The Reproductive Cycle in the Marsupial Dasyurus viverrinus.

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With Plates 6 to 8.

Contents.

Introduction.

In recent years a great deal of attention has been paid to the reproductive processes in the Mammalia, and in particular to the phenomena connected with the œstral cycle. Observations relating to the latter, however, have been almost entirely based on Eutherian mammals, and, indeed, largely on Primates (Heape (**8**, **9**, **10**), Van Herwerden (**12**), Hitschmann and Adler (**15**), and others) and various domesticated or semi-domesticated Eutheria (e.g. sheep (**16**), dog (**19**), ferret (**17**), Marshall). Having at our disposal a large volume of records relating to the breeding habits of Dasyurus, as well as an abundant supply of material, we have thought that an inquiry into the reproductive cycle in this member of the Marsupialia (in many respects a more primitive group than the Eutheria), might not only be of interest, but might, perchance, throw light on some of the problems relating to the Eutherian œstrous cycle which still await solution. We venture to hope that the account of the reproductive cycle which we are now able to present will be found to fulfil, in some measure, expectations in this latter regard.

We desire to say here that our work has been greatly facilitated by the circumstance that some of the phenomena relating to reproduction in Dasyurus have already been described in greater or less detail (Hill (**13**, **14**), O'Donoghue (**20**, **21**), Sandes (**22**)).

The breeding season has been divided up into periods for the

purposes of description, and the terminology employed is, with slight modification, that suggested by Heape (**11**). As the œstral cycle in Dasyurus differs considerably from that of the Eutherian mammal, it has been found necessary to introduce two new terms, viz. Post-œstrus, to designate the period which intervenes between œstrus and ovulation; and Pseudo-pregnancy, to designate the period which, in the non-pregnant animal, follows ovulation, and in which the changes in the ovary, mammary glands and uteri are essentially similar to those in the pregnant female.

We wish to express our thanks to Mr. F. Pittock, of the Zoological Department of this College, for invaluable help in the preparation of the photomicrographs on Plates 6–8.

Material.

The information relating to the breeding habits of the animal was obtained largely from the records mentioned above. These records relate to 170 females, which fall into two classes—pregnant and non-pregnant. Of the non-pregnant females killed, 13 were prior to ovulation, 19 were after ovulation, and in 6 there was no record of ovulation. Of the pregnant animals, 37 had less than twenty embryos, 35 had more than twenty embryos, in 25 there was no definite record of the number of embryos, and 35 were post-partum. Examples of individual records relating to these females have been given previously (**20**), and further examples are given later.

For the purposes of the present paper, the uteri of sixteen females were cut in serial section in order to study the histological changes occurring therein. These uteri were, with two exceptions, from non-pregnant animals both before and after ovulation, and were fixed either in picro-corrosive-acetic acid, strong Flemming's fluid, or in Hermann's fluid, all of which gave very good fixation, Flemming's fluid being particularly good for the cilia in the uterine glands. The sections (about 8 μ thick) were stained with hæmatoxylin

and eosin. These sections were compared with those of pregnant and post-partum uteri already in the possession of one of us, whilst we also had access to the numerous preparations of ovaries and mammary glands of Dasyurus, which formed the basis of the papers of O'Donoghue (**20, 21**) and Sandes (**22**) on the corpus luteum and the growth of the mammary apparatus. We are thus in the fortunate position of being able to take into consideration and to correlate the changes which occur during the œstral and pregnancy cycles in the several parts of the reproductive and accessory organs more accurately and in greater detail than, we believe, has yet been done for any Eutherian mammal.

Anœstrus.

The Australian native cat, Dasyurus viverrinus, is a small marsupial somewhat resembling a civet in external appearance. It is readily obtainable and fairly easy to keep alive and breed from in captivity.

In the female, the pouch[1] is a well-marked structure which, during the anœstral period, appears as a small, somewhat circular depression, situated in the median line towards the posterior end of the abdomen. It is about 10 mm. in diameter by 5 mm. in depth and its boundary is slightly less marked at the anterior end. The interior of the pouch, which in the resting animal is dry and dirty, contains as a rule six teats (vide **20**). The teats are arranged in pairs on either side of the middle line and each teat has a thickened ledge around its base. The skin over the teat itself is generally free from the sebaceous glands characteristic of the skin as a whole, and which are particularly well marked in the lining of the pouch. Examination of sections through the teat shows that the ledge around its base marks the place where the sebaceous glands of the pouch leave off. The teat with its ledge is situated in a depression, which, in its turn, is surrounded by a raised ridge very nearly circular in shape.

[1] c.f. O. Katz, 'Zitschr. wiss.,' Bd. xxxvi, 1882.

The ridges around the posterior pair of teats are continuous in the middle line, whereas those at the anterior end are separated from one another by a considerable interval. There is also a space between the median walls of the ridges of the middle pair of teats, so that the three pairs are approximately arranged in the form of a horse-shoe with the open end situated anteriorly (see Text-fig. 1, A). The floor of the pouch between the ridges surrounding the teats is loose and folded and frequently falls into a raised fold along the middle line.

The number of teats is subject to slight variation; thus in

TEXT-FIG. 1.

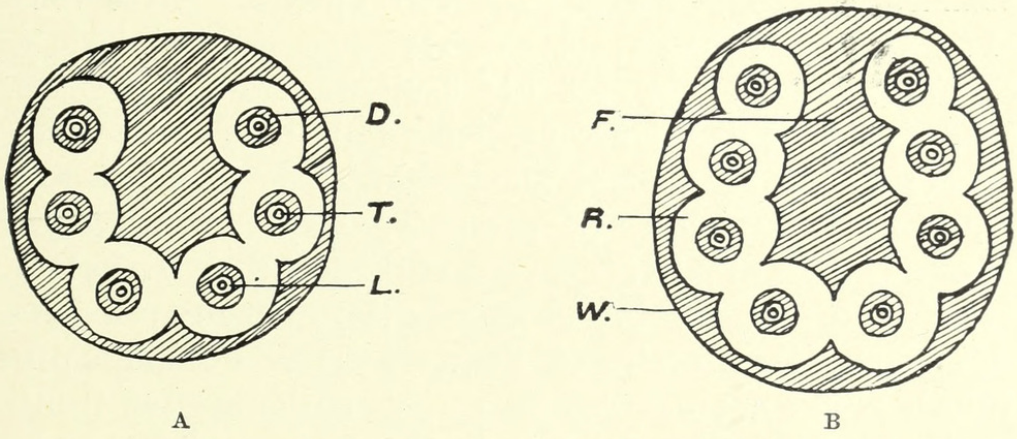


Diagram to show the arrangement of the teats within the pouch, (A) in the normal female with six teats; (B) in the females with eight teats. T. Teat. L. Ledge surrounding teat. D. Depression. R. Raised ridge. F. Floor of pouch. W. Pouch-wall.

over 170 females examined one pouch contained five teats, two pouches contained seven teats and five contained eight teats[1], but all the remainder had the normal number—six.

According to Bresslau (**4**, p. 672 et seq.) the mammary (nipple) primordia take their origin in Dasyurus as in other Marsupials from a pair of laterally situated primary anlagen, each consisting of an epidermal thickening and an underlying area of condensed cutis. As a rule, three pairs of mammary primordia are formed, three from each primary anlage, but

[1] Klatsch (quoted by Bresslau (**4**)) also records the occurrence of 8 teats in one individual.

in two pouch-young Bresslau records the presence of four pairs, whilst in a third he found three primordia on one side, four on the other. In the pouch-young, the mammary primordia are arranged symmetrically in two slightly curved longitudinal rows, the two primordia of the posterior pair lying nearest the middle line, those of the anterior pair most remote from it, so that the two rows already show the same horse-shoe shaped arrangement as is characteristic of the adult.

Round each mammary primordium, which has meantime grown into the cutis as a knob-shaped epidermal projection, a ring-shaped epidermal thickening arises and this later hollows out to form a circular groove—the marsupial pocket—bounded by a circular wall. The lateral portions of the walls of the three pockets on each side then join so as to form a continuous lateral pouch fold and finally the two folds become continuous with each other in front and behind, and so produce the circular wall of the pouch. The occurrence of supernumerary nipples (Hypermasty, Hyperthely) in D. viverrinus is of interest in view of the fact that D. hallucatus, according to Oldfield Thomas (**28**) normally possesses eight teats, that species of Phascologale and Sminthopsis (which genera are regarded by various authorities as being more primitive than Dasyurus), have ten, whilst higher numbers still are met with amongst the Didelphzidæ (Peramys-henseli, 17–25). These facts, as Bresslau (**4**, p. 805–8) has pointed out, would appear to indicate that Dasyurus, like many other marsupials, has suffered, in the course of phylogeny, a reduction in the number of teats within the pouch, which reduction has affected those more anteriorly situated. In the case of the pouch with only five teats, it seems as if six mammary rudiments were included in the pouch, but for some reason or other the anterior one on the right side failed to develop.

When the young are born, they are transferred to the pouch, presumably by the mother with the aid of her lips, and, as they become permanently fixed to the teats for some time, the number of teats determines the maximum number of young that can be reared in one litter.

Dasyurus has only one breeding season[1] in the year, which extends over the winter months, i.e. from the end of May to the first fortnight in August.

In our records there are two cases of females ovulating in May; one killed on the 21st had eggs which had just entered the uteri, and the other, killed on the 31st, showed corpora lutea in a very early stage. The two latest records of pregnant females are on the 2nd and 6th of August, and in both cases the embryos were in an advanced condition.

The male does not appear to experience an obvious period of rut, such as Semon (**25**) describes for the male Phascolarctos. We have, however, frequently observed that the substitution of another male for the one previously with a female frequently resulted in copulation (cf. record, p. 151). The records show that copulation may extend intermittently over a period of two to three days. In the majority of cases, however, there is only a single record of coition, generally lasting for several hours.

The method of copulation in Dasyurus is similar to that described by Selenka for Didelphys (**23**, p. 105), the male mounting on the back of the female and laying hold of the skin of the dorsum of the neck with his jaws. The penis, when erected, is extremely long and attenuated and possesses a markedly bifid glans, its two divisions doubtless being inserted into the corresponding lateral vaginal canals. The records show that the spermatozoa may remain alive in the Fallopian tubes (where they occur in large bunches in the gland lumina opening into same) for at least two weeks.

Pro-œstrus.

So far as our observations extend, there is no evidence of any animal ovulating twice in the same season, nor is there

[1] This term is used in the sense defined by Heape (**11**), i.e. "to denote the whole of that consecutive period during which any male or female mammal is concerned in the production of young," and does not include the period of suckling.

any record of a female once served, again copulating during that season. As a matter of fact, our experience has been that a female, once copulation has been effected, will invariably fight any male subsequently introduced into the breeding cage. Moreover, study of the ovaries in section has shown that ovulation, even in females which have not become pregnant, is not followed by the growth of a second batch of follicles. The œstral cycle in Dasyurus is thus a simple one, and as only one such cycle occurs in the breeding season, the animal is monœstrous. Our records show that Trichosurus, Phascolarctos (cf. Semon (**25**) and Caldwell) (**5**)), Phascolomys and probably the Macropods (cf. Caldwell, loc. cit.) breed only once a year, but as to whether they are monœstrous, we have no definite evidence of our own to offer.

Pro-œstrus commences early in June and appears to occupy a period of time varying from four or five to perhaps as long as ten or twelve days. It is marked by an œdematous swelling of the lips of the cloacal aperture, and at the same time the pouch enlarges somewhat and becomes tumid, whilst its interior sometimes becomes slightly moist. The tumidity is mainly the result of the enlargement of the sebaceous glands of the pouch area. The sweat-glands also hypertrophy and become more coiled. The sebaceous and the sweat-glands soon become active; in the former the cells start to undergo autolytic disintegration, and in the latter they become granular. The gland lumina increase and become filled by secretion, which, on being discharged, causes the interior of the pouch to become moist and somewhat sticky (vide **20**).

Practically no change takes place in the mammary glands during this period.

In the ovary, the Graafian follicles gradually enlarge, and as they approach maturity, they form prominences on its surface.

Uterine Changes.

We have examined sections of the uteri of three females in the pro-œstral condition.

Case 1 (17. vi. '99) (Pl. 6, fig. 1).—No record beyond a label in the bottle stating that this female was "probably getting into heat." The above date shows that she was killed towards the beginning of the breeding season. The uteri measured 11 mm. by 7·5 mm. by 7 mm. in diameter. Sections show that, apart from slight increase in size and in vascularity, they are practically in the resting condition. The mucosa averages 1·4 mm. in thickness. The uterine epithelium consists of low columnar cells with close-set nuclei, and has a thickness of ·012 mm.

The uterine glands vary in diameter from ·032–·048 mm. They are distinctly coiled in the basal half of the mucosa and possess small but distinct lumina. The connective tissue matrix of the mucosa is for the most part dense and compact. Numbers of leucocytes occur in it. The blood-vessels extend up between the glands and are already considerably enlarged.

Case 2 (No. 2, 21. v. '03) (Pl. 6, fig. 2).—This female was killed on arrival. The pouch appeared reddish and was slightly moist. In the ovary occurred practically full-grown ova, with peripheral vesicular nuclei. The mucosa averages about 1·5 mm. in thickness and the uterine epithelium, ·016 mm. The uterine glands are in a more active condition than in Case 1. They are more markedly coiled, are thicker and possess wider lumina, but the mucosa as a whole is less vascular.

Case 3 (22. vi. '01).—No record beyond statement "getting into heat." Left uterus, 11 mm. by 11 mm. by 6 mm. Ova with peripheral vesicular nuclei and not quite full-grown. Graafian follicles small, with retinacula.

The mucosa has a maximum thickness of 2·2 mm. The uterine epithelium has increased somewhat in thickness, now measuring ·02 mm. and is evidently in active growth, mitoses being not infrequently met with. The nuclei of its cells are narrow, elongated and closely packed, and situated at different levels.

The basal two-thirds of the uterine glands are markedly

coiled and form compact elongate masses, separated from each other by narrow strands of connective tissue carrying blood-vessels. The lumina of the glands are only occasionally patent. The gland epithelium is in active growth, mitoses being frequent.

The connective tissue, especially in the upper third of the mucosa, is loose and œdematous in character and has numerous leucocytes dispersed through it. Immediately below the uterine epithelium is a narrow dense zone. The blood-vessels are enlarging, the mucosa being more vascular than in Case 2.

From the foregoing, it is clear that during pro-œstrus the uteri as a whole enlarge, the mucosa increases in thickness and becomes more vascular, whilst the uterine glands grow in length and become markedly coiled. The uterine epithelium also thickens.

Œstrus.

Pro-œstrus is followed by œstrus, i. e. the period of desire on the part of the female, and although in one case this extended over three days, it appears usually to last only for one or perhaps two days. As in the higher mammals, it is only at this time that coition occurs. Selenka (**23**, p. 104), speaking of Didelphys marsupialis, states that, "die Brunst des Weibchens dauert jedesmal nur 3–5 Stunden!"

Our records show that whilst in one case [1] ovulation may have coincided with œstrus, the latter practically invariably precedes the former. We have records of five females in which copulation had occurred (in three of them, five days previously), whilst ovulation had not yet taken place, as shown by examination of the ovaries. Furthermore, we have records showing that unsegmented ova were obtained "from the uteri, four (2-celled eggs also present along with unsegmented), five, six, seven and eight days after coitus, 2-celled eggs six and seven days after, 4-celled eggs eleven

[1] This case is referred to in detail on p. 149.

and eighteen days after" (Hill, **14**, p. 3). It is thus evident that ovulation is not generally coincident with œstrus, but follows after a longer or shorter interval, amounting in some cases to six or seven or even more days.

Dasyurus thus affords a marked contrast to the Eutheria, in the majority of which œstrus and ovulation are stated to be generally coincident (cf. Marshall (**18**), p. 135 et seq.).

During this period the changes in the ovary and pouch still continue.

Uterine Changes.

Case 1 (12. vi. '02), Pl. 7, fig. 3.—Female killed on arrival. Pouch, reddish, not moist, and only slightly tumid. Cloacal margins distinctly swollen and tumid. Lateral vaginal canals, uteri and Fallopian tubes enlarged, the uteri measuring 15 mm. × 13 mm. No trace of semen. Ovaries with prominent Graafian follicles, ova full-grown with peripheral nuclei, shortly before first polar mitosis.

The mucosa has a maximum thickness of 2·5 mm. The uterine epithelium (·04 mm. in thickness) is formed of very narrow columnar cells with, for the most part, basally situated nuclei, alternating in arrangement. The uterine glands (·048–·06 mm. in diameter) are markedly convoluted over the major portion of their extent. Their epithelial walls are formed of columnar cells with basally situated nuclei and with their inner ends distinctly ciliated, the cilia working outwards (Pl. 8, fig. 10). Cilia would appear to be present over the entire extent of the gland lumina, though in some cases they are less distinct and perhaps absent at the basal extremities of the glands. We have observed this ciliated condition of the uterine glands also in Perameles. It is a feature of interest inasmuch as it affords an instance of a rather uncommon condition, viz. a glandular epithelium, composed of similar cells uniformly ciliated.

The connective tissue, except immediately below the uterine epithelium, where it is dense and very cellular, is in the form

of a very delicate reticulum, the meshes of which are occupied by a lightly staining coagulum. Leucocytes are present throughout the extent of the mucosa, being especially abundant in its more superficial region, below the uterine epithelium.

The mucosa is not specially vascular.

Case 2 (9.vi.'01).—Female killed on arrival. Pouch with reddish hairs. Not cleaned and not much enlarged. Left uterus 12·5 mm. by 12·5 mm. by 7 mm., right uterus 14 mm. by 16·5 mm. by 7 mm. Ovaries with prominent Graafian follicles, ova full grown with peripheral nuclei. It is noted that this female would not associate with the male. Copulation may therefore have already taken place, but the genital organs were not examined for sperms.

The uterus agrees closely as regards its histological condition with that of the preceding case. The mucosa measures in thickness from 2·4 to 2·7 mm., and the uterine epithelium ·032 mm. The glands generally resemble those of Case 1 and are ciliated.

An important advance is seen in the presence of numerous capillaries in the connective tissue close below the uterine epithelium, some of which lie in actual contact with the deep surface of the latter.

Making allowance for individual variation these two cases show that the growth changes initiated during pro-œstrus are continued without interruption throughout this period.

Post-œstrus.

In the higher mammals, œstrus marks the climax of the pro-œstral changes, and is, according to Heape (**11**) and Marshall (loc. cit.), the period at which ovulation occurs. It is followed in the non-pregnant female by metœstrum, a period during which the various parts of the reproductive and accessory organs return to a condition of rest.

In Dasyurus, however, we meet with a markedly different state of affairs, since we find, as pointed out above, that ovulation as a general rule does not take place until some days

after œstrus. For this period which intervenes between œstrus and ovulation, we propose the term "post-œstrus."

During this post-œstral period the changes initiated during pro-œstrus are continued, and are perhaps most marked in the ovary. The Graafian follicles continue to enlarge. The ova undergo the first meiotic division, resulting in the separation of the first polar body, and the spindle for the second division is laid down. Our records yield instances of two females, both killed five days after copulation, in one of which the first polar spindle was established, whilst in the other, the first polar body was already separated and the second polar spindle formed. These ovarian changes culminate in the rupture of the follicle and the discharge of the ovum.

The tumidity of the cloacal lips gradually disappears.

Uterine Changes.

Case 1 (No. 8, 23 . vii . '02) (Pl. 6, fig. 4).—This female came to hand on July 11th. On the 18th the cloacal margins were greatly swollen, and copulation, extending over two hours, took place. Five days after, i. e. on the 23rd, she was killed, the pouch being small and only slightly tumid. The uteri were pale in colour and somewhat enlarged, measuring 16·5 mm. by 12 mm. by 5·5 mm. Examination of the ovary revealed the presence of ripe follicles containing ova in which the first polar spindle was already formed.

The mucosa has a maximum thickness of 2·4 mm. and the uterine epithelium, ·032–·036 mm. The uterine glands have increased somewhat in diameter, and some of them are markedly enlarged, especially towards their basal ends. Where such enlargement has occurred the gland epithelium has become reduced in thickness and appears of the low columnar type. In a few of the glands the lumen is occupied by a shrunken coagulum. The gland epithelium is ciliated.

The connective tissue differs from that of œstral Case 1 in being much more cellular and compact. Leucocytes are specially abundant in the superficial portion of the mucosa,

Capillaries are present here and there below the uterine epithelium, but are not specially abundant.

Case 2 (No. 14, 26 . vii . '02).—The following is our note-book record of this female:

24 . vi . '02.—Received.

4 . vii . '02.—Cloacal margins commencing to swell.

11 . vii . '02.—Cloacal margins still swollen.

21 . vii . '02.—Copulation.

24 . vii . '02.—Pouch slightly tumid, not moist.

26 . vii . '02.—Pouch slightly tumid; killed, i. e. five days after copulation.

Examination of the ovaries revealed the presence of mature follicles containing ripe ova in which the first polar body was already separated and the second polar spindle established.

The mucosa has a maximum thickness of 2·4 mm. The uterine epithelium has made little or no progress, and appears as a low columnar epithelium (·02 mm. in thickness) with close-set ovalish nuclei. Here and there, between the ordinary epithelial cells, there occur single very narrow cells with darkly staining cytoplasm and compressed deeply staining nuclei.

The uterine glands (averaging ·048 mm. in diameter) do not differ essentially from those of œstral Case 2, and are rather less advanced than those of the preceding uterus. They are well convoluted and lined by the usual ciliated columnar epithelium. Their lumina are for the most part distinct but small.

The connective tissue is more œdematous than that of the preceding case, and contains in places much coagulum. Numerous leucocytes are present in it. The mucosa is, on the whole, more vascular than that of Case 1, though the superficial capillaries are not yet greatly developed.

So far as can be judged from these two cases, it would appear that during this post-œstral period, no very marked advance is made by the uterine mucosa, the uteri being, on the whole, very similar to those of the œstral period.

Ovulation.

It has been pointed out by Ancel and Bouin (1) that the Eutherian mammals may be divided into two classes according to the conditions under which they ovulate. In the first group ovulation is spontaneous—i. e. it takes place whether there has been coition or not (this condition appears to be the more general); whilst in the second group ovulation is non-spontaneous—i. e. in the normal course of events, copulation is necessary to provoke it.

In Dasyurus, ovulation is spontaneous and quite independent of copulation. We have records of nine females which ovulated after being under observation for periods varying from two to thirty-seven days, during which no copulation was seen to take place. Four of these females were under observation for twenty days and upwards, whilst in three of them, the unfertilised ova were found in the uteri. On the other hand, as pointed out above, we have records of five animals in which copulation had taken place but ovulation had not, three of these females having been killed five days after copulation. This evidence shows conclusively that ovulation and copulation are perfectly independent one of the other, and that the former is in no way provoked by the latter, as is the case, for example, in the rabbit.

A further point in connection with ovulation is worthy of attention, although it has already been noted elsewhere (Hill (**14**)), and that is the remarkable number of eggs discharged from the ovary at each ovulation. In one case twenty-eight eggs, in two others thirty eggs, and in yet another thirty-five vesicles were obtained from the two uteri. "There can be no doubt that Dasyurus, like various other marsupials—e. g. Perameles, Macropus, etc.—has suffered a progressive reduction in the number of young reared, but, even making due allowance for that, the excess in production over requirements would still be remarkable enough" [1] (Hill (**14**)).

[1] Examination of the ovaries of D. maculatus with full-grown follicles shows that the same excessive production of ova holds true also for this species.

Ovulation may be succeeded by one of two states. On the one hand, if the ova be fertilised, there follows a period of pregnancy, which in its turn is succeeded by a nursing period. On the other hand, in the absence of copulation or fertilisation, there follows an interval in which the various organs undergo a series of marked alterations, essentially of the same nature as those normally undergone in the pregnant and post-partum female. To this period, therefore, we propose to give the name of "pseudo-pregnancy."

Pregnancy.

If fertilisation is effected, ovulation is normally succeeded by pregnancy. The ova are fertilised in the upper parts of the Fallopian tubes, and the second polar body is there given off. They pass down the tubes, apparently with considerable rapidity, into the uteri, where cleavage begins (Hill (**14**)).

We have already directed attention to the fact "that there is no correlation between the number of ova shed during ovulation and the accommodation available in the pouch (Hill (**14**)), the number of ova shed at one period being, as a rule, far in excess of the normal number of teats (six). Our records show that out of seventy-two pregnant females in which there is a definite record of the number of eggs or embryos, thirty-five had more than twenty, and the remaining thirty-seven less than twenty. Moreover, whilst amongst the thirty-five one had as many as thirty-five vesicles, in only three of the remaining thirty-seven were there less than six embryos, and in one of these only one uterus was pregnant. Although a proportion of the eggs may for one reason or other fail to develop normally, it would appear to happen generally that a larger number of young are born than can possibly survive, the pouch accommodation being strictly limited. Our records afford two specific instances of females which were examined shortly after parturition. In one of these, eighteen young were found, of which eleven occurred adhering to the hairs round and directly below the opening of the pouch, six were attached one to each nipple, and one

occurred free in the pouch alongside an attached one. In the other, ten young were found, six attached, three free in the pouch, and one outside considerably shrivelled.

Gestation Period.

Attention has already been called to the fact that ovulation is entirely independent of copulation, a fact which renders it extremely difficult, if not impossible, to determine accurately the length of the gestation period. One of us has already recorded that "the shortest period observed between coitus and the birth of the young was a little over eight days," hence it was concluded "that the time of gestation does not exceed this period" (**14**). Selenka, it may be noted, gives the gestation period of Didelphys as barely eight days (**23**). As regards our eight-day record, we think it is desirable to give further details. Copulation was observed on June 7th, about ten a.m. It lasted a comparatively short time. The female was killed on June 15th at six p.m. A single young one (recently born, and measuring 7·5 mm. greatest length in the fresh state) was found in the pouch, which was greatly enlarged and possessed prominent sebaceous glands. Our notebook record continues, "assuming that copulation had not taken place before receipt of this female, we can put down the gestation period as a little over eight days." There are two points in this record which should be noted: first, the brevity of the copulation, and second, the question of copulation previous to receipt. The recollection of one of us regarding this particular female is that she was brought in by the collector, placed at once with the male, a short copulation, or an attempt at the same, ensuing almost immediately.

In view of the impossibility of entirely excluding the occurrence of copulation previously, we cannot regard the foregoing evidence as absolutely conclusive as to the length of the gestation period. At the same time it should not be forgotten that a female once served will not under normal circumstances again copulate. It is of course quite possible

that the circumstances in this particular case were not normal, since this female was no doubt in a cowed and frightened condition when placed in the cage with the male.

We have another record of a female[1] in which the young were born sixteen days after copulation. This particular female was received on June 20th. Copulation occurred on the 24th, and parturition on July 10th. This record we regard as perfectly trustworthy. The question as to when ovulation occurred, however, cannot be answered with any degree of accuracy. We have already stated that ovulation occurs at a variable period after œstrus. In our records we find that the shortest time intervening between copulation and the finding of unsegmented eggs in the uteri is four days (in this particular case the eggs were in the unsegmented and 2-celled stages), and the longest time, eight days. Between these limits we have records of the following: five days after, no ovulation (three cases); five days after, unsegmented ova (one case); five days after, unsegmented and 2-celled eggs (one case); five days after, 4-celled eggs (one case); six days after, unsegmented ova (one case); six days after, 2-celled eggs (one case); seven days after, unsegmented ova (one case).

The average interval between copulation and ovulation would thus appear to vary round about five or six days. If, now, we subtract five from the sixteen-day record given above, we have a gestation period of about eleven days. From the evidence we think we are justified in stating that the gestation period in Dasyurus is not less than eight, and does not exceed fourteen days.

General Changes.

The ovarian changes following ovulation, i.e. those resulting in the formation of the corpora lutea, have been fully

[1] This female is the one previously referred to (ante, p. 148) as having given birth to eighteen young.

dealt with by Sandes (**22**). It need only here be mentioned that the corpora lutea essentially resemble those of Eutheria in their mode of development and structure. Sandes (p. 380) states that "the corpus luteum forms quickly, within three days [after ovulation], and persists [not only throughout pregnancy but] during the greater part of the time that the animal is lactating, ultimately disappearing when the young animal is capable of leading an independent existence." The changes in the pouch, including its sebaceous, sweat, and mammary glands have been described elsewhere (O'Donoghue (**20**)), whilst a preliminary account of the arrangement of the fœtal membranes, the placentation, and the mode of parturition, has also been published (Hill (**13**)).

Early Pregnant Uteri.

Case 1 (No. 15, 19 . vii . '01) (Pl. 6, fig. 5).—We give here for purposes of comparison a brief account of the uterus of a pregnant female with ova at the stage before the separation of the yolk-body and shortly after entering the uteri.

The record of this female is a very complete one, extending over a period of forty-one days. We reproduce it here:

8 . vi . '01.—Resting; so on to 6 . vii . '01.

9 . vii . '01.—Cloacal margins very slightly swollen.

10 . vii . '01.—Fresh male in; no copulation.

11 . vii . '02.—Cloacal margins swollen, but not greatly.

13 . vii . '02.—Cloacal margins swollen. Fresh male in; copulation.

17 . vii . '02.—Pouch hardly altered; cloacal swelling still present.

18 . vii . '02.—Pouch very slightly tumid, very dirty.

19 . vii . '02.—Pouch distinctly tumid, not cleaned.

Killed, i.e. six days after copulation.

The uteri measured 1·3 cm. by 1·3 cm. by ·7 cm., the left containing ten ova and the right fifteen.

The mucosa has a maximum thickness of 2·1 mm. The uterine epithelium (·016–·018 mm. thick) is little advanced,

appearing as a low columnar epithelium with close-set nuclei, plump and rich in chromatin. The uterine glands (·04–·05 mm. in diameter) are well convoluted, luminated throughout, and ciliated. The connective tissue is markedly œdematous in the superficial half of the mucosa, and particularly rich in leucocytes; in the deeper part of the same, between the basal coils of the glands, it is more cellular. Below the uterine epithelium there is the usual compact zone of connective tissue, which is rich in leucocytes and fairly vascular.

Case 2 (No. 13, 25 . vii . '02).—Female killed four days after final copulation, with 1 and 2-celled ova and abnormals in the uteri. The uterus is essentially similar to that of Case 1. Mitoses in the uterine and glandular epithelium are not infrequent.

The Nursing Period.

Parturition in mammals is followed by a period termed by Heape (**11**) the "nursing period," during which the young are nourished by the milk secreted in the mammary glands of the mother. As is known, there is a noteworthy difference in the way in which the milk is obtained by the young in the Eutheria and Metatheria. In the former the young are perfectly free and seek the teat of the mother, from which they suck the milk. The new-born marsupial, on the other hand, which is brought forth in a much less advanced condition than the Eutherian, is transferred to the pouch, presumably by the mother with the aid of her lips, and becoming fast to a teat, the milk is pumped down its throat.

The nursing period in the marsupial falls into two distinct phases:

(1) A period of fixation, during which the lips of the young one are fused in such a manner that it is firmly attached to the teat, which it cannot leave. During this period the milk is said to be forced periodically into its mouth by the contraction of the muscles of the pouch area of the parent. This period in Dasyurus lasts from seven to eight weeks (**13**).

(2) A free period, during which the lips of the young animal are no longer fused, so that it can leave the teat at will, but is still entirely dependent on its mother for food. In Dasyurus, this period extends over eight or nine weeks. The total time occupied by these two periods, which together form the true nursing period, is about four months in Dasyurus (**13**).

After this period, the young move about freely away from the mother, and begin to eat, although they still may make occasional use of the pouch.

This marks the conclusion of the reproductive cycle, and the various organs now return to an anœstrous condition, or state of rest, until the following year.

Pseudo-Pregnancy.

Ovulation is followed by the formation of corpora lutea. According to Ancel and Bouin (**1**), in animals in which ovulation is spontaneous, two distinct kinds of corpora lutea are formed; (*a*) in the pregnant animal, a gestative corpus luteum (corpus luteum verum), which persists for a long time, and (*b*), in the non-pregnant animal, a periodic corpus luteum (corpus luteum spurium), which is not so fully developed as the former, and has only a transitory existence. No such distinction is possible in Dasyurus, for the structure of the corpus luteum is identical, whether pregnancy follows ovulation or not, and there is evidence to show that in the non-pregnant animal the corpus luteum persists for a considerable time, some weeks at least, and even then shows no sign of degeneration (vide **21**).

The pouch continues to enlarge after ovulation, and the sweat and sebaceous glands of the pouch area reach a state of development and activity comparable to that attained in the pregnant animal. In some cases, it was observed that the female started to clean out the pouch in the same way as does the pregnant female, in preparation for the reception of the young.

The most striking changes during this period, however, occur in the mammary glands. These changes have already been fully described (**20**), so that here it is only necessary to emphasise the fact that the glands hypertrophy in precisely the same way as do those of the pregnant animal, and ultimately reach a state of development, which is at least as advanced as that in a female thirty-six hours after the birth of the young.

Uterine Changes.

Case 1 (31 . v . '01).—This female died in captivity the day after its arrival. The uteri were slightly enlarged, but no ova were found. Examination of the ovaries reveals the presence of corpora lutea in the last stages of growth, there being present in their central region small spaces not yet filled either by lutein cells or by connective tissue. Ovulation must have occurred some days previously.

The uterine mucosa measures 2–3 mm. in maximum thickness. The uterine epithelium (·027 mm. thick) is formed of narrow columnar cells with closely packed small nuclei, ovalish or rounded in form, occupying the mid-region of the layer. The uterine glands appear crowded together and are markedly convoluted. The connective tissue is compact below the epithelium and fairly so between the glands. The mucosa is not specially vascular.

This case is of importance as showing the condition of the uterus in a female some time after ovulation and before the onset of the degenerative and regenerative changes in the mucosa.

Our next two cases are of special interest as showing stages in these degenerative and regenerative changes in the non-pregnant uterus after ovulation, changes which we are convinced are the homologues of those seen in the normal post-partum uterus.

Of Cases 2 and 3 we have no further record beyond the statement that the uteri were opened but nothing was found.

Case 2 (vii.06) Pl. 7, fig. 6. Corpora lutea are present in the ovaries. They are at a stage towards the end of the growth period, and in their degree of development are intermediate between those of Case 1 and Case 3.

The mucosa has a maximum thickness of 2·9 mm. The uterine epithelium appears as a layer of quite low cubical cells (·006—·009 mm. in thickness), which essentially resembles that of a uterus seven days post-partum. Vacuolar spaces occur here and there in the epithelium, but the nuclei on the whole are plump and rich in chromatin and show no obvious signs of degeneration. It is therefore probable that the uterine epithelium has already been reconstituted.

The uterine glands are in a very interesting condition, the appearances presented strikingly recalling those seen in the glands of post-partum uteri. Over the major portion of the thickened area of the mucosa, the basal portions of the glands are markedly convoluted and greatly hypertrophied but show no signs of degenerative change. They average in diameter ·072 mm. (varying from ·056—·1 mm.), and are lined by an epithelium composed of very narrow, high columnar cells (·024—·04 mm. in height), with small basally situated nuclei. The gland lumina are distinct, but small, and in many cases contain a homogeneous secretion which stains deeply with esoin. The convolutions of the glands are very compactly arranged, with little or no connective tissue between them.

Comparison of these hypertrophied glands with those of a pregnant uterus with blastocysts 1·5 mm. in diameter shows that whilst the gland convolutions in the latter are not so compactly arranged, the glands themselves are slightly thicker (·08—·096 mm. in diameter, epithelium ·032 mm.) and the gland lumina are much larger.

In the more superficial region of the thickened area of the mucosa, as well as round its periphery, the glands and the portions of them situated therein present a very different appearance. They are much less convoluted and much less compactly arranged. Moreover, instead of possessing thick glandular walls and small lumina, they are lined by an

epithelium of the quite low cubical type (·006—·015 mm. in thickness) and possess large and distinct lumina. These latter are not empty, but contain either a homogeneous coagulum in which cells may be embedded, or, as is more often the case, they are occupied by a more or less compact mass of cells (Pl. 7, fig. 9). The cells possess large, rounded or polygonal cell-bodies which stain deeply with eosin, and nuclei which are occasionally found in division and which are quite similar to the nuclei of the surrounding epithelial cells.

The transition between these altered portions of the glands and the more deeply situated unaltered or rather hypertrophied parts is somewhat abrupt, so that it is difficult to obtain very definite evidence as to how the intra-luminar cells originate. We are of opinion, however, that they are derived from the hypertrophied gland epithelium by a kind of desquamation process, and that they increase in size after their separation. If that view is correct, then we must regard the epithelium of the thin-walled portions of the glands as being in process of regeneration. In this connection, it is worthy of note that mitoses are not rare in the epithelium, and that the superficial portions of the glands close below the openings into the uterine cavity are ciliated.

The connective tissue is no longer œdematous, but appears as a compact, richly cellular tissue. Leucocytes are not specially abundant, but are most numerous in the more superficial region of the mucosa, where there are also present numbers of capillaries of varying size. It is worthy of note that there are no blood extravasations.

The uteri in this Case, with the uterine epithelium and much of the gland epithelium regenerated, may therefore be described as well on the way towards the resting condition. The uterine epithelium resembles that of the seven days' post-partum uterus, the glands, those of the two days' post-partum uterus.

Case 3 (1900) (Pl. 8, fig. 7).—The ovaries show corpora lutea at the end of the growth period, just older than those of Case 2.

The mucosa has a maximum thickness of about 3·5 mm. and presents a markedly folded surface. The uterine epithelium appears as an irregular folded layer of low columnar cells and shows evident signs of degenerative changes, e.g. irregularities in contour both of its cells and nuclei, occurrence of vacuoles in the cytoplasm, presence of degenerated nuclei and of diffused darkly staining (chromatinic ?) granules. Moreover, blood extravasations from the superficial capillaries would appear to have taken place through clefts formed by the breaking down of the epithelial cells. These extravasations appear to have been slight, but are undoubted. Besides corpuscles, there are present in them, cell remnants and small, darkly staining granules. In this female, accordingly, the uterine epithelium, unlike the preceding case, has not yet been reconstituted.

The uterine glands are in essentially the same condition as those of Case 2. In the deep portion of the central region of the thickened area of the mucosa, the parts of the glands therein situated appear for the most part closely packed and hypertrophied. The gland epithelium is formed by high, columnar, pale-staining cells, often vacuolated, and with small basally situated nuclei. The gland lumina are distinct and in some cases contain a staining coagulum.

Elsewhere the glands are in process of being reconstituted. They are lined by a low flattened to cubical epithelium and their lumina are occupied either by coagulum with embedded cells or by cellular masses, which differ from those of Case 2 only in that the cells are in course of degeneration.

The connective tissue differs strikingly from that of Case 2, being in the form of a fine reticular tissue, œdematous and extremely rich in leucocytes.

Numerous enlarged blood-vessels are present in the mucosa, whilst in actual contact with the under surface of the uterine epithelium there are numbers of fine capillaries such as occur normally in this position in the late pregnant uterus.

Case 4 (31 . vii . '99) (Pl. 7, fig. 8.—The record is as follows :

14 . vii . '99.—Female brought in.

15 . vii . '99.—Put with male ; no record of copulation.

17 . vii . '99.—Pouch large, dirty.

20 . vii . '99.—Pouch large, fairly clean, teats red, sebaceous glands just appearing.

22 . vii . '99
24 . vii . '99
26 . vii . '99
28 . vii . '99
} Pouch relaxed, but not quite clean, glands small.

31 . vii . '99.—Pouch quite relaxed, moist, glands fairly well marked; apparently pregnant.

Killed, but nothing found in the uteri, which were enlarged. The ovaries were sectioned and show full-grown corpora lutea, somewhat older than those of Case 3.

The mucosa is thickened and its surface is thrown into well-marked folds. The uterine epithelium is very similar to that of Case 3, and is for the most part in process of degeneration. In places it appears as a practically unaltered layer of cubical cells, but over most of its extent, it exhibits evident signs of degenerative change. It is very irregular both in contour and thickness and is invaded by leucocytes. Moreover, cells may be seen in process of desquamation from its outer surface, and in places, it appears actually to have broken down, since extravasated blood, in which occur degenerate cell-products, is present in small quantities in the uterine cavity close to the surface of the epithelium.

The uterine glands have been reconstituted throughout their extent. They are lined by a quite low epithelium, and their lumina are occupied more or less completely by cellular masses (containing leucocytes), in a more or less advanced stage of degeneration.

The connective tissue is very cellular and is invaded by large numbers of leucocytes.

Making allowance for individual variations due to age or other causes, the foregoing Cases afford evidence of the occurrence of progressive, regressive and regenerative changes in the uterine mucosa, during this period of pseudo-pregnancy.

(a) Progressive Changes.—The mucosa as a whole undergoes marked thickening, its vessels enlarge, and in particular a rich plexus of capillaries is established immediately below the uterine epithelium. The uterine glands hypertrophy, their epithelial cells assume an elongate columnar form and secrete actively.

These changes are identical with those seen in normally pregnant uteri. The only noteworthy difference in the progressive alterations in the pseudo- and normal pregnant uterus concerns the uterine epithelium, the cells of which in the former fail to become transformed into the high columnar elements characteristic of the latter.

(b) Regressive Changes.—The uterine epithelium does not appear to be shed as a whole, but undergoes partial degeneration and desquamation, and it is noteworthy that these degenerative changes may so effect the immediately underlying capillaries as to lead to their rupture and the consequent appearance of extravasated blood in small quantities in the uterine cavity.

We have so far not observed such extravasation in normal post-partum uteri, though it is possible that this may occasionally occur. On the other hand, the very marked extravasations into the connective tissue which are found in post-partum uteri are not met with in the pseudo-pregnant cases.

The uterine gland epithelium also appears to undergo a process of desquamation—at all events cells are shed from it into the gland lumina, where they form cellular masses which eventually degenerate. Comparison of our preparations of pseudo-pregnant uteri with those of normal post-partum uteri demonstrates beyond all possibility of doubt that the regressive changes in the glands are identical in the two. In post-partum uteri, we find the same reduction of the gland epithelium from the high columnar to the low cubical type taking place, and accompanied, as in the pseudo-pregnant uterus, by the appearance in the gland lumina, of masses of cells which eventually degenerate.

(c) Regenerative Changes.—The uterine epithelium

is reconstituted, and the low cubical epithelium of the uterine glands persists like that of the post-partum uterus to form the lining of the resting glands. The connective tissue reassumes its more compact form, and the mucosa as a whole undergoes marked decrease in thickness and returns to the condition of rest.

It is difficult, indeed, impossible, to ascertain the exact duration of this period of pseudo-pregnancy, but so far as we can judge with the aid of our records, it probably extends over about two weeks. We have, for example, a very full record of a female, apparently in heat about June 28th and killed on July 15th, i. e. seventeen days after heat, and of another female in heat on June 9th (copulation on June 9th to 12th) and killed on June 30th, i. e. twenty-one days after heat. In both, nothing was found in the uteri. In both, the ovaries showed old corpora lutea, the pouch was greatly enlarged the mammary and other glands being well-developed, whilst the uteri were extremely large and vascular (measuring in the first-mentioned female by 4 by 1 cm. and in the second 3·9 by 4·1 by 1·6 cm. in diameter).

Metœstrus.

After the changes described in the preceding section have taken place, the whole of the reproductive organs gradually return to a state of rest. This metœstral period corresponds functionally to the similarly named one in the Eutheria, only there is the striking difference that, whereas in the unimpregnated Eutherian mammal, metœstrum follows immediately after ovulation, in the unimpregated marsupial, the two are separated by post-œstrus and pseudo-pregnancy, the two periods together occupying at least a fortnight, and probably longer.

Summary.

Dasyurus is monœstrous and has one breeding season a year, which begins at the end of May or early in June and

lasts into the first fortnight in August (i.e. it extends over the winter months).

The male does not appear to experience a marked rutting season.

Copulation is similar to that of Didelphys (Selenka), and the sperms can remain alive in the Fallopian tubes for at least two weeks.

Anœtrus.—The anœstral period lasts more than half the year.

Pro-œstrus.—Pro-œstrus appears to extend over a varying period of from four to twelve days.

During this time, the lips of the cloaca become swollen, the pouch enlarges somewhat and becomes slightly tumid and moist, and the Graafian follicles increase in size and become vesicular. The uterine mucosa increases in thickness and becomes more vascular, its glands lengthen and become convoluted and the uterine epithelium also tends to thicken.

Œstrus.—Œstrus lasts usually for one or two days and is the period during which copulation occurs.

The changes already initiated during pro-œstrus in the various parts of the reproductive system are continued without interruption.

Post-œstrus.—Post-œstrus, which term we employ to designate the period following œstrus and terminated by ovulation, occupies as a rule about five or six days.

The tumidity of the cloacal lips disappears, but the changes in the pouch and uterus still continue, not, however, very actively.

In the ovary (1) the ova give off the first polar body and the spindle for the second meiotic division is formed.

(2) The follicles attain maturity and ultimately rupture, setting free the ova.

Ovulation.—Ovulation marks the end of this period and occurs generally about five or six days after œstrus. It is spontaneous and independent of copulation and is remarkable because of the large number of ova liberated. Ovulation is succeeded (a) by pregnancy or (b) by pseudo-pregnancy.

Pregnancy.—Fertilisation is effected in the upper part of the Fallopian tube and the second polar body is there given off.

As a rule more young are born than can possibly survive owing to the limited accommodation in the pouch.

The gestation period is not less than eight and not more than fourteen days, but the interval between copulation and birth is usually considerably longer.

Corpora lutea are formed and persist throughout the greater part of the time that the animal is lactating.

Nursing Period.—This period may be divided into two phases.

(1) Period of Fixation.—A period, lasting for seven or eight weeks, during which the young are firmly attached to the teats.

(2) Free Period.—A period of eight or nine weeks when the young are free in the pouch but dependent on the mother for food.

After this time the various organs gradually return to a state of rest.

Pseudo-pregnancy.—We have applied this term to the period following ovulation in cases where the ova have failed to develop, because of the occurrence in it of a series of changes in the reproductive organs essentially identical with those met with in pregnant females.

Corpora lutea are formed in the ovaries which are identical with those in the pregnant female.

The pouch enlarges, and its sweat and sebaceous glands reach a state of hypertrophy and functional activity comparable to that in the pregnant female.

The mammary glands also enlarge and reach a state of development equal to that in a female thirty-six hours after parturition.

The uteri enlarge and become vascular, often to a marked degree.

The uterine mucosa undergoes a series of changes, progressive, regressive and regenerative.

THE ŒSTRAL CYCLE IN THE NON-PREGNANT FEMALE DASYURUS.

	General symptoms.	Uterine changes.	Ovarian changes.
ANŒSTRUS . . .	Rest.	Rest.	Rest.
PRO-ŒSTRUS . .	Swelling of cloacal lips. Pouch commences to become tumid.	Commencement of constructive stage. Hypertrophy of mucosa with increase of vascularity. Proliferation in uterine epithelium and glands.	Growth of follicle and contained ovum.
ŒSTRUS . . .	Period of desire.	Continuance of above.	Continuance of above.
POST-ŒSTRUS . .	Pouch tumid and slightly moist, growth of sweat and sebaceous glands of pouch area. Regression of cloacal swelling.	Continuance of above but not very actively.	Follicles attain maturity. Separation of first polar body and formation of second polar spindle. Ovulation.
PSEUDO-PREGNANCY .	Further enlargement of pouch accompanied by growth of mammary glands as in pregnancy.	Continuance of above. (a) Progressive changes in uterine glands especially. (b) Regressive (destructive) changes, e.g. degeneration and desquamation of uterine and gland epithelium. Extravasation of blood. (c) Regenerative (repair) changes. Recuperation of mucosa.	Formation and growth of corpus luteum as in pregnancy.
METŒSTRUS . .	Return to rest.	Return to rest.	Return to rest.

ANŒSTRUS.—As Dasyurus is monœstrous, metœstrus is followed by anœstrus, which lasts until the following year.

THE ŒSTRAL CYCLE IN THE NON-PREGNANT FEMALE EUTHERIAN.

	General symptoms.	Uterine changes.	Ovarian changes.
ANŒSTRUS . .	Rest.	Rest.	Rest.
PRO-ŒSTRUS . .	Swelling and flushing of vagina. General symptoms termed by breeders "coming in heat."	Constructive stage: Swelling of mucosa. Proliferation in uterine epithelium and glands. Hyperæmia and congestion. Destructive stage: Increased hyperæmia. Breakdown of vessels and formation of blood lacunæ.	Growth of Graafian follicle.
	Appearance of menstrual discharge if present.	Rupture of lacunæ, degeneration of uterine and glandular epithelia. Formation of menstrual clot if present.	Maturation of ovum.
ŒSTRUS . . .	Period of desire. Vagina still swollen and flushed.	Stage of repair: Recuperation of various uterine elements.	Discharge of ova (in animals with spontaneous ovulation).
METŒSTRUS .	Return to rest.	Return to rest.	Formation of corpus luteum spurium (in animals with spontaneous ovulation).

One of two conditions may then ensue. (1) The various organs may remain for a few days in a stage of comparative inactivity, but almost immediately recommence the changes indicative of Pro-œstrus. This short interval is termed diœstrus, and the animal is said to be Polyœstrous. (2) The various organs may remain for a long time in a state of rest, this being the proper Anœstrus, and the animal is said to be Monœstrous.

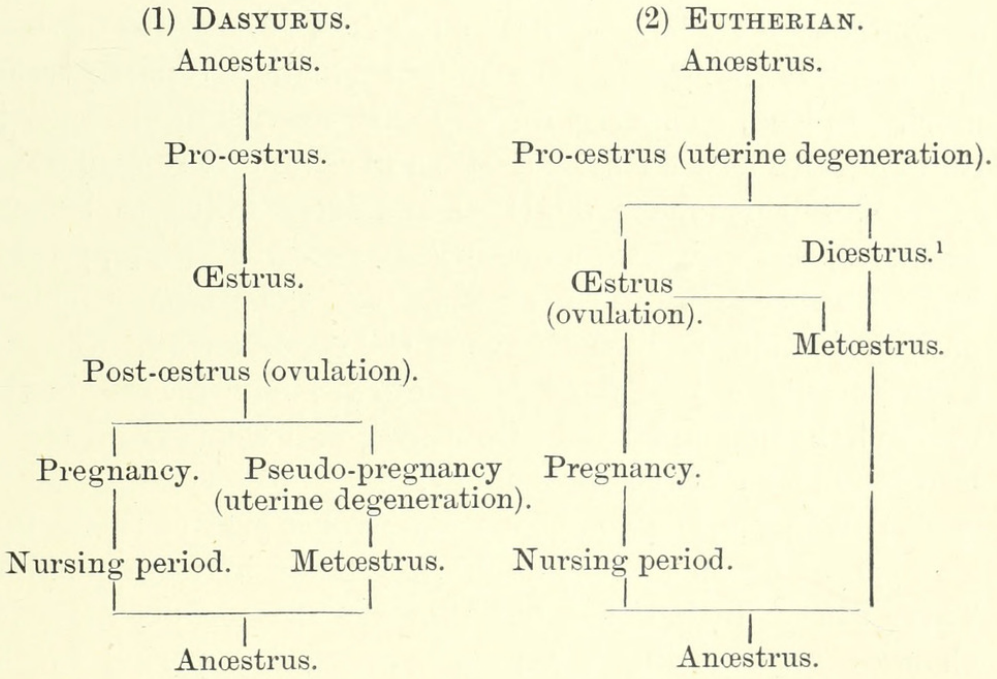
Metœstrus.—This is an indefinite period during which the whole of the reproductive organs return to a state of rest.

Conclusions.

Comparison of the Œstral Cycles in the Metatheria and Eutheria.

A comparison of the tables of the œstral cycle in the non-pregnant Dasyurus and in the non-pregnant Eutherian (pp. 163–164), following the schemes of Heape (**11**) and Marshall (**18**), discloses certain rather striking and important differences between them. These differences are seen at a glance in the

Scheme of Reproductive Cycle in—



[1] This period only occurs in polyœstrous animals.

scheme which we have drawn up of the reproductive cycle in the Marsupial (as represented by Dasyurus), and what may be taken to be the usual cycle in the Eutherian mammal as set forth by Heape and Marshall. They concern more especially the time-relations of the degenerative uterine changes

and of ovulation to the cycle as a whole. Thus, taking œstrus as an easily recognisable and obviously equivalent event in the two cycles, we see that, whilst in the Marsupial, ovulation only occurs at an interval of some days after œstrus, in the Eutherian, ovulation either coincides with, or follows immediately after œstrus. We have emphasised this important difference by applying the term "post-œstrus" to the period intervening between œstrus and ovulation.

Furthermore, there is a very striking discrepancy in the time of occurrence of the degenerative changes in the uterine mucosa in the two. In the marsupial, these succeed ovulation; in the Eutherian they not only precede ovulation, but also œstrus. This, we regard as the most striking difference in the two cycles.

In the preceding pages we have produced evidence demonstrative of the essential similarity in Dasyurus of these degenerative changes in the pseudo-pregnant and post-partum uteri. Indeed, comparison of the entire series of changes in the organs directly concerned with and related to reproduction in females which have ovulated and have failed to become pregnant, and which we have already described (ante, pp. 21–28), with those seen in the same parts of the pregnant female, entirely justifies us in recognising the occurrence of what we have termed a period of pseudo-pregnancy in the œstral cycle of the marsupial and in affirming that that period represents the phase of true pregnancy.

That being so, the question arises, What part of the Eutherian cycle corresponds to pseudo-pregnancy in the marsupial? There can be little doubt but that the uterine degenerative changes seen during the pseudo-pregnancy period in the marsupial are equivalent to those which take place in the Eutherian uterus during pro-œstrus, a conclusion which we think, in view of the evidence herein presented, will meet with general acceptance.

These pro-œstral changes in the Eutherian uterus condition the appearance in some members of the sub-class (e.g. Primates) of a sanguineous discharge—the menstrual

flow. This latter we regard as the morphological and physiological equivalent of the degenerated epithelial elements and blood extravasations met with in the pseudo-pregnant uterus of the marsupial.[1] We are therefore in complete agreement with those writers (Van Herwerden (**12**), Grosser (**7**), Hitschmann and Adler (**15**), Beard (**3**) and others) who hold that "menstruation . . . is the expression of changes in the uterine mucous membrane which are associated with preparations for the reception of a fertilised ovum," and that "menstruation itself is only a secondary process—a degeneration of the mucous membrane which from a failure of pregnancy has not been able to fulfil its purpose" (Grosser, loc. cit., pp. 97 and 102).

Our observations afford no support for the view that "menstruation is identical with 'heat'" (Heape (**11**) p. 59), nor for the view "that menstruation in the Primates is the physiological homologue of the pro-œstrum in the lower mammalia" (Marshall (**18**) p. 162).

As is generally recognised, menstruation is simply the outcome of degenerative uterine changes, and although these are manifested at the end of the pro-œstral period in Eutheria, our observations demonstrate that in the marsupial, they succeed both œstrus and ovulation, and cannot therefore be considered as forming any part of the pro-œstral phase.

Now, bearing in mind the lowly position which the Marsupials occupy in the mammalian series, and taking into

[1] Wiltshire states (**27**, p. 397), on the authority of Bartlett, that in kangaroos in the gardens of the Zoological Society, "a mattery, slimy" discharge from the cloaca, slightly tinged with a reddish colour, had been observed by the keeper in females at the time of "heat." We have one record of the occurrence of an apparently corresponding discharge from the cloacal aperture in Dasyurus. The discharge is described in our notes as "a whitish glairy secretion, consisting of refractive granules, round and angular," and is regarded as the secretion of the cloacal glands. We are not inclined to attach any importance to its occurrence in the present connection, but it is quite possible that the cloacal glands may become more active during proœstrus.

account the close similarity which is apparent between the cyclical changes in the pregnant and non-pregnant marsupial, it can hardly be doubted that the œstral cycle in this group is not only simpler but much more primitive than that of Eutheria, so far as known. If this be admitted, then it follows that, in the Eutheria, the inclusion in the pro-œstral period, of the degenerative uterine changes which condition menstruation is a purely secondary phenomenon, the result of a thrusting forward of these events to a much earlier period in the œstrous cycle as compared with the marsupial.

So far, then, as concerns the uterine changes during pseudo-pregnancy, we conclude that these are represented in the Eutheria by the alterations which occur in the mucosa during the latter part of pro-œstrus, and which, in some forms, culminate in menstruation.[1]

What induced this remarkable dislocation of events in the Eutherian œstrous cycle is a problem by no means easy of solution, but we venture to suggest that it may perhaps be brought into relation with the omission or marked shortening in the Eutheria, of the post-œstral period whereby ovulation is directly transferred to œstrus.

It is legitimate to assume that the shortening of the cycle in this way may have induced an increased growth of the mucosa during the pro-œstral period, and that this growth in its turn may have directly conditioned the earlier occurrence of the degenerative and regenerative changes, with the result that these latter now came into operation before instead of after the ovulation to which they were primitively related. Whether or not there be anything in these suggestions, there can be little doubt that the precocious onset of the degene-

[1] The histological changes in the premenstrual uterus of the human subject have been fully described by Hitschmann and Adler in their important paper (15) published in 1908. These observers demonstrate that the changes in the premenstrual uterus are identical with those seen in the early pregnant uterus. To this paper, which contains a very full bibliography, the reader is referred for further details.

rative changes in the mucosa, followed as they are by regenerative growth, is of the nature of an adaptation, of obvious advantage, as is generally recognised, from the point of view of early placental formation (embedding, trophoblastic attachment, etc.).

Whilst, then, we believe that the uterine changes characteristic of the pseudo-pregnant period in the marsupial have been shifted forwards to pro-œstrus in the Eutheria, it remains to be pointed out that amongst the Eutheria, alterations in the ovary and mammary gland corresponding to those seen in the pseudo-pregnant marsupial have indeed been recognised, but do not appear to have been brought into correlation with the œstral cycle.

Various authors (e. g. Ancel and Bouin (**1**)) have described the formation of corpora lutea spuria, so-called, which in animals ovulating spontaneously, last for a shorter time than in the pregnant female.

Further, as regards the mammary glands, it has been stated (Frank and Unger (**6**) and others) that a growth of these occurs during the œstral cycle, and according to Ancel and Bouin (**2**), only after the formation of corpora lutea. One of us (O'D.) has evidence confirmatory of this latter statement so far as concerns the rabbit.

These changes in the ovary and mammary glands have thus retained their original position in the cycle, i. e. they occur after œstrus and ovulation as in the marsupial.

The Monœstrous and Polyœstrous Conditions.

According to Heape (**11**), monœstrous mammals are those which experience a single œstrus during each sexual season, i. e. in which the anœstrous cycle alone occurs, whilst polyœstrous mammals are those "whose sexual season is occupied by a series of diœstrous cycles, or in other words, those who experience a series of recurrent œstri" (p. 9). Heape questions "if in the present state of our knowledge it is possible to determine which is the original of these two con-

ditions" (Heape, loc. cit., p. 18). We propose here to consider this question very briefly in the light of such evidence as we have of the œstral cycle in the Marsupials and Monotremes, which in respect of their reproductive phenomena generally (but making exception of the pouch of the Marsupials) are undoubtedly more primitive than the Eutheria.

We have already shown earlier in this paper that the œstrous cycle in Dasyurus occurs only once in the breeding season, i. e. that Dasyurus is monœstrous. As regards other Marsupials we have no definite evidence, but our records, and those of others (Semon (**25**) Caldwell (**5**)), indicate that Trichosurus, Phascolarctus and Phascolomys breed but once a year, each species having its own particular breeding time, regularly recurring. We think in view of the positive evidence we have concerning Dasyurus, the cautious statement of Heape (loc. cit., p. 21) that "among certain wild animals which are known to undergo parturition only during a very circumscribed time, the monœstrous condition may be assumed as probable . . . ," may be accepted and applied to the forms mentioned.

Selenka, however, makes the definite statement in regard to Didelphys marsupialis (**23**, p. 104)—"Die Brunst der Weibchen tritt normaler Weise nur ein Mal im Jahre ein. Wenn aber den Mutterthieren die Jungen kurz nach dem Gebären aus dem Beutel fortgenommen wurden oder wenn die Begattung, was öfter vorkam, aus Mangel an Geschicklichkeit der Männchen nicht gelang, so können die Weibchen 4–6 Wochen später zum zweiten male im Jahre brünstig werden, spätestens jedoch Anfang Juni."

Further, with reference to Hypsiprymnus cuniculus, he states (**24**, p. 174)—"Die herrannahende Brunst . . . zu verschiedenen Jahreszeiten, im Frühling, Herbst und Winter beobachtet wurde."

Wiltshire, again (**27**, p. 397), states on the authority of Bartlett that the kangaroos in the Gardens of the Zoological Society "display sexual excitement in September . . . and also in our spring month of April."

We have had no experience of Didelphys, but cannot regard Selenka's statement as indubitable in the absence of any record of the condition of the ovaries, whilst as regards Macropus spp., we can only say that our records indicate that in New South Wales at all events they certainly breed during the summer months (December to February). Irrespective, however, of the number of breeding seasons per year, we have no evidence as to whether the macropods are monœstrous or not.

As regards the Monotremes, our own experience confirms the statement of Caldwell (**5**) and Semon (**26**)[1] to the effect that the breeding season recurs but once annually. In view of the facts that at most two eggs in the single functional ovary reach maturity at the same time, and that the breeding season is of such short duration that it would appear to be impossible for a second set of eggs to become full-grown within its limits, we consider it justifiable to assume that Monotremes are, like Dasyurus, monœstrous.

In view of these considerations and of others relative to the breeding habits of reptiles which we do not think it necessary to bring forward here, and in view, moreover, of the lowly position occupied by the monotremes and marsupials in the mammalian series, we find it difficult to avoid the conclusion that the monœstrous condition is the primitive one, and that the polyœstrous condition has been secondarily derived from it. This latter condition where it occurs amongst the Eutheria is obviously advantageous, since it permits of the production of a larger number of young, or at least provides greater opportunity for successful impregnation.

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EXPLANATION OF PLATES 6, 7 AND 8.

Illustrating Prof. J. P. Hill and Dr. Chas. H. O'Donoghue's paper on "The Reproductive Cycle in the Marsupial, Dasyurus viverrinus."

[All the photo-micrographs are from uteri of Dasyurus and are taken at a magnification of 30 diameters except figs. 9 and 10, the magnification of which is 250 diameters.]

PLATE 6.

Fig. 1.—Uterus, pro-œstral Case 1 (17 . vi . '99).

Fig. 2.—Uterus, pro-œstral Case 2 (2 . 21 . v . '03).

Fig. 4.—Uterus, post-œstral Case 1 (8, 23 . vii . '02).

Fig. 5.—Uterus, early pregnant Case 1 (15, 19 . vii . '01).

PLATE 7.

Fig. 3.—Uterus, œstral Case 1 (12 . vi . '02).

Fig. 6.—Uterus, pseudo-pregnant Case 2 (vii . '06).

Fig. 8.—Uterus, pseudo-pregnant Case 4 (31 . vii . '99).

Fig. 9.—Portion of mucosa of same uterus as fig. 6, showing uterine glands lined by low cubical epithelium and with their lumina filled by masses of cells.

PLATE 8.

Fig. 7.—Uterus of pseudo-pregnant Case 3 (1900).

Fig. 10.—Portion of a uterine gland from the same uterus as fig. 3, showing the ciliated gland epithelium.