

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

I. THE CHANGES IN THE NON-PREGNANT GUINEA PIG.

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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INTRODUCTION.

While the literature dealing with the growth and function of the mammary gland is extensive, the mechanism determining the growth processes as well as secretion is incompletely known. Growth and function of the mammary gland are so closely interrelated with the cyclic changes of the uterus and ovaries that an understanding of the latter is a prerequisite for the former.

During the last 10 years one of the writers has brought to a conclusion the analysis of the mechanism of the mammalian, uterine, and ovarian cycle in its principal outlines. On the basis of the previous studies it is now possible to attempt a further analysis of processes of growth and secretion in the mammary gland. While the results in this and the succeeding paper clear up certain phases of the problem, it will be necessary in further studies to complete the analysis.¹

The conclusions embodied in this and the succeeding paper are based on the examination of the mammary glands in guinea pigs. In almost all the animals examined the period of heat had been

¹During the summer of 1915 a number of the mammary glands were prepared for microscopic examination in the Pathological Laboratory of the St. Louis University Medical School. The facilities of this laboratory were generously placed at our disposal by the authorities of the School and by the Director of the laboratory, Dr. Ralph L. Thompson. We take this opportunity to express our appreciation of the assistance accorded us.

observed, and the period of the sexual cycle of the animal was therefore known. It was confirmed by microscopic examination of the ovaries and uterus in the majority of cases; the former were invariably cut in serial sections and thus a comparison between the condition of the ovaries and uterus on the one hand, and of the mammary gland on the other, was made possible. In addition, the majority of the animals were subjected to certain experimental procedures, under ether anesthesia, at a known period of the sexual cycle, in order to determine the significance of the ovaries as a whole, of the corpora lutea, of the uterus, of the deciduomata, with and without pregnancy, and of lutein injection for the condition of the mammary gland.

In order to avoid the necessity of describing in detail in each case the state of the mammary gland as revealed on microscopic examination, we characterized the mammary gland as proliferating, as resting, or as intermediate. We disregarded almost entirely the amount of gland tissue which we found at the time of examination. The latter depended not so much on the stage of the cycle in the mammary gland and on the experimental interferences as apparently on more or less accidental factors, particularly on the time which had elapsed since the last pregnancy preceding the time of examination. We shall describe briefly the different types of mammary glands.

Proliferating Mammary Gland.—The acini and large ducts are of variable size, sometimes very large, and even cystically dilated. The epithelium is high cuboidal or almost cylindrical; in some instances it is only medium cuboidal. The epithelial nuclei are vesicular. There are mitoses present in the acini, sometimes in large numbers, in other glands they are less frequent; in some cases they are only rarely found. The amount of colloid-like material within the acini or ducts varies in different glands. There may be papilla-like protrusions into the large ducts. The connective tissue between the acini is scant, more or less cellular, with large, sometimes with smaller vesicular nuclei. Lymphocytes and polynuclear leukocytes are usually found in the stroma. There is usually much fluid present in the stroma. In some glands mitoses are found in the fibroblasts of the stroma; in others they are absent. The amount and character of the stroma are not identical in all glands;

there may occasionally be some stronger development of fibrous tissue, especially in certain parts of the gland; the gland may be proliferating only in parts.

Resting Mammary Gland.—We usually find small acini, occasionally cystically dilated acini, lined by low cuboidal or flat epithelium, with dark staining, condensed or small vesicular nuclei. The gland ducts have likewise low epithelium. Desquamated epithelial cells may be present in the lumen of the acini. Colloid-like material may be present or absent. Mitotic proliferation is entirely lacking. The connective tissue between the acini or around the ducts is usually densely fibrous and often present in a relatively large amount. The nuclei in the stroma are small and compressed. Mitoses are absent. Polynuclear leukocytes and lymphocytes are usually not seen.

Intermediate Mammary Gland.—While the types of proliferative and resting mammary gland are fairly sharply defined, this is not so in the same degree in the third type, the intermediate mammary gland. Here mitoses are always absent, but the structure of the gland and stroma does not approach the inactivity of the resting gland, but approaches more or less that of the proliferating gland. The gland epithelium is higher than in the resting gland, and the stroma, in parts or throughout, is more cellular than in the resting gland; the nuclei are more vesicular, and the quantity of dense hyaline connective tissue, throughout the gland or at certain places, is much diminished. Lymphocytes and leukocytes are often present, but the characteristic feature of the proliferating gland, the mitoses, are absent. The intermediate gland may otherwise bear a greater resemblance to the proliferating or to the resting gland and we may therefore make further subdivisions, designating some glands as intermediate to resting, others as better than intermediate.

Normal Cycle of the Mammary Gland in the Non-Pregnant Animal.
Period from Time of Heat to 5 Days after Ovulation.

In the guinea pig the stage of heat can be recognized by examination of the ovaries and uterus. A detailed description of these changes has been previously given by one of us.² In the observations

² Loeb, L., *J. Morphol.*, 1911, xxii, 37; *Virchows Arch. path. Anat.*, 1911, ccvi, 278; *Biol. Bull.*, 1914, xxvii, 1.

reported in this communication the diagnosis of the period of heat is based on the state of these organs as shown by microscopical examination. In some cases the objective signs of heat were not yet completely present, but even in these cases, knowing that the interval since the preceding ovulation corresponds to a full period of the sexual cycle, and finding those changes in the uterus and ovaries which approach the characteristics of heat, we were in a position to diagnose an impending heat.

Mammary Gland near the Stage of Heat or during Heat.—We examined the mammary gland in seven guinea pigs which were either in heat or near heat. In all of them we found proliferating glands; in no case was the gland in a resting or intermediate condition. In the guinea pig the period of heat is usually followed by ovulation. The latter generally occurs 6 to 12 hours after copulation, but in the guinea pig a preceding copulation is not, as it is in some animals, a prerequisite for ovulation. The time immediately preceding or following ovulation can be recognized by a microscopic examination of the ovaries, which shows greatly enlarged mature follicles either near the stage of rupture or directly following rupture.

Mammary Gland near Ovulation or within 24 Hours after Ovulation.—We examined the mammary glands of eight guinea pigs which were either at the point of ovulation or in which ovulation had taken place within the past 24 hours. In five of these animals the glands were proliferating. In three of the latter animals incisions had previously been made into the uterus and deciduomata had developed, which at the time of examination had become necrotic. Ovulation, however, had not been delayed by the presence of necrotic deciduomata. In two other cases the gland showed an intermediate character, and in one case we found a gland in the resting state. In the latter instance, incisions had been made in the uterus, and deciduomata had developed and caused a delay in ovulation. It was $26\frac{1}{2}$ days after the previous ovulation before a new ovulation took place; the deciduomata were found necrotic soon after ovulation. In this case of delayed ovulation a resting gland was found in spite of the period of the sexual cycle at which the examination had taken place. Here apparently the delay in ovulation caused by the pres-

ence of deciduomata was responsible for the lack of progressive changes in the mammary gland.

Mammary Gland from 24 to 72 Hours after Ovulation.—We examined sixteen guinea pigs in this period of the sexual cycle. In seven we found a proliferating gland. In one of these a necrotic deciduoma was present, but ovulation had taken place at the proper time. In seven animals the glands were intermediate; in two of these we found necrotic deciduomata but there had not been any delay in ovulation. In two animals the glands were resting, or slightly better. In both of these necrotic deciduomata were present. In one of the animals ovulation had been delayed, and in the second it had taken place at the proper time. We found, therefore, a proliferating gland in not quite one-half of the animals, but a resting gland only in a small minority.

Mammary Gland from 72 Hours to the End of the 5th Day after Ovulation.—At this stage of the sexual cycle we find six proliferating glands. No incisions in the uterus had been made and no deciduomata had, therefore, developed. In five animals the glands were intermediate; ovulation had taken place at the proper time in three, and the remaining two died a few days after ovulation. In no case had incisions been made into the uterus. In five animals the glands were somewhat better than resting, but not quite intermediate. At this period a little more than one-third of the glands were proliferating and almost one-third were slightly better than resting.

If we summarize the results, we find that during a period of the sexual cycle including the time preceding heat until 5 days after ovulation, there is a gradual decrease in the activity of the mammary glands. At the time of heat all the glands proliferate, and from 3 to 5 days after ovulation, little more than one-third are proliferating and almost one-third are near the resting state. There is a maximum at the period of heat and from then on there is a gradual decline to the end of this period. In some instances in which a delay of ovulation had been experimentally produced, the mammary gland seemed to show less activity during this period than in the animals in which ovulation took place at the usual time. Our observations, however, merely suggest the latter conclusion.

Period from 5 to 15 Days after Ovulation.

Mammary Gland from the Beginning of the 6th to the End of the 12th Day after Ovulation.—Twenty-nine animals in this period of the sexual cycle were examined. There was in addition one guinea pig which presumably belonged to this stage. Of these, fifteen or sixteen animals showed an intermediate, and fourteen an approximately resting condition of the mammary gland; the majority of the latter were slightly better than resting. In no case was a necrotic deciduoma present in the uterus. A proliferating gland was not found during this period. Almost half the glands were, therefore, in a resting, and the rest in an intermediate condition.

Mammary Gland from the Beginning of the 13th to the End of the 15th Day after Ovulation.—Four animals were examined in this stage of the sexual cycle. One showed a proliferating mammary gland. In this case a foreign body had been introduced into the uterus $2\frac{1}{2}$ days after heat; 6 days after heat the foreign body was removed and incisions were made into the uterus. Three good corpora lutea were found in the ovaries. In three other animals the mammary glands were resting or slightly better than resting. In the first of these a good corpus luteum and mature follicles were present. In the second we found good corpora lutea as well as a small deciduoma and mature follicles. In the third we found partly preserved, partly necrotic deciduomatous tissue, and lutein injections had been made.

In this stage of the sexual cycle the mammary gland is, therefore, on the whole inactive. We may furthermore conclude that at this stage of the cycle neither mature follicles nor well preserved corpora lutea nor the presence of a small deciduoma are usually able to stimulate the mammary gland to activity, although in one case where good corpora lutea were present, proliferation had taken place.

If we summarize the findings during this whole period, we may conclude that the time from the 6th to the 15th day after ovulation represents the lowest level in the activity curve of the mammary gland, although good corpora lutea are usually present and mature follicles may appear in the second half of this period.

Period from 16 to 27 Days after Ovulation.

The normal sexual cycle in the guinea pig varies between 15 and 19 days. This series includes, therefore, not only guinea pigs in the last stage of the sexual cycle, but also animals in which normal ovulation had been delayed.

Mammary Gland from the Beginning of the 16th to the End of the 19th Day.—In this period we find five proliferating glands. In three of these cases the uterus had been extirpated and the corpora lutea were well preserved. In the two other cases vacuolar corpora lutea were present. Mature follicles were noted in two of the five cases. In four cases the mammary glands were resting. In one of these animals the ovaries were hypotypical; in three, good deciduomata were present. In two of the latter the ovaries had not been examined; and in the third the corpora lutea showed beginning vacuolization and mature follicles were present. In two animals the glands were intermediate. In one of these the structure of the gland approached that of a proliferating gland, but mitoses were not definitely observed. The condition of the uterus and ovaries indicated that this animal was near the period of heat. In the second case vacuolar corpora lutea and reactive follicles were present. But there was not yet any other indication of approaching heat. In this case the mammary gland at one place approached the proliferating stage. We find then that at this stage of the sexual cycle approximately one-half the animals had a proliferating gland. Proliferation occurs at this period in cases in which, while a new ovulation is not approaching, the corpora lutea are well preserved (probably as the result of the extirpation of the uterus).

In a few other cases in which apparently the stage of heat is approaching, we find good intermediate or even proliferating glands. Apparently the same factors which prepare uterus and ovaries for heat, exert also some effect on the mammary gland. Notwithstanding the presence of good deciduomata and of mature follicles, the gland may still be resting in certain other cases. In one the ovaries were hypotypical, a condition which explains the inactivity of the mammary gland which is accompanied by an inactive state of the uterus.

Mammary Gland from the Beginning of the 20th to the 27th Day.— This period does not occur under normal conditions. Under normal conditions a new ovulation usually precedes this period. But if through incisions in the uterus, made 4 to 8 days after ovulation, deciduomata are experimentally produced, a new ovulation is often delayed, and the sexual period prolonged. The mammary glands of twenty-four animals were examined during this period; of these fifteen were resting glands, five were proliferating, and four were intermediate. The five proliferating glands were found in animals 21 to 25 days after previous ovulation. In the first animal the thyroid had been entirely or almost entirely extirpated. Corpora lutea as well as mature follicles were present. In the second incisions had been made in the uterus; the developing deciduomata had become necrotic, and the corpora lutea showed vacuolization. In this case there was little proliferation present. Proliferation in this case may have started at an earlier period, while deciduomata and corpora lutea were still in good condition. In the third animal incisions had been made in the uterus, infection of the uterus took place, and although the animal was sick, the corpora lutea were well preserved. In the fourth instance the uterus had been completely extirpated. Deciduomata were not present, but as usual under these conditions the corpora lutea were preserved and there were mature follicles present. In the fifth the corpora lutea were necrotic, but the state of the mature follicles suggested that the animal was near ovulation. In three of the guinea pigs, therefore, well preserved corpora lutea were present. Sickness of the animal due to infection of the uterus does not necessarily prevent proliferation of the mammary gland. In one of the remaining animals impending ovulation was probably responsible for the proliferation. The four animals in which one intermediate gland was found had all ovulated 21 to 23 days previously. In all these guinea pigs the corpora lutea were vacuolar, and there were in addition necrotic deciduomata. In two of the animals mature follicles were found.

The fifteen guinea pigs with resting glands or with glands slightly better than resting had ovulated during a period varying between 21 and 27 days preceding the examination. Of these the majority, namely eleven, showed degenerated corpora lutea; of the remaining

four two had good corpora lutea, in two others the corpora lutea were partly preserved; these four animals, however, had deciduomata which were entirely or partly necrotic. Of those animals which had vacuolar, retrogressing corpora lutea, five showed in addition necrotic deciduomata. Mature follicles were found in the ovaries of two of the guinea pigs, without, however, showing any indications that an ovulation was imminent.

We see then that in those animals in which there was a resting mammary gland degenerating corpora lutea were present in the majority of cases. In a few instances in which the corpora lutea were partly preserved, deciduomata were necrotic, while in two animals mature follicles were found. In none of these animals was there any indication of an imminent heat. It is also of interest to note that all the animals in which more than 23 days had elapsed since the previous ovulation, in which, therefore, the delay in ovulation was especially great, the mammary gland was in a more or less resting stage. These facts are of interest in considering the succeeding set of animals.

Mammary Gland in Guinea Pigs in Which Living Deciduomata and Well Preserved Corpora Lutea Are Present.

It was advisable to make a separate group of these animals in order to determine whether the presence of experimentally produced deciduomata without the accompaniment of embryonic structures, together with the presence of good corpora lutea, affect the mammary gland. It was especially of interest to compare these animals with others of the corresponding period of the sexual cycle in which pregnancy was found.

We examined the mammary gland in twenty-five animals belonging to this group. Twenty-one of the animals have not been included in the various groups of the normal sexual cycle; in these ovulation had occurred later than 16 days previous to examination, while the four animals in which ovulation had occurred from 12 to 16 days previously have been referred to above.

Mammary Gland from the 9th to the End of the 13th Day of the Sexual Cycle.—Seven animals belong to this class. One had a proliferating

gland; it has been mentioned among the animals in the normal sexual cycle. Two animals, both of which were sick, had a resting gland. Of four in which the gland was intermediate, one showed mature follicles. We see, therefore, that at this stage of the cycle the glands as a rule show no proliferation notwithstanding the presence of good deciduomata and well developed corpora lutea.

Mammary Gland from the Beginning of the 14th to the End of the 17th Day.—In three animals the mammary gland was proliferating. In two the corpora lutea showed a slight peripheral vacuolization, and in the third the corpus luteum was good throughout. In two of these three animals mature follicles were present. Three animals had an intermediate gland. In one of these the corpora lutea showed the beginning of vacuolization. In another the ovaries had not been examined, but there is little doubt that the corpora lutea were at this period still fairly well preserved. Mature follicles were also present in one animal. One-half the guinea pigs, therefore, had a proliferating, while the remainder showed an intermediate gland.

Mammary Gland from the Beginning of the 18th to the End of the 20th Day.—All three animals showed a proliferating gland. The corpora lutea were well preserved, and mature follicles were present in all of them.

If we combine the second and third groups, we have nine guinea pigs of which two-thirds show a proliferating, and the remaining one-third an intermediate gland. If we compare this result with the condition in animals of the normal cycle without the presence of good deciduomata, and in approximately the corresponding period of the sexual cycle, we find much greater activity in the mammary gland of animals in which good deciduomata are present. Without good deciduomata during an approximately corresponding period we find the majority of glands in a resting condition and only a little more than one-third proliferating.

Mammary Gland from the Beginning of the 21st to the End of the 23rd Day.—Nine animals were examined at this period. In six with good deciduomata and corpora lutea which were well preserved or showed just beginning peripheral vacuolization, the mammary gland was proliferating. In all but one of these animals mature follicles were present in the ovaries. In one animal with good corpora lutea

and with deciduomata that showed slight degeneration, it was doubtful whether the mammary gland was proliferating or intermediate. In this case also mature follicles were present. In the last two guinea pigs in which there were also good deciduomata and good corpora lutea present, the mammary gland was in one case intermediate, in the other not quite intermediate.

Mammary Gland from the Beginning of the 24th to the Beginning of the 27th Day.—In all these four animals good deciduomata and corpora lutea that were well preserved or showed merely beginning peripheral vacuolization were present. All had mature follicles. Three showed a proliferating gland, one a gland that was better than intermediate.

From the 21st to the 27th day the mammary glands of thirteen animals were examined. Nine of these were definitely proliferating, one perhaps proliferating, and the remaining three were intermediate. The majority were, therefore, proliferating; none were resting in this group. If we compare these glands with the mammary glands of animals of the corresponding period, in which the deciduomata were mostly necrotic, we find a great difference. In the latter the majority were in a resting condition. It is probable that both preservation of corpora lutea as well as deciduomata contributed to this result. In the former group of corresponding age in several instances we found a good corpus luteum without deciduomata, combined with a proliferating gland. However, in cases in which necrotic deciduomata were present, the mammary gland was usually resting, while in this group with good deciduomata the mammary gland was usually proliferating. We may then conclude that in cases in which the sexual cycle is artificially prolonged, well preserved deciduomata are usually associated with proliferating glands, while degenerating deciduomata which are usually combined with vacuolar corpora lutea, are associated with resting glands. Good corpora lutea and good deciduomata may, therefore, at this period of the sexual cycle cause proliferation of the mammary gland without the presence of pregnancy.

Mature follicles, such as are found even in cases in which vacuolar corpora lutea and necrotic deciduomata are present, are not able to cause proliferation in the mammary gland. Even at an earlier date, in the period from the 16th day on, the presence of good cor-

pora lutea combined with good deciduomata in the majority of cases produced proliferation of the mammary gland.

The period from the 9th to the 14th day of the sexual cycle belongs to the refractory period of the sexual cycle. At this time even the presence of good corpora lutea combined with well preserved deciduomata could only in one case produce proliferation; but in the period from the 14th to the end of the 24th day of the sexual period, there were fifteen or sixteen proliferating glands and six which were intermediate. It is probable that the deciduomata contribute to the effect of the corpus luteum. In three out of four cases in which good corpora lutea were present, but the deciduomata necrotic, the mammary gland was resting, while in the fourth case it was proliferating notwithstanding the necrotic condition of the deciduoma and a somewhat vacuolar state of the corpus luteum.

We see then that a definite cycle of the mammary gland corresponds to the cycle in the ovary and uterus. In the first period we find proliferation of the mammary gland, which gradually diminishes as the sexual cycle progresses, then comes a resting period at a time when the corpus luteum is well developed and functioning, to be followed again by beginning proliferation at a period when the new sexual cycle in the ovary and uterus is approaching. If we prevent the onset of the new sexual cycle and artificially prolong the old cycle, then we find that proliferation of the mammary gland is usually associated with the presence of good deciduomata and corpora lutea, while in the presence of necrotic deciduomata, which are often but not always accompanied by vacuolar corpora lutea, the mammary gland usually remains resting.

Effect on the Mammary Gland of Extirpation of the Ovaries.

In nineteen guinea pigs both ovaries were extirpated,—in the majority of instances 5 to 7 days, in some, however, as early as 3 days after ovulation. In all but two incisions were made in the uterus at the time of castration. The development of deciduomata was interfered with; they either did not develop at all or remained small and had become necrotic, if the examination took place 20 days after ovulation or later. In the two animals in which no incisions had

been made in the uterus, the examination was made approximately 18 days after ovulation; the mammary gland was resting or intermediate. In one case in which incisions in the uterus had been made, on examination 14 days after ovulation, the mammary gland proved to be resting or slightly better than resting. In this guinea pig little deciduomatous tissue had been produced.

In another in which the examination took place 9 days after ovulation the gland was almost resting. In a third animal 14 to 15 days after ovulation a small deciduoma had developed which had become entirely necrotic and showed many hemorrhagic areas. The mammary gland was resting to intermediate. In a fourth and fifth instance at the examination $9\frac{1}{2}$ to $10\frac{1}{2}$ days after ovulation, the mammary gland was likewise resting to intermediate. In these five guinea pigs the mammary glands were, therefore, examined during the negative period. Proliferation could not be expected at that period even with the presence of normally functioning ovaries. In the other twelve cases examination took place 20 to 22 days after ovulation. In almost all, deciduomata had developed which had become necrotic. The mammary gland varied in these cases between the resting and intermediate condition.

As we have shown, in the presence of good corpora lutea or of a combination of good corpora lutea and good deciduomata, proliferation of the mammary gland is frequent between the 18th and 22nd days after ovulation. After extirpation of the ovaries, however, proliferation was absent during this period. We may therefore conclude that extirpation of the ovaries prevents proliferation of the mammary gland. After castration those influences emanating from the uterus and ovaries which, under certain conditions, cause a proliferation of the mammary gland, are absent. It may be that the same condition which leads to the early necrosis of the deciduomata after castration also prevents proliferation of the mammary gland. In addition, the absence of good deciduomata or the presence of necrotic deciduomata may in such cases have an additional unfavorable influence on the mammary gland.

Mammary Gland in Animals with Hypotypical Ovaries.

Loeb has formerly described a condition of the ovaries in which the follicles do not reach full size.³ Atresia sets in at a stage when the follicles are as yet of medium size or even smaller. Such ovaries he designated as hypotypical. It seems that in almost all these cases corpora lutea are either degenerating or absent. We observed ten cases of this kind in which the condition of the mammary gland was ascertained. While in the majority of the animals there can be no doubt about the character of the ovaries, it is possible that in an exceptional case an accidental transitory condition was included in this series. In all these guinea pigs the mammary gland was resting or only slightly better.

In one of the animals the ovaries were examined 6 days after ovulation. The corpora lutea had been extirpated 4 days previously. In the second case incisions had been made in the uterus and only the remnants of degenerated corpora lutea were visible. The third guinea pig died 13 days after heat; the animal was in a state of inanition; the corpora lutea showed beginning vacuolization. In the fourth animal a few small pieces had been cut out from the ovaries 5 days after ovulation; 22 days after ovulation retrogressing vacuolar corpora lutea were found. In the fifth the thyroids had been (perhaps incompletely) removed $4\frac{1}{2}$ days after ovulation; $23\frac{1}{2}$ days after ovulation vacuolar corpora lutea were found. In the sixth instance, 26 days after thyroidectomy, very much degenerated remnants of corpora lutea were found. In the seventh guinea pig the thyroids had been extirpated $5\frac{1}{2}$ days after ovulation. On examination 23 days later degenerating corpora lutea were found; no new ovulation had taken place. In both the sixth and seventh cases the animals had been sick before examination of the ovaries. In the eighth case small pieces had been excised from the ovaries almost 4 days after ovulation. 10 days later the guinea pig died. A large, well preserved, corpus luteum was present. In the ninth animal blood had been withdrawn on two occasions, and 3 days after the last bleeding the animal was killed. Only the remnants of corpora lutea were present. In the last case an incomplete extirpation of corpora lutea

³Loeb, *Zentr. Physiol.*, 1911-12, xxv, 342.

had been done 7 days after ovulation; 10 days later the examination showed the presence of vacuolar, small corpora lutea. A new ovulation had not taken place in many of these cases.

We find, then, that hypotypical ovaries are combined with an inactive state of the mammary gland and that guinea pigs with hypotypical ovaries behave like castrated guinea pigs.

Effect of Extirpation of the Corpora Lutea on the Mammary Gland.

We stated above that in cases in which the ovaries were hypotypical the corpora lutea were usually absent or degenerating, and that in these cases the mammary gland was inactive. A similar condition of the mammary gland is observed in cases in which at an early stage of the sexual cycle the corpora lutea have been extirpated without a new ovulation taking place. As one of us has shown previously, extirpation of the corpora lutea leads in the majority of cases to a hastening of the new ovulation which marks the onset of a new sexual cycle.⁴

We examined the mammary gland in thirty-three guinea pigs in which the corpora lutea had been completely extirpated. We may divide this set into two groups: in the first one, comprising thirteen animals, a new ovulation had not yet taken place at the time of examination; one of these animals has already been mentioned among the guinea pigs in which the ovaries were hypotypical. In the second group, comprising twenty animals, a new ovulation had occurred at the time of examination.

(a) In the first group we found a resting mammary gland in six animals which were examined at a period from 4 to 17 days after ovulation. In these animals the corpora lutea had been absent from 2 to 11 days. In one of these cases incisions had been made into the uterus 6 days after ovulation; on examination 10 days later, small living deciduomata were found. The examination in these six guinea pigs was made at a time, when, during the normal cycle, no proliferation usually occurs in the mammary gland.

In seven instances the mammary gland was intermediate. In four of the animals the examination took place at an early period of the

⁴ Loeb, *Deutsch. med. Woch.*, 1911, xxxvii, 17.

sexual cycle; namely, from 5 to 9 days after the ovulation which preceded the extirpation of the corpora lutea. In these four the corpora lutea had been absent from 3 to 7 days. In the remaining three cases incisions had been made into the uterus, in two 6 days, and in one $3\frac{1}{2}$ days after ovulation. In two of the animals no deciduomata developed owing to the absence of the corpora lutea, and in the third they had become necrotic at the time of examination. Examination was made in these cases $17\frac{1}{2}$ days after the ovulation which preceded the extirpation of the corpora lutea. The corpora lutea had been absent 13 or 14 days. In two of these guinea pigs mature follicles were present and one of them was in heat. Notwithstanding the presence of heat, this animal, in which deciduomata had not developed in the cut uterus, did not show a proliferating gland. In the third animal, in which we were only able to examine one ovary, it is doubtful whether a new ovulation had not taken place. We find, therefore, after extirpation of the corpora lutea an intermediate gland either in animals with mature follicles or in animals examined during the first half of the sexual cycle, the latter class representing cases in which the period of absence of corpora lutea had been relatively short. It is probable that considering the occasional presence of proliferation in the mammary gland during the corresponding period of the normal cycle and its complete absence after extirpation of the corpora lutea, that the absence of corpora lutea is partly responsible for the non-proliferating condition in the mammary gland. On the other hand, we have seen that if heat or ovulation occurs, proliferation of the mammary gland sets in during the normal cycle notwithstanding the absence of good corpora lutea. This latter conclusion is confirmed by experiments in which after extirpation of the corpora lutea, a premature new ovulation took place.

(b) The second set comprises twenty animals in which after extirpation of the corpora lutea, a new ovulation was imminent or had taken place. As stated above, after complete extirpation of the corpora lutea the new ovulation is generally accelerated.

Two animals were found near or during the stage of heat. In one of these in which the uterus had not been cut, the mammary gland was proliferating. In this case the preceding heat had occurred a little over 12 days previously, and the corpora lutea had been

absent 10 days. The second animal has already been mentioned under (a). In this case incisions had been made in the uterus, but no deciduomata developed. Examination was made $17\frac{1}{2}$ days after ovulation; corpora lutea had been absent for 14 days. The mammary gland was almost intermediate. In three cases the animals were near ovulation or ovulation had taken place within the preceding 24 hours. No incisions had been made into the uterus. Corpora lutea had been absent in these animals for 8, 9, and 14 days, respectively, and extirpation of the corpora lutea had taken place from 2 to 3 days following ovulation. In two of the animals the mammary gland was proliferating, in the third its structure was almost that of a proliferating gland, but mitoses were not found. In three animals examination took place from 1 to 3 days after ovulation. Here the second ovulation took place from 11 to 15 days after the first one, and corpora lutea had been absent during a period varying from 7 to 9 days. In all these animals the mammary gland was proliferating.

In six guinea pigs in which the ovulation following extirpation of the corpora lutea had taken place 3 to 5 days, and in seven other guinea pigs in which the second ovulation had taken place $5\frac{1}{2}$ to 6 days previous to examination, the mammary gland was found in an approximately intermediate condition. In most of these animals extirpation of the corpora lutea, which was complete in each animal, as microscopic examination showed, had been carried out 2 to 3 days after the first observed ovulation. Examination took place 11 to 15 days after extirpation of the corpora lutea and the ovulation following extirpation of the corpora lutea was accelerated in all cases. In a number of these cases incisions had been made in the uterus, but deciduomata did not usually develop. Only in animals in which the corpora lutea had been extirpated relatively late, namely, $6\frac{1}{2}$ days after ovulation, did deciduomata develop; in one of these they had become necrotic, in the second small, good deciduomata were visible at the time of examination.

These experiments confirm the conclusion at which we arrived through our observations of the normal cycle; namely, that in the guinea pig the stage of heat and ovulation and the period immediately succeeding it are accompanied by a proliferation of the mammary gland, while the later period, coincident with the development

of the corpus luteum, is not accompanied by a proliferation. We learn, furthermore, that an absence of corpora lutea during a certain period preceding heat and ovulation does not only not prevent subsequent proliferation of the gland, but is a prerequisite for this proliferation, inasmuch as without a preceding degeneration or extirpation of all the corpora lutea no new heat or ovulation can take place. This conclusion is in accordance with the fact previously demonstrated by one of us that corpora lutea, probably through chemical influence, inhibit heat and ovulation.⁴ In a similar manner the corpora lutea inhibit that proliferation of the mammary gland which is characteristic of the first period of the sexual cycle. It is probable that this particular influence of the corpora lutea on the mammary gland is not a direct, but an indirect one, the direct action being exerted on the sexual cycle of ovaries and uterus, which act on the mammary gland.

While extirpation of the ovaries prevents the onset of a new sexual cycle and therefore the concomitant proliferation of the mammary gland, extirpation of the corpora lutea hastens both of these processes. Another constituent, namely, in all probability the follicular apparatus or part of it, is responsible for the occurrence of heat, ovulation, and the proliferation of the mammary gland in the early period of the sexual cycle.

Effect of Extirpation of the Uterus on the Mammary Gland.

Loeb has previously observed that extirpation of the uterus seems to have a preserving influence on the corpora lutea.⁵ It is therefore of interest to contrast specially this group of experiments with the preceding group in which the corpora lutea were absent. In four animals the mammary gland had been examined after an extirpation of the uterus, which had been carried out at an early period of the sexual cycle. Examination took place in one case 17, in two cases 18, and in the fourth case 23 days after the preceding ovulation. In two small deciduomata were present at the lower stumps of the uterus. Excision of the uterus had, therefore, not been complete.

⁵ It is intended to investigate further this relationship between extirpation of the uterus and the life of the corpora lutea.

In the remaining cases deciduomata were not found. In all four instances the corpora lutea were well preserved and the mammary gland was proliferating. It is probable that the proliferation of the mammary gland and the preservation of the corpora lutea in these cases stand in a causal relation to each other.

SUMMARY.

1. A definite cycle exists in the mammary gland of the non-pregnant guinea pig which corresponds to the cycle in the ovary and uterus. This cycle can be presented through a curve in which the ordinates represent the degree of activity of the gland in a series of animals, and the abscissæ the time since ovulation (period of sexual cycle). The curve passes through a first maximum at the time of heat and ovulation and gradually falls. The minimum is reached on the 6th day and continues until the 15th day after ovulation. Next begins the period when a new ovulation is imminent and the number of the proliferating glands again increases. We see then that during the normal cycle the presence of well preserved, functioning corpora lutea does not lead to proliferation, neither do mature follicles have such an effect. On the other hand, the absence or degeneration of the corpora lutea is required to insure the proliferation of the mammary gland in the first period of the sexual cycle.

If the sexual period is experimentally prolonged, we find in some instances proliferation, while in others it is absent. As far as we can determine at the present time, two factors seem to favor proliferation of the mammary gland under these conditions: (1) the presence of well preserved corpora lutea, particularly if they are associated with well preserved experimentally produced deciduomata, and (2) the imminence of a new period of heat. The connection between good corpora lutea and good deciduomata and the presence of proliferating mammary glands at this stage of the sexual cycle is, however, not absolute. There are cases in which a proliferating gland is associated with some degeneration of the corpus luteum. Or on the other hand a well preserved corpus luteum is associated with a non-proliferating gland. In some of the latter cases the simultaneous presence of a necrotic deciduoma may perhaps explain the lack of proliferation in the mammary gland. However, in the majority of

cases we found the presence of good corpora lutea and good deciduomata associated with a proliferating mammary gland. Whether a living corpus luteum as such is able to produce proliferation of the gland is as yet doubtful.

2. Extirpation of the ovaries prevents not only the proliferation of the mammary gland associated with the first stage of the sexual cycle, the condition of heat and ovulation no longer taking place in castrated animals, but in all probability also inhibits the proliferation of the mammary gland which occurs under certain conditions towards the end of the sexual cycle, or in instances of experimentally prolonged sexual cycle in which well preserved corpora lutea and deciduomata are present.

3. In animals in which the ovaries were hypotypical, the mammary glands were in an inactive condition. The presence of hypotypical ovaries has the same influence on the mammary gland as castration. In the majority, but not in all of these cases well preserved corpora lutea were absent.

4. Complete extirpation of the corpora lutea seems directly or indirectly to prevent the secondary proliferation of the mammary gland, which occurs during the latter part of the sexual cycle or during an experimentally prolonged cycle, in cases in which the extirpation is not followed at once by a new ovulation. This conclusion we consider, however, merely as suggested, not yet as definitely established through our results. On the other hand, the primary proliferation of the mammary gland, during the first stage of the sexual cycle, as well as ovulation and the objective signs of heat, is accelerated through complete extirpation of the corpora lutea. Thus the effect of extirpation of the corpora lutea differs from the effect of castration, in that after the latter neither a new heat nor the primary proliferation of the mammary gland occurs. As one of the authors has pointed out previously, the absence of functioning corpora lutea and the presence of either well developed ovarian follicles or of mature follicles are necessary for the occurrence of heat and ovulation. The same conditions are prerequisites for the primary proliferation of the mammary gland.

5. In cases in which the whole or almost the whole uterus had been extirpated, the corpora lutea were well preserved and the mammary gland was proliferating.