THE CYCLIC CHANGES IN THE MAMMARY GLAND UNDER NORMAL AND PATHOLOGICAL CONDITIONS.

II. The Changes in the Pregnant Guinea Pig, the Effect of Lutein Injections, and the Correlation between the Cycle of the Uterus and Ovaries and the Cycle of the Mammary Gland.

By LEO LOEB, M.D., and CORA HESSELBERG, M.D.

(From the Department of Comparative Pathology of Washington University Medical School, St. Louis.)

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In a previous paper[1] we analyzed the cyclic changes in the mammary gland in the non-pregnant guinea pig. In this communication we shall report on our experimental studies of the mammary gland in guinea pigs during or following the period of pregnancy. We shall furthermore report on the effect of injections of lutein on the mammary gland, and in conclusion shall briefly discuss the relation between the sexual cyclic changes in the ovaries and uterus on the one hand and in the mammary gland on the other.

Mammary Gland during Different Periods of Pregnancy.

In twenty-six guinea pigs the mammary gland was examined during different periods of pregnancy.[2] Included in this series is one case of experimentally produced extra-uterine pregnancy, of which a more detailed description has been previously given by one of us[3] from another point of view. This series also included a case

[1] Loeb, L., and Hesselberg, C., *J. Exp. Med.*, 1916, xxv, 285.

[2] When the intervals indicated in the protocols had elapsed the guinea pigs were allowed to die under ether anesthesia, and the tissues taken out at operation were placed immediately into Zenker's fluid.

[3] Loeb, L., *Proc. Soc. Exp. Biol. and Med.*, 1913–14, xi, 103; *Biol. Bull.*, 1915, xxviii, 59.

in which pregnancy was present in one horn of the uterus, while in the other horn there was a beginning abortion, and a third case in which it is doubtful whether pregnancy or merely an experimental deciduoma was present. In the remaining twenty-two cases the animals had been used for certain experiments which, however, did not interfere with the progress of pregnancy, and we have every reason to assume that they did not influence the condition of the mammary gland.

In five instances the mammary gland was examined within the first 15 days after copulation. In one of these, however, the period of pregnancy may perhaps have been slightly farther advanced. In these five cases the mammary gland was found in the following condition: In one animal 6 days and in another about 11 days after copulation, the mammary gland was intermediate. In three cases the pregnancy was approximately 12 to 15 days old; in two of these cases the mammary gland was proliferating; in the third it was intermediate.

In seven guinea pigs the mammary gland was examined during a period from the beginning of the 16th to the end of the 19th day. In three of these animals the gland was proliferating, in four it was intermediate. In one of the latter cases, however, it was doubtful whether in addition to the deciduomata pregnancy was present.

In three animals the mammary gland was examined between the beginning of the 20th and the end of the 23rd day; it was found to be intermediate. There was in addition one case in which pregnancy was present in one horn and abortion was under way in the other horn 22 days after copulation. In this case the gland was proliferating.

In nine animals the pregnancy was in a further advanced stage, at a period later than 24 days after copulation; in all these guinea pigs the mammary gland was proliferating.

We see then that proliferation of the mammary gland during pregnancy becomes regular only after the 24th day, that it was absent in two cases on the 6th and 10th days; between the 12th and 24th days the gland was proliferating in five and intermediate in eight cases. In the majority, therefore, the gland is not proliferating in the early period of pregnancy.

If we compare the condition of the mammary gland during the first period of pregnancy with the corresponding period of the normal cycle not accompanied by copulation and pregnancy, we find the majority of glands in both non-proliferating. There were, however, during the normal cycle of the non-pregnant animal a considerable number of non-proliferating glands in a resting state, while during early pregnancy the non-proliferating glands were all intermediate. In this respect the mammary gland during this period of pregnancy resembles the mammary gland of non-pregnant animals of the same period in which good corpora lutea and good deciduomata were present. In this class of animals also the non-proliferative glands were not resting, but intermediate; the proportion of proliferative glands in the latter class was, however, even greater than during early pregnancy—a difference perhaps without significance and due to accident. It is probable that pregnancy as well as the presence of good deciduomata and corpora lutea improve the condition of the mammary gland even in those cases in which the stimulating effect of these conditions is not sufficiently strong to lead to proliferation. On the whole, however, in the guinea pig, pregnancy does not induce proliferation of the mammary gland to a much higher degree than the factors active during the latter part of the normal sexual cycle unaccompanied by pregnancy, and proliferation of the mammary gland during pregnancy becomes regular only at a period of time which exceeds the duration of the normal sexual cycle unaccompanied by pregnancy. In this group we may include an animal in which extra-uterine pregnancy had been produced experimentally. In this case an incision had been made into the uterus $2\frac{1}{2}$ days after ovulation; the examination took place 18 days after the first ovulation. The condition of the ovaries indicated that about 3 days previous to the time of examination a new ovulation had taken place. The mammary gland was proliferating,—a finding not unexpected at this stage of the sexual cycle.

Mammary Gland in the Period Following Pregnancy.

In five animals the mammary gland was examined at various periods after the completion of pregnancy.

Guinea Pig 1.—About 12 to 24 hours after delivery. In the ovaries recently ruptured follicles with the beginning of corpora lutea are visible. The mammary gland is large and consists of many lobules with numerous acini. The acini are of larger size than in the glands described above. The epithelium is high cuboidal almost throughout; in the lumen of the acini there is a finely granular precipitate, representing the secretion of the gland. Some acini are distended with secretion; here the gland cells are flat, evidently as the result of pressure. Occasionally there are small vacuoles in that part of the cell which adjoins the lumen. In a number of acini there are within the lumen yellow bodies, round or of variable shape and size, containing small pigment granules. The nuclei of the gland cells are usually large and vesicular. A few mitoses occur in the gland cells. In some of the larger gland cells there are apparently some double nuclei present. The connective tissue between the acini shows loose texture; it is rich in fibroblasts and contains some polynuclear leukocytes and lymphocytes and plasma cells.

We find then at this stage a secreting gland in which, however, the stimulus present during pregnancy is still active and causes slight proliferation.

Guinea Pig 2.—15 days after delivery; new pregnancy; the animal suckles her young. Much milk is present in the gland; the acini have a rather large lumen and in some cases there is a fine, loosely arranged precipitate, and in these acini the gland cells are generally flat and vacuolar. In other acini the vacuolar cells are a little higher. The nuclei form sometimes only a narrow band around the vacuoles. Also whole gland cells, with their nuclei, apparently disintegrate in the process of milk production. Some acini show irregular projections into the lumen. The acini are separated from each other by these strands of dense fibrous tissue with small nuclei. Mitoses are absent in the gland cells.

Guinea Pig 3.—19 days after delivery; new pregnancy; the animal suckles her young. This gland is similar to that in Guinea Pig 2, but the epithelial cells are, on the average, a little higher, especially in one acinus where no secretion is present. In some of the acini where secretion is present, some of the cells are pressed flat. Some cells apparently have two nuclei. In other cells vacuolization takes place and many cells swell and degenerate. In this case there is less connective tissue present than in Guinea Pig 2, and the connective tissue is richer in cells, which are larger and have larger vesicular nuclei. Mitoses are absent in this gland. A number of gland cells are cast off; occasionally a cast off cell has two nuclei. We find occasionally cell inclusions consisting of degenerated cells.

Guinea Pig 4.—29 days after delivery; new pregnancy; the animal suckles her young. This specimen represents a gland in the last period of secretion. The acini have wide lumina. Many vacuoles are visible in the gland cells. The nuclei are, on the whole, pale, and nuclear debris is seen in many acini; evidently

many cells degenerate. No mitoses can be found, but apparently a few double nuclei occur in gland cells. There is little connective tissue between the acini, but a few lymphocytes and polynuclear leukocytes are present.

We see then that after the completion of pregnancy and in the beginning of secretion, some mitotic proliferation may still be present, but that it soon ceases, probably as the result of those processes that lead to secretion. Thus while during the period of secretion, in spite of the presence of pregnancy, mitotic proliferation ceases, and the process of secretion is able to counteract the stimulating effect of pregnancy, nevertheless some proliferative stimulus appears to be active, which, however, under the conditions present during secretion, apparently merely leads to a mitotic multiplication of nuclei. This conclusion is suggested by the appearance of double nuclei in a number of gland cells. We intend, however, to investigate further the occurrence of double nuclei in secreting glands before we consider a mitotic nuclear division in secreting cells of the mammary gland as established. It also appears that during the process of secretion not only parts of the cells are transformed into material to be secreted, but that whole cells degenerate and are admixed to the secreted material.

Not only during proliferation, but also during secretion the formation of a large quantity of dense fibrous tissue is prevented in the mammary gland.

Guinea Pig 5.—25 days after delivery; new pregnancy; the animal does not suckle her young. The gland is large, but the acini are small, and they have only small lumina. The epithelium is cuboidal. In some of the larger acini some colloid-like material is present. No mitoses are seen. The connective tissue between the acini is highly cellular; dense fibrous tissue has not been produced. Some polynuclear leukocytes are present in the interstitial tissue. This gland is, therefore, still in an intermediate condition, but might have been expected within a short time to begin to proliferate.

Mammary Gland after Abortion.

In eight animals the mammary gland was examined soon after abortion had begun or had been completed. In all these cases some experimental procedure had taken place previously, which probably led directly or indirectly to the abortion.

In five instances the abortion took place between the 17th and 22nd days of pregnancy. In these cases the mammary gland did not present the picture of a secreting gland, but was either resting, intermediate, or proliferating. In two cases abortion took place in the latter part of pregnancy, 49 to 51 days after copulation. In guinea pigs the normal duration of pregnancy is approximately 65 to 66 days. In these cases the abortion produced secretion in the mammary gland, and microscopically the typical picture of secretion was present. We may therefore conclude that after proliferation has proceeded for a certain time, inhibition of the proliferative stimulus of pregnancy leads to such changes, of a more or less degenerative character in the gland cells, as precede and cause secretion. One of the animals (No. 6) again demonstrates the fact observed after the normal completion of pregnancy that at an early period of secretion there may still be present some cell proliferation in the secreting mammary gland.

The eight cases observed are briefly as follows:

Guinea Pig 7.—After incomplete extirpation of the corpora lutea, abortion took place 18 days after copulation; resting gland.

Guinea Pig 8.—Cuts had been made into the ovaries; abortion took place 19 days after last ovulation; resting gland.

Guinea Pig 9.—After incomplete extirpation of the corpora lutea, abortion had taken place 17½ days after previous ovulation; partly proliferating gland.

Guinea Pig 10.—After thyroidectomy and incomplete extirpation of the corpora lutea, abortion took place. Examination 22 days after last ovulation; intermediate gland.

Guinea Pig 11.—In a similar instance in which the mammary gland was partly proliferating, abortion had taken place only in one horn, while the other was pregnant. This case will be referred to below.

Guinea Pig 12.—Two intravenous injections of hirudin had been given to a pregnant guinea pig; beginning abortion; a proliferating gland was found. In this case the stage of pregnancy at the time of abortion is not certain.

Guinea Pig 6.—On the 49th day of pregnancy cuts were made into a mammary gland. Abortion took place. The gland was proliferating as well as secreting. Frequent mitoses were found in those areas in which there was no secretion in the acini; where there was some secretion, mitoses were still present; mitoses were seen even in acini much distended by secretion. In different lobules the degree of secretion and proliferation varied. The connective tissue was rather cellular; some polynuclear leukocytes and lymphocytes were present in the stroma. It is possible that the incisions in the uterus, made previous to abortion, were

in this case responsible for the mitotic proliferation; perhaps the stimulus supplied by pregnancy caused it.

Guinea Pig 13.—A large secreting gland without mitoses; abortion was beginning on the 41st day of pregnancy.

Effect on the Mammary Gland of Extirpation of the Ovaries during Pregnancy.

The mammary gland was examined in four guinea pigs in which the ovaries had been extirpated $3\frac{1}{2}$ to $6\frac{1}{2}$ days after copulation, and in which notwithstanding the absence of the ovaries, the pregnancy continued for some time. The mammary gland was examined $17\frac{1}{2}$ to $18\frac{1}{2}$ days after copulation. In three cases there was beginning abortion; in one of these abortion existed as yet only in one horn of the uterus, while in the other horn pregnancy still proceeded. In the fourth animal, in which the ovaries had been extirpated $5\frac{1}{2}$ days after copulation, a normal pregnancy was still present 13 days after castration. In these four instances the gland was either resting or intermediate. Proliferation was absent. From these data we can draw no definite conclusions as to the direct effect of placenta or embryo on the mammary gland, because during the stage of pregnancy at which examination took place, proliferation in the mammary gland is often absent even in normal animals.

Mammary Gland in Pregnant Animals after Extirpation of the Corpora Lutea.

In seventeen animals the corpora lutea were extirpated in most cases about 6 days, in some cases 3 to 4 days after copulation. Pregnancy proceeded usually until the time of examination, which took place 14 to $18\frac{1}{2}$ days after copulation; in some cases abortion had taken place previous to, or was proceeding at the time of the examination. We can arrange these seventeen cases into two classes according to whether or not a new ovulation had taken place at the time of examination. In the first class (*a*) comprising seven animals no new ovulation had taken place; in the second class (*b*) comprising ten animals, a new ovulation had taken place at the time of examination.

Animals in Which a New Ovulation Had Not Taken Place at the Time of Examination.—In three of these cases pregnancy was still present from $15\frac{1}{2}$ to $18\frac{1}{2}$ days after copulation, and after an absence of the corpora lutea during a period of 9 and 12 days. In three other cases abortion was just taking place, and in the last animal abortion had been completed at the time of examination. In three animals the mammary gland was intermediate, in three others not quite intermediate, and in one the mammary gland was somewhat better than intermediate, but not proliferating. In this case almost mature follicles were present in the ovaries, and it is possible that a new period of heat was impending.

Animals in Which a New Ovulation Had Taken Place at the Time of Examination.—In three guinea pigs new ovulation had taken place within 2 days preceding examination, a period of the sexual cycle during which proliferation is usually present in the mammary gland. In the first one of these animals ovulation had taken place sometime between 6 and 18 hours previously; abortion was just proceeding; the mammary gland was in part proliferating. In the second abortion had been completed at the time of examination. A new ovulation had occurred about $1\frac{1}{2}$ days preceding examination. In this guinea pig it is not certain that mitoses were present in the mammary gland, but the structure of the gland indicated that some proliferation might have been present a short time previously. In the third case pregnancy was still present, ovulation had taken place about 2 days before examination; the mammary gland was proliferating. In these three animals the corpora lutea had been absent during a period varying between 8 and 11 days. These observations confirm conclusions formulated in the preceding paper. The period directly following ovulation is accompanied by proliferative changes in the mammary gland, even if the ovulation has been accelerated experimentally, with or without a concomitant pregnancy. These observations furthermore prove that abortion does not necessarily prevent these proliferative changes.

In seven other guinea pigs ovulation had taken place about 3 to 8 days preceding examination. All were still pregnant. In one case, examined 19 days and a few hours after copulation, incisions had been made into one horn of the uterus at the time of the extirpation

of the corpora lutea. Small deciduomata developed, of which one was still alive and one necrotic at the time of examination. A new ovulation had taken place about 7 days previously and corpora lutea had been absent during a period of 6 days. In this animal the mammary gland was slightly proliferating. In the other six the mammary gland was either resting or resting to intermediate. In these instances the pregnancy was 18 to 19 days old, and the corpora lutea had been absent during a period varying between 6 and 9 days. In four of these animals a new ovulation had taken place 3 to 6 days previous to examination; the mammary gland was resting to intermediate. In two cases a new ovulation had taken place 6 to 8 days previously; in these two animals the mammary gland was in a resting stage.

We see, therefore, that the same factors determine conditions in the mammary gland if cyclic changes have been induced experimentally during pregnancy and in cases in which the normal cyclic changes have taken place without pregnancy.

If we consider the cases in which, during pregnancy, the corpora lutea or ovaries had been extirpated and examination was carried out 15 to 20 days after the last ovulation, we find that in none of the animals examined was the gland proliferating, while during the corresponding stages of either the ordinary cycle without pregnancy or during the corresponding period of pregnancy, a certain proportion of glands was found proliferating. This makes it probable that in this case also extirpation of the corpora lutea or ovaries directly or indirectly inhibited proliferative changes in the mammary gland. Extirpation of the corpora lutea during pregnancy, however, does not prevent, but on the contrary accelerates the proliferative changes in the mammary gland concomitant with a new ovulation.

Effect of Injections of Lutein on the Mammary Gland.

It seemed important to test the effect of the corpus luteum on the mammary gland in still another way; namely, directly through injections of the corpus luteum substance into the peritoneal cavity of the guinea pig.[4] 0.5 to 1 gm. of lutein was suspended in a sterile

[4] For this purpose we used Armour and Company's preparation of lutein from the cow.

bottle in 10 to 12 cc. of distilled, sterilized water and shaken in a mixing apparatus during a period varying between $2\frac{1}{2}$ and 7 hours. The fluid was then centrifuged for a few minutes and thus the undissolved residue removed. The supernatant fluid was injected intraperitoneally; about 8 to 12 cc. of fluid were given each time to one animal. Twenty-two guinea pigs were thus injected and an interval of 2 or 3 days was allowed to elapse between two succeeding injections. In seven of the animals the ovaries had been extirpated, in eight the corpora lutea had been removed, in one of the latter incompletely. In four animals incisions had been made in the uterus; similar incisions had been made in the uterus in animals in which ovaries or corpora lutea had been extirpated. In three cases the injections were made during the second half of the sexual cycle without any other experimental interference with the animal. In a few instances in which incisions had been made in the uterus previous to the injections, smaller or greater parts of the uterus became infected as the result of the injections. Some bacteria were apparently present in the lutein specimens. After injections into an uninjured peritoneal cavity, they did not lead to infection, but sometimes they infected the surface of a cut uterus. Lutein injections as such did not produce proliferation of the mammary gland, although sometimes as many as five injections were given to the same animal.

In seven cases lutein was injected in animals in which both ovaries had been extirpated; at the same time incisions were made into the uterus. Two of the animals received five, one three, two two injections, and two one injection. In a few animals the first injection of lutein was given 1 day before castration. The animals were examined 11 to 15 days after the preceding heat. In none was proliferation noticeable in the mammary gland, which was either resting or varying between the resting and intermediate state.

Injections of lutein were given to eight animals in which the corpora lutea had been extirpated. In one the extirpation had been incomplete. In one case five, in two cases four, in one case three injections of lutein were given, and in three cases one injection was given. In several guinea pigs the first injection was given 1 or 2 days before the operation. In the majority of the animals incisions had been made in the uterus at the time of extirpation of the cor-

pora lutea; in two of these an infection of the cut uterus occurred, evidently as the result of the lutein injections. In these eight animals the examination took place at the following periods.

(1) 17 days after the preceding heat; five injections had been given; the mammary gland was resting. (2) 7 days and 17 hours after the preceding heat; extirpation was incomplete; one injection had been given; the mammary gland was intermediate. (3) 13½ days after the previous heat; four injections had been given; the mammary gland was resting. (4) 16½ days after the preceding heat; four injections had been given; the mammary gland was approximately resting. (5) 6 days after the preceding heat; 3 days after the lutein injection, one injection being given; the gland was intermediate. (6) 5 days after the preceding heat and 2 days after the lutein injection, one injection being given; the mammary gland was intermediate. (7) 10½ days after the preceding heat; small deciduomata were slightly infected at some places; one injection had been given; the mammary gland was resting. (8) 14 days after the preceding heat; three injections were given; the mammary gland was almost resting.

We see that the injections of lutein did not exert any noticeable influence on the mammary gland in animals in which the corpora lutea had been extirpated.

In four animals incisions in the uterus were made some time previous to the injections of lutein.

(1) Incisions were made in the uterus during the positive period (3 to 7 days after ovulation). 17½ days after heat the first injection of lutein was made; 26½ days after heat examination took place. Good deciduomata had developed, the corpora lutea were preserved. Four injections of lutein were given. The mammary gland was resting. (2) 6½ days after ovulation incisions were made into the uterus. 18½ days after ovulation one injection of lutein was given. Examination of the animal took place 21½ days after ovulation. The uterus, in which deciduomata had developed, was markedly infected; no mature follicles, but good corpora lutea were present in the ovaries; the mammary gland was proliferating. (3) Five injections of lutein were given; 25½ days after ovulation examination was made. Large deciduomata and corpora lutea which were on the whole preserved, but showed some peripheral vacuolization, were present. A new ovulation had not taken place. The mammary gland was intermediate. (4) Four injections of lutein were given; examination 26½ days after ovulation. 11 days after ovulation the first injection of lutein was given. Living deciduomata and small corpora lutea were found. The mammary gland was proliferating.

We find proliferating mammary glands in several cases of this series. But inasmuch as this might be expected even without lutein injections, we cannot ascribe this result to the injection of lutein.

In three cases injections of lutein were made in animals in which no experimental interference had taken place. The injections were made in the latter half of the sexual cycle.

(1) Five injections were given, the first one 13½ and the last 22½ days after heat; examination took place 23½ days after heat. No new ovulation had occurred. The mammary gland was resting. (2) Four injections were given, the first 15 and the last 22 days after heat; examination was made 24 days after heat. A new ovulation had occurred about 4 days before examination. The mammary gland was found to be intermediate. (3) Four injections were given, the first 12½ and the last 19½ days after heat; 23½ days after ovulation examination was made. A new ovulation had taken place 5 to 6 days previous to examination. The mammary gland was intermediate.

In one control case four injections of 14 cc. of 0.4 per cent sodium chloride solution were made intraperitoneally, the first injection being given 11½ days and the last 21½ days after ovulation; examination 22 days after the first heat showed that a new ovulation had occurred 2½ to 3 days previous to examination. The mammary gland was therefore found to be partly proliferating.

In these cases again results were obtained which had to be solely attributed to the condition of the sexual cycle. The mammary gland was apparently not influenced by the injection of lutein. We may then conclude that as many as five consecutive injections of lutein given to the same guinea pig do not exert a noticeable stimulating effect on the mammary gland. While it is possible that a still larger number might have produced a definite effect, we should have expected to notice microscopically at least a beginning proliferation after as many as five injections. But in no case could such an effect be established. Under normal conditions the action of the corpus luteum is probably continuous; under the conditions of our experiment the action was not continuous, although a relatively large quantity had been injected each time.

It is, furthermore, possible that the effective constituent of the corpus luteum possesses a certain species specificity, and that while the corpus luteum extract of the cow is ineffective, the extract of the guinea pig corpus luteum might have been effective. Of course we have to consider the fact that even during the normal cycle proliferation in the mammary gland takes place in the absence of the

corpus luteum and that on the other hand proliferation is absent at certain periods when corpora lutea are well developed. In this respect, then, the negative result of the injections of lutein agrees with what we found at certain periods in the normal animal. However, we found indications that the corpus luteum may favor proliferation of the mammary gland under certain conditions, and our experiments prove that as many as five injections of cow's lutein are unable to produce even the beginning of proliferation.

SUMMARY.

1. In the pregnant guinea pig proliferation of the mammary gland becomes regular only at a later stage of pregnancy; namely, during the period following the 24th day of pregnancy. Previous to this period proliferation was absent in the majority of cases. Proliferation of the mammary gland during pregnancy becomes regular only at a period of time which exceeds the duration of the normal sexual cycle unaccompanied by pregnancy. It is probable that pregnancy as well as the presence of living deciduomata and corpora lutea increases the proliferative activity of the mammary gland as compared with the ordinary cycle in non-pregnant animals or in animals lacking corpora lutea and deciduomata.

2. After the completion of pregnancy and in the beginning of secretion some mitotic proliferation may still be present, but it soon ceases, probably as the result of those processes that lead to secretion. While during the period of secretion, notwithstanding the presence of a new pregnancy, mitotic proliferation soon ceases, some proliferative stimulus seems still to be active, which, however, under existing conditions apparently leads only to a mitotic multiplication of nuclei. The latter conclusion is only suggested at the present time and needs confirmation through further studies.

3. In cases in which abortion took place in the first half of pregnancy secretion in the gland was not established; secretion occurred in two animals aborting toward the latter part of pregnancy. In one of these cases, mitotic proliferation of some gland cells was associated with the microscopic appearances of secretion.

4. In guinea pigs castrated during an early period of pregnancy

in which pregnancy continued for some time, proliferative changes were absent in the mammary gland. In conjunction with a partial similar effect observed after extirpation of the corpora lutea during pregnancy, we may perhaps attribute the lack of proliferation in some of these cases to the absence of the ovaries.

5. Extirpation of the corpora lutea during pregnancy induces a new ovulation and with it the primary proliferation in the mammary gland; abortion does not necessarily prevent these proliferative changes. Extirpation of the corpora lutea during pregnancy perhaps prevents the secondary proliferative changes in the mammary gland.

6. Five injections of cow's lutein given in relatively large quantities intraperitoneally do not produce proliferation of the mammary gland in the guinea pig.

Correlation of the Cyclic Changes in the Uterus and Ovaries with Those in the Mammary Gland.

We can distinguish two phases in the cycle of the ovaries and uterus; the first phase comprises the period of heat, ovulation, and the period immediately following ovulation. In this period certain growth processes take place in the uterus. The processes taking place in the uterus and ovaries during this period are dependent on the removal of an inhibiting substance produced by the corpus luteum and on the presence of a substance produced through another constituent of the ovaries, in all probability the large follicles including the mature follicles or perhaps the latter alone. As we pointed out previously, an interstitial gland comparable to that of the rabbit does not exist in the guinea pig. Heat changes therefore depend on the absence of the corpus luteum and on the presence of another ovarian constituent.

This period is followed by the second and longer period dominated by the function of the corpus luteum. As we have shown experimentally, the secretion of the corpus luteum sensitizes the uterine mucosa in such a way that the decidual and predecidual proliferation takes place, the former after application of mechanical stimuli or after the injury produced through the embryo penetrating into the mucosa, the latter under the influences of ordinary metabolic

changes. This sensitizing influence of the corpus luteum is, however, not exerted throughout the whole remaining part of the sexual cycle, but only during a period of 6 or 7 days (from the 3rd to the 9th or 10th day after ovulation). We must either assume that the corpus luteum ceases to secrete this sensitizing substance after the 9th day after ovulation or else that after this period it secretes in addition a substance inhibiting the action of the first substance. We have shown that the corpus luteum has an inhibiting action by force of which it prevents the appearance of a new ovulation and heat. This latter substance is given off during the whole period of the sexual cycle. It is therefore probable that we have to deal with two different substances secreted by the corpus luteum.

During pregnancy conditions are essentially the same as without pregnancy. The corpus luteum exerts the same two functions. There is only one essential difference between the conditions during pregnancy and without pregnancy. If we accelerate experimentally the onset of a new sexual cycle without pregnancy, the newly formed corpus luteum causes also a new cycle in the uterine mucosa, while during pregnancy the experimental production of a new corpus luteum is not followed by a new cycle in the uterine mucosa; here some inhibiting mechanism is at work. If we now correlate the cyclic changes in the mammary gland with those in the uterus and ovaries, we find proliferative changes in the mammary gland corresponding to the first phase of the ovarian and uterine cycle. We may call this the primary growth period of the mammary gland. We have every reason to believe that the swelling of the human mammary gland during menstruation, observed by many and in the absence of microscopic studies referred to vascular changes, represents in part these growth processes.

We are able to accelerate experimentally the primary proliferation of the mammary gland; in the same way we can experimentally accelerate heat and ovulation, through removal of the inhibiting tonus exerted chemically by the corpus luteum. The primary growth period of the mammary gland can be experimentally induced as well during pregnancy as without pregnancy. Contrary to what might have been expected, we find that the growth stimulus exerted on the uterus by the corpus luteum in the first part of the second phase of

the sexual cycle does not find a counterpart in the mammary gland of the guinea pig. During the whole period of the development and activity of the corpus luteum of the non-pregnant animal the mammary gland in the majority of cases is inactive. Only during the last part of the sexual cycle may we find in some cases some proliferation of the mammary gland. Whether this is due to the approach of the succeeding period of heat or is an accumulative late effect of the corpus luteum substance cannot be determined with certainty at present. It is possible that a quantitatively stronger action of the corpus luteum at last produces proliferative changes also in the guinea pig. Thus we usually found proliferation in cases in which we artificially prolonged the period of the sexual cycle and in which well preserved corpora lutea and deciduomata were present. How much in these cases the proliferation of the mammary gland has to be attributed to a quantitatively stronger action of the corpus luteum or to the deciduoma or to the combination of both, cannot be decided definitely at the present time.

The conditions are similar during pregnancy. We find here definite growth of the mammary gland only after the passing of a period which is longer than the duration of the normal sexual period. But in a number of instances we find following the 12th day of pregnancy some proliferation in the mammary gland. Pregnancy as well as the presence of living deciduomata and well preserved corpora lutea causes an increase in the number of proliferating glands during a time corresponding to the closing period of the normal sexual cycle. We might designate this as the secondary growth period of the mammary gland. In the pregnant animal it develops into the typical hypertrophy of the mammary gland characteristic of this condition, and, indeed, represents merely its initial stage.

In the rabbit Ancel and Bouin[5] as well as Frank and Unger[6] noticed a parallelism between the presence of good corpora lutea and the

[5] Ancel, P., and Bouin, P., *J. physiol. et path. gén.*, 1911, xiii, 31.

[6] Frank, R. T., and Unger, A., *Arch. Int. Med.*, 1911, vii, 812. Shortly after the preliminary communication of our results (*Proc. Soc. Exp. Biol. and Med.*, 1915–16, xiii, 91) there appeared a preliminary note by M. Athias (*Compt. rend. Soc. biol.*, 1916, lxxix, 557) in which this author described growth and secretion in the mammary gland in castrated male guinea pigs, into which ovaries had been transplanted. At the time of examination, soon after the beginning of lactation,

growth of the mammary gland. Ancel and Bouin conclude that it is the corpus luteum which determines the growth of the mammary gland. Our investigations show that in the guinea pig the factors determining the growth of the mammary gland are complex, and that they can be considered only partly analyzed at the present time. Provided the facts observed by Ancel and Bouin in the rabbit have found a correct interpretation, we must assume that in the rabbit the response of the mammary gland to the secretions of the corpus luteum is much more prompt than in the guinea pig. We would have to assume that the gland cells in the rabbit are more labile and thus react at once, while in the guinea pig the stimulus has to accumulate during a considerable period of time before a reaction sets in. From a biological point of view such a difference between the rabbit and guinea pig could well be understood if we consider the fact that in the rabbit the duration of pregnancy is much shorter than in the guinea pig, and that therefore the service of the mammary gland is required in the rabbit at a much earlier period after copulation than in the guinea pig. While such a consideration is not a causal explanation, it renders it at least plausible that a marked difference in the power of reaction of the two kinds of mammary gland does exist.

As to the secretory function of the mammary gland, we are inclined to consider it as a phenomenon intermediate between growth on the one hand and complete degeneration on the other hand, as we find it with deciduomata and under certain conditions also in the mammary gland[7] after the growth stimuli have suddenly ceased to act. It seems that the regenerating effect of all divisions is necessary in certain tissues in order to insure their continued preservation. The peculiar constitution of the mammary gland causes instead of complete degeneration, an intermediate state of intercellular degeneration, which is, however, coupled with a degeneration of a considerable number of gland cells. Such a view is somewhat akin to that of Hildebrandt[7] without, however, being identical with the latter.

there were no well developed corpora lutea in the ovaries. The author concludes that follicles or interstitial gland, but not the corpora lutea are responsible for the growth of the mammary gland. (It is not our intention to consider extensively the literature on this subject at the present time; we may give a more complete review at a later date.)

[7] Hildebrandt, P., *Beitr. chem. Phys. u. Path.*, 1904, v, 463.