Bitch our observations tend to show that it occurs to a greater or less extent in every case. Evidence of this may be found in the fact that epithelial cells are observed lying free in the cavity of the uterus, while in some sections parts may be seen where the cells of the stroma are uncovered by a lining of epithelium. A single layer of flattened

cells, similar to those which compose the bulk of the stroma, may, as a rule, be seen in close attachment to the epithelium during the process of denudation. A similar layer of flattened cells resembling the endothelium of the capillaries may be seen immediately subjacent to the epithelium lining the cavity of the quiescent uterus.

Leucocytosis has been described by various authors as occurring during the degeneration period of menstruation, both in the human female and in the Monkey. Our own observations on the Bitch show that polymorph leucocytes are at this stage abundant both in the uterine stroma and cavity, and that large mononuclear leucocytes (hyaline corpuscles) containing blood pigment are also seen to occur.

Large cells, containing faintly staining nuclei of very considerable size with conspicuous nucleoli, may be observed at rare intervals lying in spaces in the tissue of the stroma. The origin and function of these cells is not clear.

(4) *Period of Recuperation.*—The new epithelial lining first presents itself as a single layer of flattened cells. Its manner of formation at this period is an open question. It is usually supposed in the menstruating human female that the epithelium is renewed from the portions that remain after the period of destruction or from that of the uterine glands. But Heape (1894, 1897) has put forward the view that the new epithelium in the Monkey is derived from certain elements in the subjacent stroma. In this connection we may point out that the flattened cells of the new epithelium in the Bitch bear a close resemblance to the cells of the stroma, but we cannot attach very much importance to this observation.

The mucosa at this period is highly vascular, the newly-formed capillaries being numerous and large.

A very noticeable feature of the recuperation stage is the variety of the leucocytes present in the uterine tissues. Of these we have observed the following different kinds :—

(1) Coarsely granular eosinophil leucocytes containing lobed nuclei (fig. 6, Pl. 8). These cells, which form a small percentage of the leucocytes present in the blood under ordinary conditions, are said to be found in increased numbers in trichinosis and other parasitic affections, bronchial asthma, sarcoma, osteomalacia, and various skin diseases.

(2) Basophil cells (figs. 6 and 7, Pl. 8). These cells, which are found both in the mucosa and in the muscle layers, appear to be of different kinds, varying in size, and in the size and number of the contained granules. The nuclei are simple and differ in staining reaction, having an affinity in some cases for basic stains such as methylene blue, while in other and rarer cases the nuclei take up acid stains. The granules are coarse and the cells may be very large in size. We have never seen them to contain pigment. In some cases the basophil granules are few and scattered. These basophil cells are evidently similar to the "mast cells" of Ehrlich and the "plasma cells" of Unna. It is

to be noted that mast cells are often to be found around areas of inflammation, and they are also described as occurring along with plasma cells in the stroma of tumours. Cells of the same nature are found in the peripheral circulation in cases of lymphatic and myeloid leucæmia.

(3) Large mononuclear leucocytes (macrocytes or hyaline corpuscles) (figs. 6 and 7, Pl. 8). These contain blood pigment, which gives the Prussian blue reaction.

The presence of leucocytes of so many different kinds in the uterine tissue during menstruation or heat has not hitherto been recorded so far as we are aware. HEAPE, who, as already mentioned, refers to the large number of leucocytes present in the mucosa during the destruction stage of menstruation, says that these are restricted to the vessels during the recuperation stage. HEAPE, however, makes no mention of either eosinophil or basophil leucocytes.

While we have found polymorphs frequent in the tissues during the period of destruction, they are not present to any extent while recuperation is in progress. On the other hand, it is at this stage that the basophil cells are specially numerous, and we venture to suggest that they are functionally connected with this process.

Pigment-containing leucocytes were observed many years ago by BONNET (1882) in the uterine mucosa of the Sheep, and this observation has recently been confirmed (MARSHALL, 1903). KAZZANDER (1890) denies the occurrence of these cells, adopting the view that the pigment is not contained in leucocytes, but occurs only free in the tissue.* HEAPE states that in Monkeys "all the blood corpuscles included within the newly formed epithelium are drawn again into the circulatory system." It follows that no blood pigment is formed (*cf.* also Ferret, MARSHALL, 1904). This differs considerably from what occurs in the Bitch, where pigment formation and ingestion by leucocytes is a marked feature of the destructive process. We are of opinion that while some of the blood corpuscles may find their way into the lymphatics, and so re-enter the circulation, the majority of those extravasated degenerate and give rise to pigment.

Fig. 5 (Pl. 8) represents part of a transverse section through a uterus of this period, showing large capillaries. Spermatozoa in great numbers are also to be seen in a gland shown in this figure. They were also observed in the deeper portions of the glands.

In conclusion, we see no reason to doubt the homology between the œstrous cycle in the Bitch, and the menstrual cycle in Monkeys and the human female. We differ from BEARD (1897), who holds that "very little is required in disproof" of this correspondence. As was first pointed out by HEAPE (1900), it is the procestrum alone, and not the entire heat period, which is represented by menstruation, and our observations on the histology of the Bitch's œstrous cycle are completely in accordance with this view.

* Some pigment, apparently free, is to be observed in the Bitch's uterus during recuperation.

4.—*Ovulation and the Vitality of the Spermatozoa.*

Ovulation in the Bitch takes place after bleeding from the external opening (where that occurs) has been going on for some days, or when it is almost or quite over ;* in other words, it occurs during œstrus and not during the proœstrum, or at any rate, not during the early stages of the proœstrum. It can take place quite independently of coition, the Bitch in this respect differing from the Rabbit (Heape, 1897), and the Ferret (Marshall, 1904), which in certain cases, at least, fail to ovulate in the absence of the male, but agreeing with the Mare (Heape, 1897), the Sheep under normal circumstances, the Mouse (Sobotta, 1895), and probably the majority of mammals.† But owing to the abbreviation and less pronounced characteristics of the proœstrum and œstrus in the Sheep and other animals, it is not easy to make certain in which of these two periods ovulation normally takes place. This difficulty is alluded to in the memoir on the œstrous cycle in the Sheep (Marshall, 1903).‡ But since in the Bitch ovulation takes place during œstrus, and in the Rabbit and Ferret it may only be induced after the additional stimulus of coition, and therefore not until after the commencement of œstrus, it is highly probable that the latter period is the usual time for its occurrence in the Sheep, the Mare and the majority of mammals which ovulate in the absence of the male. It follows that such animals can become pregnant as a consequence of the artificial introduction of spermatozoa into the

* This fact has been already published in Mr. Sedgwick's text-book on the "Vertebrata," which appeared last March. The information was communicated to him privately, but by inadvertence he omitted to direct attention to this. (See Mr. Sedgwick's letter in 'Nature' for June 22, 1905.)—June 27.

† Harper (1904), in a recent paper "On the Fertilization and Early Development of the Pigeon's Egg," says, that in the Pigeon, ovulation is delayed until mating. "When a pair ready for mating are together, egg-laying ordinarily ensues at the end of a rather definite period, and at the least eight days. The female functions are held in abeyance till the proper stimulus is received from a mate. The maturing of the egg is so exclusively a female function that it seems odd, at first thought, that an apparent exception should occur to the rule. Of course we know that the final maturation of the egg, or the giving off of the polar bodies, awaits in most animals the act of fertilization. But here the effect is produced upon the egg by the entrance of sperms. How mating itself, and the act of copulation could influence the ripening of the egg in the ovary is another problem. In this connection the curious fact must be mentioned that two female Pigeons placed in confinement together may both take to laying eggs. The function of ovulation is in a state of tension, so to speak, that requires only a slight stimulus, 'mental' apparently in this case, to set the mechanism working." These observations are especially interesting in view of the fact that they were apparently written in ignorance of what is known to occur in regard to ovulation in the Rabbit, the Sheep and the Ferret ; for Harper says : "from analogy with the mammal and with many birds, such as the common Fowl, we commonly think of ovulation as an exclusively female function, going on regardless of whether the eggs produced are fertilised or not." Harper is inclined to associate the adjustment of ovulation to mating in Pigeons with the monogamous habit, contrasting the conditions with those of polygamous birds with whom mating is sure to occur, and the female functions may without disadvantage be adjusted for continuous ovulation.

‡ That Sheep in some cases ovulate after coition, and consequently during œstrus and not during the proœstrum, is shown in the paper referred to (Marshall, 1903).

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uterus or vagina. This was first demonstrated to be the case with the Bitch by SPALLANZANI (1784), and subsequently by PIERRE ROSSI (SPALLANZANI, 1784), MILLAIS (HEAPE, 1897), and others, including one of us, who induced pregnancy in a Dandie Dinmont Terrier by this method. HEAPE (1897) states that in the Rabbit not only is it essential for coition to occur before ovulation can be brought about, but the presence of spermatozoa in the uterus is also necessary. This is not the case with the Bitch, which, like the normal Sheep, appears to be capable of ovulating without any external stimulus. Ovulation may occur, however, after coition, for in the individual referred to (p. 109), in which sperms were found in the uterine glands, ovulation was apparently in process of taking place just before the animal was killed, for one follicle was found which had evidently very recently ruptured, while others seemed to be on the point of rupturing, to judge from the extent of their protrusion.

It should be mentioned, however, that HEAPE (1897) found in the ovaries from the Monkeys belonging to the species *Semnopithecus entellus* and *Macacus rhesus* that ripe follicles were not always present during the menstrual periods. But it is to be observed that these ovaries were obtained during the non-breeding season. He therefore contends that whereas Monkeys of the species investigated menstruate at intervals all the year round, they ovulate as a rule only during the sexual season.*

Possibly those instances of animals in captivity, which, like the Bears in the Zoological Gardens in London, experience œstrus and copulate without pregnancy being induced (HEAPE, 1900), are to be explained on the supposition that no follicles ready for ovulation are present in the ovaries. Such an excess of sexual over reproductive activity can only be regarded as one of those "disharmonies" which METCHNIKOFF (1903) has pointed out to be so common in the generative systems of animals. It seems not unlikely also that the unusual sexual activity experienced by some animals in captivity and occasionally by wild animals is to be similarly explained. Thus, as already mentioned, the Otter in captivity and perhaps also in its natural condition, experiences a period of œstrus monthly throughout the year (COCKS, 1881 ; see also information on p. 104), yet the wild Otter, as a general rule, breeds only in winter (SOUTHWELL, 1888).

Many species of Bats are stated to ovulate at quite a different time of the year to the œstrous period, in some cases copulation occurring in the autumn and ovulation

* There still appears to be very considerable doubt regarding the normal time for the occurrence of ovulation in the human female, some authorities stating that it takes place before, some during, while others say that it occurs immediately after menstruation. There can be no doubt, however, that the two functions are to a certain extent independent, for conception and consequently ovulation are known to have occurred in young women who had not yet experienced menstruation. (HEAPE, 1897, 1898, and the various text-books on obstetrics, etc.) There are reasons, on the other hand, for concluding that primitively at any rate, the most usual period for ovulation in the human female was during a definite œstrus and after a proœstrum, as in Bitches. For the period of most acute sexual feeling in women is generally just after the end of menstruation, while according to RACIBORSKY this is also the commonest time for fertile coition. (GALABIN, 1904.)

not until the following spring (Benecke, 1879, Eimer, 1879, van Beneden and Julin, 1880), the interval being occupied partly or entirely by hibernation. During this period the sperms are said to retain their vitality in the uterus but to be in a dormant condition, the ovary also being in a state of quiescence (van der Stricht, 1901); Salvi (1901), however, describes the Bats in the Grotta dell' Inferno at Sassari as being seen to copulate also in the spring, but it is suggested that the spring coition may only take place with females which have failed to become pregnant in the previous autumn (*cf.* Duval 1899). It appears, therefore, that in Bats ovulation can take place independently of œstrus.

Ewart (1901) has described an experiment in which a virgin Rabbit was made to copulate with a male 10 days before œstrus, for parturition did not occur until 40 days after copulation, instead of the usual 30 days which represents the normal period of pregnancy in the Rabbit. It would appear, therefore, that in this case the sperms retained their capacity to conjugate for 10 days, and that ovulation in a doe Rabbit took place independently of any stimulus from coition.

No systematic investigation on the vitality of mammalian spermatozoa has been attempted so far as we are aware. A few isolated observations have, however, been put on record. Leeuwenhoeck, and subsequently Bischoff, and Prevost and Dumas (Waldeyer, 1903), state that they found moving sperms in the internal genital organs of Bitches and female Rabbits eight days after coition. Bonnet (1900) says that he found motionless sperms, which were probably therefore dead but had not yet undergone disintegration, in a Bitch $17\frac{1}{2}$ days after coition. Strassmann (1895) records the occurrence of living sperms in the human female a week after the last coition. Bossi (1891) refers to a similar case in which the sperms are said to have survived over 12 days; and Dührssen (1893) describes sperms as being supposed to have lived in a human female for three and a half weeks.

The following experiments were undertaken by us with a view to discovering the normal duration of the life of mammalian spermatozoa.*

Experiment 1.—The vas deferens of the left side in a small Scotch Terrier was ligatured, the vessels and nerves being left intact. Six days afterwards the Dog was killed and the left vas deferens above the ligature was examined microscopically, when no spermatozoa were found. The vas deferens on the right or unligatured side was similarly examined and found to contain a number of living sperms. It was noted further that the testis and epididymis on the ligatured side contained a very small quantity of fluid and very few living sperms, but many dead ones. The same organs on the unligatured side were found to contain a quantity of fluid and many living sperms.†

Experiment 2.—The right vas deferens of a Rabbit was ligatured close to the

* It appeared to us that this would be best ascertained by observing the maximum duration of life of the spermatoza in the male genital passages.

† It is to be noted that neither the Dog nor the Rabbit possess vesiculæ seminales.

testis. The next day the Rabbit was killed and the two vasa deferentia were examined, when numerous living sperms were found in each (including the part of the ligatured vas above the ligature).

Experiment 3.—A Rabbit was castrated and killed the next day. Large numbers of living sperms were found in each vas deferens.

Experiment 4.—A Rabbit was castrated and killed after an interval of three days. Many of the sperms were found to be dead, but a number alive and vigorous were obtained from each vas deferens.

Experiment 5.—The vasa deferentia of a Rabbit were both ligatured and the animal killed after four days. Large numbers of sperms were found in the vasa above the ligature and all of them were living.

Experiment 6.—A Rabbit was castrated (both testes and epididymes being removed) and killed after eight days. Large numbers of living sperms were found in the vasa, but some were dead.

Experiment 7.—A Rabbit was castrated and killed after nine days. One or two living sperms were found in the fluid obtained from the vasa deferentia. All the other sperms (a large number) were dead.

Experiment 8.—The testes and epididymes of a Rabbit were removed, and the animal killed after 10 days. A large number of living sperms were found in fluid from the vasa deferentia.

Experiment 9.—The testes and epididymes of a Rabbit were removed and the animal killed after 13 days. No living sperms were found in the fluid from the vasa, but dead ones in varying stages of degeneration were seen to occur.

In all these experiments precautions were taken to squeeze fluid from the testes and epididymes into the vasa deferentia before operating.

As a result of the experiments we may conclude that the spermatozoa of the Rabbit can live for at least 10 days in the body of the male after leaving the testis but that they die before 13 days. With regard to those cases where the sperms were found to have died after a shorter interval, it may be pointed out that it was not known how long they might have been living in the vasa deferentia at the time of operating. The fact that in some cases at any rate the spermatozoa can survive for 10 days is in conformity with the result obtained in EWART'S experiment, already mentioned, in which they appear to have lived for the same period in the genital organs of the female. On the other hand the fact that with the Rabbit and also apparently with the Dog the duration of the sperm's life after leaving the testis is limited to less than 13 days, makes it all the more remarkable that in Bats according to EIMER, VAN BENEDEN, and others, the spermatozoa regularly maintain their vitality for many months.*

* The extreme length of life possessed by some insects' sperms, which, in the case of Bees, are said to survive for four or five years, is well known. (*Cf.* VON SIEBOLD, 1837.) LANG has lately shown (1904) that sperms may live for three years in the vesiculæ seminales in Snails.—October 12.

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5.—*Some Theoretical Considerations.*

In the present section we shall consider the bearing of some of the facts related above upon the various theories which have been put forward regarding the nature and cause of menstruation.

In 1865 PFLÜGER advanced the theory that menstruation is brought about by a nervous reflex, owing its origin to the pressure of the growing Graafian follicles upon the nerve endings in the ovary. This view has received some support from STRASSMANN (1896), who claims to have induced "heat" in animals by injecting gelatine into their ovaries and so causing intra-ovarian pressure. ELIZABETH WINTERHALTER'S discovery (1896) of a sympathetic ganglion in the ovary also tends to support this theory, but VON HERFF (1896) discredits WINTERHALTER'S description, which has not so far been confirmed.

The statement by HEAPE (1897) that he found female Monkeys in which ovulation was not associated with menstruation is opposed to PFLÜGER'S hypothesis.* This theory has likewise been considerably shaken by the various obstetricians who have shown that there is not always a correspondence between the two sets of functions (ovarian and uterine) in the human female. Certain of the transplantation experiments referred to in Part II. are also in opposition to the nervous reflex theory of PFLÜGER and STRASSMANN.

The view put forward originally by SIGISMUND (1871), and adopted by HIS (1880) and many other obstetricians and embryologists, that the degenerative stage of menstruation is of the nature of an undoing of a preparation (represented by the previous growth stage) for a fertilized ovum which is not there, necessitates the assumption that ovulation precedes the degeneration stage of menstruation. We have seen that menstruation in the Primates is the physiological homologue of the proœstrum in the lower Mammalia, and that ovulation in the Bitch takes place during œstrus or after the occurrence (or at any rate the commencement) of internal bleeding. Consequently SIGISMUND'S theory becomes untenable. This difficulty in the way of the theory that "women menstruate because they do not conceive," as POWERS expressed it, is alluded to in the memoirs on the œstrous cycle in the Sheep and Ferret (MARSHALL, 1903, 1904), but with these animals the matter is somewhat complicated by the facts that with the former it is not clear whether ovulation does not sometimes occur during the proœstrum, and with the latter it may require the additional stimulus of coition before it can be induced.†

* The Pflüger-Strassmann theory, although it does not assume that ovulation must necessarily occur before menstruation or "heat" can be induced, presumably requires us to infer that ripe (or nearly ripe) follicles are present in the ovary at the commencement of each menstrual or "heat" period, and that the latter can only occur when the follicles reach a stage nearly approaching maturity and consequently acquire a definite degree of protrusion.

† RETTERER (1892), has suggested a similar theory to account for the congestion during the Bitch's proœstrum which he appears to regard as comparable to the albuminous secretion in the oviduct of a Fowl.

Beard (1897) has advanced a modification of Sigismund's theory, in which he assumes that the whole of the phenomena of menstruation represent a process which is of the nature of an "abortion of something prepared for an egg given off at or after the close of the preceding menstruation, and [that] it takes place because this egg has escaped fertilization." "Prior to the appearance of the menses the uterus has formed a decidua, which is regarded as equivalent to that which would arise when a fertilized egg became affixed to the uterus." This theory necessitates the assumption that there is no correspondence between the proœstrum of the lower mammals and menstruation in the Primates, since the degenerative stage of the proœstrum in the Bitch or Ferret can hardly be of the nature of an abortion of something prepared for an ovum which was discharged at the preceding "heat" period which occurred many months before. The difficulty becomes still greater when we consider the case of those animals referred to in the first section of this paper on the œstrous cycle, which experience œstrus only once annually or, as in some cases, even less often, for it can hardly be supposed that these animals ovulate at more frequent periods than those at which they come "on heat."* But Beard denies that there is any correspondence between "the heat period or rut of mammals" and menstruation in the higher forms, failing to distinguish the proœstrum from the period of œstrus, a distinction which was first pointed out by Heape (1900). It should be added that Beard's views on menstruation form a part of his general theory of reproduction which he regards as a process maintaining normally a perfectly regular rhythm.

The theory that the whole proœstrous process, including the degeneration and recuperation stage, is of the nature of a preparation for an embryo, does not appear to us to be opposed to any of the known facts. According to this theory "the process is viewed as a kind of surgical 'freshening' of the uterus for the reception of the ovum, whereby the latter during the healing process can be attached safely to the uterine wall" (Geddes and Thomson, 1901). This

He points out that this secretion is still produced, even though the egg is prevented from entering the oviduct. Retterer, therefore, apparently regards ovulation as occurring during the early stages of the proœstrum, a conclusion which we have shown to be erroneous, but his only statement on this point is that it occurs during "heat." He seems also to assume that ovulation is the cause of menstruation in the human female.

* In Beard's table of "ovulation intervals," periods of gestation, etc., for various mammals, the determination of the length of the "ovulation interval" in the case of polyœstrous animals like the Sheep is apparently based on the known duration of the dioestrous cycle, ovulation evidently being supposed to occur at each "heat" period. On the other hand, in the case of the Bitch, which is monœstrous and known to experience "heat" typically once every six months, the length of the ovulation interval is missed out as being an unknown quantity, although there can be little doubt that Bitches do not ovulate oftener than they come "on heat." Why evidence, which is considered sufficient for determining the ovulation intervals in polyœstrous mammals, should be regarded as inadequate in the case of monœstrous mammals does not transpire.

theory leaves the immediate cause of menstruation and "heat" quite unexplained.*

Other authors besides BEARD have laid stress on the rhythmical nature of menstruation. Thus STEVENSON (1882) has advanced the "menstrual-wave" theory, according to which the metabolic processes gradually increase in intensity until the time of the catamenia, when they suddenly decline to their lowest point, after which they gradually rise again, and the process is repeated (*cf.* JOHNSTONE, 1886, 1895; WEBSTER, 1897; GEDDES and THOMSON, 1901). According to this theory menstruation and the ovarian functions are in no way inter-dependent, but are related only in so far as each may be brought about by the same cause. This theory is disproved by the fact, which may now be regarded as well established, that menstruation and "heat" cease after ovariotomy,† the small proportion of cases in which they are said to continue being explained on the hypothesis that the ovaries had not both been entirely extirpated (*cf.* KNAUER, 1899, for Rabbits, and HALBAN, 1901, for Monkeys; *vide* also our remarks on this subject below).

In the present place it remains for us to refer to FRAENKEL'S hypothesis (1903), according to which menstruation is caused by the activity of the corpus luteum.‡ This hypothesis is based upon nine cases in which the corpus luteum was destroyed by the cautery, in eight of which the next menstruation is said to have been missed. In our opinion FRAENKEL'S theory is disproved by the fact stated above that ovulation in the Bitch does not occur until œstrus, and consequently that at the time of the proœstrum there are no corpora lutea present in the ovary, for the corpora lutea formed after one ovulation do not persist until the next heat period (probably six months after in the Bitch), and cannot, therefore, be responsible for producing a proœstrum several months subsequent to their formation. HEAPE'S observations (1897) on the absence of corpora lutea in menstruating Monkeys should be again noted in this connection.

A case has been recently reported by RIES (1904) in which FRAENKEL'S theory was put to the test. A girl was operated upon for appendicitis. The peritoneal cavity was found to contain a quantity of blood, but the appendix showed no signs of recent inflammation. The source of the hæmorrhage was found in the left ovary, which contained a freely oozing corpus luteum. The other ovary was normal. The corpus luteum in the left ovary was then completely peeled out, and the edges of the ovarian tissue united by catgut. The patient's menstrual period during that

* *Cf.* EMRYS-ROBERTS (1905), who considers the proœstrum to be of the nature of a preparation "for providing a rich pabulum to nourish the embryo" in the earliest stages of pregnancy.—June 27, 1905.

† This is not the same as saying that menstruation and "heat" are induced by ovulation. See Part II, where we deal with the subject of ovarian secretion.

‡ This is only part of FRAENKEL'S theory regarding the nature and function of the corpus luteum. We deal with this hypothesis more fully in Part II (p. 131).

month took place as usual, except that it was three days later than the expected date.*

We shall, in Part II of this paper, consider the evidence which can be adduced in support of the view that the ovary generally (and not merely the corpus luteum) is an organ producing an internal secretion, which, among other functions, causes "heat" and menstruation.

6.—*Summary.*

The Œstrous Cycle in Carnivores.—The Bitch is monœstrous and has typically two sexual seasons in the year. The wild species of the genus in their natural state have only one sexual season annually, but in captivity they may experience two sexual seasons like the Bitch. The Cape Hunting Dog (*Lycaon*) in captivity has been shown to come "on heat" usually only once a year.

The domestic Cat has three or four sexual seasons in the course of the year. We find also that it is polyœstrous, and may have four heat periods in a single sexual season. The duration of the diœstrous cycle is about a fortnight. The wild Cat probably experiences only one sexual season in the year. The male possesses a rutting season.

The Lioness, failing pregnancy, may have several annual sexual seasons, at the same time being polyœstrous. The duration of the diœstrous cycle is said to be three weeks.

Bears, Polecats, and Seals, and probably most other Carnivores, appear to be monœstrous and breed once a year, but the Otter, in captivity at any rate, is polyœstrous, having a continuous series of diœstrous cycles each of a month's duration.

The periodicity of œstrus is dependent to some extent upon environmental conditions, as illustrated by the case of the Fox. Domestication and captivity appear to favour increased frequency in the recurrence of the cycle.

The Histology of the Uterus during the Cycle.—The histological changes which the uterus undergoes may be divided into the following periods :—

(1) Period of rest		Anœstrum.
(2) Period of growth and congestion		Proœstrum.
(3) Period of destruction		Proœstrum.
(4) Period of recuperation		Œstrus. / Metœstrum.

The second period is characterised by congestion and increase in the number of capillaries. This is followed in the next period by extravasation of blood and emigration of polymorphs. External bleeding is shortly afterwards observed, having been preceded

* The operation does not appear to have retarded the menstruation, since the latter was due one day before the operation.

by a flow of mucus, containing polymorphs. There is considerable denudation of uterine epithelium, but the denudation does not extend to more than a single layer of stroma cells. In the recuperation period the epithelium is re-formed and new capillaries arise. This stage is also characterised by the large number of leucocytes that occur free in the stroma. These are of several kinds:—(1) Large mononuclear leucocytes containing iron pigment derived from the extravasated red corpuscles; (2) coarsely granular eosinophil leucocytes; and (3) basophil cells. The latter, which occur in unusual abundance both in the stroma and in the muscle layers, are often very large.

It is obvious that the changes occurring in the uterus throughout the cycle are homologous with those which are undergone in the Sheep, the Ferret, the Monkey, and the human female.

Ovulation and the Vitality of the Spermatozoa.—Ovulation in the Bitch takes place after external bleeding has been going on for several days or when it is over. It occurs during the period of œstrus, but is quite independent of coition or of the presence of spermatozoa in the uterus. Artificial insemination has been performed in Bitches by SPALLANZANI and others.

No systematic investigation as to the vitality of mammalian sperms has hitherto been attempted. It has been ascertained by us, however, that the period of survival in the male passages of Rabbits is probably not more than ten days.

Some Theoretical Considerations.—The fact that ovulation does not take place until after the proœstrum (or at any rate until after the commencement of the external bleeding stage of the proœstrum) is in opposition to the view that heat and menstruation are produced by ovulation, or by the corpus luteum. It is also contrary to the theory that the degeneration stage occurs as a result of the absence of a fertilized ovum for which the preceding growth was preparing. The theory that the destruction stage of the proœstrum is of the nature of an abortion related to an ovum released at the preceding period is also untenable, owing to the comparative infrequency of the heat periods (and therefore of the ovulation periods) in the Bitch. On the other hand, the hypothesis that the entire proœstrous process is of the nature of a preparation for the lodgment of the ovum is in accordance with the facts.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

References to Literature.

Beard. "The Span of Gestation and the Cause of Birth," Jena (1897).

Bell. "History of British Quadrupeds," 2nd ed., London (1874).

Benecke. "Ueber Reifung und Befruchtung des Eies bei den Fledermäusen," 'Zoologischer Anzeiger,' vol. 2 (1879).

Beneden (van) and Julin. "Observations sur la maturation, la fécondation, et la segmentation de l'œuf chez les Cheiroptères," 'Archives de Biologie,' vol. 1 (1880).

Bischoff. "Ueber die Ranzzeit des Fuches und die erste Entwicklung seines Eies," 'Sitz. der Math.-phys. Classe vom 13 Juni,' vol. 2 (1863).

Bonnet. "Ueber Melanose der Uterinschleimhaut bei brunstigen und kurze Zeit trächtigen Schafen," 'Deut. Zeitschr. f. Thiermedizin,' vol. 7 (1882).

Idem. "Giebt es bei Wirbeltieren Parthenogenesis?" 'Merkel and Bonnet's Ergebn. d. Anat. u. Entwick.,' vol. 9 (1900).

Idem. "Beiträge zur Embryologie des Hundes," 'Anat. Hefte,' vol. 20 (1902).

Bossi. "Étude clinique et expérimentale de l'Époque la plus favorable à la Fécondation de la Femme, etc.," 'Rivista di Obstet. e Ginecol.' (1891).

Cocks. "Note on the Breeding of the Otter," 'Proc. Z.S.' (1881).

Idem. "Habits of the Polecat," 'The Zoologist' (1891).

Idem. "Note on the Gestation of the Pine Marten," 'Proc. Z.S.' (1900).

Idem. "The Gestation of the Badger," 'The Zoologist' (1903, 1904).

Dührssen. "Lebendige Spermatozoen in der Tube," 'Centralbl. f. Gyn.' (1893).

Duval. "Études sur l'Embryologie des Cheiroptères," Première partie, Paris (1879).

Eimer. "Ueber die Fortpflanzung der Fledermäuse," 'Zoologischer Anzeiger,' vol. 2 (1879).

Ewart. "Variation: Germinal and Environmental," 'Scientific Transactions Roy. Dublin Society,' vol. 7 (1901).

Fraenkel. "Die Function des Corpus luteum," 'Arch. f. Gynäk.,' vol. 68 (1903).

Galabin. "Manual of Midwifery," 6th ed., London (1904).

Gebhard. "Ueber das Verhalten der Uterusschleimhaut bei der Menstruation: Verhandlung der Gesellschaft für Geburtshülfe und Gynäkologie zu Berlin," 'Zeits. f. Geb. und Gynäk,' vol. 32 (1895).

Geddes and Thomson. "Evolution of Sex," revised ed., London (1901).

Halban. "Ovarium und Menstruation," 'S.B. Akad. Wissenschaften,' vol. 110 Wien (1901).

Hamilton. "The Wild Cat of Europe," London (1896).

Harper. "The Fertilization and Early Development of the Pigeon's Egg," 'Amer. Jour. of Anat.,' vol. 4 (1904).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

HARTING. "The Polecat (*Mustela putorius*)," 'The Zoologist' (1891).

HEAPE. "The Menstruation of *Semnopithecus entellus*," 'Phil. Trans.,' B, vol. 185 (1894).

Idem. "The Menstruation and Ovulation of *Macacus rhesus*," 'Phil. Trans.,' B, vol. 188 (1897).

Idem. "The Artificial Insemination of Mammals, etc.," 'Proc. Roy. Soc.,' vol. 61 (1897).

Idem. "The Menstruation of Monkeys and the Human Female," 'Trans. Obstet. Soc.,' vol. 40 (1898).

Idem. "The Sexual Season of Mammals, and the Relation of the Proœstrum to Menstruation," 'Q. Jl. Micro. Sci.,' vol. 44 (1900).

Idem. "Ovulation and the Degeneration of Follicles in the Rabbit," 'Roy. Soc. Proc.,' B, vol. 76 (1905).

HERFF (VON). "Giebt is ein sympathisches Ganglion im menschlichen Ovarium?" 'Arch. f. Gynäk.,' vol. 51 (1896).

HIS. "Anatomie Menschlicher Embryonen" (1880).

JOHNSTONE. "The Menstrual Organ," 'British Gyn. Jour.' (1886).

Idem. "The Pathological Aspect of Stevenson's Wave," 'Amer. Jour. Obstet.,' vol. 31 (1895).

KAZZANDER. "Über die Pigmentation der Uterinschleimhaut des Schafes," 'Arch. f. Mikr. Anat.,' vol. 36 (1890).

KNAUER. "Die Ovarientransplantation," 'Arch. f. Gynäk.,' vol. 60 (1899).

LANG. "Über Vorversuche zu Untersuchungen über die Varietätenbildungen von *Helix hortensis* MÜLLER und *Helix nemoralis* L.," 'Festschrift zum siebzigsten Geburtstage von ERNST HAECKEL,' Jena (1904).

LEOPOLD. "Studien über die Uterusschleimhaut während der Menstruation, Schwangerschaft, und Wochenbett," 'Arch. f. Gynäk.,' vol. 11 (1877).

LIPES. "A Study of the Changes occurring in the Endometrium during the Menstrual Cycle," 'Albany Medical Annals,' vol. 25 (1904).

MARSHALL (MILNES). "Vertebrate Embryology," London (1893).

MARSHALL (FRANCIS). "The Œstrous Cycle and the Formation of the Corpus Luteum in the Sheep," 'Phil. Trans.,' B, vol. 196 (1903).

Idem. "The Œstrous Cycle in the Common Ferret," 'Q. Jl. Micro. Sci.,' vol. 48 (1904).

METCHNIKOFF. "The Nature of Man" (Mitchell's Translation), London (1903).

MILLAIS. "British Mammals," vol. 1, London (1904).

MIVART. "The Cat," London (1881).

PFLÜGER. "Über die Bedeutung u. Ursache der Menstruation," Berlin (1865).

RENGGER. "Naturgeschichte d. Säugethiere von Paraguay," Basel (1830).

RETTERER. "Sur les Modifications de la Muqueuse Utérine à l'Époque du Rut," 'C. R. de la Soc. de Biol.,' vol. 4 (1892).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Ries. "A Contribution to the Function of the Corpus Luteum," 'Amer. Jour. Obstet.' (1904).

Rink. "Danish Greenland" (1877).

Roberts (Emrys.). "A Preliminary Note upon the Question of the Nutrition of the Early Embryo," 'Roy. Soc. Proc.,' B, vol. 76 (1905).

Salvi. "Osservazioni sopra l'Accoppiamento dei Chirotteri nostrani," 'Atti della Società Toscana di Scienze Naturali,' vol. 12 (1901).

Siebold (von). "Fernere Beobachtungen über die Spermatozoen wirbelloser Tiere," 'Muller's Archiv' (1837).

Sigismund. "Ideen über das Wesen der Menstruation," 'Berliner klin. Wochenschr.' (1871).

Sobotta. "Über die Bildung des Corpus luteum bei der Maus," 'Arch. f. Mikr. Anat.,' vol. 47 (1896).

Southwell. "On the Winter Breeding of the Otter," 'The Zoologist' (1888).

Spallanzani. "Experiences sur la Génération," Geneva (1785).

Stevenson. "On the Menstrual Wave," 'Amer. Jour. Obstet.,' vol. 15 (1882).

"Stonehenge." "The Dog in Health and Disease," 4th ed., London (1887).

Stricht (van der). "L'Atrésie ovulaire et l'Atrésie folliculaire du Follicule de Graaf, dans l'Ovaire de Chauve-souris," 'Verhand. d. Anat. Gesell. in Bonn' (1901).

Strassmann. "Lehrbuch der gerichtlichen Medizin" (1895).

Idem. "Beiträg zur Lehre von der Ovulation, Menstruation, und Conception," 'Arch. f. Gynäk.,' vol. 53 (1896).

Sutton. "Menstruation in Monkeys," 'British Gyn. Jour.,' vol. 2 (1880).

Turner. "On the Placentation of Seals," 'Trans. Roy. Soc. Edin.,' vol. 27 (1875).

Idem. "Lectures on the Comparative Anatomy of the Placenta," Edin. (1876).

Waldeyer. "Die Geschlechtszellen," 'Hertwig's Handbuch der vergleich. und experim. Entwick. der Wirbeltiere,' Jena (1903).

Webster. "The Biological Basis of Menstruation," 'Montreal Med. Jour.' (1897).

Winterhalter. "Ein sympathisches Ganglion im menschlichen Ovarium," 'Arch. f. Gyn.,' vol. 51 (1896).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

DESCRIPTION OF THE PLATES.

The figures were drawn by Mr. Richard Muir, of the Pathological Department of the University of Edinburgh. Each drawing represents part of an actual section. We are much indebted to Mr. Muir for the care with which he has made the drawings, as well as for help in various other ways.

Reference Letters.

Bas., basophil-cell; bl. v., blood-vessel; cav., cavity of uterus; eos., eosinophil-cell; ep., epithelium lining cavity; ep. gl., epithelium of gland; ex. bl., extravasated blood corpuscles; gl., gland; mon., mononuclear leucocyte containing pigment; pig., pigment; polym., polymorph leucocyte; sec., cells probably indicating increased secretory activity (see p. 107); str. c., stroma cell.

Plate 7.

Fig. 1.—Transverse section, showing a portion of the uterine mucosa during Period II. The vessels are much congested. × 300 diam.

Fig. 2.—Transverse section, showing a portion of the mucosa during an early stage of Period III. × 200 diam.

Fig. 3.—The same. × 50 diam.

Plate 8.

Fig. 4.—Horizontal section, showing a portion of the mucosa during a very early stage of Period IV. The epithelium is in process of renewal. × 300 diam.

Fig. 5.—Transverse section, showing a portion of the mucosa during a late stage of Period IV. New capillaries have been formed. × 500 diam.

Fig. 6.—Transverse section, showing a portion of the mucosa during about the middle of Period IV. Wandering cells of various kinds are very numerous. × 1000 diam.

Fig. 7.—Wandering cells, seen during Period IV. × 1000 diam.

Part II.—The Ovary as an Organ of Internal Secretion.

Contents.

1.—*Introduction.*

The first part of this contribution is devoted to a consideration of the cause of heat, and the evidence that can be adduced in support of the view that this is produced by an internal secretion of the ovary.

The function of the corpus luteum is next discussed and experimental evidence is brought forward that this organ is an essential factor in uterine gestation.

2.—*The Cause of "Heat."*

It has long been known that the ovary, like the testis, exerts a profound influence over the general metabolism, and that the extirpation of the ovary, like castration in the male, leads to very distinct results. In the human female double ovariotomy before puberty, in addition to preventing the onset of puberty and the occurrence of menstruation, produces effects on the general form and appearance, as may be seen in adult women in barbarous parts of Asia where the natives practise ovariotomy on young girls. Such women are said to be devoid of many of the characteristics of their sex, and in some cases to present certain resemblances to men. Ovariotomy performed after puberty likewise produces well marked effects, of which the chief is the cessation of menstruation, sometimes accompanied by atrophy in the breasts and the internal genital organs, and a tendency to obesity (*cf.* Dixon 1901). Similar results are brought about in animals as shown by the experiments of Knauer (1899) and other investigators. Some authors, as already mentioned in Part I., have adopted the view that menstruation can occur independently of the presence of the ovaries, but there is very strong evidence to show that in the cases upon which they base their evidence, one or both of the ovaries had not been entirely removed. Instances are known of women whose ovaries were supposed to have been completely extirpated but who subsequently became pregnant (*cf.* Gordon, 1896, who describes two such cases).

So far as we are aware, Brown-Séquard (1889) was the first definitely to put forward the hypothesis that the ovary exercises its influence upon the animal meta-

bolism by means of an internal secretion which it produces. The beneficial results of his experiments with injections of testicular extracts induced him to attempt similar experiments with extracts of ovary. These he regarded as causing effects similar to those produced by testicular extracts, but not so powerful.

Since 1889 ovarian preparations have been administered medicinally in a large number of cases with more or less successful results. They have been used for menopause troubles, natural and post-operative, by MAINZER, LANDAU, BODON, JAYLE, LISSAC, MURET, SPIELMANN and ETIENNE, CHROBAK, JACOBS, TROUVENAINT, BESTION DE CAMBOULAS and SELIGMANN, all of whom report good results. Ovarian extracts have also been employed with considerable success in cases of chlorosis and amenorrhea by MAINZER, MURET, SPIELMANN and ETIENNE, FIDELI, JACOBS, TROUVENAINT, and BESTION DE CAMBOULAS. BARUCH, MOND, and FLOCKEMANN have also used the extracts, but obtained only a moderate amount of success. COHN, on the other hand, says that in his experience the results are nearly always unsatisfactory.

The fresh ovaries are themselves taken or ovarian tissue is given in the form of powder (ovarine, oöphorine, ovigenine, etc.) or fluid. The fresh ovaries or ovarian powder are eaten, but the fluid can be administered by the mouth, by the rectum, or by hypodermic injection.* The method of administering ovarian extract by the mouth is open to the criticism that the "active principle" of the substance may be altered during the metabolism of digestion. So far as we are aware no experimental evidence has been brought forward on this point.

The nature and effects of the internal secretion of the ovary are discussed at some length in a memoir by BESTION DE CAMBOULAS (1898), who describes a large number of experiments with ovarian extract on Dogs, Rabbits, and Guinea-pigs, as well as a series of clinical observations on the effects of ovarian medication. Experiments were performed on male animals as well as on females. The lethal injection was found to be about twice as much in non-pregnant females as in males, but pregnant females died after an amount equivalent to the lethal dose in males. In some cases genital excitement is said to have been produced. With non-toxic doses the females gained weight, but the males lost weight. The lesions discovered after lethal doses are said to have been congestion of viscera, and minute hæmorrhages in the dorsal and lumbar regions of the spinal cord. As already mentioned, BESTION administered ovarian extracts to his patients and obtained distinctly beneficial results. Menopause troubles are described as either completely disappearing or being much ameliorated, while rapid improvement was always seen to occur in cases of chlorosis or amenorrhœa. BESTION says that ovarian extract should never be administered to pregnant women, since it causes such grave effects when given to pregnant animals.

In our opinion the most unsatisfactory feature from an experimental point of view about ovarian medication as practised, is that the extract is obtained without regard to the condition of the ovaries which are employed. It seems hardly reasonable to expect to obtain uniform results by indiscriminately using ovaries with mature follicles (like those got from animals "on heat"), ovaries in a state of relative quiescence (like those from animals during the anœstrous period), or ovaries consisting for the most part of corpora lutea (such as those from pregnant animals). This is a point which will appear more evident when we have referred to our own experiments.

The theories of PRENANT (1898) and of FRAENKEL and COHN (1902) that the ovarian secretion is elaborated by the corpus luteum and the evidence on which these theories are based are discused in the next section of this paper.†

* *Cf.* article by ANDREWS (1904), from whom the references on ovarian medication are taken.

† In papers by HALBAN (1901) and DIXON (1901), experiments by FEODOROFF (Frommel's 'Jahrb. für Geb. und Gyn.,' 1898) are cited as affording evidence of an ovarian secretion. In these experiments

Further evidence in support of the doctrine that the ovary is an organ of internal secretion is supplied by the results of various attempts to transplant ovaries. Experiments of this nature have been undertaken by Morris (1895), Glass (1899), and Dudley (1899) on the human subject, and by Knauer (1896, 1899), Grigorieff (1897), Ribbert (1898), Marchese (1898), Rubinstein (1899), Foà (1900), Schultz (1900), Herlitzka (1900), Halban (1900, 1901) and Limon (1904) on various animals. It would appear from these experiments that ovaries, when freed from their normal nervous and vascular connections, are not only in some cases able to acquire a secondary connection with the tissue on which they are grafted but may retain their functional activity and continue to produce ova.

Morris (1895) gives an account of a woman aged 20, with an infantile uterus, who suffered from amenorrhœa and on whose fundus uteri he transplanted an ovary obtained from a person of 30. The transplantation is said to have been successful, inducing menstruation after two months.

The case described by Glass (1899) was similar. A patient, aged 29, whose ovaries had been removed, was suffering from menopause symptoms. Two years after the extirpation a healthy ovary from a girl, aged 17 (removed on account of vaginal stricture) was grafted in the normal position on the first-mentioned patient. The experiment is stated to have resulted in complete success, there being a renewal of menstruation, and the patient eight months after the operation is said to have been completely restored to health.

In the case mentioned by Dudley (1899) a double pyosalpinx was removed from a woman, aged 21, and the right ovary afterwards implanted on the fundus uteri. The patient is described as having menstruated regularly afterwards.

Knauer (1896, 1899) describes experiments with Rabbits in which he removed the ovaries and grafted them in the mesometrium or between the fascia and the muscle of the abdominal wall. He found that they could be successfully implanted on both peritoneum and muscle, but that some portion of the grafted ovary always died. The rest, however, remained functionally active and continued to produce ova which could be fertilised, with pregnancy occurring as a result. The formation of the problematical internal secretion of the ovaries is closely associated, according to Knauer, with the capacity for producing eggs, for ovaries which no longer carried out the latter function ceased also to exert an influence on the metabolism of the animal. The uterus is said to undergo atrophy in animals in which the ovaries are simply removed without being

Feodoroff claims to have induced a rise of blood pressure by injecting ovarian extract. Vincent and Sheen (1903), however, instead of obtaining such a rise, observed a slight fall of blood pressure, a result similar to that obtained by the injection of other tissue extracts. So far as we are aware, neither Feodoroff nor Vincent and Sheen took into consideration the condition of the ovaries employed with reference to œstrus or pregnancy. We, therefore, considered it desirable to repeat the experiment with ovaries obtained from animals "on heat." Extract of such ovaries was found to give, in some cases no result, either in raising or lowering the blood pressure, in others a very slight fall.

transplanted.* KNAUER draws the general conclusion that so long as a functionally active ovary is present in an animal it is immaterial in what part of the body it is situated, so far as its action on the general metabolism is concerned. KNAUER performed similar experiments on Bitches and obtained results which also pointed to the existence of an internal ovarian secretion.

HALBAN (1900) found that the uterus and mammary glands of Guinea-pigs, from whom the ovaries had been removed shortly after birth, remained undeveloped, but that if the ovaries were removed from their normal position and grafted beneath the skin the other genital organs developed normally. HALBAN (1901) also experimented upon Monkeys and found that after ovariotomy menstruation ceased, but that it continued after a grafting of the ovary, even though the ovary was transplanted in a position different from the normal one.

LIMON (1904), working upon Rabbits, grafted ovaries between the muscle layers of the abdominal wall and on the peritoneum in the same individuals. He obtained successful results, confirming those of RIBBERT, KNAUER, etc. LIMON, who describes the histology of the ovaries, giving figures, expresses the belief that an internal secretion is elaborated by the "interstitial cells" which have so close a connection with the blood-vessels of the ovary, and not by the Graafian follicles, or the transient structures known as corpora lutea.†

* Similar results have been obtained by BUYS and VANDERVELTE, who show that the uterine muscles undergo atrophy and the mucosa undergoes fibrosis after double ovariotomy in Rabbits. (MALCOLM CAMPBELL, 1905.)

† The other experiments referred to were undertaken without special reference to the ovarian secretion theory. The following is a brief account of them:—

GRIGORIEFF (1897), transplanted ovaries of Rabbits to various abnormal positions, such as the broad ligament or the peritoneum of the vesico-uterine pouch, and found that the follicles matured and ruptured in the grafted ovaries just as in normal ones.

RIBBERT (1898), working on Guinea-pigs, and RUBINSTEIN (1899), working on Rabbits, also transplanted ovaries to abnormal positions in the same individuals and obtained successful results.

SCHULTZ (1900), carried out five experiments in which he grafted the ovaries of Guinea-pigs on to the bodies of males. Portions of the ovaries underwent degeneration, but some parts survived for as long as 117 days, when follicles and ova were found in an apparently perfectly healthy state.

FOÀ (1900), describes numerous experiments upon Rabbits in which very young ovaries were grafted in the normal position upon individuals of various ages, whose own ovaries had been removed immediately before. Several of the experiments were successful, the ovaries surviving or even growing to the normal size. Experiments were also performed in which the undeveloped ovaries were grafted without removing the ovaries of the Rabbits upon which the grafts were made, and these experiments are also described as being successful in three cases out of five.

HERLITZKA'S experiments (1900), which were upon Guinea-pigs, were similar to those of FOÀ and were likewise successful in certain cases.

ARENDT tried to transplant ovaries from other individuals, but his experiments were a failure (RIBBERT, 1898). Similar attempts, which were likewise a failure, were made at an earlier period by ROMANES (1895).

In dealing with ovarian grafts, LACK'S experiments (1899), should also be noted. This investigator scraped the epithelial cells from the incised surface of the ovaries, and on diffusing the dissociated

It is apparent that the successful experiments referred to above (especially those in which the ovaries were transplanted to abnormal positions), support the doctrine that the ovary is an organ producing an internal secretion.

Further evidence that the proœstrum and œstrus are brought about by a substance circulating in the blood but not necessarily elaborated by the ovary is supplied by certain facts cited by HALBAN (1901). This author states on the evidence of KEHRER that the milk of suckling Sows is affected during the period of "heat," the young as a consequence developing unhealthy symptoms. In a similar way the milk from women is said to be affected during the menstrual periods. Also, according to YOUATT (1835) Cows can be brought "on heat" artificially by supplying them with milk obtained from other Cows which are "on heat."

GOLTZ'S experiments (1874) may also be cited as showing that "heat" is not caused by a cerebral reflex. In one experiment the spinal cord of a Bitch was transected in the lumbar region. Normal proœstrum followed by œstrus and conception occurred unaccompanied by sensation, but the Bitch showed a distinct inclination towards the Dog which she had not previously displayed. Pups were born after a full time pregnancy. In another experiment (GOLTZ and EWALD, 1896) the lumbar part of the spinal cord was completely removed from a Bitch, which subsequently came "on heat" and produced pups as in the former case. This experiment indicates that "heat" is not brought about by a spinal reflex.*

The case described by BRACHET (1837), of a woman suffering from paraplegia in the lower part of the body and legs, who conceived and became pregnant, may also be cited.

The following is an account of our own experiments on the cause of "heat":—

epithelium through the peritoneal cavity, found that the cells became implanted, but set up malignant growths.

BOUIN and ANCEL (1903), maintain that the "interstitial cells" of the testis provide an internal secretion without which the sexual characters of the male are not developed. A paper on the same subject by SHATTOCK and SELIGMANN has been communicated to the Proceedings of the Royal Society (1904), where caponisation experiments are described. In some cases the testis is said to have broken up during the operation, so that minute fragments were left behind, sometimes in the normal position and sometimes becoming dislocated and attached to the adjacent viscera or abdominal wall. Although these pieces of testis continued to produce spermatozoa, they were virtually ductless glands. In such cases the secondary male characters developed to a varying extent. "One must regard the external character of maleness as a quantity which varies proportionately with the amount of gland tissue present." SHATTOCK and SELIGMANN also did experiments upon the effects of occluding the vasa deferentia, and found that Herdwick Rams and Fowls with occluded vasa developed full secondary sexual characters. They point out that this result indicates that the formation of secondary sexual characters is not due to metabolic changes set up by a nervous reflex arising from the physical function of sperm ejaculation.

* We are indebted to Professor SHERRINGTON for supplying us with information regarding certain experiments which he has performed, and which show that heat in Bitches is not brought about by a cerebral reflex. In the experiments in question, the spinal cord was transected at the hinder end of the cervical region, and heat of normal duration and character continued to appear in the Bitches so operated upon.—June 27.

I.—*Injection of Ovarian Extract.*

1. The ovaries of a Fox-terrier, which presented a condition of œstrus and in which bleeding from the vulva had been evident for two or three days previously, were removed. Both ovaries showed large ripe unburst Graafian follicles. The ovaries were minced, pounded with sand and an extract made by means of RINGER'S fluid (NaCl 0·9, CaCl 0·024, KCl 0·042, $NaHCO_3$ 0·01 per cent.). This extract—10 cub. centims. in amount—was injected under the skin of a Fox-terrier Bitch, which was not in a condition of œstrus. On the first day after injection the vulva was observed to be slightly swollen and redder. On the third day the mucous secretion on the vulva was increased above what is normally present. The swelling, redness and increased mucous secretion thereafter passed off and by the sixth day the external genitals had resumed their normal appearance.

Similar results were obtained in four other cases where the same experiment was performed, and also in a further case where large injections of the blood serum of an œstrous Bitch were employed instead of ovarian extract. In no case was a flow of blood observed externally, but in other respects the animals showed transient symptoms of a condition of "heat."

No results were produced by injecting extracts of ovaries obtained from Bitches that were not on "heat" (three experiments).

II.—*Ovarian Grafts.*

1. The ovaries were removed from a Fox-terrier Bitch which presented a condition of œstrus, bleeding having been observed from the vulva for two days previously. The ovaries contained a number of ripe Graafian follicles and one follicle was found recently ruptured. The ovaries were grafted subcutaneously in the loose tissue of the groin of a Terrier Bitch in an anœstrous condition. On the day following the operation slight swelling and reddening of the vulva were observed. The swelling increased and by the fifth day the vulva was distinctly swollen, reddened and moistened by an increased secretion of mucus. These appearances passed off and by the ninth day the external genitals had resumed their ordinary appearance. The animal was then killed, when it was found that the ovaries implanted had been entirely absorbed.

2. The ovaries, which contained ripe Graafian follicles, were removed from an œstrous Terrier Bitch which had shown bleeding from the vulva, and were grafted into the peritoneal cavity of another Terrier Bitch whose ovaries had been extirpated three months previously.

Indications of "heat" were observed as in the preceding experiment. Further, the Bitch showed a distinct inclination towards the Dog. No bleeding was observed externally. By the fourteenth day signs of "heat" had entirely passed off and it

exhibited the marked disinclination towards the Dog characteristic of the "anœstrous" Bitch.

About a month after the operation the animal underwent an absolutely typical procestrum and œstrus. Bleeding occurred at the vulva and continued for several days, and the animal showed the other unequivocal symptoms of "heat."* That passed off and the animal again became anœstrous. About three weeks later the Bitch was killed. No trace of ovarian tissue was discovered in the normal position where the ovaries had been removed. The grafted ovaries were found *in situ*. Subsequent histological examination confirmed this observation. The ovaries, however, had undergone some fibrous degeneration in the stroma. They did not contain luteal tissue. The ova also had apparently undergone degeneration.

3. The ovaries were removed from a Fox-terrier Bitch in a condition of œstrus, blood having been apparent on the vulva for about four days previously. They showed large ripe unruptured follicles. These ovaries were grafted between the layers of abdominal muscles of a mongrel Setter whose ovaries had been removed two months previously at an œstrous period. The follicles were not ruptured during the operation of grafting. No very marked change occurred until nearly three weeks after the operation. The whole of the external genital organs then became very congested and there was a profuse mucous flow which continued for about a week. Short of external bleeding the Bitch gave every evidence of a normal procestrum. The congestion then subsided. The Bitch is still alive at the time of writing, it being our intention to observe whether heat recurs.

In the first two experiments we attribute the results following almost immediately to fluid from the ovary released during operation and becoming thereafter absorbed. The slight transient effects produced we conclude to be comparable to those produced after injection of ovarian extract (*see* p. 128). The pronounced results which followed after a longer interval in Experiments 3 and 4 may be explained on the assumption that the ovaries had by that time acquired adequate vascular connections, and had recovered from the effects of transplantation and consequent temporary impairment of nutrition.

* Professor DUNSTAN of the Royal Veterinary College, Edinburgh, informs us that it is a common practice among veterinarians to cut out the ovaries of Bitches in cases where it is desired that they should not come "on heat." We note also that a Yorkshire Terrier in our possession, whose ovaries were extirpated in December of last year has so far at any rate failed to show any signs of procestrum or œstrus. This is in accordance with the fact, about the truth of which most obstetricians agree, that women cease to menstruate after ovariotomy (see p. 123, and *cf.* HALBAN, 1901, for Monkeys). It is to be noted also that according to KNAUER (1900), removal of the ovaries in Rabbits produces a premature menopause, the uterus undergoing atrophy, while if ovariotomy is performed prior to puberty, the uterus remains infantile (see p. 125). (See postscript regarding Yorkshire Terrier.)

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3.—*The Function of the Corpus Luteum.*

Various explanations have been suggested to account for the formation and presence of the corpus luteum. One theory is that this structure is of the nature of a stop-gap, preserving the cortical circulation of the ovary by preventing an excessive formation of scar tissue (Clark, 1898, Williams, 1904).

Prenant (1898), so far as we know, was the first to put forward the view that the corpus luteum is a ductless gland, which he regarded as producing an internal secretion that exercises an influence over the general metabolism such as has been attributed to the internal secretion of the ovary. The phenomenon of chlorosis is explained as being due to the absence of this secretion. The function of the corpus luteum is also, according to this author, to prevent the occurrence of ovulation during pregnancy or between the œstrous periods.

The theory that the corpus luteum produces an internal secretion is supported by Regaud and Policard (1901), who state that in the Hedgehog it can be shown by means of special staining that droplets of such a secretion are present in the cells of the corpus luteum.

Beard (1897), in the year before Prenant's paper was published, also put forward the theory that the corpus luteum is a contrivance to suppress ovulation or render it abortive during pregnancy, and he suggested that it stops short some time before the close of pregnancy "to render an ovulation just after birth possible." In this connection it may be pointed out that in many mammals (*e.g.*, Bitch, Ferret, Sheep), if not in the majority, the sexual season does not recur until months have elapsed since the time of parturition, and that it is unlikely that ovulation takes place in the anœstrous period.*

Beard's theory is adopted by Sandes (1903), who has studied the corpus luteum in the marsupial Cat (*Dasyurus viverrinus*). Sandes states that in *Dasyurus*, as in other mammals, the corpus luteum disappears towards the end of lactation and with the approach of the next œstrous period, when the follicles begin to grow in preparation for the ensuing ovulation. He shows further, that as soon as the corpus luteum is formed, the ova in the surrounding follicles, which were up to that time in various stages of active development, begin to atrophy. This atrophy commences in the follicles in closest proximity to the newly-formed corpus luteum and is continued in the other follicles in ever widening circles. Sandes suggests that the process is brought about by mechanical pressure or is due to the internal secretion of the corpus luteum, if it has one.

Without in any way disputing the accuracy of the facts which Sandes describes, we find it difficult to understand what advantage is gained by a mechanism having a no more important object than that of securing the degeneration of the surplus ova

* Beard says, "I hold [that ovulation ensues soon after parturition] in all mammals."

within the ovary instead of externally to it, nor do we see how, according to the usually accepted doctrines of utility and natural selection, an organ having such a function could ever have been developed at all.

The theory that the corpus luteum is a "ductless gland" having the function of providing a secretion which aids in the attachment of the embryo to the uterine mucosa, was first suggested by Gustav Born of Breslau, who persuaded Ludwig Fraenkel to put this view to an experimental test. For this purpose a series of experiments upon Rabbits was undertaken (Fraenkel and Cohn, 1901, Fraenkel, 1903), the ovaries being removed from one to six days after the occurrence of copulation. The animals were subsequently killed, when it was found that the extirpation of the ovaries had prevented the fixation of the ova. In further experiments the corpora lutea are described as having been removed by being burnt out with the galvano-cautery without destroying the rest of the ovaries, and in these cases the result was similar. Control experiments were performed by removing one ovary but not the other, and by destroying some of the corpora lutea, but not all, and in the majority of instances the animals brought forth young. The results of these experiments therefore support the view that there is an intimate connection between the presence of the corpus luteum and the occurrence of pregnancy, and that this connection in a certain sense is one of cause and effect.

Apart from the experimental evidence, Fraenkel adduces certain other facts which tend to support his theory. He points out that the general structure of the corpus luteum is suggestive of its being a ductless gland, that it is formed mainly of large epithelioid cells arranged in regular rows or columns not unlike those of the cortex of the supra-renal body and surrounded by a network of capillaries, and that its increase in size until it becomes bigger than a mature follicle, is otherwise inexplicable. This increase, he shows, is out of all proportion to that of the rest of the ovary, and when the corpus luteum is most hyperæmic, the other part of the ovary is unusually anæmic, while towards the close of pregnancy, when increase in the blood supply to the generative organs is at its highest, the corpus luteum is frequently reduced to a scar. Fraenkel also lays some stress on the fact that it has been shown that the lutein cells are derived from the follicular epithelium and not from the connective tissue of the theca wall (Sobotta, 1896, etc.). He points out further, that though many cases of double ovariotomy during pregnancy without disturbance of the pregnancy have been recorded, in none of these, so far as he is aware, has the operation been performed during the early weeks.

An apparent exception, however, has lately been recorded by Essen-Möller (1904). A cystic ovarian tumour had been diagnosed in a woman of 43. On September 6, 1903, laparotomy was performed and the left ovary removed. The right ovary, which was said to contain a corpus luteum of pregnancy, is also stated to have been removed. The patient made an uninterrupted recovery, and on June 2, 1904, was delivered of a full-term child, which, however, died six days afterwards of

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congenital heart disease. Since the child was born 269 days after the removal of the ovaries, it is supposed that conception occurred shortly before the operation. This case, as far as we are aware, is unique, and in the light of our own experience (see p. 133) we venture to suggest that a small portion of the ovary containing lutein cells might have been left behind accidentally at the time of the operation (see also p. 123).

FRAENKEL has also stated in support of his theory that in "non-placental' mammals (Marsupials and Monotremes) the corpus luteum is rudimentary or does not exist at all. SANDES (1903), who has carefully described the formation of the corpus luteum in *Dasyurus*, points out that this is erroneous, stating that there is a large corpus luteum in the members of both of these groups. It must be remembered, however, that in Marsupials there is a "yolk-sac placenta," while in at least one genus (*Perameles*) a definite allantoic placenta is developed. The only description of corpora lutea in Monotremes appears to be that of POULTON (the authority to whom SANDES refers), who wrote more than 20 years ago (1884), and from whose account it would appear that the corpora lutea of these animals are certainly not typical.

A further objection that might be urged against FRAENKEL'S theory is that structures resembling corpora lutea have been found in the ovaries of certain lower vertebrates (birds, reptiles, amphibians, and fishes).* This resemblance relates chiefly to the hypertrophy of the cells of the follicular epithelium after the discharge of the ova. Such an objection does not appear to us to be a very serious one, for there is nothing improbable in the conception that rudimentary corpora lutea, providing possibly some sort of secretion should have been developed before the acquirement of the function which, on FRAENKEL'S hypothesis, is possessed by the fully formed structures which characterise the placental Mammalia.

FRAENKEL has also pointed out as an argument in favour of his theory that in ectopic gestation the uterus undergoes the usual changes, although the ovum is not in the uterine cavity. It is clear, therefore, that the changes do not occur as a consequence of the presence of the ovum. It is also pointed out that in normal pregnancy the uterine changes commence before the ovum enters the uterus.

The theory that the corpus luteum is responsible for the early development and attachment of the embryo, also receives support from those cases in which pathological conditions of the embryo are correlated with pathological conditions of the corpus luteum. For an account of such cases and a brief *résumé* of the literature of this subject, ANDREWS' paper (1904) may be consulted. (See also MALCOLM CAMPBELL, 1905.)

FRAENKEL'S general conclusions regarding the functions of the corpus luteum can be summed up as follows :—The corpus luteum is a ductless gland which is renewed every four weeks during reproductive life in the human female, and at different intervals in the various mammals. Strictly speaking, there is only one corpus

* It is hoped that a paper dealing with the literature of this subject may be published shortly.

luteum, representing the only ovarian organ of internal secretion, which is regenerated periodically in slightly different positions. Its function is to control the nutrition of the uterus from puberty to the menopause, to prevent it from lapsing into the infantile condition or undergoing atrophy, and to prepare its mucous membrane for the maintenance of the ovum. If the ovum be fertilized the corpus luteum is responsible for maintaining the raised nutrition of the uterus during gestation. If the ovum be unfertilized it merely produces the hyperæmia of menstruation, and then undergoes degeneration until it is renewed in a fresh position. Since the corpus luteum is *the* ovarian gland, "lutein" alone, and not preparations of the entire ovary, should be employed for purposes of ovarian medication, *e.g.*, after ovariotomy.

We have already given reasons for the conclusion that this extended theory of the meaning and function of the corpus luteum is untenable. The fact that in a very large number of animals œstrus, and presumably, therefore, ovulation, occur at infrequent intervals does not support it; while it has been shown in Part I. of this paper (p. 110) that in the Bitch, at any rate, ovulation does not take place until after external bleeding has begun. (See also p. 116, Part I.) These facts, however, are in no way opposed to that part of FRAENKEL'S hypothesis which assigns to the corpus luteum the function of governing fixation of the ovum, and helping to maintain its nutrition during pregnancy.

The following is an account of our own experiments on this subject:—

I.—*Bitches.*

1. The ovaries were removed from a mongrel Setter which was "on heat" and copulated three days previously. The animal never became visibly pregnant.

2. The ovaries were removed (but, as the result showed, the removal was not quite complete) from a white Fox-terrier Bitch which was "on heat" and had copulated three days previously. Pregnancy supervened, and the mammary glands developed markedly. Fifty-three days after copulation the Bitch was noticed to adopt and suckle a litter of pups belonging to another Bitch with which she was kept. The day following one pup was born, which was suckled for three days, when the pup died. Since the average period of gestation in the Bitch is about 62 days, it is to be noted that this pup was born prematurely. Its weight was 132 grammes (weight of mother, 6·83 kilos.). The Bitch was then killed, when it was found that a small portion of the right ovary containing luteal tissue had been left behind at the operation. Serial sections of this piece of tissue were cut, and these disclosed portions* of two undoubted corpora lutea.

3. The ovaries were removed from a Fox-terrier Bitch 10 days subsequent to copulation. Slight swellings on the uterus indicating pregnancy were observed at

* These, as is usual with developing corpora lutea, had no doubt undergone considerable hypertrophy since the date of the operation, which was only three days after coition, as stated above.

the operation. A month later the animal was killed, when it was found that the pregnancy had not continued. Sections through the uterus showed decidua cells, but there were no signs of embryos.

4. The ovaries were removed from a Yorkshire Terrier Bitch, which had been warded about a fortnight previously. Slight swellings were observed on the uterus at the time of operation. The pregnancy did not continue. Abortion was not observed.

5. The ovaries were removed from a black Scotch Terrier Bitch which had been pregnant for about three weeks. The usual swellings were noticed on the uterus. The pregnancy did not continue.

6. *Control Experiment.*—The ovaries of a Rough-haired Terrier, pregnant about three weeks, were exposed, incised, and cauterised superficially. The pregnancy continued, five healthy pups being born at full time (61 days after impregnation).

7. The ovaries were removed from a Rough-haired Terrier, said to be four weeks in pup, and obviously pregnant. The pregnancy did not continue.

It will be observed that the above experiments form a series, the dates of operation ranging from three days to four weeks after impregnation. In no case did the pregnancy continue after removal of the ovaries with the exception of Experiment No. 2, where luteal tissue was found *post-mortem*. The interruption of the pregnancies cannot be attributed to the shock caused by the operation, since Bitches stand abdominal operations extremely well. It will be noted that the Control Experiment No. 6 was in some respects a more serious operation than simple removal of the ovaries, and that it caused no interruption of the pregnancy.

II.—*Rats.*

1–10. The ovaries were removed from 10 female Rats which had been for several days in the company of males. No pregnancy supervened in any case. *Note*:—Actual copulation is not readily observed in Rats, but since the females are known to come on heat at very frequent intervals, there can be little doubt that the majority if not all the Rats in question had copulated.

11–19. The ovaries were removed from nine Rats in a very early stage of pregnancy (almost certainly in each case during the first week). Slight swellings on the uterus were observed in each instance. The pregnancies did not continue in any of the cases.

20. The ovaries were removed from a Rat, 13 days pregnant. Twelve young were born at full time (15 days after the operation). The young were suckled normally. The mother was killed and no trace of ovarian tissue was found.

21. The ovaries were removed from a Rat 21 days pregnant. Five young were born at full time and suckled normally.

22. The ovaries were removed from a Rat, pregnant about 21 days. A litter of young was produced at full time.

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23. *Control Experiment.*—The right ovary was removed from a Rat which had been in the company of a male for several days. There were no visible signs of pregnancy at the time of the operation. The animal was then kept separate from the male, and 16 days afterwards was killed, when it was found to be obviously pregnant. It will be seen that the retention of the left ovary (with its corpora lutea) was sufficient for the continuance of pregnancy.

The ovaries were also removed from a Guinea-pig which had been for some time with the male. The uterus was observed to be much congested and slightly enlarged and the ovaries contained corpora lutea. At the *post-mortem* examination a fortnight later it was found that the pregnancy had not continued.

All our experiments were performed under complete anæsthesia and strict aseptic precautions were adopted. The wounds always healed by first intention. It is to be noted that we removed the entire ovaries instead of attemping, as was done in some of FRAENKEL'S experiments, to extirpate the corpora lutea alone. This we did purposely, as it appeared to us very difficult if not impossible to make sure of destroying all the luteal tissue without also removing the ovaries. The ovaries during pregnancy usually consist almost entirely of corpora lutea, and any attempt to discriminite between luteal tissue and stroma while the ovary is lying in its normal position in the body-cavity seems in our judgment to be impracticable.

It will be observed from the above experiments on Rats that the corpus luteum ceases to be functional about the middle of the period of gestation in these animals.

According to DUVAL (1893) the allantoic placenta begins to supersede the trophoblast at about the middle of pregnancy in the case of the Bitch. It is probable that in Rats this occurs somewhat prior to mid-pregnancy, but we have no direct evidence on this point. It will be seen, therefore, that our experiments lend some support to the idea that an essential factor in the nourishment of the embryo through the trophoblast is contributed by the corpus luteum, and that the latter ceases to be functional during the later part of pregnancy, when the trophoblast is replaced by the allantoic placenta.

It is to be noted also that in Experiments 20 and 21 lactation occurred independently of any ovarian influence, the ovaries having been removed.

4.—*General Conclusions.*

As a result of our observations and the considerations detailed above and in the first part of this paper, we arrive tentatively at the following general conclusions:—

The Mammalian ovary, in addition to its function of producing ripe ova, is an organ elaborating an internal secretion which reacts on the general metabolism of the animal, as is shown by the effects brought about by its removal. The secretion is probably formed in greater or less quantity at all times, but it is produced in

greatest abundance at certain more or less regularly recurrent periods, when it brings about those conditions which characterise the proœstrum and œstrus. It is at these periods also in typical cases that the ova mature and the follicles discharge.

The secretory activity of the ovary can be influenced to some extent by climatic and general environmental conditions, this being shown, for instance, in those species in which the sexual season varies in relation to the habitat. Extreme cases of such variation are seen in certain animals under domestication or in captivity. The secreting power of the ovary is likewise affected by the nature and quantity of the food supply, as is shown by the effects of certain artificial methods adopted by flock-masters for increasing the fertility of their Sheep. These methods consist in supplying the ewes with additional kinds of food at the approach of the sexual season, which is thereby hastened.* It would seem, therefore, that the secretory power of the ovary undergoes a stimulation which results further in an increased production of ripe ova.†

At present it is uncertain what part of the ovary is concerned in the production of the secretion which causes heat. It would seem, however, that this secretion must be produced either by the cells of the follicular epithelium or by the interstitial cells of the stroma as suggested by Limon (1904).‡

The ovarian secretion produces a marked effect on the nervous system, as is evident from the behaviour of an animal which is "on heat." It is probable also that the process of ovulation is brought about in part through a nervous stimulus. Thus, in the Rabbit and in the Ferret it has been shown that in the absence of coition the ova may degenerate *in situ* instead of being discharged (Heape, 1905, Marshall, 1904). In these cases, therefore, it would appear that the additional stimulus of coition, which must be of a nervous nature, is necessary in order that ovulation can be induced (*cf.* also Pigeons, Harper, 1904).

After ovulation the corpus luteum is formed, and with the development of this organ the internal secretion of the ovary undergoes a change, the corpus luteum being now an active secreting agent. If the animal becomes pregnant the corpus luteum undergoes great hypertrophy, and persists as a functional ductless gland until near the middle of pregnancy when it begins to degenerate, becoming gradually reduced in size. If pregnancy does not supervene after ovulation this structure commences to degenerate within a much shorter period. The function of the corpus luteum is to provide a secretion which is a necessary factor in the nourishment of

* It is hoped that before very long a paper may be published giving an account of observations and experiments by various flockmasters bearing on this subject. (See Marshall, 1905—September 18.)

† *Cf.* Heape (1905), who proposes the term "gonadin" for the internal secretion of the generative glands.—June 27.

‡ It is interesting to note, that according to Miss Lane-Claypon (1905), the follicular epithelial cells and the interstitial cells have an identical origin, both being derived like the primordial ova from the original ingrowths of the germinal epithelium.—June 27.

the embryo during the first part of pregnancy. So long as the corpus luteum is functional, and until it becomes degenerate, it is necessary for the continuance of pregnancy.* The part of the ovary not occupied by corpora lutea (usually a very small part comparatively, the follicles not being matured) is markedly anæmic, as pointed out by FRAENKEL.

So far from the theory that the corpus luteum is the cause of heat and menstruation being correct, the direct antithesis of this view might well be nearer the truth. For, as far as we are aware, no animal is known to experience a condition of proœstrum so long as there is a corpus luteum present in one of the ovaries. By the time that this organ is fully formed the period of heat is over, while prior to the approach of the next heat period, it is in an advanced state of degeneration or is reduced to a mere scar.

The uterine changes, therefore, which characterise the successive periods comprising the œstrous cycle, are correlated in the lower mammalia with ovarian changes which are consequently also of a more or less regularly rhythmical nature.

5.—*Summary.*

The Cause of Heat.—As pointed out by BROWN-SÉQUARD, and others, the ovaries, like the testes, exercise an influence on the general metabolism of the organism throughout the reproductive period. Ovarian medication has been employed to a considerable extent for disorders associated with the female generative organs, and in the majority of cases is said to have produced beneficial results. This method of treatment, however, is in many cases purely empirical, and is adopted without regard to the condition of the ovaries from which the extract is obtained.

Several investigators have experimented with ovarian grafts both in normal and abnormal positions. From some of these experiments it would appear that portions of ovarian tissue may obtain vascular connections and produce an effect on the general metabolism comparable to that produced by ovaries in normal animals.

It has been shown by GOLTZ and others that the occurrence of œstrus is not due entirely to cerebral or spinal reflexes.

Our experiments have demonstrated that "heat," or a transient condition resembling it, can be produced by the injection of extracts made from ovaries in proœstrous or œstrous condition, and that when such ovaries are successfully grafted into an animal previously deprived of its ovaries, the condition produced is identical with a normal heat, and that irrespective of the situation of the graft.

The Function of the Corpus Luteum.—Of the various theories as to the function of the corpus luteum, that of FRAENKEL is the only one that is supported by experimental evidence. According to this theory the corpus luteum is the only ovarian organ of internal secretion, and exerts an influence on the generative

* That is to say the presence of luteal tissue is necessary during the first half of pregnancy or thereabouts (and so, possibly, while the embryo is nourished through the trophoblast).

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functions generally throughout the whole reproductive period of the animal's life. Among its other functions, according to this theory, it produces heat and menstruation and controls the attachment of the ovum and the formation of the placenta. This theory is only partially correct. Corpora lutea are not present during the proœstrum, and are, therefore, only functional subsequent to ovulation.

From our own experiments upon Bitches and Rats we draw the conclusion that the presence of luteal tissue is necessary during the first part of pregnancy, but that the corpus luteum ceases to be functional in the later stages. In these experiments we removed the ovaries from animals at various intervals after impregnation, and found that pregnancy did not continue except in those cases in which the operation was performed in the later stages of pregnancy. Control experiments in which the ovaries were damaged or partially removed were also performed, when it was found that the animals brought forth young.

General Conclusions.—The ovary is an organ providing an internal secretion which is elaborated by the follicular epithelial cells or by the interstitial cells of the stroma. This secretion circulating in the blood induces menstruation and heat. After ovulation, which takes place during œstrus, the corpus luteum is formed, and this organ provides a further secretion whose function is essential for the changes taking place during the attachment and development of the embryo in the first stages of pregnancy.

Postscript—*September* 18.

Last July the Yorkshire Terrier Bitch referred to in the second experiment on page 133, and in the footnote on page 129, was noted to show signs of being "on heat," but there was no external bleeding. Subsequently, however, she permitted a Dog to ward her. In August she was killed, when a small portion of one ovary was discovered, but the Bitch was not pregnant. The ovarian substance left behind had probably undergone hypertrophy since the operation. It is to be noted that the "heat" period (which, since there was no external bleeding, was scarcely normal in character) occurred after an unusually long interval (especially for a little Dog), the preceding œstrus having been in December. This postponement of œstrus was no doubt due to the removal (incomplete though it was) of the ovaries, the portion remaining behind having perhaps to undergo a sufficient degree of hypertrophy before "heat" could be induced. The difficulty of removing all trace of ovarian substance from Bitches has already been commented on, but it is improbable that in this experiment the corpora lutea were not completely destroyed. We are much indebted to Professor Ewart for his kindness in keeping this Terrier under observation for us at Penycuik during a part of the summer.

With reference to the not improbable connection between the corpus luteum and the trophoblast (see p. 135, and Emrys-Roberts, 'Roy. Soc. Proc.,' B, 1905), and the suggested digestive functions of the latter, Dr. Andrew Hunter kindly undertook to test luteal extract for pepsin and trypsin. The results however were negative. Dr. Hunter reports as follows:—"The substance of the corpus luteum was tested for the presence of proteolytic ferments in the following way:—Extracts of the fresh luteal substance were made with normal saline, with 0·2 per cent. HCl, and with 2 per cent. Na_2CO_3. These were added to carbol-gelatine tubes after the method of Fermi, some of the tubes being neutral, some acid, and some alkaline. They were allowed to stand at room temperature. None of them showed the slightest liquefaction, even after a period of three weeks. Gelatine plates sprinkled with fresh minced corpus

luteum also failed to show any trace of digestion. There is, therefore, no evidence that the corpus luteum contains a pepsin-like or a trypsin-like ferment."

In dealing with the possible functions of the ovary we omitted to make mention of the "auto-intoxication" theory which has been put forward (as an alternative to the internal secretion theory) to explain the influence of certain of the ductless glands upon the general metabolism. According to this theory, the organ in question is regarded as being excretory as opposed to secretory in function. We do not see that the auto-intoxication hypothesis is readily applicable to the case of the ovary, though it is no doubt possible that each of the two theories (internal secretion and auto-intoxication) may be in part true as applied both to the ovary and to certain of the other "organs of internal secretion."

References to Literature.

Andrews. "Internal Secretion of the Ovary," 'Jour. of Obstet. and Gyn.,' vol. 5 (1904).

Beard. "The Span of Gestation and the Cause of Birth," Jena (1897).

Bestion de Camboulas. "Le Suc Ovarien," Paris (1898).

Bouin and Ancel. "Recherches sur les Cellules interstitielles du Testicule des Mammifères," 'Arch. de Zool. exp.' (1903).

Brachet. "Recherches," 2nd ed., Paris (1837).

Brown-Séquard. "Des effets produits chez l'homme par des injections sous-cutanées d'un liquide retiré des testicules frais de cobaye et de chien," 'C. R. Soc. de Biol.' (1889).

Campbell (Malcolm). "Pathological Conditions of the Ovaries as a Possible Factor in the Etiology of Uterine Fibroids," 'Scottish Medical and Surgical Journal,' vol. 16 (1905).

Clark. "Ursprung, Wachstum, und Ende des Corpus Luteum, etc.," 'Arch. f. Anat. u. Phys.,' Anat. Abth. (1898).

Dixon. "The Ovary as an Organ of Internal Secretion," 'The Practitioner,' vol. 66 (1901).

Dudley. "Über intra-uterine Implantation des Ovariums," 'Intern. Gyn. Congress,' Amsterdam (1899).

Duval. "Le Placenta des Carnassiers," 'Journ. de l'Anat. et la Phys.,' vol. 29 (1893).

Essen-Möller. "Doppelseitige Ovariotomie im Anfänge der Schwangerschaft," 'Centralbl. f. Gyn.,' vol. 28 (1904).

Foà. "La Greffe des Ovaires en Relation avec Quelques Questions de Biologie Générale," 'Arch. Ital. de Biol.,' vol. 34 (1900).

Fraenkel and Cohn. "Experimentelle Untersuchungen über den Einfluss des Corpus luteum auf die Insertion des Eies," 'Anat. Anz.,' vol. 20 (1901).

Fraenkel. "Die Function des Corpus Luteum," 'Arch. f. Gynäk.,' vol. 68 (1903).

Glass. "An Experiment in Transplantation of the Entire Human Ovary," 'Medical News' (1899).

GOLTZ. "Ueber den Einfluss des Nervensystems auf die Vorgänge während der Schwangerschaft und des Gebärakts," 'Pflüger's Archiv,' vol. 9 (1874).

GOLTZ and EWALD. "Der Hund mit verkürztem Rückenmark," 'Pflüger's Archiv,' vol. 63 (1896).

GORDON. "Two Pregnancies following the Removal of both Tubes and Ovaries," 'Trans. Amer. Gyn. Soc.,' vol. 21 (1896).

GRIGORIEFF. "Die Schwangerschaft bei Transplantation der Eierstöcke," 'Centralbl. f. Gynäk.,' vol. 21 (1899).

HALBAN. "Ueber den Einfluss der Ovarien auf die Entwickelung des Genitales," 'Monatschr. f. Geburtsh. and Gynäk.,' vol. 12 (1900).

Idem. "Ovarium und Menstruation, etc.," 'S.B. Akad. Wiss., Wien,' vol. 110 (1901).

HARPER. "The Fertilization and Early Development of the Pigeon's Egg," 'Amer. Jour. of Anat.,' vol. 4 (1904).

HEAPE. "Ovulation and Degeneration of Ova in the Rabbit," 'Roy. Soc. Proc.,' B, vol. 76 (1905).

HERLITZKA. "Recherches sur la Transplantation des Ovaires," 'Arch. Ital. de Biol.,' vol. 34 (1900).

KNAUER. "Einige Versuche von Ovarientransplantation am Kaninchen," 'Centralbl. f. Gynäk.' (1896).

Idem. "Die Ovarientransplantation. Experimentelle Studie," 'Arch. f. Gynäk.,' vol. 60 (1899).

LACK. "Preliminary Note on the Experimental Production of Cancer," 'Jour. of Path. and Bact.,' vol. 6 (1899).

LANE-CLAYPON. "On the Post-natal Formation of Primordial Ova," 'Proc. Phys. Soc.,' in 'Jour. of Phys.,' vol. 32 (1905).

LIMON. "Observations sur l'état de la Glande Interstitielle dans les Ovaires Transplantés," 'Jour. de Phys. et de Path. gén.,' vol. 16 (1904).

MARCHESE. "Sulla Transplantazione del Ovaje," 'Arch. Ital. de Gyn.' (1898).

MARSHALL (FRANCIS). "The Œstrous Cycle and the Formation of the Corpus Luteum in the Sheep," 'Phil. Trans.,' B, vol. 196 (1903).

Idem. "The Œstrous Cycle in the Common Ferret," 'Q. Jl. Micro. Sci.,' vol. 48 (1904).

Idem. "Fertility in Scottish Sheep," 'Roy. Soc. Proc.,' B, vol. 76 (1905).

MARSHALL (FRANCIS) and JOLLY. "Contributions to the Physiology of Mammalian Reproduction. Part I.—The Œstrous Cycle in the Dog."

MORRIS. "The Ovarian Graft," 'New York Med. Jour.' (1895).

POULTON. "The Structures Connected with the Ovarian Ovum of Marsupialia and Monotremata," 'Q. Jl. Micro. Sci.," vol. 24 (1884).

PRENANT. "La Valeur Morphologique du Corps Jaune, etc.," 'Rev. Gén. des Sciences Pures et Appliquées' (1898).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

REGAUD and POLICARD. "Fonction Glandulaire de l'Epithelium Ovarique chez la Chienne," 'Compt. Rend. Soc. Biol.,' Paris, vol. 53 (1901).

ROMANES. "Darwin and After Darwin," Vol. 2, London (1895).

RIBBERT. "Über Transplantation von Ovarien, Hoden und Mamma," 'Arch. f. Entwickelungs-Mechanik,' vol. 7 (1898).

RUBINSTEIN. "Extirpation beider Ovarien," 'St. Petersb. mediz. Wochenschr.' (1899).

SANDES. "The Corpus Luteum of *Dasyurus Viverrinus*, etc.," 'Proc. Linnean Soc. New South Wales,' vol. 28 (1903).

SCHULTZ. "Transplantation der Ovarien auf männliche Tiere." 'Centralbl. f. all. Path. u. path. Anat.,' vol. 11 (1900).

SHATTOCK and SELIGMANN. "Observations upon the Acquirement of Secondary Sexual Characters, Indicating the Formation of an Internal Secretion by the Testicle," 'Roy. Soc. Proc.,' vol. 73 (1904).

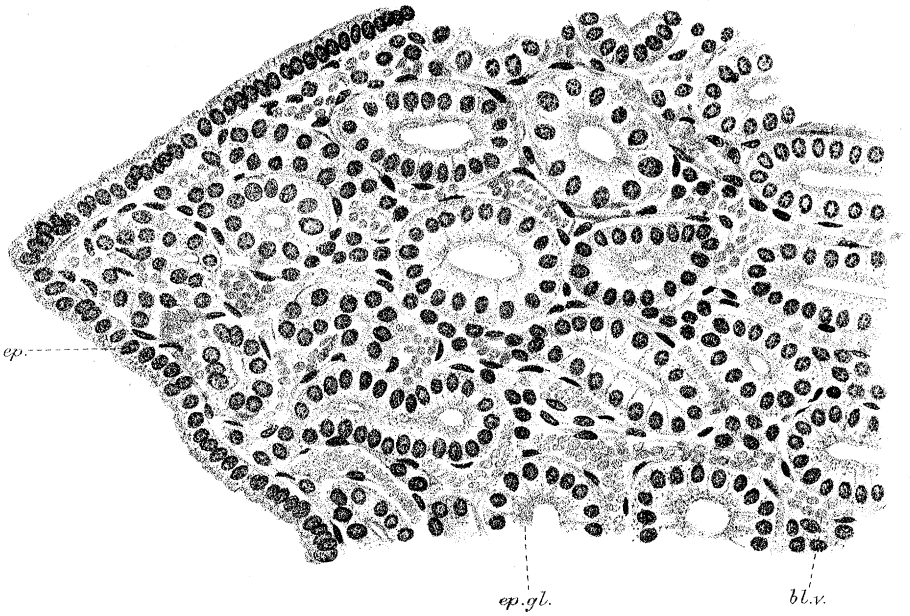
SOBOTTA. "Über die Bildung des Corpus Luteum bei der Maus," 'Arch. f. Mikr. Anat.,' vol. 47 (1896).

VINCENT and SHEEN. "The Effects of Intravascular Injections of Extracts of Animal Tissues," 'Jour. of Phys.,' vol. 29 (1903).

WILLIAMS. "Obstetrics," New York (1903).

YOUATT. "Cattle," London (1835).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES



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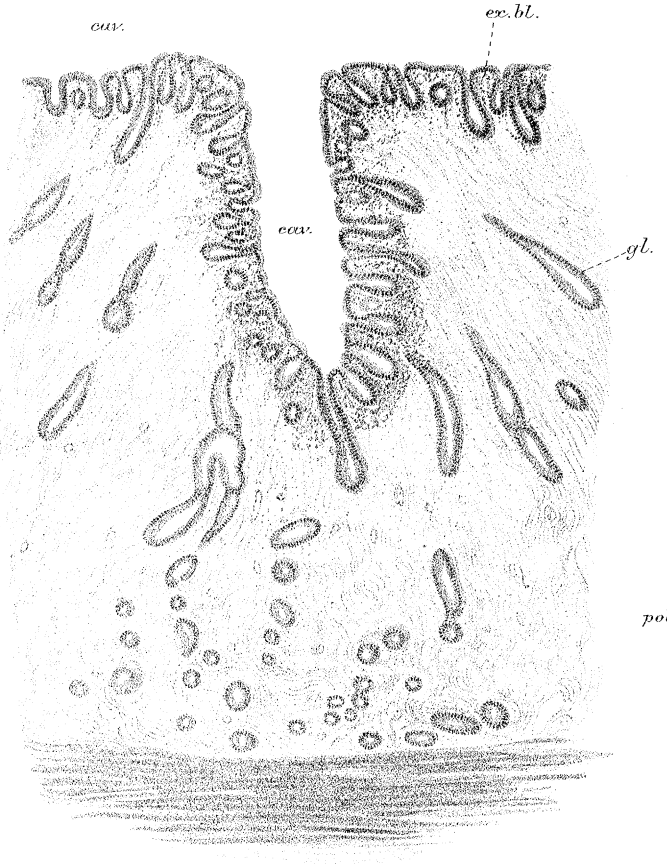

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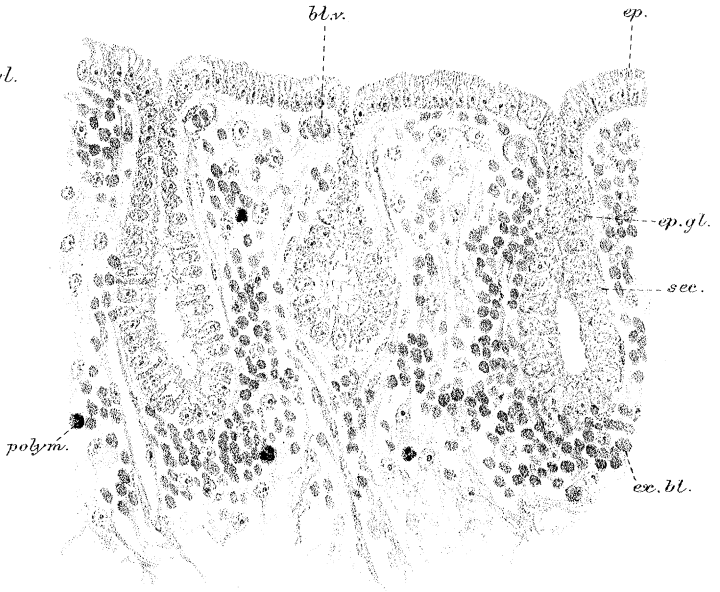

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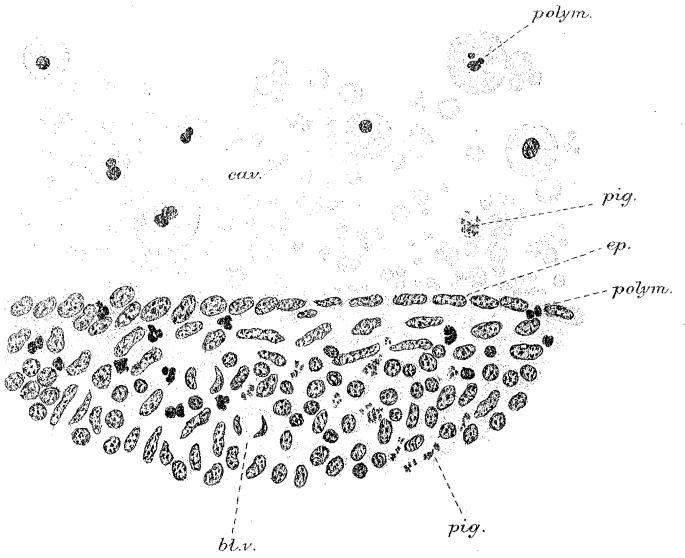

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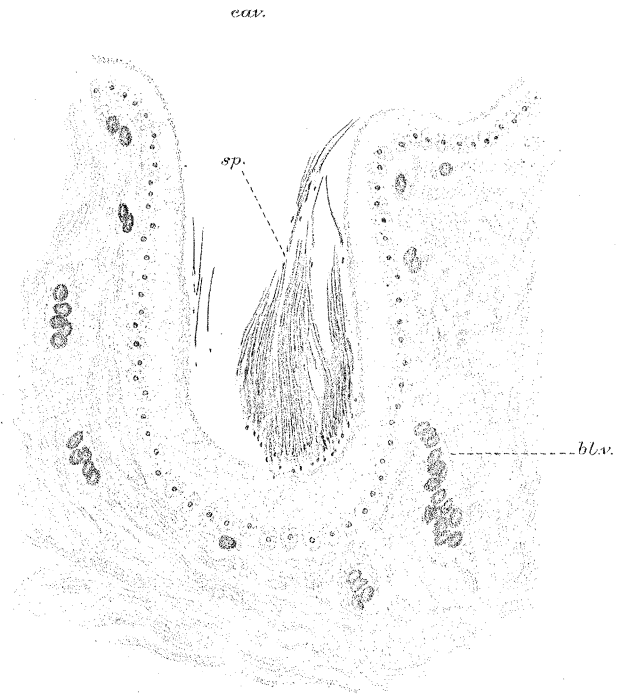

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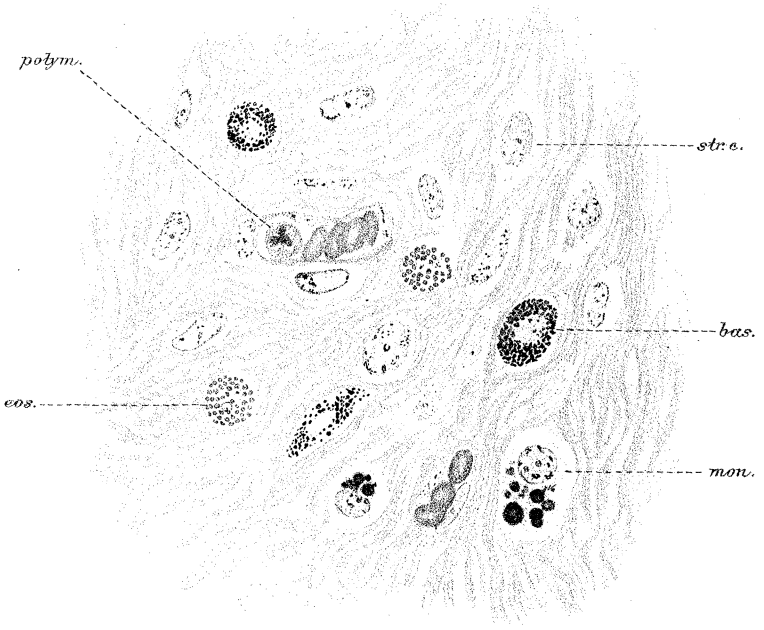

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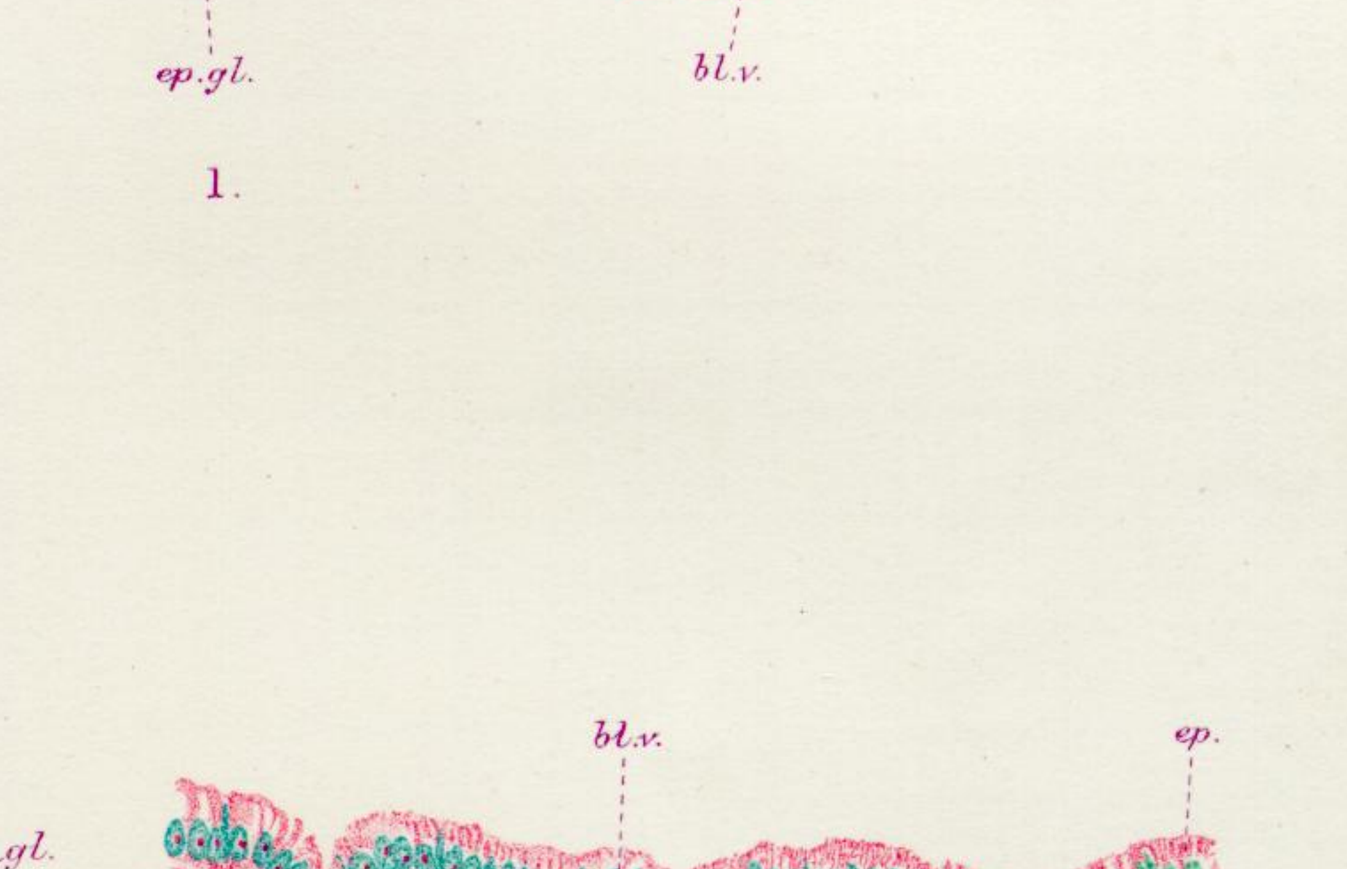

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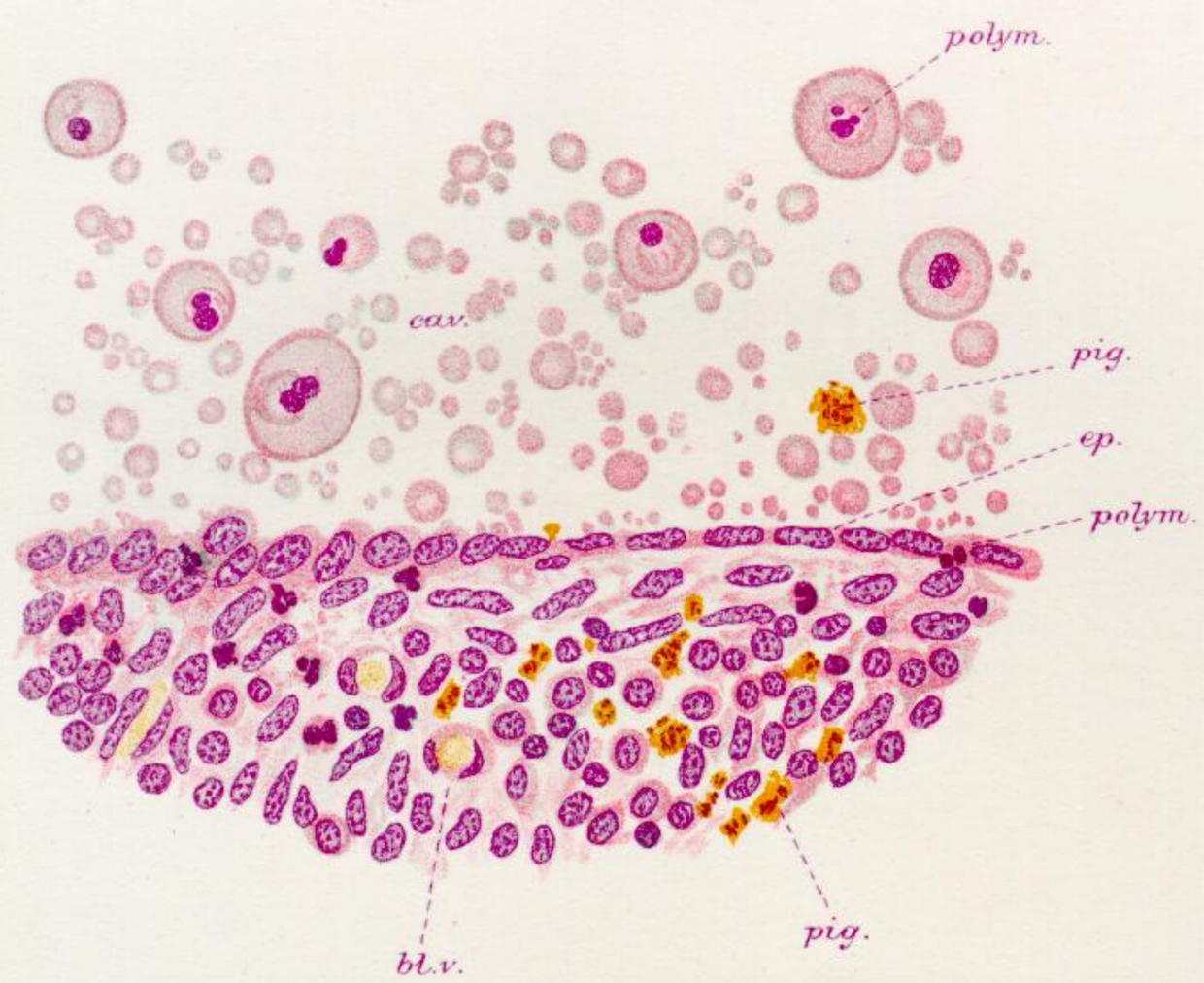
Plate 7.

Fig. 1.—Transverse section, showing a portion of the uterine mucosa during Period II. The vessels are much congested. × 300 diam.

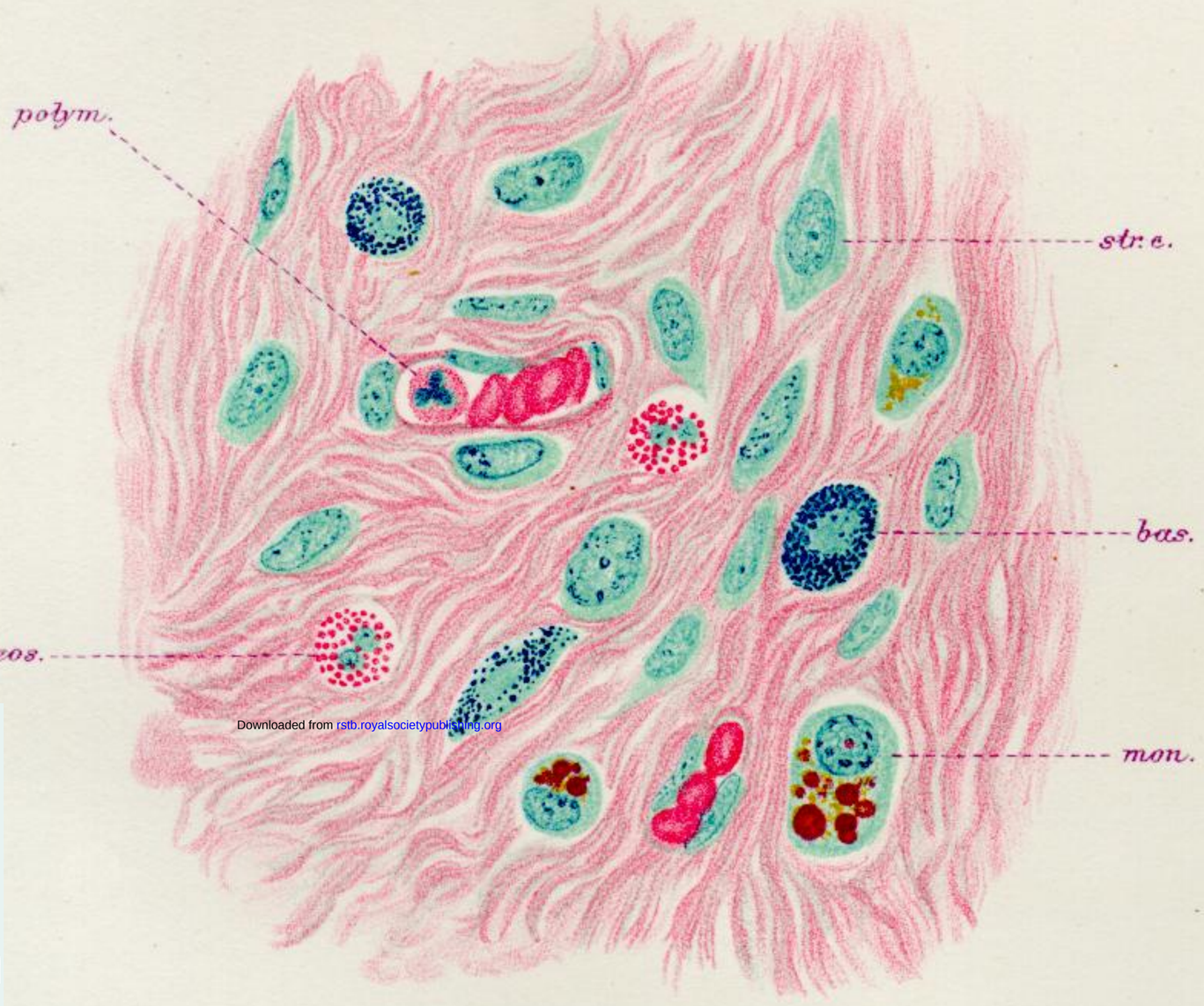
Fig. 2.—Transverse section, showing a portion of the mucosa during an early stage of Period III. × 200 diam.

Fig. 3.—The same. × 50 diam.



4.

5.



6. 7.

Plate 8.

Fig. 4.—Horizontal section, showing a portion of the mucosa during a very early stage of Period IV. The epithelium is in process of renewal. × 300 diam.

Fig. 5.—Transverse section, showing a portion of the mucosa during a late stage of Period IV. New capillaries have been formed. × 500 diam.

Fig. 6.—Transverse section, showing a portion of the mucosa during about the middle of Period IV. Wandering cells of various kinds are very numerous. × 1000 diam.

Fig. 7.—Wandering cells, seen during Period IV. × 1000 diam.