On the Causes Responsible for the Developmental Progress of the Mammary Glands in the Rabbit during the Latter Part of Pregnancy.

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[Plate 23.]

The parts played by the various reproductive organs in causing the growth of the mammary gland and the secretion of milk have been the subject of much recent investigation. Most of the work has been done with the rabbit, and the changes which occur in its mammary glands have been studied very minutely.*

The causes of the growth changes in the gland during the first part of pregnancy are now conclusively shown to be due to the influence of the corpus luteum.† These changes in the rabbit culminate at about the 16th day after coitus; after this time in pseudo-pregnant rabbits (*i.e.*, rabbits which have had coitus and developed corpora lutea, but have not become pregnant) the gland undergoes atrophy. If the growth changes of the mammary glands of a series of pregnant and pseudo-pregnant rabbits be compared, it will be seen that the changes are similar until about the 16th day, at which period in the pseudo-pregnant condition the gland begins to atrophy, while in the pregnant animal the gland becomes much thicker, increasing rapidly in weight from the 24th to the 30th day.

The object of the investigations described in the present paper was to determine the causes of this further development of the mammary gland (called by Ancel and Bouin‡ the glandular phase) which takes place after the 16th day of pregnancy.

In the present paper it is shown that, contrary to the commonly accepted opinion, the corpus luteum in the rabbit does not become atrophied during the latter part of pregnancy, but maintains its size till late in gestation and even into the period of lactation, the cause of this development originating in the fœtus. It is also shown that the changes in the mammary gland in the second half of pregnancy are correlated with the further development of the corpora lutea, and consequently that the origin of the

* Schil, 'Recherches sur la Glande Mammaire,' Nancy, 1912.

† Ancel and Bouin, 'Compt. rend. Soc. Biol.,' vol. 66 (1909). For further references see Hammond and Marshall, 'Roy. Soc. Proc.,' B, vol. 87 (1914).

‡ Ancel and Bouin, 'Journ. de Phys. et de Path. générale,' vol. 13 (1911).

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influence which causes the growth changes and also the glandular phase of the gland is essentially the same and not different, as Ancel and Bouin have supposed.*

Many methods of research have been used to eliminate the various factors which might possibly cause this development. These are described under the headings of the various tissues or organs which were suspected of being the originators of the stimulus.

The Myometrial Gland.

Ancel and Bouin† state that the myometrial gland occurs in the uterus of the rabbit during the second half of pregnancy. They describe it as consisting of clumps of cells of an epithelioid appearance, lying under the placental cells and between the muscle cells of the circular and longitudinal muscular coats in close proximity to the blood-vessels. They suggest that it is a gland of internal secretion which takes on the functions of the corpus luteum during the second half of pregnancy, controlling the glandular phase of the mammary gland‡ and also the tolerance of the uterus for the fœtus.§

Fraenkel‖ has verified their observations as to the presence of the gland in rabbits. He could not find it, however, in the uteri of many other species of animal examined. On this account, and also on account of its slender vascularity (for a gland of internal secretion), he does not accept their theory as to its function.

Mercier¶ identifies the myometrial gland with cells described by him as nephro-phagocytes, and states that the normal connective tissue of mammals contains these cells, which have the property of phagocytosis, absorbing solid substances and fixing soluble substances injected into the organism. He found that these cells occur only in the pregnant and not in the normal uterus.**

The uteri of many rabbits between the 16th and 30th days of pregnancy have been examined by cutting serial sections across a large number of pieces from each uterus. Epithelioid cells, which correspond to those described by Ancel and Bouin as the myometrial gland, have been found only in one or two cases. In every case they have been found beneath the small yellow patches which are seen in the region of the uterine mucosa

* Ancel and Bouin, 'Journ. de Phys. et de Path. générale,' vol. 13 (1911).
† Ancel and Bouin, 'Compt. rend. Assoc. des Anat.,' 13e. réunion, Paris, 1911.
‡ Ancel and Bouin, 'Compt. rend. Soc. Biol.,' vol. 72 (1912).
§ Ancel and Bouin, 'Compt. rend. de l'Acad. des Sci.,' vol. 154 (1912).
‖ Fraenkel, 'Arch. für Gynaek.,' vol. 99 (1913).
¶ Mercier, 'Compt. rend. Soc. Biol.,' vol. 74 (1913).
** Mercier, 'Compt. rend. Soc. Biol.,' vol. 72 (1912).

opposite the placenta. Since these pustules consist of giant cells (fig. 1) (which are supposed to be detached trophoblast cells) surrounded by a mass of decidual cells, it seems quite possible that the myometrial cells are fœtal cells which have wandered to the muscular coat.

As the cells of the myometrial gland do not occur constantly in all pregnant rabbits, so far as we have been able to investigate, we must conclude that they cannot have the important functions which Ancel and Bouin ascribe to them.

The Maternal Placenta.

Many investigators have attributed to the placenta the property of causing milk secretion (Halban,* Basch†), and, although as a primary cause of the growth changes of the mammary gland in the first half of pregnancy it is shown to be unnecessary, yet it seemed quite possible that it might be responsible for the glandular phase of the second half of pregnancy.

Injection experiments with placental extracts were not tried, since there is great difficulty in getting fresh extracts sterile, and the process of boiling is open to the objection that the active principle may be thereby destroyed. The maternal placenta was, however, caused to form in rabbits by the use of the methods adopted by Loeb‡ when studying tissue growth in the uterus of the guinea-pig.

Pseudo-pregnant doe rabbits, obtained by coitus with a vasectomised buck, were taken at about the 7th day (the time at which the ovum normally becomes fixed in the uterine wall), and the uterus was stimulated, in some cases by snipping out pieces of the wall from each horn, or in other cases by slitting the uterus for the whole of its length and then cutting small pieces away from the edges.

The rabbits operated on in this way form decidual cells in the parts of the uterus which have been stimulated by cutting or by contact with a foreign body (figs. 2, 3, and 4). These masses of decidual cells are practically identical in appearance with those of the maternal placenta.

In each case the duration of pseudo-pregnancy dates from the day on which coitus took place with the vasectomised buck.

The results of the experiments are given below.

* Halban, 'Arch. für Gynaek.,' vol. 75 (1905).

† Basch, 'Monatsch. f. Kinderheilk.,' vol. 8 (1909).

‡ Loeb, 'Jour. Amer. Med. Assoc.,' vol. 50 (1908), and 'Arch. f. Entwick. der Organ.,' vol. 32 (1911).

(A) *Cases in which no Corpora lutea were Present in the Ovaries.**

(1) Multiparous rabbit. Small pieces of muscle, with the attached mucosa, were removed from the uterine walls at short distances apart (to represent fœtal attachments) on the 9th day after coitus. Killed on the 25th day. Mammary glands consisted mostly of ducts, with a little alveolar tissue. Openings in the uterus had closed up, and there was no decidual formation. The glands were small and the blood-vessels were not enlarged.

In the following cases (2–5) the rabbits had never been pregnant :—

(2) Small pieces of uterus removed on the 9th day after coitus. Killed on the 25th day. Ovaries very small. Uterus closed and infantile : no decidual formation. Mammary gland consisted of ducts only.

(3) Small pieces of the uterus removed on the 9th day after coitus. Killed on the 28th day. Some very old corpora lutea seen in the ovaries, which could not have been the result of the last coitus. Uterus closed; glands small; one fold of mucosa containing a few decidual cells. Mammary glands atrophic, with a little milk in the ducts.

(4) Uterine horns slit for whole length and pieces removed on the 7th day after coitus. Killed on the 30th day. Uterus closed on one side but open on the other. Mucosa showed small glands and congested capillaries, but with very little proliferation of the connective tissues. Mammary glands consisted of ducts only.

(5) Uterine horns slit and pieces removed on the 7th day. Killed on the 31st day. Uterus closed in most places. Very few glands and blood-vessels present, but one fold of the mucosa had swollen slightly. Mammary gland consisted of ducts only.

(B) *Cases in which Corpora lutea were Formed in the Ovaries.*

(6) Rabbit not previously pregnant. Uterine horns slit and pieces removed on the 8th day of pseudo-pregnancy. Killed on the 11th day. Uterus open wide, glands of the mucosa large and active, capillaries dilated, and decidual cell formation beginning in the connective tissue. Mammary glands beginning to hypertrophy, but not thickened.

(7) Multiparous rabbit. Uterine horn slit and pieces removed on the 7th day. Killed on 17th day. Uterus wide open, with large growths on the mucosa, consisting of masses of decidual cells containing hyaline material. Glands atrophic but blood-vessels dilated. Mammary glands well developed but not thickened.

* It has been noted in a former paper ('Roy. Soc. Proc.,' B, vol. 87 (1914)) that coitus is not necessarily followed by ovulation in the rabbit.

(8) Multiparous rabbit. Small pieces of uterus removed on the 8th day. Killed on the 18th day. Uterus not closed in places, but with outgrowing mucosa (fig. 2). Glands of mucosa moderately well developed, one large fold and one small one, with well developed decidual cells enclosing capillaries (fig. 3). Mammary glands well developed but not thick; milk present in the ducts.

(9) Rabbit not previously pregnant. Small pieces of the uterus removed on the 7th day. Killed on the 22nd day. Uterus open in places and decidual cells formed. Glands small, blood-vessels numerous and very dilated. Mammary glands showing signs of atrophy but not thickened.

(10) Rabbit not previously pregnant. Uterine horns slit and pieces removed on the 8th day. Killed on the 24th day. Uterus wide open (fig. 4). Glands small and capillaries dilated, and forming a plexus below the surface of the mucosa (fig. 5). Decidual cells well developed. Mammary glands well developed but not thickened.

(11) Multiparous rabbit. Uterine horns slit and pieces removed on the 7th day. Killed on the 25th day. Uterus wide open, uterine glands small, and capillaries dilated. Decidual cells formed in connective tissues, which contained small masses of hyaline material. Mammary glands well developed but not thickened.

(12) Multiparous rabbit. Small pieces of uterus removed on 9th day. Killed on the 25th day. Uterus closed, glands moderately well developed, and capillaries dilated. A few decidual cells were formed at the ends of the folds of the mucosa. Mammary glands not thickened, but contained milk, which could be expressed from the nipples.

13) Multiparous rabbit. Small pieces of the uterus removed on the 10th day. Killed on the 31st day. Uterus almost closed. Glands of the mucosa small, connective tissue dense, with slight congestion of the blood-vessels. Mammary gland in the atrophic stage.

In those cases in which, after a sterile copulation, no corpora lutea were found there was very little or no formation of decidual cells, thus confirming the opinion expressed in a former paper* that the raised nutrition of the uterus brought about by the formation of the corpus luteum is necessary for the fixation of the ova. Where corpora lutea were developed, the connective tissue cells hypertrophied and formed decidual cells, these often enclosing a large amount of hyaline material. In several cases the blood-vessels were dilated and formed a plexus supplying the surface of the mucosa (fig. 4).

* Hammond and Marshall, 'Roy. Soc. Proc.,' B, vol. 87 (1914).

Contrary to the findings of Ancel and Bouin,* we have never discovered any traces of the myometrial gland in the uteri of rabbits which have been operated on as in this series.

The effect of the formation of the decidual cells on the mammary gland is negative, no growth or glandular development taking place beyond what occurs in normal pseudo-pregnancy (see Table II).

These experiments, which were confirmed by the subsequent investigations in which the fœtuses were removed (see below), show that the maternal placenta is not the source of the stimulus which causes the glandular phase of the mammary gland.

The Fœtus.

Lane-Claypon and Starling† thought that the growth of the mammary gland in rabbits was probably due to the presence of the fœtus, and other investigators have confirmed their results. Although the actual results of their experiments have been rendered doubtful through the discovery of the action of the corpus luteum, it has not been shown that the fœtus is without effect on the mammary gland during the second half of pregnancy.

Experiments were therefore undertaken to determine the effect of removal of the fœtuses from pregnant rabbits, leaving the rest of the generative tract —ovaries, uterus, and placentæ—intact.

Weymeersch‡ has shown that the placenta persists in the uterus for some time after the embryo has been removed. Also observation of the placentæ of fœtuses which had atrophied at an early stage during development showed§ that once started the placentæ may persist during the whole course of gestation independently of the attachment of the living fœtus.

The fœtuses have been removed from pregnant rabbits between the 13th and 15th days. An incision was made through the uterus and fœtal membranes, and the fœtus withdrawn by cutting through the umbilical cord. In each case the removal resulted in the arrest of the growth of the mammary gland, and was followed shortly afterwards by the secretion of milk.

The details of these experiments are given below :—

Rabbit 1.—Fœtuses (3 and 4) removed on the 13th day after coitus. Milk expressed from nipples on the 20th day. Killed on the 25th day. Placenta still attached to the uterine wall, but showing signs of atrophy. Mammary gland not thickened.

* Ancel and Bouin, 'Compt. rend. Soc. Biol.,' vol. 72 (1912).

† Lane-Claypon and Starling, 'Roy. Soc. Proc.,' B, vol. 87 (1906).

‡ Weymeersch, 'Ann. Soc. Roy. de Science med. nat. Bruxelles,' vol. 70 (1912).

§ Hammond, 'Journ. Agric. Sci.,' vol. 6 (1914).

Rabbit 2.—Fœtuses (3 and 5) removed on the 13th day after coitus. Milk expressed on the 21st day. Killed on the 27th day. Placentæ attached to the uterine wall, three well developed, but the remainder showing signs of atrophy. Mammary glands not thickened.

Rabbit 3.—Fœtuses (4 and 5) removed on the 13th day after coitus. No milk on the 15th day. Fur pulled out and nest made on the 31st day. Killed on 32nd day, when milk was squeezed from nipples. Seven placentæ found loose in body cavity. Mammary glands not thickened.

Rabbit 4.—Fœtuses (3 and 5) removed on the 15th day after coitus. Making nest on the 20th day. Killed on the 22nd day, when milky fluid was expressed from nipples. Seven placentæ found loose in body cavity. Mammary glands not thickened.

Rabbit 5.—Fœtuses (3 and 5) removed on the 15th day after coitus. Making nest on the 20th day. Colostrum expressed on the 23rd day. Killed on the 30th day. Three atrophic placentæ attached to uterus, and four placentæ found loose in the body cavity. Mammary glands not thickened.

Rabbit 6.—Fœtuses (5 and 5) removed on the 15th day after coitus. Milk expressed on the 19th day. Killed on the 28th day. Three atrophic placentæ attached to the uterus and six free in the body cavity. Mammary glands not thickened.

It is concluded from these experiments that the fœtus is a necessary factor in causing the secondary growth changes in the mammary gland. In these cases the placentæ were retained and, in some of the animals, in a fresh condition. The results confirm those of Biedl and Koenigstein,* who found that implantation of placentæ into non-pregnant rabbits was without effect on mammary secretion, but that implantation of the fœtus resulted in the growth of the gland and the secretion of milk.

There are several facts, however, which indicate that the action of the fœtus on the mammary gland is not a direct one. Halban† has pointed out that the origin of the stimulus which causes milk secretion must be situated outside the fœtus, since the act of parturition sometimes results not only in the secretion of milk by the mother but also by the fœtus—the so-called "witch's milk."

Moreover, many cases are known in which a woman has produced a healthy, vigorous child, and yet the mammary glands secreted very little milk. Also, as O'Donoghue‡ has pointed out, milk is secreted in the rabbit several days

* Biedl and Koenigstein, 'Zeits. f. Exp. Path. u. Therap.,' vol. 8 (1911).

† Halban, 'Arch. f. Gynaek.,' vol. 72 (1905).

‡ O'Donoghue, 'Quart. Journ. Micro. Science,' vol. 57 (1911).

before parturition, but does not occur in man and Dasyurus till several hours after it, so that the secretion of milk does not coincide with the expulsion of the fœtus. We have observed a case of a goat in which milk was secreted in large quantities (800 c.c. per day) for three weeks before the kids were born. This goat was served on September 28 and aborted on November 6; it was served again on November 20 and came into milk on April 1, the kids being born on the 21st of that month.

A further interesting fact which seems to be correlated with the diminished activity of the corpus luteum is the instinct of nest making. Often at the end of pseudo-pregnancy, at the time the animal is also "on heat," or a short time after the removal of the ovaries or fœtuses, fur is plucked out and a nest is made in preparation for the expected young.

The Corpus Luteum.

The conclusion arrived at from the previous experiments was that the growth changes of the mammary gland in the second half of pregnancy are dependent on the fœtus, but probably not directly. Since we know that the presence of the fœtus (in those animals which ovulate spontaneously) causes the corpus luteum spurium to develop into the corpus luteum of pregnancy, we should expect much the same thing to happen in the rabbit. If it does so, we might expect that the further development of the mammary gland is brought about as a result of the further growth of the corpus luteum, for it seems more likely that the mammary gland should be controlled from one point than from two, as Ancel and Bouin suggest.

The supposition that the corpus luteum of the Eutheria remains in an active state during the second half of pregnancy is not in accordance with the commonly accepted views, but O'Donoghue* has shown that the whole of the process of growth of the mammary gland and of milk secretion in the marsupial cat (*Dasyurus*) is under the influence of the corpus luteum.

He states that, in many cases after coitus where pregnancy did not supervene, the growth and activity of the mammary glands reached a stage of development indistinguishable from that which occurs in mammals a few days after parturition.

Sandes† also states that he found that the formation of the corpus luteum in Dasyurus was rapid, and that it persisted during the greater part of the time that the animal was lactating, and only disappeared when the young animals became independent.

* O'Donoghue, 'Quart. Journ. Mic. Sci.,' vol. 62 (1911).

† Sandes, 'Proc. Linn. Soc. New South Wales,' vol. 28 (1903).

Watson* has also shown that the corpora lutea of the rat during the lactation period are very large and bigger than corpora lutea spuria. He was inclined to believe that they were corpora lutea of pregnancy, which had not degenerated in the latter part of gestation.

In order to test this supposition, the corpora lutea of a series of rabbits in various conditions as regards sexual activity (pregnant, pseudo-pregnant, with decidual tissue produced, and with fœtuses removed) were measured as a test of their state of development. The method has been to cut free-hand sections of the corpora lutea in the ovaries, which in almost every case had been preserved in formalin. Three or four sections showing the greatest diameter from each corpus luteum were picked out. Their diameter was accurately measured under the low power of the microscope by means of a scale attached to the mechanical stage. In every case the largest diameter was measured. As a rule three or four corpora lutea from the ovaries of each rabbit have been measured in this way, and the results arranged so that each figure given in the Table (I) below represents the average of about sixteen measurements.

The figures given are no doubt influenced to some extent by the size and age of the rabbit, but, nevertheless, they show quite clearly that the corpus luteum of pregnancy undergoes a further development than that of the pseudo-pregnant condition.

The latter portion of the Table shows that this further development of the corpus luteum is not correlated either with the growth of the maternal placenta (as shown by the animals in which this condition was experimentally produced), or with the formation of the fœtal placenta or membranes (as shown by the removal experiments).

In order to correlate with these observations the effect of the same treatment on the mammary gland, a similar Table has been made out for this gland. The pseudo-pregnant growth of the gland is mostly in the lateral direction, so that in stained and cleared specimens one can easily see the changes occurring; but in the second stage of development the growth is rather in thickness, so that other means have to be taken to demonstrate its growth during this period.

The mammary glands have been dissected out, attached to their usual muscular band, and fixed in alcohol. The four centre glands, with the muscle beneath them, have then been cut out and weighed. This is not such a satisfactory mode of measurement as that used for the corpus luteum, for it is more dependent on the size of the animal and the amount of muscle taken with the gland. It is sufficiently accurate, however, to show that the degree

* Watson, 'Proc. Phys. Soc.,' 'Journ. of Physiol.,' vol. 34 (1906).

Table I.—Size of Corpora Lutea.

State of rabbit.	Days after coitus.														
	3–4.	5–6.	7–8.	9–10.	11–12.	13–14.	15–16.	17–18.	19–20.	21–22.	23–24.	25–26.	27–28.	29–30.	31–32.	33–34.
Pregnant.....	7·5	10·0	—	—	13·2	15·0	12·8	13·0 13·9 14·1 12·3 14·3 12·4	—	—	14·7 14·7	13·5 12·1 13·1 13·7	14·3 14·1 14·3 11·4 12·5	13·0 15·0 13·7 13·8	—	—
Average.....	7·5	10·0	—	—	13·2	15·0	12·8	13·3	—	—	14·7	13·1	13·3	13·9	—	—
Pseudo-pregnant ...	—	—	—	9·2	—	13·2	11·6	10·8 11·6 8·0	—	—	9·2 11·2	—	6·7 8·5	—	—	—
Average.....	—	—	—	9·2	—	13·2	11·6	10·1	—	—	10·2	—	7·6	—	—	—
With experimentally produced decidual cells	—	—	—	—	10·5	—	—	12·2 11·6	—	—	—	8·8 7·5	6·7	—	7·2	—
Average.....	—	—	—	—	10·5	—	—	11·9	—	—	—	8·1	6·7	—	7·2	—
With fœtus removed	—	—	—	—	—	—	—	—	—	9·2	—	8·3	8·2 8·1	7·6	—	8·7
Average.....	—	—	—	—	—	—	—	—	—	9·2	—	8·3	8·2	7·6	—	8·7

Table II.—Weight of Mammary Gland (Grm.).

State of rabbit.	Days after coitus.														
	3-4.	5-6.	7-8.	9-10.	11-12.	13-14.	15-16.	17-18.	19-20.	21-22.	23-24.	25-26.	27-28.	29-30.	31-32.	33-34.
Pregnant.....	13·5	11·0	—	12·5	—	—	—	18·6 18·6 11·5 14·0	—	—	16·7 20·5	32·3 30·2	38·4 29·7 45·0 37·3 27·7 39·8	49·7 31·4 41·6 45·3	—	—
Average.....	13·5	11·0	—	12·5	—	—	—	15·7	—	—	18·6	31·2	36·3	42·0	—	—
Pseudo-pregnant ...	—	—	—	—	—	—	—	11·0 16·0	—	—	—	16·0	12·6	—	—	—
Average.....	—	—	—	—	—	—	—	13·5	—	—	—	16·0	12·6	—	—	—
With experimentally produced decidual cells	—	—	—	—	8·5	—	—	21·0 14·0	—	8·5	11·5	15·5 18·5	16·5	10·5	10·5	—
Average.....	—	—	—	—	8·5	—	—	17·5	—	8·5	11·5	17·0	16·5	10·5	10·5	—
With fœtus removed	—	—	—	—	—	—	—	—	—	24·0	—	19·5	14·5 18·0	14·5	—	12·0
Average.....	—	—	—	—	—	—	—	—	—	24·0	—	19·5	16·2	14·5	—	12·0

Fig. 1.

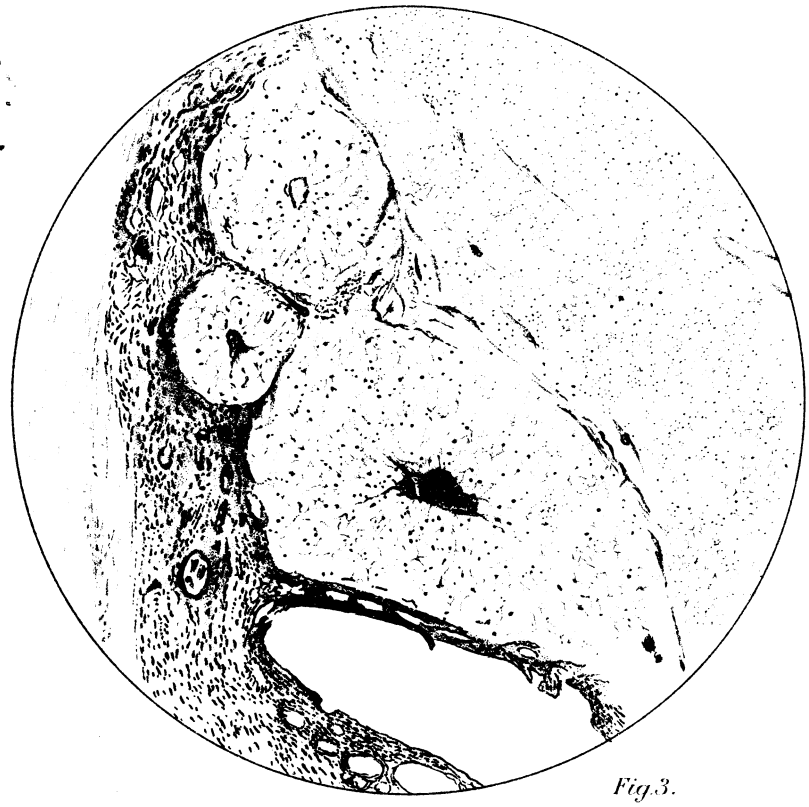

Fig. 3.

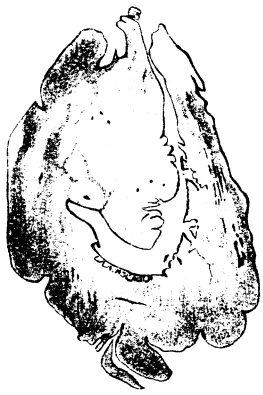

Fig. 2.

Fig. 4.

Fig. 5.

of development of the gland in the pregnant and the pseudo-pregnant condition is quite distinct.

Table II shows the results obtained. Comparing this with Table I it is seen that there is a definite correlation between the development of the corpus luteum and the increase in weight of the mammary gland.

Summary and Conclusions.

1. Experimental results show that the development of the mammary gland of the rabbit during the second half of pregnancy is under the same influence as that which controls the development during the first half, namely, the corpus luteum.

2. Contrary to the generally accepted opinion, this gland (the corpus luteum) is active during the second half of pregnancy.

3. The further development of the corpus luteum, which takes place during the latter part of pregnancy, is due to the influence of the fœtus.

4. The experiments do not uphold the view of Ancel and Bouin that the glandular phase of the mammary gland is due to something entirely different from that which causes the growth-changes, but confirm the views expressed in an earlier paper by Hammond and Marshall, in which it was shown that milk secretion in pseudo-pregnancy takes place in correlation with the involution of the corpus luteum.

Apparently the secretion of milk results whenever the influence causing the glandular growth (the corpus luteum) is removed or lessened in amount, provided that the initial development has gone far enough.

The experiments described above were performed in 1913–14 at the School of Agriculture and Field Laboratories, Milton Road, Cambridge. The expenses were, for the most part, defrayed by a research grant from the Board of Agriculture and Fisheries out of funds placed at their disposal by the Development Commissioners.

I am greatly indebted to Dr. F. H. A. Marshall for the help he has given me in preparing this paper.

DESCRIPTION OF PLATE.

[The figures were drawn by the late Edwin Wilson, of Cambridge.]

Fig. 1.—Giant cells referred to on p. 536. [This section also passed through the myometrial gland, and it was intended that the cells of this gland should be shown on the figure, but, unfortunately, owing to inability to supervise the drawing, as a result of the outbreak of war, and the subsequent death of the artist, the figure was not completed.]

Fig 2.—Experimentally produced Placenta—Section of Uterus, showing Outgrowing Lobe of Mucosa, where small piece has been removed by operation. (Low power.)

Fig. 3.—Experimentally produced Placenta—Connective Tissue forming Decidual Cells which enclose Blood Capillaries. (Same section as fig. 2, more highly magnified.)

Fig. 4.—Experimentally produced Placenta—Part of Section of Uterus, which has been slit open, showing Growth of Placental Tissue in upper portion of section. (Low power.)

Fig. 5.—Experimentally produced Placenta (same section as fig. 4, more highly magnified)—Plexus of Blood-vessels below the Surface of the Mucosa.

On the Post-Œstrous Changes occurring in the Generative Organs and Mammary Glands of the Non-Pregnant Dog.

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[Plates 24–26.]

The œstrous cycle in animals which come "on heat" at relatively infrequent intervals (*i.e.* moncestrous animals) has been divided by Heape into the following four periods:—

(1) Anœstrum (period of rest).

(2) Pro-œstrum (periods of growth and destruction).

(3) Œstrus (period of desire).

(4) Metœstrum (period of recuperation, occurring only in the absence of pregnancy).

This scheme of classification was adopted by Marshall and Jolly in describing the changes occurring in the generative organs of the Bitch. It has been shown that ovulation normally takes place during œstrus at or near the termination of the sanguineous discharge which characterises the pro-œstrum. This observation has since been confirmed by Keller. Ovulation occurs spontaneously in bitches, the additional stimulus of coitus being unnecessary.

Fig.1.

Fig.3.

Fig.2.

Fig.4.

Fig.5.