The Functional Correlation between the Ovaries, Uterus, and Mammary Glands in the Rabbit, with Observations on the Œstrous Cycle.

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(Communicated by Dr. F. G. Hopkins, F.R.S. Received January 22,—Read March 5, 1914.)

[Plates 17 and 18.]

Recent experimental work has resulted in proving that there is a definite functional correlation between the growth of the corpora lutea in the ovaries and the hypertrophy of the mammary glands (Ancel and Bouin and O'Donoghue). In the present paper experiments are described showing that this hypertrophy in rabbits that have never been pregnant may be so considerable as to lead to the production of milk, the secretion of which may be temporarily increased by the injection of pituitary extract. Further experiments are recorded showing that the uterus is not a necessary factor in the development of the mammary gland. The influence of experimentally produced corpora lutea upon the uterus is also described.

The Influence of the Ovaries upon the Mammary Glands.

It is well known that the mammary glands in man begin to undergo enlargement at the time of puberty in correlation with the increase in ovarian activity. Apart from this pubertal growth which is more or less permanent, there is known frequently to be a slight swelling of the glands at each menstrual period. A similar process takes place in the sow and probably in other mammals at the "heat" periods (Marshall). In the virgin rabbit we have noticed a growth of the mammary ducts in six cases prior to ovulation, but the cell proliferation, though quite definite, did not extend to the glandular tissue. Experiments were undertaken to determine if the growth could be increased by injecting fœtus extract, with a view to bringing further evidence to bear upon the hypothesis, put forward by Starling and Lane-Claypon, that the anabolic changes involved in mammary hypertrophy are dependent upon a fœtal hormone. The results, however, were negative in each case.

The following are the details of this series of observations. The extract was made by grinding the fresh fœtuses with sand and extracting with Ringer's fluid. The extract was then boiled and filtered. In the first three experiments described below 39 rabbit fœtuses were employed:—

(1) Fœtus extract was injected for 15 days into a virgin rabbit aged 5 months. The rabbit was then killed, when it was found that the ovaries contained a few follicles apparently ripe or nearly ripe, and the uterus a few glands. The mammary development was limited to ducts which were about 1 cm. long (fig. 1).

(2) In another virgin rabbit of the same age and treated identically the ovaries showed a degenerate follicle and a few follicles apparently ripe. The uterine glands were slightly developed, and the ducts of the mammary glands were fairly well developed, being about $1\frac{1}{2}$ cm. long.

(3) Another virgin rabbit of the same age and treated identically gave similar results to the last (No. 2).

(4) A virgin rabbit, aged 5 months, was allowed to undergo a sterile copulation with a buck from which a portion of each vas deferens had been removed. It was killed 12 days after copulation. Contrary to expectation, no corpora lutea were found in the ovaries, but there was one large follicle. The uterus contained a few glands, and the ducts of the mammæ were about $\frac{1}{2}$ cm. long.

(5) A virgin rabbit, 7 months old, was allowed to undergo a sterile coition with a vasectomised buck. It was killed 12 days afterwards. As in the last case (No. 4) no corpora lutea were found, but protruding follicles were present. The uterus had a few glands. The ducts of the mammary glands were well developed, being about $2\frac{1}{2}$ cm. long.

(6) Another virgin, 7 months old, was allowed to undergo a sterile coition. It was killed 24 days later. There were no corpora lutea, but many protruding follicles, and the mammary ducts were about 2 cm. long.

It is thus seen that prior to ovulation the mammary development was limited to a slight cell proliferation in the ducts, and that the growth was not augmented by the injection of boiled fœtus extract. On the other hand, after ovulation (at least in the rabbit) definite mammary hypertrophy sets in, as will be described below.

Probably in the majority of mammals ovulation takes place spontaneously during œstrus. This is the case in the mare, the cow, the sow, the sheep (at least ordinarily), and the bitch. On the other hand, in the rabbit, the cat, and the ferret, ovulation, as a general rule, only occurs as a result of a stimulus set up by sexual intercourse. To which of these categories man belongs is still an open question.

It is generally believed that whereas the corpus luteum verum (or corpus luteum of pregnancy) and the so-called corpus luteum spurium (which is developed when pregnancy does not follow ovulation) are identical by origin, the structure formed after ovulation does not hypertrophy to the same

extent as when pregnancy supervenes, but on the other hand undergoes retrogression after a few days. Ancel and Bouin, however, assert that in such animals as the rabbit the corpus luteum, when formed, undergoes the same amount of hypertrophy irrespective of the occurrence of gestation, and that since these animals do not normally ovulate excepting after coition the presence of corpora lutea is nearly always associated with the pregnant condition. Further, they put forward the view, for which a considerable body of evidence has been adduced, that in such animals as the rabbit the corpora lutea provide the exciting cause for the growth of the mammary glands during the first part of pregnancy.

In order to test this hypothesis they carried out experiments in which the Graafian follicles of rabbits were ruptured under such conditions that pregnancy could not supervene. The method usually adopted was to ligature the vasa deferentia of the male rabbits. This operation although inhibiting pregnancy, since spermatozoa cannot be ejaculated, does not prevent the occurrence of coition. Since coition without seminal ejaculation is generally sufficient to induce ovulation in the doe, corpora lutea could be formed just as though pregnancy had supervened. Ancel and Bouin found that the growth of the corpora lutea produced in this way was accompanied by a hypertrophy of the mammary glands which continued for about 15 days or until the corpora lutea began to undergo retrogressive changes. It was naturally concluded that the growth of the mammary glands was brought about by the activity of the corpora lutea. The further development of the mammary glands in pregnant rabbits is ascribed by Ancel and Bouin to the activity of a different gland, which is described as lying between the stroma and muscular layers of the uterus, and is designated the myometrial gland.

Frank and Unger have described a case of a virgin rabbit with corpora lutea in the ovaries and a breast development such as is usually characteristic of the end of the first third of pregnancy.

Furthermore, O'Donoghue has investigated the relation of artificially produced corpora lutea to the mammary glands. He took female rabbits in a condition of œstrus, and ruptured the Graafian follicles mechanically. In many cases corpora lutea were formed, and when this happened their presence was associated with a growth of the mammary glands. The amount of growth in 14 or 15 days is stated to have been about equivalent to that shown by the normal pregnant rabbit in 12 days. If, however, the artificial rupture of the follicles was not followed by the formation of corpora lutea the mammary glands did not show any hypertrophy. O'Donoghue had previously adduced evidence that the corpora lutea and mammary glands are functionally correlated in *Dasyurus*.

The following is an account of our experiments. In Experiments 7–17 the animals were all virgin prior to the occurrence of the recorded coition. The uterine changes are described separately below in dealing with the question as to the influence of the corpora lutea upon the uterus.

(7) A rabbit, 6 months old, was killed 3 days after a sterile copulation with a buck from which portions of each vas deferens had been removed. The ovaries contained corpora lutea. The ducts of the mammary glands were well developed, and there were slight traces of alveolar formation.

(8) A rabbit, 7 months old, was killed 5 days after a sterile copulation. There were corpora lutea of two ages present in the ovaries, and the mammary glands were well developed with the alveoli containing a secretion that appeared to be milk.

(9) A rabbit, 8 months old, in which the Fallopian tubes had been ligatured, was killed 9 days after a sterile copulation. The ovaries contained corpora lutea. The alveoli of the mammary glands were in process of formation.

(10) A rabbit, 8 months old, in which the Fallopian tubes had been ligatured, was killed 12 days after a sterile copulation. Sections through the ovaries showed that ovulation must have occurred in this case some considerable time (probably about 25 days) previously, since the corpora lutea were old and degenerate, and not recognisable on the surface of the ovaries. The mammary glands showed signs of involution, but milk was present in both the large and the small ducts. Milk could be expressed from the nipples before killing.

(11) A virgin rabbit, aged 7 months, was found to have ovulated spontaneously, this being very unusual in rabbits as above mentioned.* The ovaries contained corpora lutea, apparently about 14 days old. There were numerous alveoli found in the mammary glands (fig. 2).

(12) A rabbit, aged 15 months, from which portions of the Fallopian tubes had been removed, was killed 16 days after a sterile coition. The alveoli of the mammary glands were well developed.

(13) A rabbit, aged 8 months, was killed 24 days after a sterile coition with a vasectomised buck. Old corpora lutea were found in sections through one of the ovaries. The alveoli of the mammary glands were well developed and active, containing a granular milky secretion. Milk could be squeezed from the nipples. The milk was examined microscopically and

* This rabbit was in a cage with another female. Doe rabbits in a state of œstrus when kept together have been observed to "jump" one another after the manner of cows when on heat, and it is possible that the stimulus set up in this way may be sufficient to induce ovulation.

stained with Sudan II, when fat globules were seen. The fluid when collected had the appearance of ordinary milk, and yielded a flocculent precipitate when treated with dilute acetic acid.

(14) A rabbit, 10 months old, was injected every day with boiled fœtus extract together with boiled placenta extract from the 11th to the 24th days, after a sterile copulation, the Fallopian tubes of the rabbit having been previously cut and portions removed. The rabbit was killed on the 27th day. Old corpora lutea were found in sections through the ovary. The alveoli of the mammary glands showed signs of atrophy, but the ducts and some of the alveoli contained milk. Before killing, a serous milky fluid was expressed from the nipples. The milk was tested as before, and found to contain some fat and albumen.

(15) A virgin rabbit, 11 months old, was rendered sterile by the Fallopian tubes being severed. It was then injected with boiled extract of uterus from the 11th to the 24th days, after a sterile coition. The rabbit copulated again on the 29th day, and was immediately afterwards killed. Old corpora lutea were found in the deeper parts of the ovary and several apparently ripe follicles on the surface. The mammary glands contained milk. Previously milk had been expressed from the nipples on the 19th, 21st, 27th, and 29th days after the sterile coition. The milk of this rabbit, collected in a test-tube, had the appearance of normal milk, and samples under the microscope were seen to contain globules of fat.

(16) A rabbit was injected with boiled uterus extract from the 11th to the 24th day after a sterile coition. Milk was expressed from the nipples on the 19th, 21st, 27th, and 29th days. The milk was collected and examined as in the previous case (No. 15). The rabbit copulated a second time with a vasectomised buck on the 29th day; 30 days later the rabbit copulated again and on the same day milk was expressed from the nipples; 28 days later milk was again expressed from the nipples. Next day the rabbit copulated again. Then on the same day 1 c.c. of pituitary extract was injected and the animal killed. The mammary glands were well developed, but showed signs of involution. They were full of milk. The ovaries contained old corpora lutea and ripe follicles.

(17) A rabbit was injected daily with boiled placenta and fœtus extract from the 11th to the 24th day after a sterile coition. Milk was expressed from the nipples on the 19th, 21st, 27th, and 29th days, and was collected and examined as in the preceding cases. On the 29th day (June 16) the rabbit again underwent a sterile copulation; 28 days later (July 14) although not pregnant, the rabbit plucked its fur from its breast and made a nest as if preparing for parturition. On the same day milk was expressed

from the nipples. Two days later (July 16) the animal again underwent a sterile coition. On the 17th day afterwards an attempt was made to express milk from the nipples but none could be obtained. On the 22nd day a little serous fluid was obtained, and on the 28th day a considerable quantity of fluid. The same day (August 13) the rabbit copulated a fourth time; 24 days later (September 6) no fluid could be expressed from the nipples, but two days later (September 8) a few drops of serous fluid were obtained. The rabbit copulated again (September 24) and is still alive.

(18) A multiparous rabbit underwent a sterile copulation with a vasectomised buck. On the 15th day after copulation no milk could be expressed from the nipples. On the 20th day milk could be obtained in considerable quantity. On the 22nd day the rabbit was again on heat and after undergoing copulation was killed. The mammary glands were found to be full of milk. Old corpora lutea and numerous degenerate follicles were found in sections through the ovary.

(19) This experiment was with a multiparous rabbit and the result was similar to that of the preceding experiment, there being no milk on the 15th day, but some milk on the 20th and 22nd days, on the latter of which the rabbit copulated again with a vasectomised buck (August 15). Twenty-two days later (September 6) a few drops of milk were obtained, and two days later quite a lot of milk was drawn off. The rabbit is still alive.

(20) This experiment was upon a multiparous rabbit which was exceptional in that no milk could be expressed from the nipples at any time during the period between two successive copulations with a vasectomised buck. After copulating a third time fluid could be expressed from the nipples on the 22nd day and on the 24th day.

(21) A multiparous rabbit was killed 17 days after a sterile copulation with a vasectomised buck. The mammary glands were found to be well developed but they contained no milk.

It is thus seen that in pseudo-pregnant rabbits (that is, in rabbits in which corpus luteum formation followed upon sterile coition) milk first made its appearance about the 19th day after copulation. At about this period the mammary hypertrophy appeared to have become complete and retrogressive changes set in, anabolism giving place to katabolism, at any rate to a considerable extent. These changes took place in the absence of any observed activity on the part of the myometrial gland, and it must be assumed that this gland is not an essential factor in mammary development. Moreover the immediate secretion of milk in considerable quantity followed by the characteristic changes in the tissue of the mammary glands could be induced by the injection of pituitary extract in the same kind of way as in normal lactation.

The interval between two œstrous periods (that is the interval occupied either partly or wholly by pseudo-pregnancy) was from 22 to 30 days, the period of gestation in the normal rabbit being 30 days.

Whether or not the corpus luteum plays any part in mammary growth or secretion in the latter stages of normal pregnancy is a point which has not been determined.

In normal pregnancy the development of the glands is undoubtedly greater than anything that occurs in pseudo-pregnancy, and it would seem probable that some further factor is concerned in bringing about this growth. This factor is possibly to be sought for either in the placenta, as suggested by Basch, or in the myometrial gland, as supposed by Ancel and Bouin. Nevertheless, it is clear that the presence of corpora lutea alone, apart from the existence of any subsidiary factor, suffices to stimulate gland growth to such a degree of completion as to result in the secretion of milk.

As mentioned already, Ancel and Bouin distinguish between the corpora lutea of pregnancy and the so-called "periodic corpora lutea" which only occur in animals that ovulate spontaneously. The artificially produced corpora lutea in the rabbit are regarded as belonging to the former kind. Moreover in those animals (like the rabbit) which only ovulate after coition the interstitial cells are supposed to take the place of the periodic corpora lutea. It may be doubted whether the distinction made between the two kinds of corpora lutea by Ancel and Bouin should be insisted upon. In the first place the corpora lutea are all formed in precisely the same way from the discharged follicles, while according to Biedl the ovarian interstitial cells in rodents arise from connective tissue which grows inwards so as to fill up the cavities of degenerate follicles. Such cells are designated by Seitz "theca lutein cells" since they arise in the theca interna of the follicles, and subsequently develop into cells resembling those of corpora lutea. Miss Lane-Claypon, however, states that the ovarian interstitial cells are derived, like the follicular epithelial cells, from the germinal epithelium.*

Furthermore, from the account given by Hill and O'Donoghue it would seem that the corpora lutea in *Dasyurus* always undergo the same degree of development irrespectively of the occurrence of pregnancy. They describe an animal as being seen to clean out its pouch for the reception of young, although it had not become pregnant, thus showing that in *Dasyurus* the cyclical changes of the sexual organs, which are apparently consequent upon ovarian changes, may even extend to the instincts associated with parturition and the nursing of the young, although pregnancy had not taken place.

* I have noted the presence of interstitial cells in the ovary of the rabbit prior to the maturation of any follicles.—J. H.

A case of a rabbit which prepared a bed for a litter and secreted milk at the termination of the pseudo-pregnant period has been recorded above. Cases have also been reported by various observers of similar instincts in bitches, which have been described as making preparations for parturition and secreting milk nine weeks after coition although they had failed to become pregnant. Thus Heape records instances of bitches which had been "lined" but had "missed" having pups, yet had secreted milk at the time when they were due to whelp, in sufficient quantity to admit of their rearing litters belonging to other bitches. Cases have also been recorded by Noel Paton. Moreover, several such cases of bitches which did not conceive but yet have afterwards yielded milk have been recently reported to the authors.

It is suggested that in these animals the building up of the mammary glands and the resulting secretion of milk may have taken place in response to a stimulus arising in corpora lutea which developed after œstrus and possibly persisted for an abnormal length of time. If this explanation is correct it is clear that no essential distinction can be drawn between the corpora lutea of pregnancy and the periodic corpora lutea in regard to their functional relation to the mammary glands.

Our observations lend no support to the theories of Starling and Lane-Claypon, Foà, Biedl and Koenigstein, who have supposed that the mammary glands are built up under the influence of a hormone arising in the fœtus, neither are they confirmatory of the view put forward by Halban, who regards the placenta as a factor in mammary growth. Our experimental results are, at first sight, somewhat difficult to reconcile with the facts observed by Ott and Scott, and Schäfer and Mackenzie, who found that corpus luteum extract (like that of pituitary) when injected into the circulation has an immediate galactogogue action. It must be borne in mind, however, that the sudden injection of considerable quantities of corpus luteum extract into the circulation is not a process which occurs in nature, and consequently we might expect its effect upon the mammary tissue to be different from that of small quantities of the problematical hormone when continuously secreted over a long period.

The Effect of Hysterectomy without Ovariotomy.

Experiments were also undertaken to ascertain whether or not the uterus is an essential factor in mammary growth. As already mentioned, Ancel and Bouin have expressed the opinion that in the later stages of pregnancy the myometrial gland of the uterus is an exciting cause in mammary development. It occurred to us that it was possible that the uterus might also be an essential factor in bringing about mammary development in the

earlier stages of pregnancy, and that the corpora lutea might be unable to exert their influence upon the mammæ excepting through the mediation of the uterus. The changes which the uterus undergoes (to be described below) as a result of the formation of the corpora lutea lent a certain amount of evidence in favour of this view. It had, however, been shown that the removal of the uterus in young rabbits has no effect upon the subsequent growth of the ovaries, for animals so operated upon after becoming mature are capable of copulation, ovulation, and the formation of corpora lutea just as though they had not undergone hysterectomy; but the effects (if any) of the removal upon breast development were not recorded (Carmichael and Marshall).

The following is an account of our experiments:—

(22) The uterus was removed from a virgin rabbit when 10 months old. Subsequently the animal copulated and was killed 25 days after copulation. No remains were found of the uterus or Fallopian tubes, and one ovary was missing, presumably having become absorbed as a result of vascular interference at the time of the hysterectomy operation. The other was normal and contained nine corpora lutea. The alveoli of the mammary glands showed signs of atrophy, but it was clear that they had undergone a considerable growth previously. Both alveoli and ducts contained a secretion.

(23) The uterus was removed from a virgin rabbit when 3 months old. After it had reached maturity it was allowed to copulate several times, and killed 12 days after the last copulation. One ovary contained four corpora lutea, the other having undergone atrophy. No remains of uterus or Fallopian tubes could be found. The mammary glands showed a great development of alveoli but no milk was present (fig. 3).

(24) The uterus was removed from a virgin rabbit when 3 months old. After it had reached maturity it was allowed to copulate several times, and was killed 9 days after the last copulation. The left ovary contained several corpora lutea. Small pieces of the Fallopian tube were found attached to it. The right ovary had undergone partial atrophy presumably as a result of vascular interference, and there was a small piece of the right Fallopian tube with a cyst. The mammary glands were well developed, the alveoli being filled with a secretion.

(25) The uterus was removed from a virgin rabbit when 3 months old. The rabbit subsequently copulated. A little serous fluid could be squeezed from the nipples on the 22nd and 27th days after copulation. The rabbit copulated again on the 28th day (August 14). A few drops of fluid were expressed 25 days afterwards (September 8), when it copulated again and was immediately killed. The mammary glands were well developed. The

alveoli and ducts were full of a milky secretion. Both ovaries contained corpora lutea. There were no remains of tubes or uterus.

(26) The uterus was removed from a virgin rabbit when 3 months old. On the 27th day after copulation (which took place when maturity was reached) fluid could be squeezed from the nipples. On the 28th day the rabbit was killed, when it was found that the alveoli of the mammary glands were well developed. The ovaries contained corpora lutea. There was a small piece of one Fallopian tube left.

(27) The uterus was removed from a virgin rabbit when 3 months old. It reached maturity, copulated, as in the preceding cases, and was killed 17 days later. No remains of uterus or tubes could be found. The ovaries contained eight (three and five) corpora lutea. The mammary glands were well developed, the ducts and alveoli being filled with a secretion.

These experiments show that mammary development occurring in rabbits as a result of the formation of experimentally produced corpora lutea takes place independently of any uterine influence. Thus the uterus is not a factor in mammary growth any more than in ovarian growth. The experiments show further that the presence of one ovary, with its contained corpora lutea, is sufficient to bring about the mammary hypertrophy.

The Influence of the Corpora Lutea upon the Uterus.

It has been concluded by Fraenkel and others that the corpus luteum is an essential factor in the fixation of the fertilised ovum to the uterine wall and in the nourishment of the embryo during the first stages of pregnancy. This conclusion is based on the results of ovariotomy during early pregnancy and on a large number of control experiments. Whether or not the evidence is sufficient to justify the theory being stated in precisely this form, it would seem clear that the development of the corpus luteum is functionally connected with the contemporaneous hypertrophy of the uterine wall during the first stages of gestation, since the raised nutrition of the uterus is dependent upon the presence of the corpus luteum (Marshall and Jolly). Ancel and Bouin state that in the case of the rabbit the non-pregnant uterus undergoes hypertrophic changes when corpora lutea are developed. This has been called in question by Dubreuil and Regaud, but Niskoubina's observations are confirmatory of those of Ancel and Bouin.

The following is an account of our observations upon the changes undergone by the non-pregnant uterus after ovulation consequent upon sterile coition (excepting in the case of Experiment 11 where the rabbit had ovulated spontaneously). The condition of the ovaries and mammary glands has been already described. The numbers of the experiments provide a

means of identifying the individual rabbits previously referred to. The respective ages of the rabbits, which prior to the occurrence of the recorded coition were all virgins, have also been given above.

(7) In a rabbit killed 3 days after sterile coition the uterine glands were just commencing to undergo active growth.

(8) In a rabbit of 5 days the glands were considerably developed and the muscular walls had undergone some thickening.

(9) In a rabbit of 9 days the process had been carried further (fig. 5).

(10) In a rabbit of 12 days the uterine glands were more numerous and smaller than those of No. 9. They were also more closely packed, and the uterus showed congestion. The muscular walls were very thick. It is to be noted again that the ovaries contained very old corpora lutea (see above).

(11) This rabbit had ovulated and corpora lutea were present, apparently about 14 days old. The uterus showed a great development of glands which were elongated and formed a spongy-looking mass at the base of the folds. The muscular coat was thickened.

(12) In a rabbit killed 16 days after sterile coition the uterine glands were enlarged and spongy-looking. The capillaries in the stroma between the glands were distended. The muscular layers were very thick.

(13) In a rabbit of 24 days the uterine glands were smaller than those of No. 12, but still very active. The folds of the mucosa contained a large amount of extravasated blood, showing that the congestion had resulted in a breaking down of the blood-vessels. The muscular coat was moderately thick (fig. 6).

The changes outlined above presented an essential similiarity to those described by Hill and O'Donoghue for the pseudo-pregnant marsupial cat. There is a strikingly close likeness between the appearances which we have just described (as shown in sections through the rabbit's uterus during the successive stages) and the figures published in Hill and O'Donoghue's paper on *Dasyurus*. In view of this great similarity there can hardly be reason to doubt that the changes which take place in the rabbit's uterus after sterile coition are physiologically homologous with the changes which occur in the uterus of *Dasyurus* during the period of pseudo-pregnancy. As will be shown subsequently the recognition of this fact, which has not hitherto been pointed out, materially affects the views entertained by the above-mentioned authors regarding the nature of the homology between the œstrous cycle of the marsupial and that of the Eutherian mammal.

Lastly, the hypertrophic changes which take place in the uterus during pseudo-pregnancy are clearly comparable to those which occur in true

pregnancy in association with the development of the embryo, whose presence necessitates the maintenance of a raised nutrition on the part of the organ which protects it and through which it derives nourishment. That the corpora lutea are a factor in preserving this raised nutrition seems to have been established beyond question.

The Œstrous Cycle.

According to Heape a period of five or six months (*i.e.* spring and summer) is the usual duration of the sexual season in the domesticated rabbit. Heape says further: "No doubt if they are kept warm, carefully fed, and their breeding carefully regulated throughout the spring and summer, they may exhibit œstrus also in winter, but it must be recollected that here we are treating of œstrus independently of pregnancy, which is a very different matter."

Our experience has been different from that of Heape, for many of our rabbits, kept in hutches in an outhouse and without any artificial heating, have bred in the winter months, though not with the same frequency as in spring and summer. The following is a record:—

	Percentage breeding.
Of 12 rabbits which copulated about Dec. 14, 5 had young	41·7
„ 24 „ „ Mar. 22, 14 „	58·3
„ 21 „ „ May 18, 17 „	81·0
„ 8 „ „ June 14, 8 „	100·0

Half of these rabbits had been treated with Yohimbine, administered by the mouth for several days before copulation, but the drug, although in other cases it caused a pronounced congestion of the uterus, did not increase the breeding powers or affect the fecundity, as compared with the other rabbits which were kept as controls.

Prof. Punnett, who has kindly supplied us with further information concerning the recurrence of œstrus in rabbits, finds that when kept in a moderate temperature, produced when necessary by artificial heating, not only is there very little, if any, restriction of the sexual season to a particular time of the year, but that copulation in the winter is followed by pregnancy. The following is a record of the œstrous periods (so far as observed) and times of litters for one of Prof. Punnett's rabbits from October, 1910, to May, 1912:—

Put to male.	First notes made on litter.
Sept. 27, 1910	Oct. 29, 1910
Jan. 26, 1911	Feb. 27, 1911
Mar. 29, 1911	Apr. 30, 1911
June 2, 1911	July 4, 1911
July 15, 1911	Aug. 19, 1911
Sept. 2, 1911	Oct. 6, 1911
Oct. 25, 1911	Nov. 27, 1911
Jan. 12, 1912	Feb. 15, 1912
Feb. 15, 1912	Mar. 17, 1912
Apr. 11, 1912	May 13, 1912
May 17, 1912	June 19, 1912

Heape states that 10–15 days is the average duration of the diœstrous cycle, but that some individuals exhibit heat at intervals of three weeks.

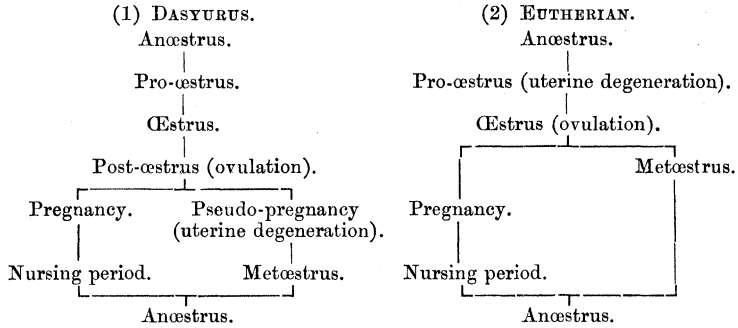
The pro-œstrum is stated to last from one to four days, and œstrus for about a day or longer. During the pro-œstrum the vulva tends to become swollen and purple in colour, and this appearance may continue during œstrus. There is no external bleeding, and it is difficult or impossible to state when the pro-œstrum ends and œstrus begins. It would seem that the two periods are much abbreviated, as in the case of the sheep and many other animals in which the uterine changes characterising the heat periods are slight, as compared with those of the dog or the monkey.

The uterus may show undoubted congestion at the heat period, but we have never observed any breaking down of vessels or extravasation of blood in the non-pregnant rabbit's uterus, excepting near the end of the pseudo-pregnant period. It is possible that these (or some of these) cases represented the commencement of a pro-œstrous period. Apart altogether from these instances congestion presenting a close similarity to that observed in the case of the pro-œstrous sheep was found to occur in the rabbit's uterus at the time of heat. Pigment formation has not been noticed. Its absence from the uterus of the rabbit suggests that in this animal blood extravasation does not ordinarily take place in the pro-œstrous or œstrous periods. The glands do not show very much evidence of activity during the heat period, and their degree of development is very much less than that shown in the earlier stages of the pseudo-pregnant period.

Theoretical.

Many of the observations described above have an important bearing upon certain statements made by Hill and O'Donoghue in a recent paper on the œstrous cycle in the marsupial cat, *Dasyurus viverrinus*. According to these authors ovulation in *Dasyurus* occurs at an interval of some days after œstrus, there being a definite post-œstrous period terminating in ovulation.

Further, it is stated that the degenerative changes in the uterine mucosa of the marsupial instead of preceding ovulation, as they do in the dog, take place after ovulation during a period which, in the non-pregnant animal, is designated the period of pseudo-pregnancy. The differences in the reproductive cycles are shown in the following scheme drawn up by Hill and O'Donoghue:—*



Hill and O'Donoghue express the opinion that the degenerative changes seen in *Dasyurus* during the pseudo-pregnant period are equivalent to those which take place in the Eutherian during the pro-œstrum. They suggest that the shortening of the cycle in the Eutherian may have induced an increased growth of the mucosa during the pro-œstrum, and that this in time may have conditioned the earlier recurrence of the degenerative and regenerative changes, with the result that these have been shifted forward so as to occur prior to ovulation instead of after it. On the other hand, Hill and O'Donoghue appear to hold the view, which seems to us scarcely consistent with the suggestion just quoted, that menstruation in man is a degeneration of the uterine mucous membrane, due to its being unable to fulfil its purpose owing to the absence of a fertilised ovum. They state, further, that their observations "afford no support to the view that 'menstruation is identical with heat' nor for the view that 'menstruation in the Primates is the physiological homologue of the pro-œstrum in the lower Mammalia.'" Thus they appear to regard the condition existing in the Primates as directly comparable to that occurring in *Dasyurus*, and different from the condition found in the dog.

Our own observations on the rabbit indicate that the changes in the non-pregnant uterine mucosa which take place concurrently with the development

* In the scheme drawn up Hill and O'Donoghue "Dicestrus" is unaccountably inserted for the non-pregnant Eutherian between "Pro-œstrus" and "Metœstrus." In the scheme as given above, this is omitted, since the dicestrous period, when it occurs in polycestrous animals, supervenes after metœstrum and not before.

of the corpora lutea are essentially similar to those described for *Dasyurus* in the period of pseudo-pregnancy. The close likeness between the sections of the rabbit's uterus and the figures given in Hill and O'Donoghue's paper has been commented on above. Moreover, the processes which take place in the ovaries and mammary glands are also clearly of an identical nature in the two animals. We suggest, therefore, that the uterine changes which go on in the pseudo-pregnant uterus in the marsupial are not comparable to the pro-œstrous changes of the Eutherian, as Hill and O'Donoghue suppose, but are identical with those in the pseudo-pregnant rabbit's uterus, both being dependent upon the formation of corpora lutea in the ovaries. It is possible, however, that the uterine congestion occurring near the close of the pseudo-pregnant period is of the nature of a pro-œstrous congestion, since pseudo-pregnancy (like true pregnancy) would probably in some cases have been followed by another œstrous period, had the animals been permitted to live.

It has been shown by Hill and O'Donoghue that in the marsupial cat there is only one sort of corpus luteum, the duration of which is presumably always the same. In the rabbit, also, there is only one kind of corpus luteum occurring in correlation with either pregnancy or a condition comparable to pseudo-pregnancy. The existence of only one kind of corpus luteum (which lasted for an identical period, irrespectively of whether or not ovulation was succeeded by pregnancy) was no doubt the condition common to all primitive mammals, and it seems probable that the shortening of the duration of the "periodic corpus luteum"* was associated with the development of the polyœstrous habit from a state of monœstrum. For it is known that ovulation cannot ordinarily occur in the presence of fully developed corpora lutea, which, if they persist, cause follicular atrophy and inhibit the development of ripe ova. Consequently it would be disadvantageous for such animals to have periodic corpora lutea persisting for as long a period as corpora lutea associated with pregnancy.

In monœstrous animals, such as the dog, the persistence of the corpus luteum over a period equivalent to pregnancy would not be detrimental to fecundity, while we have shown above that there is evidence (derived from numerous cases where bitches have been known to secrete milk nine weeks after œstrus) that even in the dog such a persistence may occur. Moreover, the great variability which different individual dogs experience in the recurrence of œstrus is suggestive of a variation in the period over which the corpus luteum persists. It may be that in monœstrous animals the primitive condition occurring in *Dasyurus*, in which there is one sort of corpus luteum only, continues to exist or is reverted to in certain individuals.

* Or corpus luteum spurium.

Summary and Conclusions.

(1) The development of the corpus luteum of pregnancy, or of pseudo-pregnancy, in the rabbit is functionally correlated with the hypertrophy of the mammary glands, as already shown by Ancel and Bouin, and by O'Donoghue.

(2) This hypertrophy is followed on about the 19th day after coition, in pseudo-pregnant rabbits, by a definite secretion of milk, the quantity of which may be temporarily augmented by the injection of pituitary extract, just as in normal lactation.

(3) The mammary hypertrophy can take place in rabbits from which the uterus has been removed while still immature, thus showing that the uterus is not an essential factor in the development of the mammary glands.

(4) The development of the corpora lutea of pseudo-pregnancy is further correlated with uterine hypertrophy and hyperæmia followed by extravasation of blood.

(5) These uterine changes are clearly comparable to those which occur in true pregnancy, and afford a confirmation of the view that the corpora lutea are a necessary factor in causing and maintaining the raised nutrition of the uterus during the first part of the period of gestation.

(6) The changes which take place in the rabbit's uterus during pseudo-pregnancy are homologous with those which occur in the uterus of the marsupial cat during pseudo-pregnancy, and these latter are not pro-œstrous in character (at any rate, in the earlier stages) as Hill and O'Donoghue suppose.

(7) The domesticated rabbit is capable of breeding throughout the whole year, but less frequently in winter than in spring or summer. If corpora lutea of pseudo-pregnancy are produced, the recurrence of œstrus is postponed until these are in an advanced stage of retrogression.

(8) The shortening of the duration of the so-called corpus luteum spurium of many mammals has probably been brought about in correlation with the acquirement of the polyœstrous condition.

The injections referred to in this paper were done by J. Hammond; the operations by F. H. A. Marshall. The work was carried out at the Field Laboratories, Cambridge, in connection with the School of Agriculture. The expenses have been defrayed by a grant made by the Board of Agriculture and Fisheries out of money allotted to it, for purposes of research, by the Development Commissioners.

[*Postscript, March* 6, 1914.—In describing the results of hysterectomy we omitted to mention that Foges found that the uterus was not a factor in the pubertal growth of the mammary glands.

Aschner and Grigoriu in a recent paper describe the effects of injecting placental extract into virgin guinea-pigs. Development of the glands followed, and this was succeeded by milk secretion. In the guinea-pig ovulation may take place spontaneously, so that it is probable that there was some gland development before the injections were made. Ovarian or placental extract was found to cause hyperæmia and other changes in the uterus.

Fellner has lately described marked changes in the uterus and mammary glands of the rabbit after injecting extracts of corpus luteum and placenta. The organs affected are said to have undergone a considerable hypertrophy, but milk production could not be induced.

Steinach has recorded experiments on guinea-pigs in which the ovaries of females were transplanted into males and produced breast development.

Doncaster in a very recent paper on sterility in cats records a case of what may be regarded as milk production following upon pseudo-pregnancy. Longley had previously observed that the cat, like the rabbit, normally ovulates only after coition. One of Mr. Doncaster's cats after copulating with a tortoise-shell male failed to become pregnant. It occurred, however, to one of the present writers that since copulation had taken place it was probable that corpora lutea had been formed though unaccompanied by pregnancy. It seemed possible, therefore, in the light of our experiences with rabbits that the cat in question might secrete milk. This was found to be the case four weeks after the last copulation, and Doncaster records that the secretion continued for about two weeks subsequently.]

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DESCRIPTION OF PLATES.

Plate 17.

Fig. 1.—Microphotograph of mammary tissue of virgin rabbit (Experiment 1, p. 423). The mammary development is limited to a few ducts.

Fig. 2.—Microphotograph of mammary glands of virgin rabbit which had ovulated spontaneously about 14 days previously (Experiment 11, p. 425). The glands contained numerous alveoli.

Fig. 3.—Microphotograph of mammary glands of rabbit from which the uterus had been removed while still a virgin. It was killed 12 days after copulation (Experiment 23, p. 430). The glands showed a great development of alveoli.

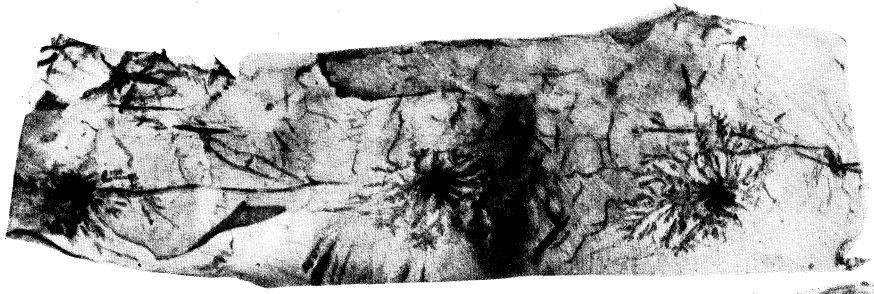
Plate 18.

Fig. 4.—Section through portion of mammary gland of rabbit 24 days after sterile coition (Experiment 13, p. 425). The alveoli contain milk. × 78.

Fig. 5.—Section through uterine mucosa of rabbit nine days after sterile coition (Experiment 9, p. 432). The glands are very greatly developed. × 35.

Fig. 6.—Section through uterine mucosa of rabbit 24 days after sterile coition (Experiment 13, p. 432). A large quantity of extravasated blood is present. The glands are still somewhat enlarged. × 35.

Figs. 4–6 were drawn by Mr. Edwin Wilson, of Cambridge.



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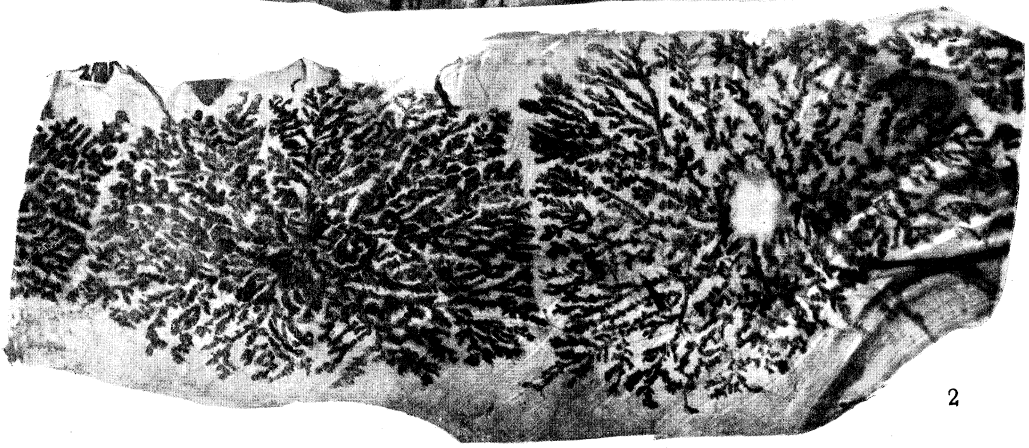

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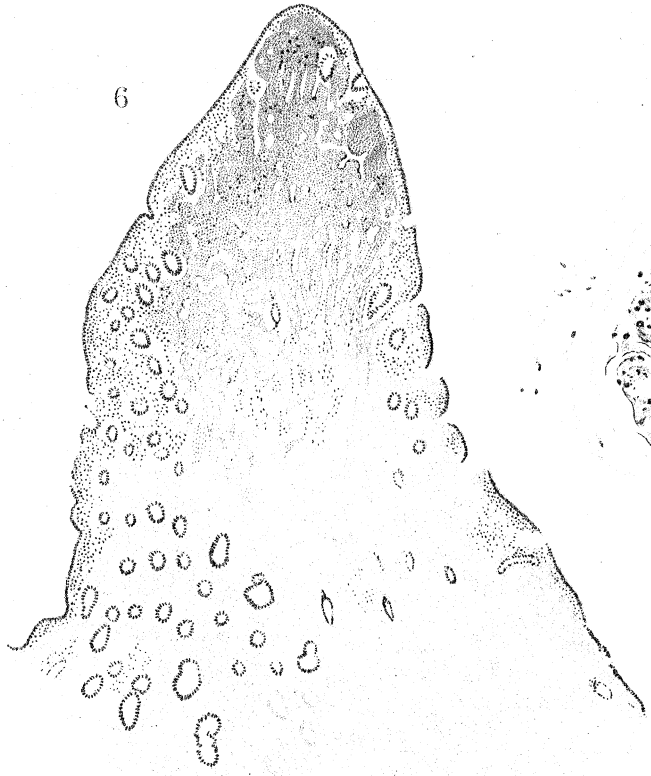

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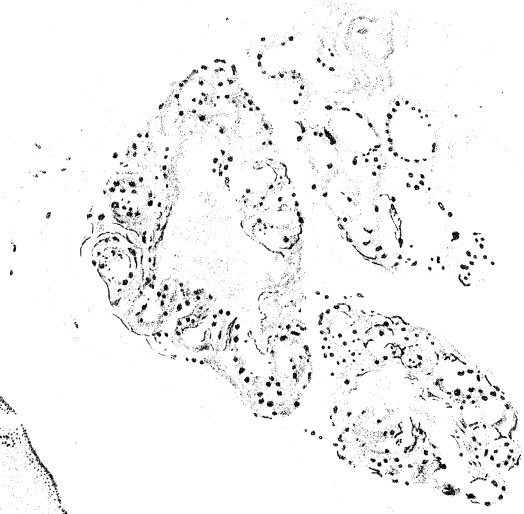

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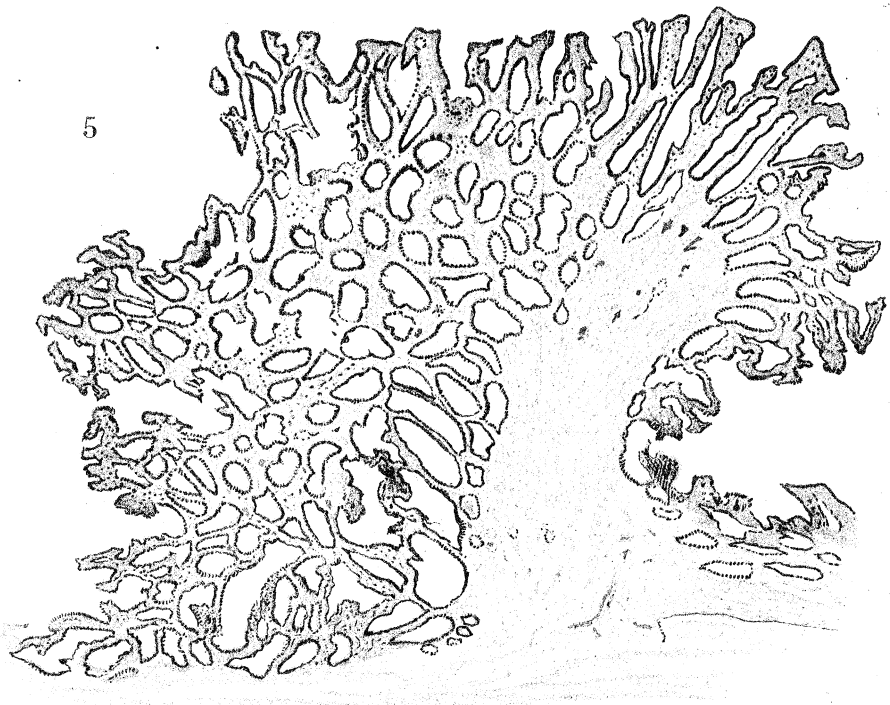

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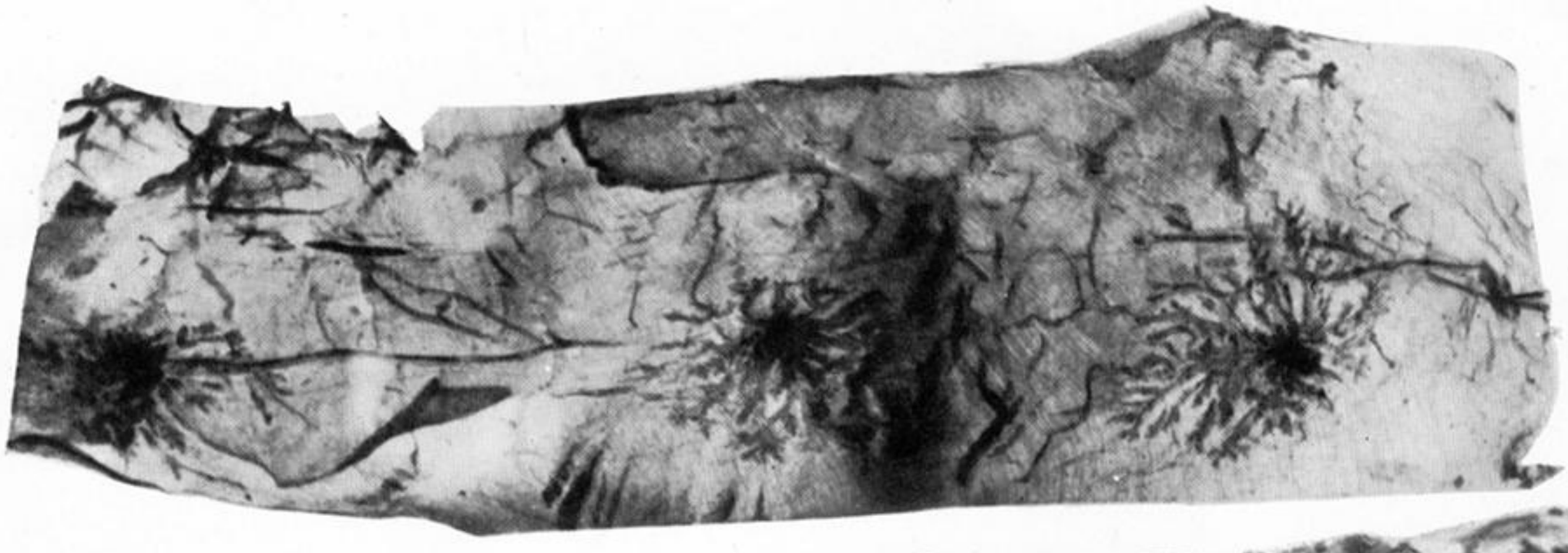

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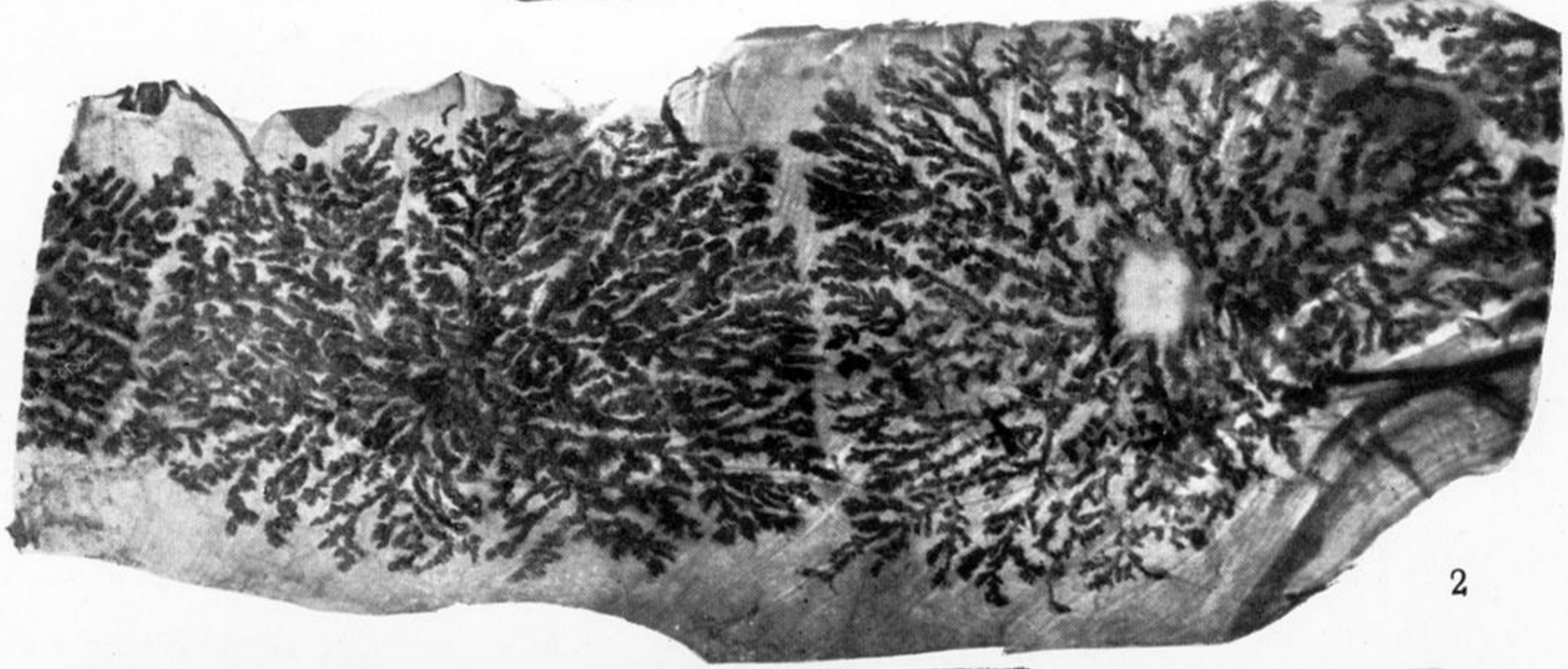

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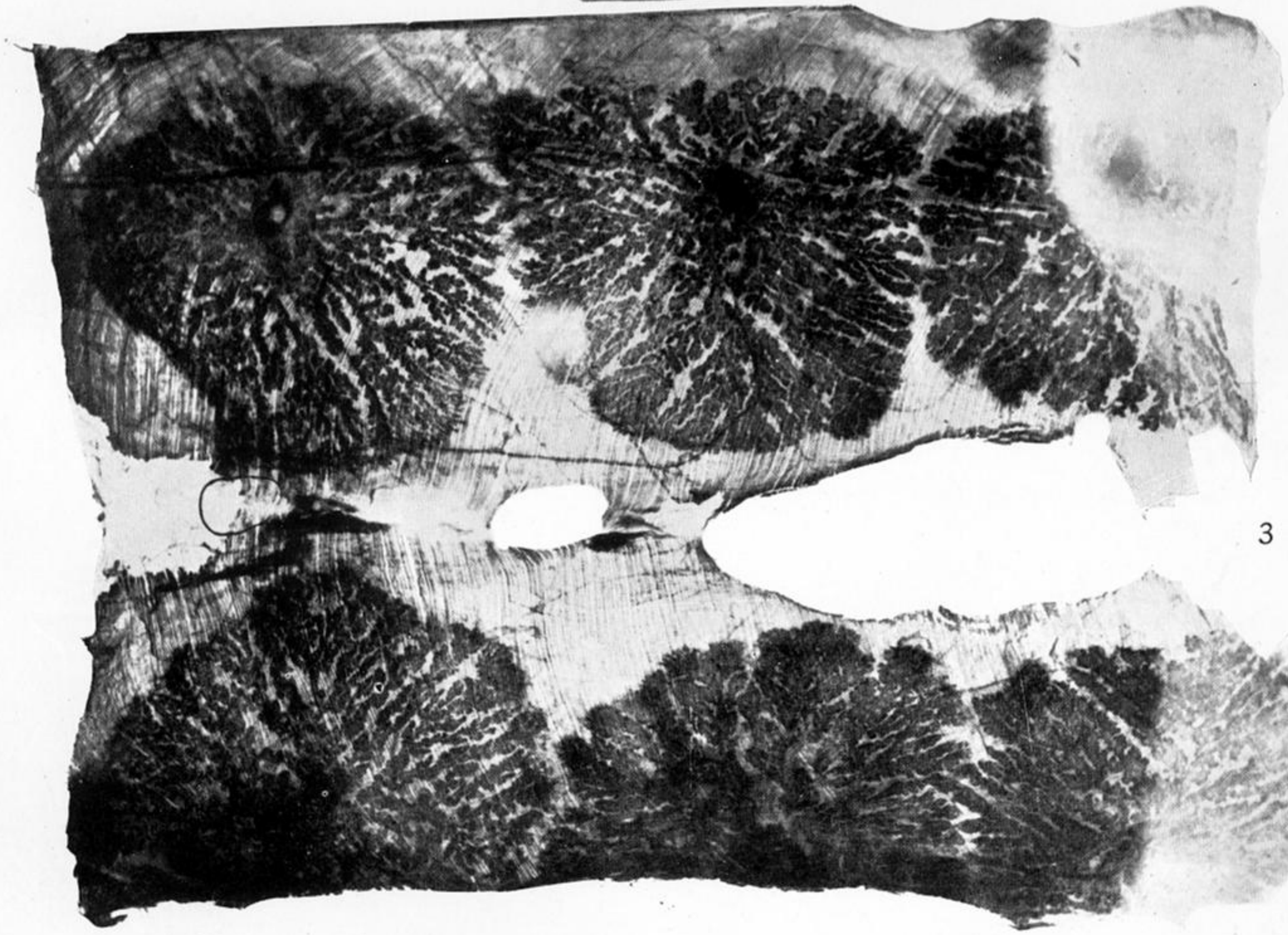

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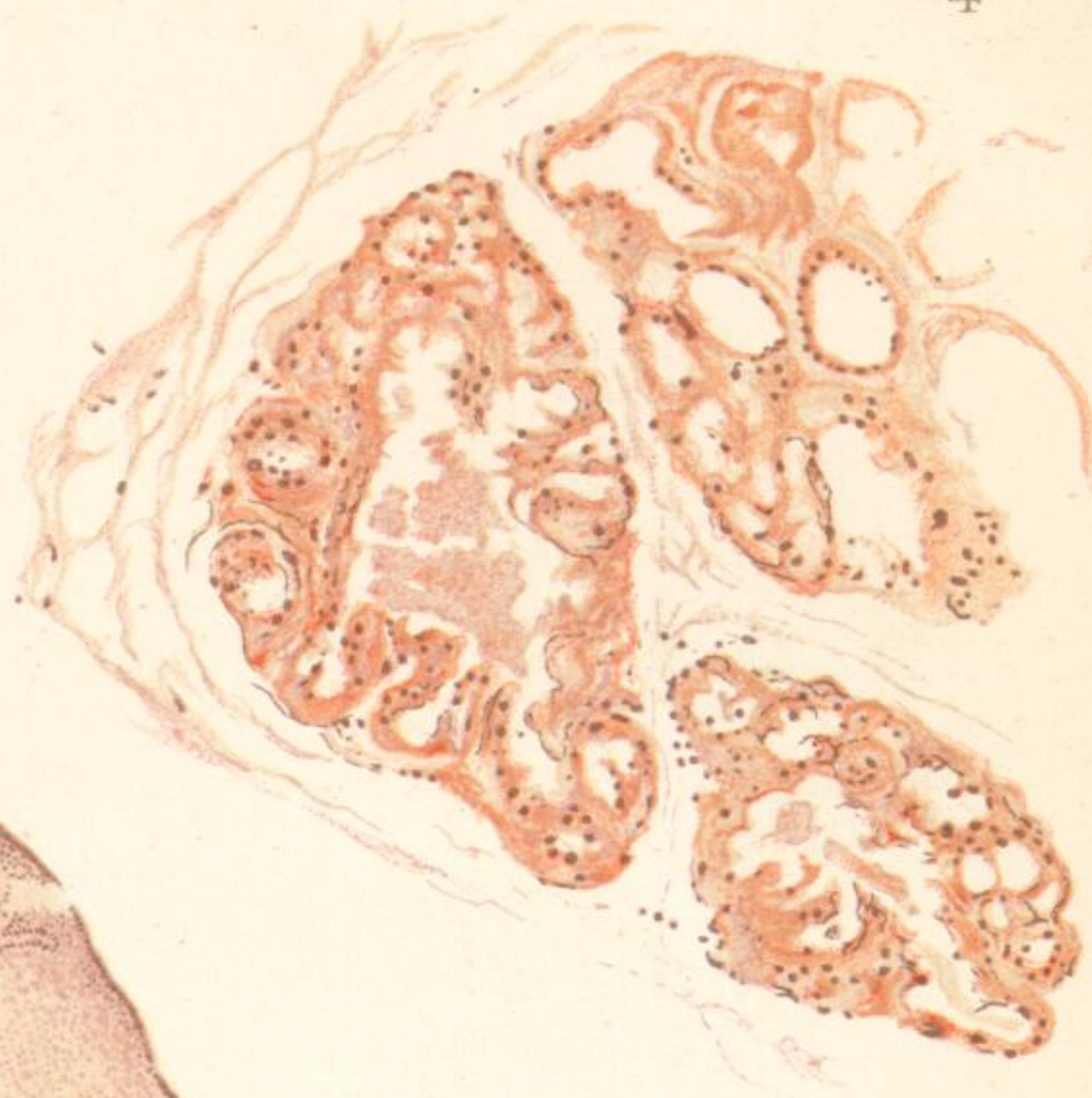

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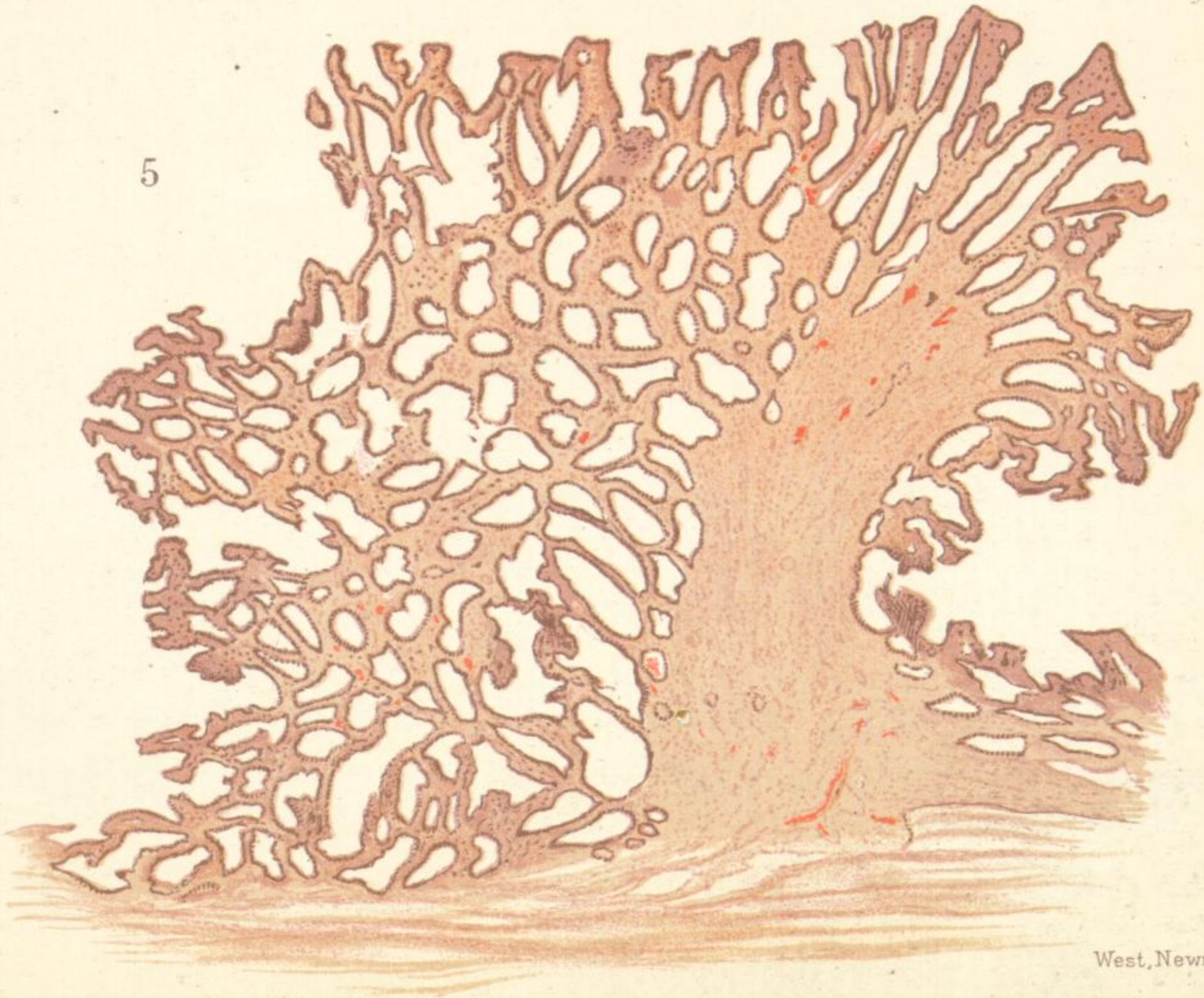

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