THE UTERINE CYCLE OF PREGNANCY AND PSEUDO-PREGNANCY AS IT IS IN THE DIPROTODONT MARSUPIAL *BETTONGIA CUNICULUS*.

WITH NOTES ON OTHER REPRODUCTIVE PHENOMENA IN THIS MARSUPIAL.

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(Plates xix–xxi; three Text-figures.)

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Contents.

INTRODUCTION.

Little attention has been paid to the investigation of reproductive phenomena in the diprotodont marsupials, and the present communication is an endeavour to lessen the gap in our knowledge by describing the uterine cycle in one of them, *Bettongia cuniculus*. Opportunity is also being taken to offer some remarks on the breeding season, pouch-phenomena, foetal nutrition, the gestation period and other matters having to do with reproduction in this marsupial.

The observations herein set down can be regarded as being preliminary only, it being intended that the reproductive cycle in its various phases will be more intensely investigated later.

Bettongia cuniculus is one of the so-called "rat kangaroos" and is a diprotodont marsupial of small size found in Tasmania. There is some difficulty in securing specimens at present and it is inevitable that, with the spread of settlement, its numbers will be even more seriously depleted in the near future.

Collection of material of *Bettongia* has proceeded since 1924 and has been supported by funds supplied by the Trustees of the Ralston Bequest and more recently by a grant from the Grants Committee of the Royal Society. To both these bodies I take the opportunity to express my heartfelt thanks for their generous assistance.

Much of this work was carried out in vacation time in the Zoology Department of the University of Sydney and I have to express my sincere thanks to Professor W. J. Dakin for placing the resources of his department at my disposal.

The fixatives employed in the preserving of uteri, ovaries and associated organs have been of several kinds, mainly, however, corrosive sublimate with acetic acid and Bouin's picro-formol-acetic solution. Carnoy's mixture of acetic alcohol with chloroform and Sansom's modification of the same have been employed where a quickly penetrating fluid has been required as, for example, in the fixation of

whole uteri with contained embryos. In this latter case, in order to preserve as far as possible the relationships of the foetal membranes to the uterine wall, the process of double embedding with paraffin and celloidin has been resorted to.

Reproductive Phenomena in Bettongia.

(*a.*) *Number of Pouch Young.*

Bettongia cuniculus agrees with *Potorous tridactylus*, *Phascolomys*, *Trichosurus* and various species of Macropodinae in that it produces but one young at a birth. There being four teats in the pouch, three of them are unoccupied at a time. In the respect that there are insufficient young born at one time to occupy all the teats of the pouch, *Bettongia*, with those marsupials above mentioned and some others, differs very considerably from another group in which the number of newly-born is normally greater than the pouch can accommodate. This phenomenon was first noted by J. P. Hill (1910) in *Dasyurus*, and was shown afterwards to occur in *Didelphys* (Hartman, 1920; Hill, 1918) and probably happens also in *Sarcophilus* (Flynn, 1921).

Pseudochirus cooki, with respect to the number of young born, occupies a position intermediate between these two groups (Flynn, 1921). Possessing four teats, two are functionless. These never become enlarged and are never used by the pouch young. There may be as many as six young produced at a birth in this animal; usually there are three; rarely there is but one. In this way *Pseudochirus cooki* may be recognized as an example now living of a marsupial showing progressive reduction of the number of teats and of young. Where, as in *Trichosurus*, *Phascolarctos*, *Phascolomys*, various species of Macropodinae and others, the number of young ones born is normally less than the number of teats available, the phenomenon might be termed Hypogony.

Bearing in mind the suggestion of Hill and O'Donoghue (1913) that marsupials show a progressive reduction in the number of teats present in the pouch—a statement with which one must agree—it is also obvious that, in the Class, there is also to be found a progressive reduction in the number of young born, and that this reduction has reached its limit in the hypogonous marsupials in which it has proceeded in advance of teat reduction.

(*b.*) *Breeding Habits, etc.*

I have been able to collect females of *Bettongia* over the greater part of the year, and find that its breeding season lasts at least from March to December, that is, over the autumn, winter, spring and early summer months. I have not yet had the opportunity of collecting animals during January and February, but there is every possibility that during these months the animal rests from its breeding activities.

Bettongia is polyoestrous. This is shown by the fact that most of the females obtained over the above-mentioned breeding season are found to be pregnant, by the condition of the ovaries, and by the fact that quite a large number of the pregnant females possess at the same time a pouch young. Thus, of eighty-one females taken in the breeding season of 1924, no less than seventy were pregnant, and of these twenty-six had in addition a young one in the pouch. Of the other pregnant animals, some eight were in a condition that showed that the young animal still occasionally visited the pouch to be suckled.

This shows that, of the seventy pregnant females gathered in the breeding season of 1924, at least thirty-four had given birth to an embryo some short time previously.

Evidence of the condition of polyoestrum is given by the following notes taken from my laboratory note-book. These examples are only two of many.

Example 1. *Bett.* A., 1/10/27.

"Pouch, fairly well haired pouch young present, left anterior gland and teat well developed, other three resting and small.

"Uteri, both about equal in size and of similar appearance.

"Ovaries, left ovary with fairly recent corpus luteum, whitish in colour, right ovary with one very old corpus luteum."

On opening the left uterus a small blastocyst was found.

Example 2. *Bett.*, 15/10/27.

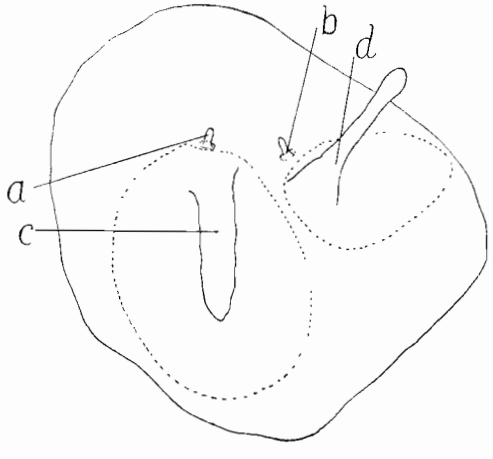
"Pouch, pouch young present, very small.

"Teats, the young one attached to the anterior right teat, anterior left and posterior right teats very small and undeveloped, posterior left teat and gland very large giving indication of the fact that this teat and gland had up till recently been used by a young one that had been visiting the pouch for the purpose of being suckled.

"Uteri, small, slightly congested, both about the same size.

"Ovaries, left ovary with a recent corpus luteum, the right ovary with an old corpus."

A small blastocyst was found in the left uterus. These organs showed that there had been three successive pregnancies.



Text-fig. 1.—Direct evidence of the condition of polyoestrum as shown by the arrangement of the nipples in the pouch of specimen *Bett.*, 10/8/28. The mammary glands are indicated in their extent by broken lines. The right posterior gland is strongly secreting and its condition and the size and form of its nipple (*c*) show that a young one still visits the mother to use it. To the left posterior nipple (*d*) was attached a pouch foetus measuring 25 mm. in direct length. The anterior nipples (*a* and *b*) and glands are small and non-functioning. An early blastocyst was found in the right uterus. There is, therefore, definite evidence of three successive pregnancies.

Not only are these examples definite evidence of the condition of polyoestrum, but they also bring to mind a suggestion made some time ago by O'Donoghue (1916, pp. 442 and 457), that in *Phascolarctos* and *Trichosurus vulpecula*, in which animals only one young one is produced at a birth, ovulation is alternate from each ovary.

As will be seen from the body of this paper, pregnancy in *Bettongia* is normally alternate and it is very likely that in *Phascolarctos* and *Trichosurus* this condition also holds. While one of the uteri is pregnant, the other is "pseudo-pregnant" and, for this reason, it is impossible to know by external examination of the uteri alone, until mid-pregnancy, which of the uteri is the gravid one.

The condition of "pseudo-pregnancy" in *Bettongia* is of a somewhat different type and has a correspondingly different history from that which supervenes in all marsupials when the spontaneously discharged ovum is not fertilized.

This subject will be discussed later in this paper. A full and complete understanding of it will finally depend, however, on the results obtained by a thorough examination of the ovaries at various stages. This work is being carried on by Miss M. Garde, B.Sc., of the School of Anatomy, University of Sydney.

(*c.*) *Periods of Gestation and of Lactation.*

On the length of these periods I have at present very little information based on direct evidence. So far, I have been unable to get *Bettongia* to breed in captivity, so that coitus has never been observed. The same difficulty arises with *Potorous tridactylus*, as has already been pointed out by Misses Altmann and Ellery (1925, p. 466). It does not seem possible that the period of gestation in such a marsupial as *Bettongia*, in which pregnancy is unilateral and ovulation spontaneous, could ever be correctly determined. An approximate calculation might be made by opening animals under anaesthesia and determining the age of the corpus luteum. This I have not yet attempted. The few cases which I have had under observation point to about six weeks as being the approximate length of gestation in both *Bettongia cuniculus* and *Potorous tridactylus*.

Records relating to the length of time which elapses between coitus and parturition in the Macropods exist in widely scattered publications and are so conflicting as to suggest that considerable research and observation are necessary before the matter can be cleared up. Wood Jones (1923, p. 74) quotes a case in which a male and female *Macropus rufus* were kept for some time in the Zoological Gardens in Philadelphia, the male dying on October 28, 1908. Seven weeks after the male died an embryo was observed in the pouch of the female. This young suckled till November 7, 1909, but on September 25, 1909, another, apparently new-born, embryo was noticed in the pouch.

The interval between coitus and parturition in this case could not have been less than eleven months. It is hardly likely that the gestation period in the Macropods would extend over more than six weeks or so, and it is evident that more information is needed as to the length of time the spermatozoa may remain alive and retain their fertilizing power in the body of the female in these animals.

With regard to the period of lactation, the recording of observations is difficult in the case of *Bettongia*, since in most cases the pouch young disappear a few days after the animals are put in the yard. However, since there is never more than one foetus found in the pouch at one time and since in pregnant animals

there is present in many instances a pouch foetus, it is obvious that in normal cases the duration of the lactation period is about equal to the length of the gestation period, i.e., about six weeks. It is also worthy of note that the lactation period of one pregnancy is overlapped by the gestation period of the next succeeding one.

(d.) Pregnancy and Pseudo-pregnancy.

In all viviparous mammals, it is agreed, the growth and shedding of the ovum have a profoundly stimulating effect on the uterus and upon the remainder of the female reproductive organs. It is now universally admitted that this effect is due to hormones secreted by the growing follicle and the corpus luteum, but the whole series of occurrences is not yet sufficiently investigated, and it is obvious that other glandular structures may also be effective in this stimulation.

In those mammals in which ovulation is spontaneous, normal pregnancy may be expected to supervene if fertilization follows ovulation; on the other hand, should fertilization fail, the uteri and other female organs enter into a condition which simulates pregnancy. This condition, noticed first by Hill and O'Donoghue (1913) in *Dasyurus*, was called by these investigators "pseudo-pregnancy". It is a most significant phenomenon, since it shows definitely the influence of ovarian structures, before and after ovulation, in activating the uterus. Pseudo-pregnancy is, naturally, a temporary condition and the transition to normal anoestrum or dioestrum consists in the reconstitution of the uterine and gland epithelia and in the resorption of the distending lymph material found in the connective tissue of the mucosa (Hill and O'Donoghue, pp. 159-160).

In *Bettongia*, ovulation is spontaneous, as might be expected, but is unilateral, and only one ovum is shed at a time. Either pregnancy or pseudo-pregnancy may follow, according as this ovum is fertilized or not. Unfertilized ova are quite rare. Ordinary pseudo-pregnancy in this animal is of the same character as in the case of *Dasyurus* (Hill and O'Donoghue) and has not been examined in any detail for the purposes of this paper.

Pregnancy is obviously unilateral and the pregnant uterus proceeds to undergo the usual changes associated with this condition and which have their climax in the act of parturition. More interesting, however, is the fact that the contralateral (non-pregnant) uterus also undergoes changes by which it is not to be distinguished either by external observation or by microscopical examination from the pregnant uterus until the time of mid-pregnancy. The non-pregnant uterus is therefore in a condition of pseudo-pregnancy. But this pseudo-pregnancy is of a somewhat different type from that occurring in the case of ovulation not followed by fertilization. In the latter type, the pseudo-pregnant uterus returns more or less gradually to the condition of anoestrum or dioestrum, this return being caused apparently by the waning hormonic influence of the ovary. In *Bettongia*, however, the history of the unilaterally pseudo-pregnant uterus is peculiar and significant. It does not attempt to return immediately to the condition of dioestrum, but remains through the latter half of pregnancy in a well stimulated condition, exhibiting a turgid mucosa, with a well-developed lymph and blood supply. Just before parturition occurs in the contralateral uterus, there is a convulsive expulsion of the contained lymph from the connective tissue of the mucosa of the pseudo-pregnant uterus.

The significance of this behaviour is to be found apparently in two causes: (*a*) the presence of an embryo in the pregnant uterus, (*b*) the stimulation of the

uterine muscles which results in parturition. These will be discussed later in this paper.

THE UTERINE CYCLE OF PREGNANCY AND PSEUDO-PREGNANCY IN BETTONGIA.

Stage 1. *Bett.* E., 15/9/26 (stage of anoestrum). (Plate xix, figs. 1, 2.)

It is remarkable at what an early stage the young *Bettongia* is able to receive the male. Coitus seems to occur soon after the young animal is reasonably free of the pouch. For this reason it is difficult to obtain resting uteri from free animals during the breeding season. Consequently I have taken as an example of the anoestrous condition the uterus of a young *Bettongia* which had not yet entirely severed its connection with the pouch.

Each of the two uteri measured 7 × 3·5 mm. Sections show that the wall has the usual layers found in the uterine wall of other marsupials. The uterine epithelium consists of a single layer of columnar cells of moderate height with somewhat large nuclei. The latter are found near the centre of the cell, not basally situated as is the case with the nuclei of the gland epithelium. The nuclei are oval in shape and to a great extent fill the centre of the cell. The shape and position of the nucleus vary according to the direction in which the cell is cut. Narrow cells with thin nuclei mostly prove to be cells cut along one edge. The cytoplasm of the cell is finely granular. The nucleus has a well defined membrane and is coarsely granular, often with one large chromatic particle standing out from the rest. The thickness of the epithelium varies between 0·019 and 0·025 mm.

The uterine epithelium in the anoestrous stage is strongly and almost uniformly ciliated. I have not been able to find in literature any reference to the fact that the surface epithelium of the uterus of any marsupial is ciliated at any stage. Hartman states (1923, p. 372) that his preparations of the anoestrous uteri in the opossum do not allow him to say whether any of the cells of the epithelium or of the glands possess cilia. Hill and O'Donoghue (1913) refer to gland ciliation only, so we may infer that no surface ciliation is present in the uterus of *Dasyurus*.

The uterine glands are also strongly ciliated, even to their bases. The glands are of comparatively large diameter, having a diameter of from 0·046 to 0·058 mm. The glands are straight and are lined by fairly high columnar cells in which, however, the nuclei are basally situated. They have small but distinct lumina, these, particularly in their basal portions, being occluded by a mass of material which, when examined, proves to be closely aggregated cilia, these being just as strongly developed in the lower portions of the glands as in the upper.

Of the remainder of the mucosa there is nothing to be said other than that it is in the resting condition. It is not markedly supplied with blood vessels or with lymphatic material. There is a slight condensation of the connective tissue below the uterine epithelium to form a sub-epithelial layer.

Stage 2. Oestrous Stage. (Plate xix, figs. 3, 4.)

As an example of this stage will be taken the sterile uterus of specimen *Bett.* F., 29/8/24. In this specimen the two uteri were empty, but there was a very small young one in the pouch, newly born. This foetus measured 14·8 mm. direct length. There was in the pouch also a larger pouch-foetus which had been dead

for some time and which had become quite putrescent. No doubt if this latter one had lived it would have been by this time free of the pouch.

Examination of the uteri showed that the smaller foetus had come from the left uterus which showed remains of the foetal membranes, not yet completely absorbed. The right uterus, from which the larger foetus had been born, has now recovered and has progressed to a stage closely corresponding to oestrus.

The epithelium of the right uterus is very high and the nuclei are plentiful and are arranged at several heights so as to resemble the similar stage in the pig (see Corner, 1921, Plate 4, figs. 25, 26). Mitotic figures are very common in the epithelium and there are instances of the vacuolar degeneration to which Corner refers. The epithelium measures from 0·021 to 0·028 mm. in thickness, so that it is appreciably thicker than in the anoestrous stage, but cilia are not nearly so plentiful on the surface epithelium.

The cells of the gland epithelium are extremely active in division, mitotic figures being very common. In a single cross-section of a gland as many as three cells may be seen in division. A gland section containing two divisions is shown on Plate xix, fig. 4. Cilia are abundantly present in the glands, and they often project profusely from the gland mouth into the uterine lumen. The gland lumina are larger than in the previous stage and there is sometimes present a little secretion. The diameter of an average gland is 0·46 mm. Before dividing, the gland cell falls out of line, approaching nearer to the gland lumen. After division the two cells apparently work back again to their place in the gland epithelium. It is of note to mention that, contrary to what occurs in the pig (Corner, 1921), mitoses are just as numerous in the deeper portions of the glands as in the more superficial.

Each gland cell has clear protoplasm and the nucleus here again largely fills the cell. The thickness of the gland epithelium is from 0·020 to 0·021 mm.

There is at this stage an appreciable increase in the amount of infiltrated lymphatic material in the mucosa and through it, also, many blood vessels extend and come to lie just below the epithelium.

Polymorphs occur fairly plentifully at this stage, being found in the connective tissue of the mucosa, in the gland epithelium and in the uterine epithelium as well as being free in the gland and uterine lumina.

In another small female (*Bett.*, 4/12/29) the uteri were in a condition either of late pro-oestrous or early oestrous. She was a very small animal, obviously not long free of the pouch, and it is surprising to find her organs in a condition of preparatory activity. The two uteri are small, about the size given in the stage of anoestrum, left 7 × 3·2 mm., right 7 × 3·0 mm. The uterine wall shows a slight advance on the previous stage. The uterine epithelium consists of a single layer of columnar cells, but the cilia are much less numerous and are not so easily stained. There is a tendency for the epithelial cells to become crowded in places so that they become arranged at several heights. Occasional mitoses occur in the epithelial cells. The uterine epithelium has an average depth of 0·023 mm.

The mucosa measures on the average 0·80 mm. from the top of the folds to the muscularis, so that it is thicker than in the anoestrum stage. There has been a certain amount of infiltration of lymphatic material, so that the glands are now being forced somewhat farther apart. The inter-glandular connective tissue is becoming more attenuated and fluid laden.

Glands.—These present, in general, the same characteristics as in the previous stage, with a diameter from 0·054 to 0·060 mm. They are very strongly ciliated and their activity is shown by the presence, fairly commonly, of mitoses in the gland epithelium.

Stage 3. *Bett.* F., 19/9/24. (Pl. xix, fig. 5.)

In this specimen the left uterus contained a small blastocyst in a very early unilaminar stage. There was also a well-grown foetus in the pouch. The uteri and blastocyst were fixed in picro-nitro-osmic solution.

In external appearance and in microscopic structure the two uteri are identical.

The uterine epithelium has now become appreciably thicker, measuring from 0·026 mm. to 0·04 mm. Mitotic divisions have by this time completely ceased and the number of nuclei present in the epithelium is now very large, so that they appear to overlap even in thin sections. They are arranged at about three levels. The free ends of the epithelium cells are slightly rounded as in the pig at this stage (Corner, 1921) and the protoplasm is finely granular. Cilia are not nearly so plentiful. The epithelium gives every evidence of being in a state of active secretion and the exposed surfaces of its cells are coated with amorphous material exuded from them into the uterine lumen.

A feature of the epithelium is that the distance between the nucleus and the base of the cell has increased, due to the intense vacuolation of the basal cytoplasm. This portion of the cytoplasm is quite distinct and owes its distinctive appearance no doubt to the absorption of fluid material from the underlying stroma, this material being on its way to the interior of the uterus.

The glands are still fairly crowded, but there is opportunity for lymphatic material to be well distributed through the mucosa. The lower portions of the glands are much convoluted and the coils are closely crowded. In their upper courses the glands are straight. The glands are ciliated right to their bases. The cilia are not so plentiful or so strong as in the previous stage. Mitoses in the gland cells have entirely ceased. The thickness of the gland epithelium is about 0·024 mm. The relative proportion of the amount of cytoplasm to nucleus in the gland cells is greater than in the previous stage, and there is a strong tendency for the cytoplasm to become vacuolated. The nuclei are in general quite basally situated in the gland epithelium, but there has commenced in this stage a phenomenon which seems to be of the greatest importance and which is much more marked in subsequent stages.

This consists in some of the gland nuclei leaving their basal positions and passing toward the gland lumen. In the process, they lose their staining qualities and sometimes are with difficulty distinguishable from the general cell protoplasm. In the process of desquamation which the gland cells undergo, especially in the next stage, these nuclei go to swell the amount of cellular secretion present in the gland lumina. This secretion, although fairly abundant just now in the deeper portions of the glands, is much more marked in subsequent stages. On occasions it is possible to see the very pale nuclei working their way into the lumina of the glands.

Stage 4. *Bett.* A., 6/9/24. (Pl. xix, fig. 6.)

A small unilaminar blastocyst, somewhat more advanced than in the last stage, was present in the right uterus. The two uteri, which were identical in external appearance and in microscopical structure, were fixed in Bouin's solution.

The uterine epithelium.—This is appreciably lower than in Stage 3, measuring on an average 0·020 mm. in thickness. The changes initiated in the previous stage are continued in this.

Structurally, the uterine epithelium of this stage bears a very close resemblance to that of the pig eight days after ovulation (Corner, 1921, Plate 4, fig. 28). An interesting and prominent feature is the presence of abundance of narrow cells with dark-staining nuclei whose nature and function have caused much discussion. These are the so-called "intercalar cells", or "cells with pycnotic nuclei" to which Corner refers in the pig (1921), which Hill and O'Donoghue have recorded in the uteri of *Dasyurus* (1913, p. 146), and which are also shown by Hartman to occur in the uterus of *Didelphys* (1923, p. 375). They have been long regarded as being cells undergoing extinction but, in the case of *Bettongia*, they appear rather to be wandering cells which, in pushing their way between the epithelial cells, have been forced to take a narrow shape while they resume a more rounded one on arrival in the uterine lumen. Corner refers to darkly-staining rounded cells which force themselves between the epithelial cells at their bases in the case of the pig. The basement membrane at this stage is very indefinite and may be said to have disappeared.

No mitoses are observable in the epithelial cells either of the uterus or of the glands. In the latter the migration and alteration of the gland nuclei are in full activity.

The cilia have practically the same arrangement as in the last stage. They are not very common on the uterine epithelium and the ciliated cells are usually somewhat depressed below the general surface. The glands, however, are ciliated right to their bases.

Stage 5. *Bett.* B., 30/8/24. (Pl. xix, fig. 7 and Pl. xx, fig. 8.)

A fully formed bilaminar blastocyst was found in the right uterus. The two uteri are identical in appearance and structure. They have both increased in size and turgidity.

Epithelium.—This shows a considerable advance on the previous stage. Due to the thickening of the mucosa, the formation of uterine folds and the general increase of area covered by the epithelium without any compensating new cell formation, the epithelium has become of a low columnar type, its thickness being now about 0·010 to 0·014 mm. The nuclei do not overlap any longer in the sections, but lie side by side. The exposed edges of the epithelial cells are plentifully coated with secretion and the whole layer is in an active secretory condition. Cell boundaries are not always distinct in this layer. Some of the cells are ciliated.

Leucocytes are abundantly present in some places, particularly in the superficial layers of the stroma. Polymorphs are to be found in the superficial epithelium, and in that of the glands, also in the stroma.

The subepithelial layer is particularly well defined at this stage, due to the large number of capillaries which now form its dominant feature.

Infiltration of the stroma with lymphatic material has gone on apace. Its arrangement is such that there is a superficial portion of the stroma which is rather spongy and in which the gland tubes are widely separated and are fairly straight.

Contrasted with this there is a deep compact layer of the mucosa not so much infiltrated and in which the glands are tortuous and more closely set.

There is also a considerable difference in the histology of the glands in these two regions of the mucosa.

In the deeper portion, the gland epithelium has increased greatly in thickness, to 0·040 mm. The cytoplasmic portion of the cell has grown largely in volume and is profusely vacuolated. In many cases the rounded nucleus is contained in a small mass of protoplasm connected to the cell wall by strands.

Migration of the nuclei is very active, and there seems to be no doubt that their substance passes into the lumen of the gland together with the frayed-off ends of the gland cells. Nevertheless, many of the gland cells still retain their ciliated condition.

In their superficial portions the epithelium of the glands is not nearly so thick (0·033 mm.). Further, the nuclei are smaller and deeply staining. The cytoplasm is but little vacuolated. The transition from the deeper portion of the gland to the more superficial region is somewhat abrupt.

Stage 6. *Bett.*, 2/9/24. (Pl. xx, figs. 9-13.)

A blastocyst of the primitive streak stage was present in the left uterus.

In this stage, although externally the two uteri are similar, microscopically they are very different.

Structure of the right (non-pregnant) uterus.—The epithelium shows considerable signs of disintegration and degeneration. Where intact it is similar to the epithelium of the last stage, but its connection with the underlying tissues is very loose, so that in patches it is liable to break away and float in the cavity of the uterus. Other degenerative processes are also in operation. Epithelial cells swell, their nuclei lose their staining properties and the whole cell either breaks away completely or even bursts, throwing the contained material into the uterine lumen. Vacuolar degeneration *in situ* is common. Associated with these processes is the presence of numbers of leucocytes in the epithelium. The place of the cast-off or degenerated epithelial cells is apparently taken by connective tissue cells of the mucosa, these cells moving up into place and forming a new single-layered epithelium.

Blood vessels are plentiful and the breaking away of the epithelial cells causes blood extravasations, but these are rare and not of great extent.

The mucosa is tremendously infiltrated with fluid material, so much so that it resembles a mass of fluid in which the blood vessels and glands are suspended.

The glands are also undergoing degeneration and reconstitution, these phenomena being confined at this stage to their more superficial portions. Here, vacuolar degeneration, desquamation and migration of the gland cells and nuclei are in progress, although not so actively as in the uterine epithelium. With these phenomena is associated the presence of leucocytes of various kinds, the number of which is remarkable.

The result of all this is that each gland becomes divided into two portions, a lower secretory portion, convoluted and lined by large vacuolated columnar cells with vesicular nuclei, and a more superficial duct-like portion, non-secretory, lined by smaller cells, with granular non-vacuolated protoplasm and containing small dark-staining nuclei. The separation of each gland into these two portions is very distinct.

Left (pregnant) uterus.—There are no signs of degeneration in the uterine epithelium. Each gland consists of two portions as in the non-pregnant uterus, but

the alteration has not gone so far, nor is the division between the two portions of each gland so abrupt. Leucocytes, though present, are not nearly so numerous as in the non-pregnant uterus at this stage.

The two uteri, pregnant and non-pregnant, at this time agree, then, in the division of each uterine gland into two portions, a superficial non-secretory and a deeper secretory portion. They agree also in the fact that the mucosa is largely infiltrated with lymphatic material. The non-pregnant uterus, however, shows a wide-spread destruction and regeneration of the superficial epithelium associated with the presence of numerous leucocytes. These features are absent in the pregnant uterus.

Stage 7. *Bett.* E., 14/9/24. (Plate xx, fig. 14.)

The right uterus contained an embryo in the medullary plate stage.

The two uteri are very dissimilar in external appearance, the measurements being: right uterus 17 × 9 mm., the left 12 × 5·5 mm.

Left (non-pregnant) uterus.—The epithelium varies in thickness from 0·012 to 0·0121 mm. and in most places is completely restored, although here and there are still to be found signs of degeneration. The basement membrane in most places is very distinct. Leucocytes are much rarer than in the previous stage. In the glands the division into superficial non-secreting and deeper secreting portions is quite marked. In the latter portion the glands have a diameter of 0·48 mm. and possess large and vacuolated cells. More superficially they measure 0·32 mm. in diameter and the cells possess small darkly-staining nuclei and the cytoplasm is granular and but little vacuolated. Cilia are very plentiful.

Right (pregnant) uterus.—The cavity of the uterus is almost circular owing to the presence of the blastocyst which fits snugly against the epithelium, obliterating many of the folds. The blastocyst is separated from the epithelium by the shell membrane which, although very thin, has not yet disappeared. The blastocyst is orientated so that its embryonic pole is towards the mesial side of the uterus and so that the embryo is arranged transversely to the length of the uterus. The anterior end of the future embryo is placed towards the dorsal side. In order to retain as much as possible the true relations of the uterus and embryo, the organs with the contained embryo were fixed intact in Carnoy's solution.

Although the inner surface of the uterus is practically smooth, there are still a few grooves left and these are in the neighbourhood of the embryo. The grooves disappear at the lower pole of the vesicle.

The epithelium consists of a single layer of cells which are cubical in form and possess large spherical nuclei. There is a tendency for the epithelium here and there to thicken slightly and penetrate downwards between the subepithelial blood-vessels. Many of the epithelial cells possess the excretory processes which are characteristic of similar cells in the American opossum (Hartman, 1923, p. 374) and in the pig (Corner, 1921, p. 139). Corner considers these processes to consist of extruded cytoplasm, contrary to Geist, who believes that they represent secreted material on its way into the lumen of the uterus.

Whatever may be the case in the pig, it appears that in *Bettongia* the processes consist of amorphous material extruded actually from the epithelial cells.

Cilia are present, though rarely, on the surface of the uterine epithelium. Underlying the epithelium there is an almost continuous layer of capillaries which,

with the particular stroma cells, makes a very definite subepithelial layer. Between this and the overlying epithelium there is a narrow clear zone which consists of aggregated lymphatic material collected probably on its way to the lumen.

Glands are well developed and are ciliated right to their bases. The glands are actively secreting and many are greatly dilated. Each gland presents the same two divisions already referred to. A feature of the stroma is the presence of great numbers of deeply-staining granules which are specially congregated just below the surface epithelium.

Stage 8. *Bett* G., 11/9/24. (Pl. xx, fig. 15; Pl. xxi, fig. 16.)

The two uteri measured as follows: right 21 × 11 mm., left 10 × 5·5 mm. The embryo contained in the right uterus measured approximately 3·5 mm. in direct length.

Left (non-pregnant) uterus.—The cavity of this uterus is compressed and its inner surface is marked by fairly deep longitudinal grooves.

The cells of the epithelium are now arranged to form a somewhat irregular columnar layer of medium height. Cilia are now found more plentifully distributed on the surface and take the stain more thoroughly. Blood-vessels have been largely withdrawn from the vicinity of the surface epithelium. Polymorphonuclear leucocytes are observable in the epithelium. The basement membrane and subepithelial layer are distinct. The stroma is still infiltrated with lymph. Occasionally there are leucocytes to be found in it.

Glands.—These have become much less active than in previous stages, although in some of them is to be found secreted material which often contains whole cells. Leucocytes are also to be seen in the gland lumina occasionally. In all except one region the gland cells take the character of non-secretory cells of low cubical type with dense nuclei. In these the average height of the cells is from 0·0132 to 0·0148 mm., whereas towards the mesial side of the uterus where the deeper parts of the glands still consist of vacuolated columnar cells, the height of the cells is on the average 0·020 mm. The glands are ciliated to their bases.

Right (pregnant) uterus.—In order to retain the embryo in its natural position, the right uterus was divided by a vertical longitudinal incision. The mesial half is shown from its inner aspect on Plate xxi, figure 16. The embryo in its membranes is contained in this mesial half. It is arranged fairly transversely, the head being dorsally directed. The anterior portion of the body is strongly bent on the remainder and is contained in the proamnion. In the figure the embryo, viewed from underneath, is hidden by the yolk-sac splanchnopleure (*y. spl.*) which is invaginated towards the observer into the cavity of the yolk-sac (*y.s. cav.*). The yolk-sac splanchnopleure passes up at the side of the embryo and is then carried outward towards the uterine wall where it unites with the chorion and is then deflected downwards as the yolk-sac wall. This is in extremely close contact with the inner surface of the uterine wall, so that the large cavity in the figure is the cavity of the yolk-sac, not of the uterus as would first appear. The upper portion of the omphalopleure consists of the vascular omphalopleure. The lower limit of this is shown by a light line representing the sinus terminalis (*s.t.*). The remainder of the yolk-sac wall consists of the bilaminar omphalopleure which is in close and intimate contact with the uterine epithelium.

As has already been suggested by Jenkinson (1913, p. 193), the amnion in Marsupials is formed by folds. In *Bettongia* it is the head-fold which appears

first. The foetal membranes of a corresponding stage have been figured by Selenka (1886-91, Plate xxxii, figure 3). The arrangement of the foetal membranes of *Bettongia* is that indicated by Semon in his description of the second marsupialian type (1894). It is similar to that of all Macropods so far described, except *Halmaturus ruficollis* in which Caldwell (1884) testifies to the presence of an allantochorion and of a union between the bilaminar omphalopleure and the uterine wall. With this exception the allantois in all Macropods is small, its vascular supply is poorly developed and no part of its wall reaches at any stage to the chorion.

At this stage there is, in *Bettongia*, a large proamnion, but it is not so voluminous as is shown in Selenka's diagram, in which more than half the body is enclosed in proamnion.

The chorionic ectoderm consists of a layer of cells which exceed greatly in size the cells of the uterine epithelium which are apposed to them. Their nuclei are round or oval and usually stain very darkly. The structure of these cells is rather characteristic, for the nucleus, placed in the centre of the cell, is surrounded by much vacuolated protoplasm. There is intense absorption going on in this layer. It will be referred to later. The chorionic mesoderm is a thin layer consisting of much smaller flattened cells, with correspondingly flattened nuclei, well spaced apart and connected by thin strands of protoplasm.

The trophoblast of the vascular omphalopleure is similar to that of the chorion, but the cells have a tendency to be somewhat larger. There are many places where the contact between this layer and the uterine epithelium is very intimate. The two are undoubtedly united in places, but can always be distinguished. There is nothing of the nature of an interpenetration of one layer by the other. The union occurs on the tops of the small folds which are so characteristic of the inner wall of the uterus. Into the deeper folds the vascular omphalopleure at this stage does not pass. The mesoderm in this region is very delicate, but is abundantly supplied with blood-vessels which make an almost complete layer underlying the trophoblastic ectoderm. The entoderm cells are usually flat and delicate, but are sometjmes large, never, however, becoming as large as the ectoderm cells.

The trophoblast of the bilaminar omphalopleure resembles in general that of the other regions, but the cells of the entoderm of this region differ markedly from those elsewhere. Instead of being flattened, they are robust cells of the characteristic shape made familiar in the drawings of Selenka. These cells have somewhat narrow bases, but their apices are expanded and rounded. Typically each cell is separated from its neighbours by narrow spaces across which continuity is assured by delicate strands of protoplasm.

Maternal Structures.—The inner uterine surface may be distinguished into three regions, one in contact with the chorion of the embryo, a second in contact with the vascular omphalopleure, and a third in contact with the bilaminar omphalopleure.

The inner surface of the uterus is covered with fine corrugations due to the swollen condition of the blood-vessels which lie just below the epithelium. Sections of the uterus show that the mucosa is very variable in thickness. At the lower pole of the yolk-sac it may be as little as 0·15 mm., whereas it may be as much as 0·5 mm. in other places.

Uterine Epithelium.—In the region corresponding to the chorion of the embryo, the uterine epithelium is quite thin with round or oval nuclei fairly

deeply stained. Division between the cells is made out with difficulty. The layer is raised into numerous minute rugosities caused by the underlying blood-vessels. The nuclei being spaced widely apart in some places, there remains between them only a thin and delicate film of protoplasm covering the capillary. The epithelium of the other regions of the uterus shows very little departure in its structure from what has just been described. Occasionally in any region there may be found in the uterine epithelium a ciliated cell, its cilia being bathed by the secretion which fills the space between the epithelium and the trophoblast.

The Mucosa.—The connective tissue of the mucosa consists of a very delicate reticulum with stroma cells distributed at wide intervals. There is abundant fluid in the meshes of the net-work. Blood-vessels, sometimes of great size, are plentiful in the mucosa and their minute branches come to lie immediately below the epithelium as a definite and practically continuous layer of capillaries. The presence of these in abundance causes the corrugations of the epithelium previously referred to.

The glands are of small diameter, measuring on an average 0·056 mm. Their lumen is small and there appears to be little glandular secretion. Their epithelial cells possess small, deeply staining, basally situated nuclei. Many of the cells, basal as well as superficial, are ciliated but the cilia are very delicate.

Through the stroma are scattered leucocytes of various kinds and these are to be found especially just below the epithelium.

The Placenta.—The placenta is an organ consisting of the intimate apposition or fusion of the foetal membranes with the uterine wall for the purpose of carrying out physiological processes (and, it may be added, certain mechanical ones) destined for the well-being of the embryo (Assheton, 1909).

With this conception in mind, it can be stated at the outset that *Bettongia* possesses a very efficient placenta formed by the intimate apposition throughout its extent of the trophoblast to the uterine epithelium. The allantois remains small, but very efficient absorptive and nutritive functions are carried out in the regions of the chorion, vascular omphalopleure and bilaminar omphalopleure respectively. Actual fusion between maternal and foetal tissues at this stage is practically confined to the region of the vascular omphalopleure—a condition recalling that in *Dasyurus* and *Phascolarctos*—but this union is very slight and occurs at relatively few points. There is no actual penetration of maternal by foetal tissue. In the region of the chorion, the space between this membrane and the uterine epithelium is entirely filled by a liquid material, actively secreted by the maternal tissues and just as actively absorbed by the chorionic ectoderm. This secretion occupies all the spaces and folds between the two tissues. It is mainly a transudate from the mucosa, and to a very limited extent a secretion from the uterine glands. Beyond an occasional leucocyte, there is very little of a cellular nature in it. Here and there, however, occur patches of pigment, particularly in the neighbourhood of the mouths of some of the glands. It would be expected that this is the result of the breaking down of extravasated haematids, but I am not able to observe any such extravasations into the mucosa or the passage of haematids into the gland lumina.

A feature of this region, as it is of others, is the formation of pigment in the blood-vessels themselves. This, due probably to the degeneration of the red corpuscles, is confined to the superficial vessels and no doubt is of embryotrophic significance.

In the region of the vascular omphalopleure (omphaloplacental region) the relationship of the trophoblast to the maternal epithelium is much more intimate than in the chorionic area, and there are numbers of places where actual union of the two layers occurs. Where this happens, the foetal and maternal blood-streams are separated by the endothelium of the foetal vessel, the trophoblast, the uterine epithelium and the endothelium of the maternal vessel. Sometimes connective tissue is interposed between the maternal vessels and the uterine epithelium. Usually there is a narrow space between the foetal and maternal tissues and the space is filled with secretion similar to that found in the chorionic area. In the omphaloplacental region, however, with this fluid medium there is abundance of other material. The same formation of pigment occurs in the superficial vessels of this region as in the chorionic area. Patches of similar pigment appear in the embryotrophic secretion and are also in evidence in the cytoplasm of the trophoblast cells. Many of the leucocytes of the foetal circulation show dense masses of pigment in their substance. The activity of the absorption by the trophoblast cells of the fluid transudate is such that it is often impossible to define the boundary between the two media, the cytoplasm on the one hand and the secretion on the other. There is also to be found a considerable quantity of cellular debris, many leucocytes and often whole cells. These cells undergo immediate and intense disorganization so that, soon, all that is evident of the original cell is the nucleolus set in a pale non-staining mass of protoplasm. The whole of the colourable materials of the cell, with the exception of the nucleolus, seem to have been dispersed. These nucleoli form a very characteristic feature of the embryotrophe and of the contents of the megalokaryocytic trophoblast cells. Most of the cells which appear in this way in the uterine milk are cells of the uterine epithelium which have been loosened from their moorings and in this way have formed an integral part of the nutritive material of the embryo. This phenomenon is much more evident in the region of the bilaminar omphalopleure.

The cells of both layers of the bilaminar omphalopleure in the metrioplacental region are tremendously vacuolated. This points to the active absorption of carbohydrates in this portion of the yolk-sac placenta.

In many places in this region there are to be found clumps of cells projecting from the surface of the uterine epithelium. Sooner or later these become loosened and fall away into the secretion between the foetal and maternal epithelia. They soon lose their identity and in a short while the only recognizable part of each original cell is the nucleolus. As in the region of the vascular omphalopleure, these nucleoli can be recognized in the cytoplasm of the trophoblast cells.

Many leucocytes are to be seen in the embryotrophe of this region.

Evidence of active absorption is to be seen in the fact that the free apices of the cells of the yolk-sac entoderm are covered with caps of secreted material.

Stage 9. *Bett.* B., 11/9/24. (Pl. xxi, figs. 17, 18, 19.)

Right uterus contains an embryo near full term with the following measurements: direct length 14·2 mm., head length 7 mm.

Description of non-pregnant (left) uterus.—The uterus has a lumen which is narrow and compressed, presenting narrow longitudinal grooves. This is a highly significant and important stage in the cycle of the pseudo-pregnant uterus, since active resorption and removal of the infiltrated lymphatic material are in progress.

Epithelium.—This consists of a columnar epithelium of medium height with the cells and contained nuclei fairly closely packed. The nucleus in each case fills most of the cell and is oval in shape. Occasionally the nuclei are arranged at more than one height. Mitoses occasionally occur. Here and there are to be seen also degenerating epithelial cells. No doubt their remains go to swell the secretion present in the uterine lumen. Cilia are only rarely present. The epithelium measures in average thickness about 0·048 mm.

Stroma.—Below the epithelium there is a marked condensation of the stroma to form a sub-epithelial layer. Everywhere the stroma contains abundance of lymph material, particularly at the apices of the folds. The blood-vessels of the mucosa are much swollen. Leucocytes, mainly of the large mononuclear type, are fairly plentiful in the stroma.

Glands.—It is in these structures that the greatest activity is shown at this stage, since they serve as the most important agents in the removal of the contained lymph from the stroma. The glands are of all diameters, ranging from 0·16 to as much as 0·80 mm. Usually the gland epithelium is high and strongly ciliated, but in the case of the much expanded glands the epithelium is low and cubical, an expression of the internal pressure to which these glands are being subjected. The oval, somewhat vesicular nuclei are mostly basally situated.

The cavities of the glands are filled with abundance of lymphatic material which is being poured into the uterine cavity. With this is to be found cellular debris associated with whole cells and portions of the gland epithelium.

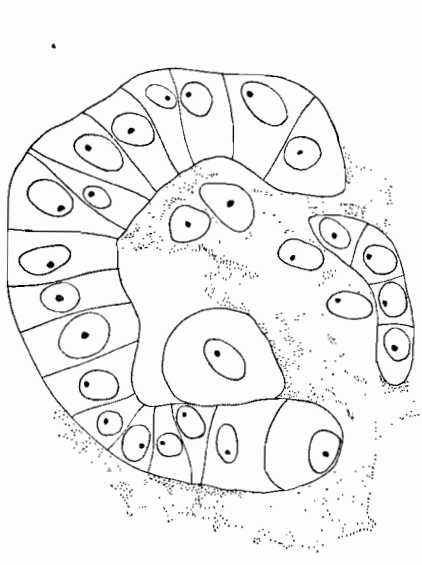
The weight of passage of the fluid lymphatic material into the glands is remarkable. In many cases it transudes in such a way as to cause little disturbance to the integrity of the gland epithelium. Often its passage is of such a kind as to cause the epithelium to break down and cells are thus carried into the gland lumina and added to the gland secretion. In Text-figure 2 one of these breaks is shown. Here the external pressure has caused a gap in the wall and the flood of lymph has carried some of the epithelial cells with it. In other cases, as is shown in Text-figure 3, a larger area of the epithelium becomes invaginated and at last loses its connection with the wall altogether. The appearance is then presented of a ball or short rod of coagulated secretion wrapped in an envelope of flattened and distorted epithelial cells contained within the gland lumen.

These phenomena recall very vividly the formation of cellular secretion by invagination of the gland walls as recorded by Kolster and Bonnet for the horse and dog (see Jenkinson, 1913, fig. 142). If a gland be unable to dispose of the secreted material quickly enough or should its duct for some reason become blocked up, its cavity becomes distended to the relatively enormous dimensions mentioned above.

Leucocytes are a prominent feature of the gland secretion and are to be seen in many places making their way through the gland epithelium.

Lymph also makes its way to the uterine lumen directly through the uterine epithelium, without, however, greatly disturbing the latter. Obviously the pressure which would force the lymph into the glands with such destructive effect is considerable, and one can only liken the process to that of water being squeezed from a sponge. It appears, therefore, as if the force is exercised by the contraction of the circular musculature of the uterus which at this stage must have been subjected to some considerable stimulus. It is of some significance that this should happen in the pseudo-pregnant uterus at a stage which corresponds to a few hours before parturition in the pregnant uterus.

Pregnant uterus.—This contained an embryo, near full term, of the measurements given above. The allantois is quite small and does not enter into relationship with the chorion.



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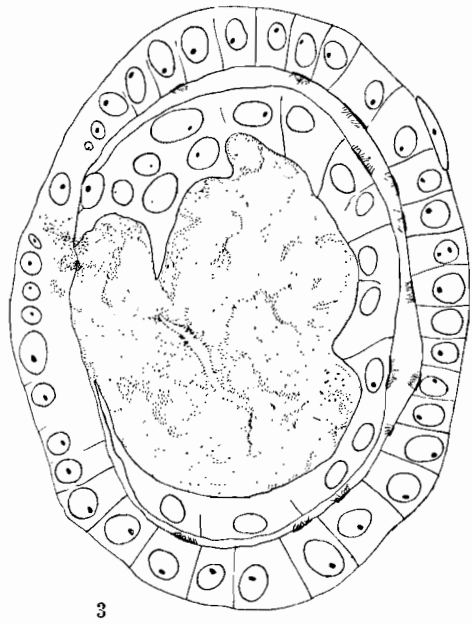

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Text-fig. 2.—One of the glands of the left non-pregnant uterus of specimen *Bett.*, 11/9/24. The pressure of the lymph has caused a break in the wall and some of the epithelial cells have been carried inward with the flow of lymph.

Text-fig. 3.—A gland from the same uterus as the gland shown in Text-fig. 2. Here the invading lymph stream has actually invaginated a portion of the gland wall. Notice the reversed position of the cilia of the invaginated epithelium.

The wall of the uterus is greatly folded and into the grooves and crannies so formed the wall of the vesicle follows. In some cases these grooves approach very closely to the muscularis, there being only a small thickness of mucosa left between the two. Here the mucosa may measure only 0·2 mm. in thickness, while to the top of the folds it may easily be ten times as much. The mucosa is very uniform in appearance, consisting of a homogeneous plasma-like mass in which the glands and blood-vessels appear to float. Stroma cells are very few and are much scattered. Leucocytes are present, some bearing pigment, and there are occasionally to be seen erythrocytes. The lymph plasma is particularly aggregated in the tops of the folds where it stains deeply with eosin. The blood vascular supply shows its best development in the superficial region of the stroma where it takes the form of a very rich and continuous layer of capillaries. In these there is to be found a similar formation of pigment to that which occurs in the previous pregnant stage. Here, however, it is not so marked.

Epithelium.—Opposite the chorion the maternal epithelial cells are very thin and strap-like, with nuclei which cause the cell to bulge at its centre. This layer

is for the most part, then, no more than a thin investment covering the sub-epithelial capillaries. In other regions of the uterus the epithelium presents much the same characteristics.

Glands.—These are small, measuring about 0·055 mm. in diameter. The nuclei of the gland epithelium are small and condensed. Ciliation is not very marked. Cilia are not always present and, where they are found, neither the cilia themselves nor their basal granules stain very well. Further, there is no great evidence of secretory activity in the glands. In some there is present a homogeneous liquid secretion, to which may be added occasionally cellular detritus. Sometimes an expanded gland is to be met with, but the impression given is that glandular secretion does not play a very important rôle in embryotrophic processes at this stage. However, wherever a gland is found pouring its secretion into the uterine lumen, the trophoblast cells can be seen to be actively absorbing the secreted material by means of pseudo-podial processes.

Foetal structures.—Over the full extent of the vesicle, with very little exception, the trophoblast is closely and intimately applied to the uterine epithelium. It is a case of union without intergrowth. This particularly occurs in the region of the vascular omphalopleure. In some places, especially over the mouths of active glands, the maternal and foetal tissues are separated by spaces which are filled with secretion.

The Chorion.—The trophoblast cells here, as over the remainder of the vesicle, are of very great size. Their nuclei are also large and are chromatically rich. These cells are true megalokaryocytes and the presence in their cytoplasm of pigment, of vacuoles, and of various granules with the inclusion of broken down cells and of leucocytes points to the same active processes of absorption being carried on as in the previous stage. Occasionally, an extravasation of blood occurs and, where this happens, active ingestion of the haematids by the trophoblast cells occurs.

The mesoderm of this region is a flattened layer with the nuclei spaced at intervals.

In the mesoderm of the vascular omphalopleure is contained a continuous layer of blood-vessels, and it is probably in this region that the most active processes of absorption are being carried on. The two blood-streams, foetal and maternal, are separated by the two epithelia, themselves very thin, and the two endothelia, of even greater tenuity. To these may be added, but not always, a small proportion of maternal connective tissue. The enormous size of some of the megalokaryocytes is remarkable. Their contents are similar to those of the megalokaryocytes of the chorionic area.

Bilaminar omphalopleure.—Here the trophoblast presents the same features as in other regions. The entoderm cells are large and extensively vacuolated. This, as I have already suggested in the case of *Perameles* (1923, p. 150), is probably due to the absorption of carbohydrate in this region of the yolk-sac placenta.

Stage 10. *Bett.* F., 29/8/24. (Post-partum.) (Pl. xxi, fig. 20.)

This is the animal in which the right uterus served as an example of the stage representing the condition of oestrus. In the pouch was a newly-born embryo measuring 14·8 mm. in direct length.

This uterus contains remnants of the foetal membranes which are being actively absorbed. Mostly they are more or less free in the uterine lumen where absorption is in progress by the aid of enormous numbers of polymorphonuclear leucocytes.

Maternal structures.—It is remarkable with what rapidity repair has been effected. In some situations the uterus is almost normal, while in others regeneration processes are being carried on with the utmost rapidity.

The epithelium has undergone a remarkable transformation from its condition just before parturition. In the latter stage, it consisted of thin flattened cells placed end to end, thus forming a thin investment for the underlying blood-vessels and connective tissue. Now, however, where thoroughly regenerated, the epithelium consists of fairly high plump cells with the nuclei arranged in two or even three layers. Where regeneration is in progress many of the original epithelial cells are undergoing degeneration *in situ*. This consists mainly in the absolute dissolution of the cell which soon becomes an amorphous darkly staining mass (Pl. xxi, fig. 20, *d.c.*).

Regeneration of the epithelium is brought about partly by the growth and multiplication *in situ* of a number of the original cells. Partly also it results from the immigration of underlying stroma cells. Such cells undergo marked hypertrophy and subsequent division (Pl. xxi, fig. 20, *str. c.*). The formation of new epithelium by radial growth from the gland mouths as recorded by Hill for *Perameles* (Hill, 1897, p. 422) does not occur in *Bettongia*.

Where the epithelium is practically normal the cells are fairly high and the nuclei are arranged at one, two or three heights. The cytoplasm is vacuolated while the nucleus is vesicular, its chromatin being confined to one or two conspicuous particles.

Active proliferation is still being carried on, mitoses being very frequent. Cilia are quite common and are very robust, their basal granules being easily stained.

Stroma.—Although in some places the stroma is still largely infiltrated, in others it has practically returned to its normal condition. In such situations the glands are quite closely packed. Further, the stroma cells form a fairly compact layer below the epithelium. Although there is no doubt that these cells have increased somewhat in size and in many cases undergone division, there is no evidence of as great a growth and multiplication as is instanced by Hill in the case of *Perameles* at a corresponding stage (Hill, 1897, p. 240). In the blood-vessels, there is some thickening of the walls and in some cases this increase in thickness is almost or completely sufficient to obliterate the lumen of the vessel.

The glands have undergone considerable alteration. The gland cells have increased in size and their protoplasm has become greatly vacuolated. The nuclei have now become larger and more vesicular with a well-defined nuclear membrane. The glands are in many cases still engaged in the removal of lymphatic material and cell remnants. They are well ciliated, cilia being best observed at or near the mouths of the glands. In their basal portions are to be found many polymorphonuclear leucocytes. These are also to be seen in the stroma, and in the vessels.

Summary of Conclusions.

1. Breeding Habits, etc.

(*a*). The breeding season of *Bettongia* is known with certainty to extend over ten months of the year, from the beginning of March to the third week in December.

(*b*). *Bettongia* is polyoestrous.

(*c*). Ovulation is spontaneous and unilateral, one ovum being discharged at each ovulation.

(*d*). Pregnancy is unilateral and under normal conditions occurs alternately in each uterus.

(*e*). Gestation and lactation periods are each of about six weeks' duration.

(*f*). When breeding is active, the gestation and lactation periods overlap, i.e., a new pregnancy may occur while there is still present a pouch foetus.

2. Pregnancy and "Pseudo-pregnancy".

(*g*). While one uterus is pregnant the contra-lateral one enters into and remains in a condition of pseudo-pregnancy. This condition of pseudo-pregnancy persists till parturition, probably being brought about by the same hormones which control the pregnant uterus.

(*h*). During the first half of pregnancy the pregnant and pseudo-pregnant uteri are to all intents and purposes identical, both macroscopically and microscopically. Although ovulation is unilateral, the two uteri undergo quite similar changes during pro-oestrus, oestrus and early pregnancy.

(*i*). Ciliation of the epithelium of the uterus and glands is strongly developed in anoestrum, but later the surface ciliation tends to become suppressed. Gland ciliation persists quite well through the whole cycle.

(*j*). The uterine epithelium proliferates actively during pro-oestrus and oestrus, with the result that the cells become greatly crowded and are arranged at several heights. Thereafter there is little or no new cell formation. The increase in the extent of the internal surface causes the epithelium to become definitely single-layered. Towards the end of pregnancy this layer becomes very delicate, with the nuclei spaced widely apart.

(*k*). Other features of pro-oestrus and oestrus are increase of vascularity, tremendous oedema of the mucosa with infiltration of lymphatic material, and, following upon this, active secretion by the glands of liquid material and of cellular debris, the latter resulting largely from thrown-off gland epithelial cells.

3. "Pseudo-pregnancy".

(*l*). From mid-pregnancy onward the pseudo-pregnant uterus decreases slightly in size. This is due very little to loss of infiltrated material. The large difference in dimensions between the two uteri is mainly due to the mechanical effect of the presence of a growing embryo in the pregnant uterus.

(*m*). Mid-pregnancy is marked by the occurrence of well-marked regeneration processes in the epithelium of the pseudo-pregnant uterus and of the superficial portions of its glands.

(*n*). Infiltration of the mucosa persists until just before the stage of parturition.

(*o*). In the later stages the glands gradually lose their secretory function, becoming more or less inactive.

(*p*). A few hours before parturition occurs the whole wall of the pseudo-pregnant uterus becomes greatly disturbed. This is apparently due to stimulation of the uterine muscles, whose contractions cause the infiltrated material held in the mucosa to break through the walls of the glands and so be removed.

(*q*). Immediately after parturition has occurred and while the breeding season lasts, the pseudo-pregnant uterus enters into the stage of pro-oestrus.

4. Pregnancy.

(i.) Maternal Structures.

(*r*). As in the pseudo-pregnant uterus, infiltration of the mucosa endures throughout and the glands appear to lose their secretory importance.

(*s*). Embryotrophic material is present in abundance and consists mainly of a transudate from the epithelium with included epithelial cells, cellular debris, leucocytes and haematids.

(ii.) Foetal Structures.

(*t*). The amnion in *Bettongia* arises by folds, of which the head fold appears first.

(*u*). The allantois remains small and no allanto-chorion is formed.

(*v*). An efficient placenta is developed by the close apposition of the trophoblast to the uterine epithelium. Actual union without penetration occurs, being most marked and appearing first in the region of the vascular omphalopleure.

5. Parturition.

(*w*). Parturition occurs when the embryo measures about 14·5 mm. in direct length.

(*x*). The foetal membranes are retained in the uterus at parturition and are absorbed with the aid of maternal leucocytes.

Discussion.

The genus *Bettongia* belongs to what is acknowledged to be a very specialized family of marsupials, the Macropodinae, and for that reason may be expected to exhibit highly specialized characteristics superimposed upon others which can be recognized as primitive.

In this discussion, I will confine myself to three points: (*a*) the condition of polyoestrum, (*b*) the placental arrangements, (*c*) the control of pregnancy.

(*a*). *The condition of polyoestrum.*

In Hill's monograph on the development of the Native Cat, *Dasyurus* (1910), it is stated that *Dasyurus* has only one breeding season in the year. Later (1913), Hill and O'Donoghue repeated this statement, and drew the inference that the monoestrous condition is the primitive one. However, still later (1918), the result of investigations on the breeding habits of *Didelphys aurita* raised doubts in Hill's mind as to the accuracy of his statement for *Dasyurus*. What is of some

importance to us at the present juncture, also, is that he states, with regard to *Macropus ruficollis*, originally believed by him to be monoestrous, that it breeds "during August–September, and again during December–February, as soon as the young one has vacated the pouch or even before" (p. 102). This last statement shows that Hill had recognized the overlapping of the gestatory and lactatory periods in a Macropod.

As regards *Didelphys virginiana*, Hartman at first believed (1916) that this marsupial was monoestrous, but his later researches showed that in this belief he was wrong and that the Virginian opossum is definitely polyoestrous.

It is highly probable that *Dasyurus* is also polyoestrous, although I have no observations to offer on this point myself, and that the polyoestrous condition, as suggested by Hartman, is the more primitive.

The condition of polyoestrum as found in *Bettongia* offers considerable differences in detail from the conditions found in *Didelphys*. In the latter animal, at ovulation, a large number of ova are shed, some from each ovary. Should these be unfertilized, pseudo-pregnancy supervenes which lasts for some eleven days. After this comes a short dioestrous period which is followed again by the next pro-oestrus.

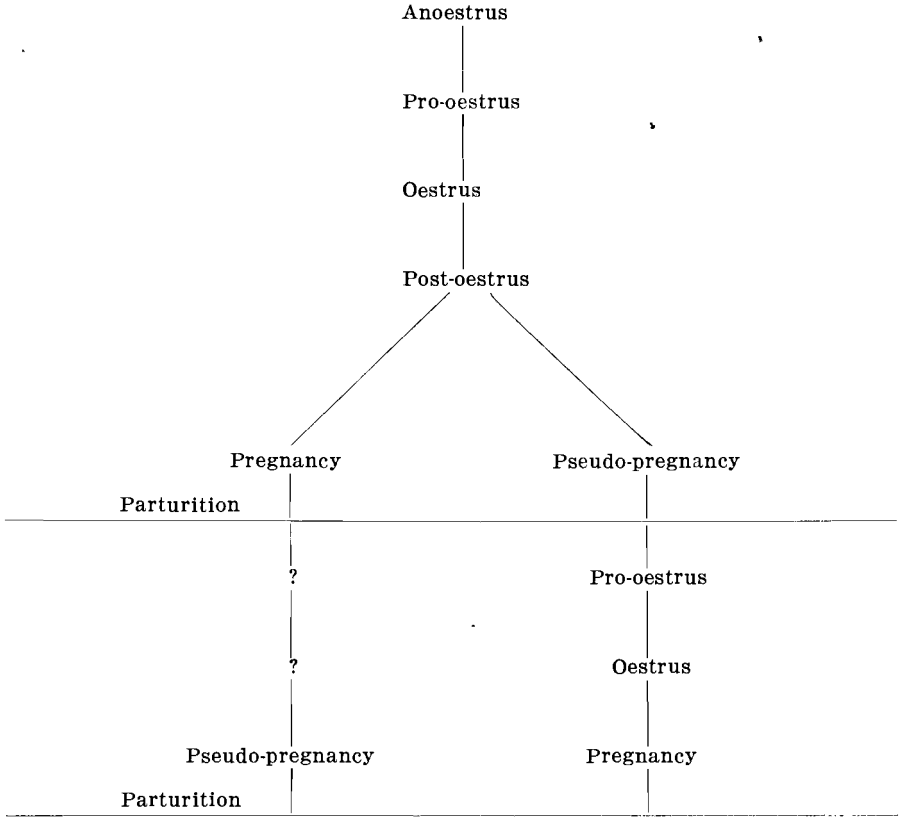
During the season, then, the cycle in *Didelphys virginiana* is as follows:

Anoestrus (or Dioestrus)
|
Pro-oestrus
|
Oestrus
|
Post-oestrus
|
Pseudo-pregnancy or Pregnancy
|
Dioestrus (or Anoestrus)

In *Bettongia* ovulation is unilateral and one ovum only is discharged. Except for this detail the non-pregnant cycle is not very different from that in *Didelphys*. After ovulation, in the non-pregnant female, psuedo-pregnancy of the two uteri supervenes (controlled apparently by the single corpus luteum) after which the organs return to the condition of dioestrus.

In a female undergoing successive pregnancies during the breeding season, the cycle becomes somewhat complex, since, while one uterus is pregnant, the other is pseudo-pregnant, and this goes on alternately throughout the season under normal conditions. The pseudo-pregnant uterus, so soon as parturition occurs

in the pregnant uterus, passes on to the pro-oestrous and oestrous stages. The cycle is therefore of the following type (commencing from anoestrus):



Cycle of Pregnancy in Bettongia.

It will be seen from the above diagram that the cycle of pregnancy in *Bettongia* is very different from any yet described for a mammal and in fact is only possible in a monotocous marsupial. While the whole cycle is deserving of more intense and detailed study, it will also be seen that the post-partum changes undergone by the ex-pregnant uterus, at a stage in the cycle when the ex-pseudo-pregnant uterus is being influenced by pro-oestrous and oestrous stimuli, is, in the present state of our knowledge with regard to the origin of these stimuli, one particularly deserving of attention.

Finally, it may be conceded, I think, without difficulty, that in view of the nature of the cycle in *Didelphys*, the curious cycle found in *Bettongia* is a type of polyoestrum which must have been secondarily acquired simultaneously with the development of the monotocous condition from one primitively polytocous.

(*b*). *Placental arrangements in* Bettongia.

The evidence adduced by Hill (1897, 1899), Flynn (1923) and others is unquestionably in favour of the ancestral marsupial having been a placental

mammal. It is also unquestionable that *Bettongia* is a genus very far removed in many respects from the primitive type, and it might be expected that this specialization will be indicated in its placental arrangements. This is so, since in this marsupial the allantois remains quite small and an allanto-chorion, consequently an allantoplacenta, is never developed. Nevertheless, nutrition, respiration, and elimination of the waste-products of the foetus have to be arranged for and this is done by the development of a yolk-sac placenta of a very efficient type. This consists of the close apposition and, in places, of actual union, of the trophoblast with the prepared uterine epithelium. Definite union is particularly to be found in the region of the vascular omphalopleure. Between the foetal and maternal tissues, where separated, there occurs abundant "uterine milk" consisting of cell debris, leucocytes, haematids, lymph and other material, all being actively absorbed and ingested by the trophoblast cells.

As in other marsupials, at parturition, the foetal membranes remain behind in the uterus to be absorbed.

(*c*). *The control of pregnancy.*

It will be seen from the foregoing that the phenomenon of pregnancy (and pseudo-pregnancy) in *Bettongia* offers for solution interesting and complex problems, the elucidation of which may well help in the understanding of the sexual cycle in mammals in general.

The first half of pregnancy in this animal is characterized by identical changes in the two uteri, respectively pregnant and pseudo-pregnant. There are to be found, *inter alia*, definite identity in the alterations in the form of the epithelium of the uterine lining and of the glands, in the amount of lymph infiltration into the stroma and in the intensity of vascularization of the uterine tissue.

During the later stages of pregnancy, macroscopically and microscopically, the two uteri differ greatly. Macroscopically, the pregnant uterus increases greatly in size, this increase being due to the presence of the growing embryo. There is no increase in the amount of lymph infiltration over that found in the contralateral pseudo-pregnant uterus. At about mid-pregnancy, the surface epithelium, and that of the superficial portion of the glands, in the pseudo-pregnant uterus, undergo involution, but it is to these structures that involution is confined, for the stroma remains of the same turgidity and of the same vascularity until just near the stage of parturition. It would, therefore, seem as if two factors, at least, are concerned in the control of the uteri during pregnancy.

Detailed experimentation will be necessary before any certainty can be arrived at as to what these factors are and how they act.

As pointed out above, a further stimulus is evident just before parturition when the lymphatic contents of the wall of the pseudo-pregnant uterus are squeezed out and the wall collapses.

There are many other outstanding problems awaiting solution in this animal. The relation of pro-oestrus and oestrus to lactation, the overlapping of lactatory and gestatory periods, and the occurrence of ovulation (no doubt preceded by pro-oestrus and oestrus) in the immediate post-partum period are a few of the prominent questions whose solution, I am convinced, will help to a better understanding of the sexual cycle in the higher mammals.

In speaking of the function of the corpora lutea, Parkes (1929, p. 175) says: "The ideal subject for experiments of this nature would be a small monotocous animal in which the ovary containing the corpus luteum could be readily removed, leaving intact the second ovary containing no corpus luteum, to carry on the other ovarian functions." In this, as in other respects (for example, the possession of two separate uteri), *Bettongia* seems to be eminently suitable, and for our knowledge of these and allied phenomena investigation and experimentation upon this animal hold great hope for the future.

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EXPLANATION OF PLATES XIX–XXI.

b.m., basement membrane; *cap.*, capillary; *ch. ect.*, chorionic ectoderm; *ch. mes.*, chorionic mesoderm; *cil.*, cilia; *c.m.*, cellular material in active ingestion by the trophoblast; *d.c.*, degenerating cell; *ent.*, entoderm; *foet. cap.*, foetal capillary; *gl. ep.*, gland epithelium; *haem.*, haematids; *leuc.*, leucocyte; *m. cap.*, maternal capillary; *mit.*, cell in mitosis; *mgc.*, migrating gland nuclei; *plm.*, polymorphonuclear leucocyte; *proa.*, proamnion; *sec.*, secretion; *s.t.*, sinus terminalis; *str. c.*, stroma cell; *troph.*, trophoblast; *u.e.*, uterine epithelium; *u.m.*, material secreted into uterine lumen (uterine milk);

w.c., wandering (?) cell; *x* (fig. 8), remains of a degenerated gland nucleus as it passes into the gland lumen; *y.s.cav.*, yolk-sac cavity; *y.s.spl.*, yolk-sac splanchnopleure.

Plate xix.

Figs. 1, 2.—Stage 1. *Bett.* E., 15/9/26. 1. Uterine epithelium with underlying stroma. 2. Transverse section of uterine gland.

Figs. 3, 4.—Stage 2. *Bett.* F., 29/8/24 (right uterus). 3. Portion of the uterine epithelium and underlying stroma. 4. Cross-section of uterine gland.

Fig. 5.—Stage 3. *Bett.* F., 19/9/24. Portion of uterine epithelium and underlying stroma of left uterus.

Fig. 6.—Stage 4. *Bett.* A., 6/9/24. Portion of uterine epithelium and underlying stroma of right uterus.

Fig. 7.—Stage 5. *Bett.* B., 30/8/24. Portion of uterine epithelium and underlying stroma (right uterus).

Plate xx.

Fig. 8.—Stage 5. *Bett.* B., 30/8/24. Cross-section of a uterine gland showing inward movement of nuclei accompanied by degeneration. At *x* is shown the last remnants of a nucleus as it passes into the lumen. At *y* is a portion of a gland cell about to be desquamated into the gland cavity.

Figs. 9-13.—Stage 6. *Bett.*, 2/9/24. 9. Portion of the epithelium of the right, non-pregnant uterus showing degeneration of epithelial cells and the movement upward of stroma cells to help form a new epithelium. 10 and 11. Degeneration *in situ* of epithelial cells of the non-pregnant uterus, the latter figure showing the contents of the cell being thrown into the uterine cavity. 12. Cross-section of the upper portion of a gland in the non-pregnant uterus, showing active involution in this region. 13. Epithelium of pregnant uterus with underlying stroma.

Fig. 14.—Stage 7. *Bett.* E., 14/9/24. Portion of the epithelium of the pregnant uterus with underlying stroma.

Fig. 15.—Stage 8. *Bett.* G., 11/9/24. Portion of the epithelium of the non-pregnant uterus with underlying stroma.

Plate xxi.

Fig. 16.—Stage 8. *Bett.* G., 11/9/24. Internal view of the mesial half of the pregnant uterus.

Figs. 17-19.—Stage 9. *Bett.* B., 11/9/24. 17. Section showing active absorption, by the trophoblast cells of the vascular omphalopleure, of secretion from a uterine gland. 18. Section showing an area in which the chorion is fused to the uterine epithelium and in which active ingestion by the chorionic ectoderm of extravasated blood corpuscles and other cellular material is in progress. 19. Close apposition of the bilaminar omphalopleure to the uterine epithelium and ingestion by the former of haematids and other cellular material.

Fig. 20.—Stage 10. *Bett.* F., 29/8/24. This shows degeneration of uterine epithelium and the movement upward of stroma cells to form new epithelium. Notice cells in mitotic division and the enlargement of the endothelial cells of the maternal capillaries.