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Estudios sobre el ovario del espermófilo, con especial mención del cuerpo amarillo.

En el presente trabajo se considera el ciclo de cambios que ocurren anualmente en los ovarios del espermófilo, dando detalladas descripciones histológicas del cuerpo amarillo en cada uno de los estados de su desarrollo. Se han empleado coloraciones específicas para poner de manifiesto los caracteres nucleares y protoplásmicos de las células amarillas. En el ciclo vital del cuerpo amarillo se reconocen tres fases: Primera, una fase que se caracteriza por la presencia de un gran número de gránulos rojos, indudablemente gránulos de secreción, en el protoplasma de las células amarillas. Esta fase comprende, prácticamente, todo el periodo de la preñez. Segunda, la fase lipóide, así, llamada por la abundancia de gotitas de substancia lipóide en el protoplasma de dichas células. Esta fase comienza algún tiempo antes del parto y dura próximamente unas seis semanas después de este, que es también próximamente el tiempo que requiere para completarse la involución normal del útero. Tercera, la fase de regresión. Se dan a conocer también ciertos estudios experimentales, tales como los efectos de la ovariectomía sencilla y doble, practicada sobre animales preñados y no preñados. La ovariectomía sencilla produce resultados negativos. La ovariectomía doble en animales no preñados causa una atrofia funcional del útero, que se manifiesta muy gradualmente. En las hembras preñadas esta operación da lugar a abortos, excepto cuando se practica cuando la preñez está muy avanzada. Como resultado de los estudios histológicos y experimentales, la autora llega a la conclusión de que los cuerpos amarillos producen dos secreciones internas que presiden sobre los cambios que tienen lugar en el útero a consecuencia de la preñez. La primera secreción produce la implantación normal y desarrollo del embrión, y la secreción lipóide ulterior ayuda a la involución normal del útero.

STUDIES ON THE OVARY OF THE SPERMOPHILE (SPERMOPHILUS CITELLUS TRIDECIMLINEATUS) WITH SPECIAL REFERENCE TO THE CORPUS LUTEUM¹

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LITERATURE

The first papers written on the corpus luteum of the ovary had to do largely with its formation, particularly with the origin of the luteal cells. Were these cells connective-tissue elements

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from the internal theca of the follicle according to the hypothesis of von Baer, or were they epithelial in nature and derived from the membrana granulosa of the follicle as advocated by Bischoff? Marshall, in his book, "The Physiology of Reproduction" ('10), carefully reviews this early literature.

It was not until Sobotta ('96) published the first of his series of papers on the corpus luteum that the discussion over the origin of the luteal cells began to come to an end. Sobotta's study on the corpus luteum of the mouse was the first systematic record of the transformation of the follicle into a corpus luteum and the latter's subsequent development. He describes, first, the follicle about to burst; then, one just after bursting; one, one-half hour afterward, and others at succeeding short intervals up to seventy-two hours after bursting. All the descriptions are very detailed, every change in the structure as it proceeds in its development being noted. Sobotta traces the origin of the luteal cells to the epithelial cells of the granulosa of the follicle. The internal theca cells, he says, expend themselves utterly in the formation of connective tissue and blood-vessels. The external theca remains as it was.

Sobotta believed the function of the corpora lutea was to maintain a constant equal tension in the ovaries, which shows how little thought he gave to this phase of the problem. He was all absorbed in the formation of the luteal structure. This paper of Sobotta's was followed shortly by a very similar one on the rabbit, in which he confirmed all of his findings in the mouse. These papers started a real investigation of the corpus luteum, and for the following ten or fifteen years there were any number of papers written regarding it. A few writers continued the discussion as to the origin of the luteal cells. Some criticised Sobotta, trying to disprove his statements. Among these was Clarke ('98), who contributed an account of the formation of the corpus luteum in the sow. His studies were made on serial sections of pig's corpora lutea and follicles in different but not subsequent stages of development. The sections in each set were treated alternately to a process of tryptic digestion and a picro-fuchsin stain. From a study of these sections he concludes that the

luteal cells are of a connective-tissue origin and that the function of the corpus luteum is to preserve the ovarian circulation.

Sobotta ('99) published his third paper on the corpus luteum of mammals. Honore ('00) was one of the first to confirm Sobotta's findings. He wrote concerning the corpus luteum in the rabbit. Marshall ('01) published a paper on the corpus luteum in the sheep, coming to practically the same conclusions as Sobotta. Cohn ('03) further confirmed Sobotta's work on the rabbit. Jankowski ('04) published a paper in which he came to entirely different conclusions. They are as follows: "He (Sobotta) simply lets the internal theca vanish. If one layer must vanish, it would be the one for whom the conditions after the follicle bursting are very unfavorable and that is the case with the epithelium of the follicle. The corpus luteum is not an epithelial but a connective tissue structure." Sobotta ('06) published a fourth paper on the formation of the corpus luteum in the guinea-pig, again confirming his former work.

Such an amount of histologic investigation over the formation of the corpus luteum could not very well go on without arousing much interest in regard to the physiologic function of the structure. The men who upheld the connective-tissue origin held many curious ideas as to its function, all of which tended toward making its action more or less mechanical. Several thought the only function of the luteal structures was to prevent ovulation. Clarke thought its function was to preserve the ovarian circulation.

On the the other hand, those who accepted Sobotta's conclusions began looking for a much more important function for the corpus luteum. If the luteal cells were epithelial and each was so intimately in contact with the blood stream, and the whole organ had so much the appearance of a gland, why couldn't it be a gland of internal secretion? Several histologists began studying the cells for evidences of a secretory product. Regaud and Policard in 1901 were the first to publish any results. They stained sections of ovaries of the dog, that had been fixed in acetic potassium bichromate, with a copper-hematoxylin method of Weigert. They described some black secretion droplets in

the luteal cells. In Cohn's paper previously mentioned, he describes some granules which he thinks may be the same as those described by Regaud and Policard. He also gives his reasons for believing that the fatty osmic-stained droplets, seen in the luteal cells in greatest abundance when the cells reach their maximum hypertrophy, are real secretion droplets and not evidences of fatty degeneration as had been previously contended.

Along with the attempt to discover by a special histologic technic, evidences of secretory products in the luteal cells, a number of men sought to discover, by animal experimentation, proofs of the glandular action of the corpus luteum. The uterus and the mammary glands, in the minds of all, were the most closely associated of all the organs of the body, with the ovaries. Ovulation had been observed to be closely related to menstruation in the human and to the heat periods in animals. Complete double ovariectomy was known to stop menstruation, cause an atrophy of the uterus, and, in young women, to bring on menopause symptoms. In the very young castration had prevented the development of the uterus and mammary glands. Double ovariectomy in pregnant women, especially in the first months, was known to be followed by abortion.

Various attempts had been made to overcome the bad effects of ovariectomy by the transplantation of ovaries and by ovarian medication. These were reported to have given good results, at least partially.

All these facts seemed to speak for the production of an internal secretion in the ovaries which affected the uterus and mammary glands.

Fraenkel ('06) was the first to attempt to prove by a series of experimental studies on rabbits and cats that the corpora lutea in the ovaries were responsible for the various effects produced on these organs.

He begins his papers as follows: "The corpus luteum must, from its structure and development, be a gland of internal secretion, made to insure the implanting and development of the fertilized egg in the uterus. The corpus luteum maintains

the state of nutrition of the uterus during the years of sexual activity." He thought it ruled over the phenomena of rut as well as pregnancy.

About this same time Marshall and Jolly published a series of experiments very similar to Fraenkel's. They used dogs and rabbits, and their conclusions were much like those of Fraenkel.

Daels ('08) published a paper in which he gives his several objections to Fraenkel's theories and records a series of experiments on guinea-pigs. In his first series of experiments he tried to determine the influence of bilateral ovariectomy on the pregnant animal, concluding that this operation in the pregnant animal always interrupts pregnancy during more than the first half of its duration. He also tried giving Fraenkel's lutein tablets, with no results. He had better results from a product of the whole ovary.

Ancel and Bouin ('08-'09) contributed several papers to the literature on the corpus luteum. They believed with Fraenkel that the corpus luteum produced rut and the other changes incident to pregnancy. They performed a series of experiments on rabbits in which they produced an unfertile coitus either between a normal female and a male in which a part of the vas deferens had been resected, and a female in which a part of the uterus had been resected and a normal male. They wished by these experiments to eliminate any action of the egg and the placenta on any changes taking place in the uterus incident to ovulation. They describe structural changes in the uterus and mammary glands for a period equal to the period of activity of the yellow body.

In this same year ('09) two other Frenchmen, Regaud and Dubreuil, published several articles. They were particularly interested in the cause of rut and ovulation. They made a systematic study of a large number of uteri and ovaries in different phases of the genital cycle and concluded that rut is independent of the corpora lutea, and that it is improbable that the corpora lutea plays a rôle in originating the pregestative changes in the uterus, for the graphic curve of their development is much later chronologically than the curve of its changes. They claim that

coitus only will bring about ovulation. The congestive phenomena which they notice in the ovaries during rut will not produce the rupture of a single follicle without coitus.

Niskoubina, the same year ('09), published a series of studies which confirmed the observations of Ancel and Bouin. He first made a histologic study of a series of ovaries removed at varying intervals after coitus and then did some experiments similar to Fraenkel's to determine the period during which the ovaries seem to exert an influence on the pregnant uterus. From his experimental studies, he concludes: "The corpus luteum exercises an obvious action on the physiology of pregnancy. It puts the uterus in a condition necessary to assume the development of the fertilized egg. This action lasts during the first half of pregnancy, after which it ceases to act."

Loeb ('08) published a paper in which he states that deciduomata can be produced experimentally in the uterine mucosa of guinea-pigs by making a number of transverse and longitudinal cuts so as to break the continuity of the tissue. He states that this can happen only during a certain definite period after copulation, between the fourth and the eighth days. This is the time when freshly formed corpora lutea are present in the ovaries. These changes were not excited by the presence of ova, since they took place when the uterus was ligated and the passage of ova prevented. If the ovaries were removed, deciduomata could not be produced. He concluded, then, that the ovaries at certain periods after ovulation elaborate a predisposing substance, in the presence of which indifferent stimuli may produce deciduomata.

Parhon, Dumitresco and Nissipesco ('09) published a paper on the lipoids of the ovary. From various staining reactions on sections of ovaries and from chemical reactions of a powder made from ovaries, they conclude that in the interstitial cells of the ovary and in those of the yellow body fats are found which differ in many characteristics from the fat of the adipose tissue.

Mulon ('10) reported that the fat in the corpus luteum, which stains only faintly with osmic acid, was similar to other fats found in the adrenal and other organs of the body which form a

class of fats different from the ordinary body fat. He thought these so-called glandular fats had to do with the neutralization of the glandular excretions or the ordinary poisons formed in the cellular activity. The specific action of the fat of the corpus luteum was to neutralize the poisonous products formed by the developing embryo.

Miller ('10, '14) published some studies on human corpora lutea. He tried out many fat stains on fresh corpora lutea and claimed they contain no fat. He says the negative result of the fat reaction on fresh corpora lutea makes it possible to tell the difference easily between these and other ovarian structures. When the involution of the yellow body begins, the neutral fat reaction begins. The peripheral parts show the fat reaction first. In the corpus luteum of pregnancy the reaction to neutral fat remains negative to the end of pregnancy. He says that in a corpus luteum of five days the fat reaction was negative, in one of six to eight days there was a little, in one of eleven days more, and in one of twelve to sixteen days the cells were rich in fat (not neutral fat).

Meyer ('11, '13) wrote on the human corpus luteum. In his first paper he described the development and regression of the human corpus luteum of pregnancy, which confirms Sobotta's and Cohn's work.

Van der Stricht ('12) published the results of his studies on the corpora lutea and the interstitial cells of the ovary of the bat. This is one of the most valuable studies ever published on the corpus luteum, because the ovary of the bat, with its contained structures, is one of the simplest ever studied. No confusion arises from old corpora lutea; these are gone before the new ones are formed, as the periods of ovulation are so far apart, occurring each spring only. The author describes two secretions in the luteal cells. The first is a serous secretion which is very like the liquor of the follicle. This is secreted by the cells from the time of bursting until about the time the egg enters the uterine horns. The second is a lipid secretion. Beginning some time after the bursting, there is a slow elaboration of fatty granules from the depth of the cytoplasm of the luteal cells, the amount of which increases as the cells increase in size.

He says: "Far be it from our idea of admitting two absolutely distinct phases for serous secretion and lipid secretion. On the contrary, at a moment in the development of the corpus luteum these two processes coexist, but the first is especially marked at the beginning of gestation and the second exists alone during the following period." He concludes that: "the serous secretion exercises its influence on the transformations of the uterine mucosa of the first phase of gestation during the displacement of the egg and that the lipid secretion intervenes principally to provoke the arrest and fixation of the blastocyst and the formation of the placenta." Van der Stricht has not been able to demonstrate this serous secretion in the luteal cells, but because it is present in the central cavity of the young corpus luteum and in the lymphatics when they are first formed, he thinks the cells must be secreting it. Both the serous secretion and the lipid secretion are carried away, he says, by the lymphatics.

Corner ('16) published a paper on the corpus luteum of pregnancy in swine. He claims to have found in the corpora lutea of pregnancy, beside the cells which are descendants of the granulosa cells and the cells which are descendants of the theca cells, two more types of cells which can be found at all stages of pregnancy.

Livon ('09) published the results of the effect of injecting luteal extract into guinea-pigs. He writes as follows: "We have employed an extract of the corpora lutea of the sow and the cow, a product that I have today called the Product A. Injected into the peritoneal cavity of guinea-pigs, we find a toxic action varying with the rapidity of the absorption and with the individual. The toxic dose obtained generally is 20 to 30 centigrams per kilogram of animal. The animals die presenting general tremors, dyspnea, convulsions and uttering weak cries."

Champy and Gley ('11) are said to have been the first to show that the corpus luteum from pregnant cows was exceedingly active, whereas that from non-pregnant animals possessed little physiologic action.

Hare ('12) reported very favorably on the clinical use of corpus luteum extract.

Frank and Rosenbloom ('15) published the results of some experimental work done on rabbits with extracts of the placenta and the corpus luteum. They claim to have gotten better results than former investigators because they used a more concentrated and an alcoholic (fat solvent) solution of the active substance of the luteal extract. This active substance, they say, "is not a lipoid but is carried along the lipoids." They state that the only corpus luteum substance extracted, which was found to be active, was derived from pregnant animals.

Dannreuther ('14) reported his results obtained clinically from the use of corpus luteum extract. He calls attention to the necessity of using the extract of pregnant animals only.

Up to date, the most noteworthy publication concerning the clinical value of the extract of the corpus luteum is that of Culbertson ('16), entitled, "A study of the menopause." He regards the climacteric as a "functional disarrangement on the part of the endocrine glands, the ovarian secretion having ceased." His theory concerning the value of luteal extract in the treatment of menopause disorders is as follows:

"Thus the chief characteristic stamping the vasomotor disturbances of the climacteric seems to be a disarrangement of the systolic-diastolic relation producing elevation in the pulse pressure. In blood-pressure estimations, then, we find a fairly reliable measure of the vasomotor disturbances of the menopause, as will be shown, a satisfactory method of treatment.

"If we accept the propositions thus far laid down, that the cessation of ovarian activity leads to a functional over-efficiency on the part of the pituitary and adrenal glands and that this, in turn, produces an arterial hypertension, the corollary is that by the administration of corpus luteum extract, the pressor substances will be neutralized and the tension will decrease."

In summing up, it may be stated that up to 1906 practically all the literature on corpus luteum had to do with the histologic origin and consequent structure at various succeeding periods in its life cycle. The writings of Sobotta and Cohn practically established the epithelial nature of the luteal cells and the glandular character of the structure as a whole. From 1906 to

1912 most of the papers written were reports of experimental studies undertaken to prove that the corpus luteum is the gland of internal secretion in the ovaries, and that, through this secretion, the luteal structure produces specific effects on other organs, particularly the uterus. In spite of the many criticisms directed against his work, Fraenkel still stands preëminent among the experimental workers who established beyond a doubt the foregoing hypothesis.

Since 1910, efforts have been put forth by investigators actually to demonstrate this secretion in the corpus luteum. Van der Stricht comes nearer the goal than any others. The greater part of recent literature, however, concerns the extract of the corpus luteum, its chemical constituency, its physiologic action, and its clinical value.

THE OVARIAN CYCLE

In the summer of 1914, while studying microscopic sections of the various tissues and organs of the spermophile, the relatively immense size of the ovaries, compared with those observed the previous spring, strikingly presented itself. On further comparison, it was very evident that this great increase in size had been brought about by a growth in the corpora lutea only. One ovary contained eleven of these bodies; there remained only a framework of ovarian stroma with a few atretic follicles.

With the particular stain the luteal cells bore a marked resemblance to the cells of the cortex of the adrenal. The former were much larger, but the shape of both, their arrangement in columns, the position and appearance of the nuclei, and the presence of lipid droplets in the protoplasm accounted for the likenesses. In fact, the luteal cells resemble secreting cells.

From observations that had been going on, it was known that these spermophiles had given birth to young about a month before. According to most writers, degeneration of the luteal cells begins not later than birth. Here were what looked like actively secreting cells a month after birth. Thereupon it was decided to try out some differential stains on these luteal cells at every stage in their life history, and to study the complete

ovarian cycle in the spermophile with a view to gaining some accurate information of the origin, development, life history, and function of the corpora lutea of the ovary.

In the spring of 1915, numbers of spermophiles were captured. But not having realized how very soon the animals become impregnated after coming out of hibernation, no strenuous efforts were made to obtain them until they were quite numerous in the fields, and consequently they were found to be either in advanced stages of pregnancy or lactating. However, all the animals that could be gotten were used for a study of the ovarian cycle through the summer and fall, until hibernation began.

Several animals were sacrificed each week. They were killed quickly with ether and bleeding. The ovaries were immediately placed in one of several fixatives, 10 per cent formalin, Zenker's fluid with acetic acid, Bensley's formalin Zenker, and Bensley's acetic acid bichromate. Many stains were experimented with. After considerable study of the fixed and stained sections, it was decided that for the problem in hand, two fixatives seemed best, Bensley's formalin Zenker and Bensley's acetic osmic bichromate—the former particularly for the nuclear structures and the latter for the elements in the protoplasm. In all the work of the past spring ('16), one ovary of each animal sacrificed was routinely placed in formalin Zenker and the other in acetic osmic bichromate.

Of the sections fixed in zenker, the best results were obtained with a modified Weigert stain (copper-chrome-hematoxylin), Ehrlich's hematoxylin and eosin, Mallory's connective-tissue stain, and Bensley's acid fuchsin and methyl green. A few sections of each series were prepared with these stains.

Of the sections fixed in acetic osmic bichromate, one of each series was stained with the Weigart stain and several with the acid fuchsin and methyl green of Bensley. Complete paraffin serial sections were made of all the ovaries studied.

The spermophiles went into hibernation about the middle of October although many of them became partially torpid earlier than this. The next spring it was determined to get them early enough. The frost was not out of the ground until about

April 15. As a result of strenuous efforts, the females were obtained while in rut and every day through the period of pregnancy, which was found to be twenty-eight days. Two or more were sacrificed each day.

The ovarian cycle was now completed. Ovaries were at hand for every week of the year except during the hibernation period when only a sufficient number of animals was sacrificed to make sure there were no changes taking place in these organs. For the period of pregnancy, a time which is especially related to ovarian activity, there were ovaries for even fractions of a day.

The period of rut evidently follows immediately on the awakening of the spermophiles in the spring. Ovulation follows on coitus. Most of the females are impregnated in a very short time. The period of pregnancy follows. From the time of ovulation until about September 1 the ovaries contain corpora lutea. These approach their greatest size about July 1. From September 1 to 15 the large corpora lutea have disappeared. With a disappearance of the corpora lutea, there is a very noticeable rapid growth of the follicles together with a noticeable decrease in size. The ovaries which in July consisted almost entirely of large corpora lutea with a small amount of ovarian stroma containing a few atretic follicles, by September 15 contained no corpora lutea, but instead, many medium and good-sized growing follicles containing very little liquor folliculi, but filled with mitotic figures.

In this paper descriptions will be presented, gross and microscopic, of the ovaries which, as the year goes around, show the characteristic changes of their cycle.

A typical ovary of the early fall will be presented first; second, one of the late fall just before hibernation, with its inactivity, and, third, one of the early spring, showing the characteristics of the rutting season. For the fourth period, the period of pregnancy, several ovaries will be described, marking the successive changes occurring in the corpora lutea principally. Finally, will be presented descriptions of a number of ovaries of the summer months, which show the final stages in the life history of the corpora lutea and the corresponding notable differences in the rest of the ovary.

Experiment 379-15 (spermophile 200). Captured during the spring of 1915. The ovaries were removed surgically September 14, 1915. Weight, 150 grams.

Microscopic observations of an ovary. Fixative, formalin zenker. Stain, hematoxylin and eosin (fig. 10). There are nine good-sized follicles, the larger of which measure about 0.4×0.4 mm. There are twice as many follicles half as large. There is a goodly number of small hyalinized follicles. Around the periphery of the ovary are numbers of primordial ova. The larger follicles are approaching maturity, but are still growing. They contain many mitotic figures and very little liquor folliculi. They are located through the cortex of the ovary, only three being near the surface. None of these larger follicles appear to be atretic. Through the medullary portion of the ovary are conspicuous clumps of interstitial cells.

The spermophiles begin to become torpid about September 15, but they are active by spells for some time after this, depending on weather conditions. In the laboratory, some are active until November 15.

Experiment 503-15 (spermophile 254). Captured during the spring of 1915. Sacrificed November 15, 1915. The animal had been hibernating six days.

Microscopic observations of an ovary. Fixative, formalin zenker. Stain, hematoxylin and eosin. This ovary appears very similar to that of spermophile 200. The larger follicles are about the same in number, size, and location. There are about the same number of smaller and hyalinized follicles as well as primordial ova. The size of the ovaries as a whole, however, has decreased. This is probably due to a marked decrease in the size of the blood-vessels and sinuses. The clumps of interstitial cells are much less conspicuous. The ovaries appear to have prepared themselves for their long period of functional inactivity.

Experiment 246-16 (spermophile 291). Captured April 25, 1916. Was injured in being caught, so was sacrificed immediately. Weight, 170 grams.

Gross observations of the uterus. The animal is in rut. The rutting season evidently lasts about two weeks. During this time practically all the females become impregnated. It may be stated here that the laboratory animals come out of hibernation much earlier. Those killed from the 1st to the 15th of March appeared to be in rut and one killed April 11 was found to be pregnant, showing that weather conditions set the time of the commencement of their sexual activities. The large size of the uterus is immediately noted (fig. 7). There has been an increase in length and breadth. It is twice as large as the inactive uterus. The cervix shows the greatest increase in size and it is filled with a thick mucoid substance. The walls of the vagina are swollen. It is very evident that the great increase in the size of the whole organ is due to a marked increase in the fluid content, which gives it a pale appearance. (For measurements see table.)

Gross observations of the ovaries. The ovaries of rut show several changes from those of the fall. They are larger and seem to be slightly congested. On the surface of each ovary may be seen several slightly raised, tiny, colorless, cyst-like bodies which are the mature follicles.

Microscopic observations of the ovaries. Fixative, formalin zenker. Stain, hematoxylin and eosin. Serial sections show ten larger follicles, all of which lie at the surface of the ovary and several of which are projecting slightly from the surface. One of these follicles measures 0.5 mm. x 0.4 mm., and another 0.5 mm. x 0.6 mm. None of them shows atretic changes and all are apparently mature. There is considerable liquor folliculi, and few mitotic figures among the granulosa cells (fig. 12).

The internal and external theca of these large follicles are distinct and comparatively thick layers. There is a well-marked membrane between the stratum granulosum and the internal theca. Acetic osmic bichromate sections stained with acid fuchsin and methyl green show a red secretion along this membrane and between the cells of the granulosum near it. The cells of the granulosum seem to have an increased amount of protoplasm which makes them larger.

Of the smaller follicles, a few are in good condition, but the greater number have become hyalinized. The hyalinization of so many of the smaller follicles gives a characteristic appearance to the ovaries of the spring (fig. 11). Indeed, it would seem that a few mature follicles have been produced at the expense of many. The primordial ova are few in number. The interstitial cells are not at all conspicuous.

Toward the end of the rutting season many uteri present a different picture. They are smaller and instead of appearing edematous appear congested.

Experiment 256-16 (spermophile 298). Date of capture April 27, 1916. Sacrificed April 28, 1916. Weight, 105 grams. (For measurements of uterus see table.)

Gross observations of the uterus. The blood-vessels to the uterus are all much congested and the organ itself shows some congestion throughout. There is one especially congested area in each horn about 1 cm. from their point of union. There is another specially congested area in the body near the point of union of the horns.

As there are no corpora lutea in the ovaries of this spermophile, the uterus is still one of rut, not pregnancy. What brings about this change in the uterus is not evident. The congested condition is, however, without doubt preparatory to the reception of the fertilized ova.

This brings us in the life cycle to the ovaries of pregnancy. It has been shown that the ovaries of the fall, winter, and early spring contain no corpora lutea. Occasionally some remains of these bodies of the previous year may be found, but this is very unusual. Thus when coitus takes place during rut and the

follicles burst and become transformed into corpora lutea, these new bodies are the only corpora lutea in the ovary. They are all produced simultaneously and they also develop simultaneously if they are not abnormal in some way.

The picture of the ovary changes, then, when fertilization brings on pregnancy. From this time on until the period of the growing follicles is reached in the late summer, the ovaries contain corpora lutea. These are the predominating structures in the ovaries of the spring and summer. The changes which take place in these organs during the period have to do with the corpora lutea principally. The following descriptions of ovaries will be attempts at picturing them with corpora lutea of various ages. As this study is very largely concerned with these luteal structures, they will be described in considerable detail. Probably the ovaries which contained the very youngest corpora lutea seen were those in which ovulation took place in the laboratory, the animals being sacrificed very shortly afterward.

Experiment 268-13 (spermophile 303). Captured May 3, 1916. Sacrificed the same day. Weight, 112 grams.

Gross observations. There are no visible signs of pregnancy in the uterus except congestion. The ovaries contain several slightly raised, small spherical bodies which resemble mature follicles except that they are red or pink instead of colorless.

Microscopic observations of the left ovary. Fixative, acetic osmic bichromate. Stain, acid fuchsin and methyl green. This ovary contains five luteal bodies, three of which are normal and two of which are not. Beside these, there are several growing medium-sized follicles, no large ones, quite a number of small atretic follicles and a few primordial ova. The interstitial cells are not as conspicuous in this ovary as they were in some of the ovaries of early pregnancy. All the blood-vessels and sinuses in the medullary portion of the ovary are very much dilated. Most of the ovary is made up of the five corpora lutea. Serial sections of the three normal ones show them to be of different sizes from 0.7 mm. x 0.8 mm. in diameter to 1.3 mm. x 0.1 mm., depending on the amount of blood which they contain, for practically all the young corpora lutea contain blood in their centers (figs. 13 and 17). A hemorrhage from a blood-vessel in the wall of the follicle must occur as the follicle bursts. The exact point of this bursting cannot be made sure of in any of these structures. Each luteal body is surrounded by a very thin connective-tissue capsule, no doubt the same theca externa which surrounded the follicle. From this thin capsule to the central core of blood are massed the luteal cells. They

seem to have no definite arrangement. Among them may be seen numerous fibroblasts and endothelial cells. These are most numerous about the periphery of the central mass of blood. Some are already making their way into it. The cells all seem to have their axes directed radially as if they were approaching the central mass of blood from the periphery of the luteal body. The luteal cells are of various sizes and shapes. Some are spindle-form and some polygonal, but the majority are spherical or oval. There is one specific characteristic of all young luteal cells and that is the existence of spherical granules in their protoplasm. In the sections fixed in Bensley's acetic osmic bichromate and stained with acid fuchsin and methyl green, these granules are strikingly brought out (fig. 26). They are colored a brilliant red. Their sizes vary somewhat, but they are all spherical. The protoplasm of some cells is so full of these granules that it resembles a homogeneous red secretion, but on examination with very high power, the separate granules may be seen. In many cells where the granules do not fill the protoplasm, they are grouped about the nucleus, leaving a narrow clear zone about the periphery of the cell. The nuclei take the green stain and are strikingly brought out against the red granules. Each nucleus contains one or two good-sized bright, red staining nucleoli. The chromatin threads stain green and do not show very well with this stain. For the nuclear characteristics, another ovary stained differently will be described.

The two abnormal luteal structures of this ovary are very interesting. One of them is a luteal cyst (fig. 23). There is only a single layer of luteal cells next to the thin capsule. No fibroblasts or endothelial cells are present. The whole body is filled with a transparent greenish-colored fluid which has every appearance of being of the same composition as the liquor folliculi.

The other structure (fig. 21) is much more normal. The center contains what resembles the above green-tinged fluid containing numerous red blood corpuscles. Masses of normal luteal cells are found most of the way around the body. But on one side is a mass of follicle or granulosa cells persisting untransformed. In several places, as in figure 22, some granulosa cells are found among the luteal cells. This peculiar luteal structure appears to give striking evidence to the theory that luteal cells are simply transformed granulosa cells. The differences between them are well brought out in the picture.

It may be well to state here that other fixatives and stains bring out these same specific luteal-cell characteristics, particularly the granules in the protoplasm. With a formalin zenker fixative and the acid fuchsin and methyl green stain, the granules appear the same in every way except in color. They are pink instead of red. With this same fixative and a copper-chrome hematoxylin stain, the granules appear brownish-black. With an acetic osmic bichromate fixative and the copper-chrome hematoxylin stain, the granules appear bluish-black. The nuclear characteristics of the early luteal cells are best brought out with a formalin zenker fixative and a hematoxylin and eosin stain.

Experiment 275-16 (spermophile 310). Captured May 1, 1916. One ovary was removed and the uterus ligated May 4, 1916.

Gross observations. There are no recognizable signs of pregnancy in the uterus, but the ovaries contain what resemble young corpora lutea.

Microscopic observations of the ovary removed. Fixative, formalin zenker. Stain, hematoxylin and eosin (fig. 13).

The nuclei of these early luteal cells are strikingly like those of the follicle cells. The nuclei of the latter have several small nucleoli with quite conspicuous chromatin strands. The nuclei of most of these early luteal cells also have several small nucleoli with numerous chromatin strands. But in some cells, the nucleus is much larger and contains only one or two larger and darker nucleoli, while the chromatin strands are finer and fewer.

A very few of the luteal cells in the early luteal structures show mitotic figures. There were none at all in those of spermophile 303. Of all the early corpora lutea studied, mitotic figures were found in these structures in the ovaries of only three spermophiles. These were apparently the earliest luteal bodies found. If mitosis occurs, as a rule it occurs just after the bursting of the follicle. It may be that the luteal cells which show mitotic figures are the transformed follicle cells undergoing mitosis as the bursting occurred.

There is one abnormal early luteal structure in the ovary (spermophile 310) which deserves mention, as it seems quite common and furnishes further proof that the luteal cells are simply transformed granulosa cells (fig. 24). The structure with this staining appears at first glance like a mature follicle. The liquor folliculi is present and the ovum lies over at one side of the central cavity against the surrounding cells, which resemble the granulosa cells of the follicle. On closer observation, however, it will be seen that the cells which were thought to be the epithelial cells of the follicle are larger, richer in protoplasm, and more irregular in shape and size. Scattered through them are numerous fibroblasts and endothelial cells. These fibroblasts are quite numerous about the ovum, as if they were attempting to wall it off. The ovum has been stripped of its own rim of granulosa cells and appears to be undergoing degeneration. The internal theca is missing. In fact, what we have here is a corpus luteum formed in a follicle which, if it burst, did not throw out enough of its contents to get rid of the ovum. Practically all of the epithelial cells must then have been retained. Where are they if they are not the luteal cells? The only missing cells are those of the internal theca and the only new cells are the fibroblasts and endothelial cells. Does it not appear reasonable that the internal theca cells which are of the same origin as the connective-tissue cells expend themselves in the formation of the new fibroblasts and endothelial cells? It is hard to account for these abnormal structures. Several of them showed blood in the central cavity, as if the normal hemorrhage had occurred into them. Possibly they are formed in the mature follicles that are ready to burst

and undergo the same changes incident to this phenomenon as the others except that, on account of not occupying a position close enough to the surface of the ovary, they are not able to discharge their contents.

Experiment 293-16 (spermophile 328). Captured May 4, 1916. Both ovaries were removed May 6, 1916. Weight, 119 grams.

Gross observations. The fetuses in the uterus measure about 2 mm. in length, which makes the luteal bodies in the ovaries older than those previously described.

Microscopic observations of the right ovary. Fixative, formalin zenker. Stain, acid fuchsin and methyl green. This ovary contained six corpora lutea. These luteal bodies appear differently, due principally to the rapid growth which has been going on among the fibroblasts and endothelial cells. These ovaries suffered some congestion through the manipulation of removal, and this helps to show the great numbers of capillaries and blood-vessels that have been formed in a short time. The central mass of blood is undergoing rapid organization. No doubt the presence of this blood with its serum and fibrin is the great attractive force which aids in the complete formation of the luteal body. Fibroblasts and endothelial cells are always attracted by serum and fibrin. As soon as the hemorrhage occurs in the follicle, they start in to organize it. This is evident from the radial direction which the axis of the fibroblasts all take very early. As they go into the center, the transformed epithelial cells of the follicle are carried in by them. Endothelial cells grow in, and so very early there is formed in the corpus luteum a complex system of blood-vessels and capillaries, as is seen in sections of this ovary.

In one of the corpora lutea in this ovary, the hemorrhage was so extensive that instead of trying to organize it, the fibroblasts have formed a wall around it. Since the fibroblasts have not penetrated very far, there is only a narrow rim of luteal cells. This structure is what is ordinarily called a hemorrhagic luteal cyst (fig. 25). Beside the corpora lutea in a section of this ovary, one notices readily the large clumps of interstitial cells through the medullary portion. These are, as a rule, conspicuous in the ovary of early pregnancy.

Microscopic observations of the left ovary. Fixative, formalin zenker. Stain, hematoxylin and eosin. This ovary contains only two corpora lutea. There are present in it several large, apparently mature follicles. The number of such follicles in an ovary evidently depends on the number of corpora lutea. Where there are a good many of the latter, the follicles evidently cannot grow. When there are only a few corpora lutea in an ovary, one or two follicles may reach the size of 0.5 mm. x 0.5 mm., or 0.5 mm. x 0.7 mm. These will, of course, degenerate as ovulation takes place only once a year, during the rutting season which has just gone by. And as there are practically no large follicles ever seen in the ovaries of July which contain the largest luteal bodies, they must degenerate before this time. Perhaps the pressure of even one large, growing corpus luteum is enough to bring this about.

Experiment 264-16 (spermophile 302). Captured May 1, 1916, and sacrificed the same day.

Gross observations. This animal is definitely pregnant, the fetuses measuring 7 mm. in length. The blood-vessels going to the uterus and the ovary are very much congested. They stand out sharply, showing plainly the blood supply to the two organs. The blood supply to the ovary is practically separate from that to the uterus, there being only one small anastomosing branch close to the ovary. There are what look like corpora lutea in the ovaries, but they cannot be counted with any certainty. They resemble little reddish-pink cysts sticking out from the surface of the ovary. Naturally, from the size of the fetuses, the corpora lutea in these ovaries are older than those of spermophile 328.

Microscopic observations of the left ovary. Fixative, acetic osmic bichromate. Stain, acid fuchsin and methyl green. There are four luteal structures in this ovary. They show some changes over the younger ones previously described. They are slightly larger, measuring about 0.7 mm. x 0.8 mm. The luteal cells have become larger and more regular in shape. Many more have assumed an oval form and all seem to be approaching this. They seem to be tending toward a radial arrangement also. This is being effected evidently by the arrangement of the connective-tissue strands. The latter are running from the capsule to the central core of blood, which is almost organized. The system of capillaries and blood-vessels is even more complex than that of the luteal body of spermophile 328. In the luteal cells themselves the red granules have increased in number and vary slightly in size. They occupy the same position in the cell. The nuclei of all the cells are larger and contain one or two large bright nucleoli with numbers of very fine chromatin strands.

Experiment 296-16 (spermophile 331). Captured and sacrificed May 6, 1916. Weight, 146 grams.

Gross observations. The fetuses in the uterus are 1 cm. in length, which lead us to expect to find changes in the corpora lutea of the ovaries.

Microscopic observations of the left ovary. Fixative, acetic osmic bichromate. Stain, acid fuchsin and methyl green. The size of the luteal structures has increased. There are two in this ovary, one measuring 0.9 mm. x 0.9 mm., and the other, 1 cm. x 0.8 mm. The most noticeable feature of this later luteal body is the absence of any blood in the center. Instead, there is a core of connective tissue. The size of this connective-tissue core depends evidently on the amount of hemorrhagic material there is to organize. In some bodies the core is much larger than in others. Very perceptible strands of connective tissue run from this central mass to the capsule, enclosing columns of luteal cells. Gross strands have developed also so that connective-tissue strands seem to be enveloping each cell. There is a complete capillary network following the arrangement of the strands of connective tissue. Good-sized blood-vessels are located about the periphery

of the structure. Several smaller ones are present in the central connective-tissue core. There are several sinuses about the periphery lined with endothelium which appear to contain lymph. The lutea cells themselves have increased in size. Many more have taken on an oval shape and are lying with their long axes perpendicular to the capsule. The same red granules are still present in the protoplasm. These do not seem to be quite as numerous in the cells, which fact is in part due, no doubt, to the increased size of the latter. The granules are now found scattered throughout the protoplasm, the clear zone about the periphery of the cell having disappeared. The granules show more difference in size than formerly, but they are still all spherical. The nuclei have not changed. In a few cells there is a clear space in the protoplasm on one side of the nucleus.

Microscopic observations of the right ovary. Fixative, formalin zenker. Stain, hematoxylin and eosin. There are seven corpora lutea which make this ovary larger than the other one. Beside these bodies, this ovary contains approximately five good-sized, growing follicles about 0.4 mm. x 0.4 mm. Four follicles nearly the same size are undergoing atretic changes and there are about twenty small follicles, some of which are degenerating. Around the edge of the ovary are a few primordial ova. Through the medullary portion are some small groups of interstitial cells. They are not nearly as conspicuous as they were earlier in pregnancy (fig. 14).

Experiment 355-16 (spermophile 375). Captured May 20, 1916. Both ovaries were removed May 22, 1916. Weight, 128 grams.

Gross observations. The fetuses in the uterus are 1.5 cm. in length.

Microscopic observations of the right ovary. Fixative, acetic osmic bichromate. Stain, acid fuchsin and methyl green. There are six corpora lutea in the right ovary, measuring about 0.8 mm. x 0.9 mm. in diameter. Something is noted in the luteal structures in this ovary which has not been seen before, namely, that there are present in the cells close to the periphery some osmic-stained droplets. The size of the corpus luteum and the size of the individual cells are about the same as that of spermophile 331. There is a slight increase in the number of red granules in the cells. The clear space next to the nucleus is present in many more cells. The osmic-stained droplets are located at the periphery of the cell. They are very large compared with the red granules. They vary somewhat in size, but not in shape; all are spherical. Of course, in sections fixed with formalin zenker and stained with the various stains which were used, these lipid droplets appeared as vacuoles. But they could be easily recognized by their corresponding size and location in the cells. For convenience, these droplets will be called lipid droplets, because they certainly are a lipid product. They do not appear in the luteal cells before the fetus is about 1.5 cm. in length or about fourteen days old, that is, until the period of pregnancy is half over. These droplets make up the 'lutein' of the corpus luteum which has been described for many years and which has given the corpus luteum its name.

When the lipoid droplets appear in the cells, the period of the red granules is waning. The latter seem to reach their crisis of abundance when the fetus measures about 8 mm. in length. But the granules are still very abundant in the cells until the lipoid droplets begin to appear. From this time on, the former grow fewer and fewer and the latter increase in number, as will be shown, until they, too, reach a crisis of abundance and then decline.

Experiment 363-16 (spermophile 383). Captured and sacrificed May 24, 1916. Weight 140 grams.

Gross observations. The animal was in labor when killed. The uterus still contains two live fetuses, four having already been born. The crown-rump measurement of a fetus is from 4 to 5 cm.

Microscopic observations of the left ovary. Fixative, acetic osmic bichromate. Stain, acid fuchsin and methyl green. The luteal structures in the ovaries of this animal show some changes over those previously described (fig. 18). There are four of them. They have increased slightly in size, measuring 0.9 mm. x 0.9 mm. and the individual cells have increased correspondingly. Still more noticeable than their increase in size is the regularity of their oval form and the uniformity with which all their axes point in a radial direction. This seems to be due to an increased amount of connective-tissue framework, which, from the first, has seemed to govern the position and shape of the cells. The increase of connective-tissue framework has been accompanied by an increase in the size of the blood-vessels and capillaries. In the luteal cells (fig. 27) the red granules have decreased still more than in the cells last described, and this is very general throughout the structure. The lipoid droplets are much more numerous in all the cells. In fact, it seems hard to tell which is the predominant product of the cells, the red granules or the black droplets. The nuclei of these cells are slightly larger than the ones of the preceding description. Otherwise, they are the same. Most writers have agreed that degeneration of the corpus luteum begins about the time of birth. None is in evidence here. Several writers have stated that the principal reason for their belief was the entrance into the cells of osmic staining droplets which they considered to be evidences of fatty degeneration in the cells. It would not seem from the nuclear characteristics of the cell nor from the color, shape, and the regular size of the droplets, that they could be fatty degeneration products, especially when compared with the true fatty degeneration which occurs much later in the life history of the corpus luteum and which will be described accordingly.

Microscopic observations of the right ovary. Fixative, formalin zenker. Stain, hematoxylin and eosin. There are two corpora lutea in this ovary. A striking feature is the number of good-sized atretic follicles. There are at least twelve. This seems to be a noticeable feature of other ovaries about this same time. In fact, the follicles which were growing when ovulation took place, evidently go on and develop if there are not too many corpora lutea in the ovary, but as

the latter structures begin to increase in size quite rapidly about the time of parturition, there seems to be a degeneration of all the mature follicles even where there are only one or two luteal structures present. There are five medium-sized and about twenty small growing normal follicles, only a few of which show atretic changes. The primordial ova are very few in number, and the interstitial cells can scarcely be distinguished from the connective-tissue cells of the stroma.

Experiment 368-16 (spermophile 387). Captured May 24, 1916. Sacrificed May 25, 1916, twelve hours after having given birth to young. Weight, 115 grams.

Microscopic observations of the left ovary. Fixative, acetic osmic bichromate. Stain, acid fuchsin and methyl green. The three luteal structures in the left ovary of the animal measure either 0.9 mm. x 1.1 cm. or 0.9 mm. x 1 cm. The appearance of the luteal structure as a whole and of the individual cells is very similar to that of spermophile 383.

Experiment 369-16 (spermophile 388). Captured May 24, 1916, and sacrificed May 26, 1916, twenty-four hours after having given birth to young. Weight, 115 grams.

Microscopic observations of the left ovary. Fixative, acetic osmic bichromate. Stain, acid fuchsin and methyl green. The corpora lutea of the ovaries of this animal measure 0.8 mm. x 0.9 mm. There is scarcely any new noticeable difference unless it is a slight increase in the number of lipoid droplets.

Experiment 370-16 (spermophile 389). Captured May 24, 1916. It gave birth to young on May 25, 1916. It was with the young until May 26, 1916, when it killed them. The next day, May 27, 1916, the animal was sacrificed. Weight, 135 grams.

Microscopic observations of the left ovary. Fixative, acetic osmic bichromate. Stain, acid fuchsin and methyl green. The corpora lutea in this ovary show a marked increase in the amount of lipoid in the luteal cells. The red granules have correspondingly decreased in number. There are no other differences except a slight increase in size of the luteal structure and the cells.

Experiment 372-16 (spermophile 391). Captured May 18, 1916, and gave birth to normal young May 22, 1916. The young were destroyed May 26, and the animal was sacrificed May 31, 1916.

Microscopic observations of the left ovary. Fixative, acetic osmic bichromate. Stain, acid fuchsin and methyl green. The corpora lutea in the ovaries show the lipoid content to be still more increased in amount and the red granules to be very scarce. There are no other differences. It might be stated here that there is some variation in the time when this lipoid change comes on. For instance, the corpora lutea of the ovaries of spermophile 390, ten days after parturition, do not contain as much lipoid as those of spermophile 391, nine days after parturition. But the majority of luteal bodies for any given time before or after parturition appear very similar.

Experiment 351-16 (spermophile 371). Captured May 20, 1916. The left ovary was removed on May 22, 1916. Weight, 135.5 grams.

Gross observations. The fetuses in the uterus are 2 cm. in length. This animal gave birth to young May 31, 1916, and was sacrificed June 17, 1916.

Microscopic observations of the right ovary. Fixative, acetic osmic bichromate. Stain, acid fuchsin and methyl green. The individual luteal cells are of about the same size and shape. The red granules are still quite abundant, but there seems to be a marked difference in the cells as to their individual content of red granules. This appears to vary with the number of lipid droplets in the cell. In a few cells where the latter are very abundant, the red granules are found only in a rim about the periphery. Where the lipid droplets are still few the cell protoplasm still contains many red granules with these few droplets scattered among them or occupying a peripheral position. Many cells have not changed at all; they have no lipid droplets. Some cells have a peculiar appearance. Their protoplasm appears honeycombed. Since none of the cells had this appearance in the unstained sections, it was concluded that the cells must have been filled with lipid which was dissolved out in the staining process and the result was this honey-combed appearance to the protoplasm. This was later proved to be the case. The lipid droplets, when they are numerous, show much less variance in size than when they are few in number in the cell. The nuclei of these cells appear very similar to those of earlier cells. In some cells there is a slight change in their position. Instead of being directly in the center, in the cells filled with lipid, the nucleus lies a little to one side of the center. The 1st of July, about thirty-five days after parturition, the corpora lutea in the ovaries were larger than at any other time.

Experiment 412-16 (spermophile 415). Captured and sacrificed July 1, 1916. Weight, 125 grams.

Gross observations. The ovaries are the largest yet seen, owing to the comparatively immense size of the corpora lutea. These are now of a yellowish-cream color and stand out prominently so that the ovary looks as if it were made up of several spherical bodies 1.5 mm. in diameter. Any other ovarian tissue except that holding the spherical bodies together can scarcely be distinguished. There are three corpora lutea in the right ovary and four in the left, which numbers correspond to the tiny white spots marking the former placental sites in the uterus.

Experiment 413-16 (spermophile 416). Captured and sacrificed on July 3, 1916. Weight, 157.3 grams.

Gross observations. The right ovary appeared grossly just like that of spermophile 415 (fig. 15).

Microscopic observations of the left ovary. Fixative, acetic osmic bichromate. Stain, acid fuchsin and methyl green. The corpora lutea are much larger than those previously described (fig. 19). They measure 1.1 mm. x 1.5 mm. in diameter (1.7 mm. x 1.3 mm., grossly some shrinkage). The luteal cells are correspondingly larger, and their

protoplasm is absolutely full of a mass of lipoid droplets. This is seen to advantage in the unstained sections. In the stained sections the cells appear more or less honeycombed, according to the amount of lipoid which has been dissolved out. These lipoid droplets are very uniform in size and are dark brown, quite a different color from the black fat droplets of fatty degeneration. The red granules are gone. Where there is any protoplasm visible, it appears granular and pinkish-gray in color (fig. 28). The nuclei of the cells appear slightly smaller than formerly, but this apparent decrease in size is evidently due to the increase in the size of the cells, for the nuclei are no smaller by measurement. The location of the nucleus in the cell is the same as described under spermophile 371, either in the center or to one side of the center. The nucleolus stands out large and bright and the chromatin strands appear as they did. There are no apparent degenerative changes. Beside the three corpora lutea in this ovary, there are six or seven medium-sized normal growing and three atretic follicles. There are no large follicles or even any of good size. A few primordial ova are present, fewer than in any of the ovaries described thus far, and no interstitial cells can be distinguished as such (fig. 15).

Experiment 439-16 (spermophile 436). Captured June 23, 1916. Sacrificed July 15, 1916. Weight, 194.2 grams.

Gross observations. The uterus still shows several tiny white spots marking the placental sites. It is otherwise normal. The ovaries appear to contain corpora lutea, but these latter are certainly much reduced in size over those of spermophile 416. They appear congested or of a reddish-yellow color.

Microscopic observations of the left ovary. Fixative, acetic osmic bichromate. Stain, acid fuchsin and methyl green. The corpora lutea are much smaller (fig. 20). They measure 0.7 mm. x 0.8 mm. and 0.9 mm. x 0.9 mm. The cells are smaller. The protoplasm of the cells contains no red granules and very few lipoid droplets or any honeycombing suggestive of these. It has a grayish, granular appearance. Something is present, however, which has not been seen before, and that is fat. Scattered here and there throughout the luteal structure, fat globules, characteristic of fatty degeneration, are present in the protoplasm of the cells. They are of various sizes and take on a characteristic black color with the osmic acid in the acetic osmic bichromate fixative. The nuclei of the cells show degenerating changes. The nucleolus has disappeared in some cells and in others appears pale and fringed. The chromatin strands are fewer and appear clumped in some cells. One striking new feature in the luteal structure is the great increase in the size of the blood-vessels and capillaries. The congestion of blood is not common to the whole ovary, but is only in the corpus luteum. The vascular change is apparently one factor in the disappearance of the luteal body.

Experiment 515-16 (spermophile 458). Captured August 18, 1916. Sacrificed August 21, 1916. Weight, 190 grams.

Gross observations. There are no evidences of placental sites in the uterus. There are no signs of corpora lutea in the ovaries.

Microscopic observations of the left ovary. Fixative, acetic osmic bichromate. Stain, acid fuchsin and methyl green. The left ovary shows three luteal structures (fig. 16). The largest measures 0.8 mm. x 0.8 mm. No red granules or lipid droplets are discernible. The greenish-gray granular protoplasm is everywhere filled with various sized fat droplets. In some cells these fat droplets are so large that they occupy nearly the whole cell, squeezing the degenerated nucleus out to one side of the cell (fig. 29). The nuclei are so degenerated, no chromatin strands or nucleolus are recognizable as such. What is left of the nucleus takes the acid fuchsin rather than the methyl green stain—so it is red. All the blood-vessels and capillaries are markedly dilated. There is a marked increase of connective tissue throughout the body. The thin capsule of connective tissue which before surrounded the luteal structure seems to have disappeared in places, making it appear as if the connective tissue of the body were continuous with that of the ovary around it. This connective-tissue invasion is evidently another factor in the disappearance of the corpus luteum. Three factors, then, are associated with the disappearance of the corpora lutea in the ovaries, cellular degeneration, vascular dilatation, and connective-tissue invasion.

A word may be added concerning the changes in the ovary outside the luteal bodies. There were in this organ six or seven good-sized, normal, growing follicles and four and five atretic ones. The most noticeable feature is the number of small hyalinized follicles. This is a noticeable feature of all the ovaries of this date which contain old corpora lutea. It would appear that as long as there are still luteal bodies in the ovary, there is very little growth in the follicles. There are a few primordial ova. The interstitial cells are not recognizable as such. By September 1 the corpora have disappeared from the ovaries and the organs again have assumed the appearance described for September 15.

There are occasional exceptions to the normal cycle. An ovary of spermophile 462, sacrificed October 30, 1916, was found with a little structure in it which appeared in every way to be a young corpus luteum. The cells contained red granules. Perhaps impregnation had occurred in the fall. This instance is mentioned because one function of the corpus luteum in spermophiles has been suggested by it. What keeps the follicles from becoming mature and ovulation from taking place at other times in the year from the spring? Evidently, the corpora

lutea help in regulating the periods between oestrus. No new follicles become mature in the fall as long as there are luteal bodies in the ovary and by the time the latter have disappeared it is too late for the follicles to become mature before the period of hibernation comes on.

A word concerning the interstitial cells may be added. These appear very prominent at two periods of the cycle, during the period of early pregnancy when the corpora lutea are young and apparently very active, and in the early fall when the follicles are developing and growing rapidly. Their significance is not evident. Several writers have claimed that the cells of the corpus luteum become the interstitial cells of the ovary. There is absolutely no basis for such a supposition as far as the spermophiles are concerned.

EXPERIMENTAL STUDIES

These experiments were made with a view to determining whether or not the results of certain experimental studies on the spermophile would substantiate the same work done on other animals.

The spermophile stand experimental surgery well. They are very satisfactory to work on as they are not nearly as susceptible to infectious diseases and are more resistant to local infections than most small laboratory animals. They are easily anesthetized. The surgical technic must be aseptic, and the animals must be placed in separate cages after the operation or they will chew one another's wounds open. They recover from the effects of the operation quickly, as a rule, and the wounds usually heal by first intention.

To all workers who have been interested in the functional relationship of the ovaries and the uterus and especially to those trying to isolate a particular function or functions for the corpora lutea, two problems have seemed of vital importance: First, what are the effects on the uterus of the removal of one or both of the ovaries? And, second, what are the effects on the ovaries of the extirpation of the uterus? These two problems

have a special bearing on the function of the corpora lutea when they deal with pregnant animals.

Another much studied problem closely related to the function of the luteal bodies attempts to explain the means by which ovulation is brought about and the results on the development of the corpora lutea if fertilization is prevented.

Following are protocols and results of experiments performed in connection with these various problems. Only those experiments which proved operative successes and in which reliable data were obtained will be included.

Series 1. Effects of the removal of the uterus on the ovaries of non-pregnant spermophiles

Experiment 230-15 (spermophile 146). Captured in the spring of 1915. Weight, 128 grams. Operated on June 11, 1915. Complete removal of the uterus.

Gross observations. There are good-sized corpora lutea in the ovaries.

Sacrificed July 20, 1915.

Gross observations. The ovaries appeared very much smaller.

Microscopic observations of the ovaries. Fixative, formalin zenker. Stain, hematoxylin and eosin. The ovaries show no degenerative changes except in the corpora lutea. These are undergoing regression. Connective tissue has heavily invaded every part of the luteal structure. The blood-vessels are numerous and good sized. There are many fat vacuoles present. The decrease in size of these ovaries is no doubt due to the decrease in the size of the corpora lutea.

Experiment 443-15 (spermophile 247). Captured in the spring of 1915. Weight, 207 grams. Operated on October 1, 1915. Complete removal of the uterus.

Died October 8, 1915.

Gross observations. Death was due to peritonitis.

Microscopic observations of the ovaries. Fixative, formalin zenker. Stain, hematoxylin and eosin. There is a marked congestion throughout the ovary. Many of the larger follicles are undergoing degeneration. Practically all the smaller follicles are markedly degenerated. This, no doubt, is a pathologic condition.

Experiment 444-15 (spermophile 248). Captured in the spring of 1915. Weight, 196 grams. Operated on October 1, 1915.

Died January 13, 1916.

Microscopic observations of the ovaries. Fixative, formalin zenker. Stain, hematoxylin and eosin. The great number of small hyalinized follicles are immediately apparent. They are so numerous that the

ovary has a lacy appearance. There are a few good-sized, apparently normal follicles and on the periphery are numbers of primordial ova.

Experiment 445-15 (spermophile 249). Captured in the spring of 1915. Weight, 110 grams. Operated on October 4, 1915. Complete removal of the uterus.

Sacrificed April 22, 1916.

Gross observations. The blood supply to the ovaries is intact. The ovaries are very small; the left so small as to leave doubt as to its identity. The right ovary appears to contain several tiny cysts.

Microscopic observations of the ovaries. Fixative, formalin zenker. Stain, hematoxylin and eosin. Tissue removed for left ovary proves to be a bit of granulation tissue. The right ovary appears to be normal. The several cysts noted grossly are large mature follicles which normally occur on the surfaces of the ovaries at this time of the year. No effects from the removal of the uterus are noted.

Discussion and summary of results. There seems to be no marked effect on the ovaries from the removal of the uterus. They seem able to repeat their life cycle as far as the follicles are concerned. It was thought that the great number of hyalinized follicles noted in the ovaries of spermophile 248 was a sign of degeneration due to removal of the uterus. However, on studying the ovaries of normal spermophiles killed about the same time for controls, there were found in the ovaries of two a great number of these hyalinized follicles (fig. 11). Evidently the hyalin degeneration occurs normally at this time of the year. It is, no doubt, part of the attempt to produce a few large mature follicles at the expense of many smaller ones.

Series 2. Effects of removal of the uterus on ovaries in pregnant animals

Experiment 270-16 (spermophile 305). Captured May 1, 1916. Weight, 98 grams. Operated on May 4, 1916. The uterus was removed to the cervix.

Gross observations. There are no signs of pregnancy in the uterus, but the right ovary contains what appears to be a hemorrhagic cyst.

Died May 9, 1916.

Gross observations. There was no apparent cause for death.

Microscopic observations of one ovary. Fixative, formalin zenker. Stain, hematoxylin and eosin. The ovary contains several corpora lutea of an early stage. One is markedly hemorrhagic. There is some degeneration apparent in the luteal bodies. Some cells are undergoing chromatolysis. There is an increased amount of connective tissue. The rest of the ovary appears to be in a very normal condition.

Experiment 294-16 (spermophile 329). Captured May 4, 1916. Weight, 117 grams. Operated on May 6, 1916. The uterus was removed to the cervix.

Gross observations. The animal appears to be in early pregnancy.

Sacrificed May 20, 1916. Weight, 130 grams.

Gross observations. There are no adhesions about the ovaries; they appear to contain several white, transparent corpora lutea.

Microscopic observations of the right ovary. Fixative, formalin zenker. Stains, hematoxylin and eosin, and acid fuchsin and methyl green. There is no apparent pathologic condition of this ovary. Even the corpora lutea appear normal. They have proceeded in their development without the uterus and now have the same appearance as others of the same age. The only detectable difference might be a smaller number of red granules in the luteal cells.

Experiment 251-16 (spermophile 348). Captured May 6, 1916. Weight, 108 grams. Operated on May 9, 1916. The uterus was removed to the cervix.

Gross observations. The animal is pregnant. There are five placental swellings in each horn, measuring 6.5 mm. in length.

Sacrificed May 19, 1916. Weight, 102 grams.

Gross observations. Everything in the abdominal cavity is in excellent condition.

Microscopic observations of the ovaries. Right ovary: fixative acetic osmic bichromate; stain, acid fuchsin and methyl green. Left ovary: fixative, formalin zenker; stain, Weigert's copper-chrome hematoxylin. The ovaries are apparently normal. The corpora lutea appear like those of the controls except that the luteal cells contain a larger number of lipoid droplets and fewer red granules. There are no signs of degeneration in the nuclei of the cells.

Experiment 314-16 (spermophile 349). Captured May 6, 1916. Weight, 122 grams. Operated on May 9, 1916. The uterus was removed to within 3 mm. of the cervix.

Gross observations. The animal is pregnant. There are four placental swellings in the right horn and five in the left about 4 mm. in length.

Sacrificed June 5, 1916. Weight, 135 grams.

Gross observations. Everything in the abdominal cavity is in excellent condition. Both ovaries were readily found and appear to contain large corpora lutea.

Microscopic observations of the ovaries. Right ovary: Fixative, formalin zenker; stain, Weigert's copper-chrome hematoxylin. Left ovary: fixative, acetic osmic bichromate; stain, acid fuchsin and methyl green and Weigert's copper-chrome hematoxylin. There is no apparent abnormality in the ovaries. The corpora lutea have developed normally. They are still in the red-granule stage. The cells contain some lipoid droplets, but are full of red granules. The lipoid droplets in these ovaries are not as numerous as would be expected, but normal ovaries vary somewhat as to the time when the lipoid droplets begin to appear in the cells.

Experiment 317-16 (spermophile 353). Captured April 25, 1916. Weight, 116 grams. Operated on May 9, 1916. The uterus was removed to the cervix.

Gross observations. The animal is pregnant and, having been kept by itself since capture, the period of pregnancy must be at least fourteen days. The placental swellings measure 13 mm. in length and 10 mm. in width.

Sacrificed May 20, 1916. Weight, 130 grams.

Microscopic observations of the ovaries. Right ovary: fixative, formalin zenker; stains, hematoxylin and eosin, and Weigert's copper-chrome hematoxylin. Left ovary: fixative, acetic osmic bichromate; stain, acid fuchsin and methyl green. There is no pathologic condition apparent in the ovaries. The corpora lutea have gone on in their normal development. They appear very similar to those of spermophiles whose fetuses are nearing parturition.

Experiment 318-16 (spermophile 353). Captured April 28, 1916. Weight, 127 grams. Operated on May 9, 1916. The uterus was removed to the cervix.

Gross observations. The animal is pregnant and, since it has been kept by itself since capture, it must have been pregnant at least eleven days. The placental swellings measure 7 mm. in length.

Sacrificed May 31, 1916. Weight, 120 grams.

Gross observations. The ovaries appear very small. The blood supply is intact and there are no adhesions. There is a mass of fat around each ovary.

Microscopic observations of the ovaries. The right ovary was carefully studied. The other was lost. Fixative, formalin zenker. Stains, hematoxylin and eosin, and acid fuchsin and methyl green. Many degeneration changes are present all through the ovary. Practically all the follicles are degenerating. The corpora lutea show many degeneration changes also. There are practically no red granules in the cells and many fatty degeneration vacuoles are present. The nuclei of the cells show degenerative changes. Their margins are indented and their nucleoli are faded and fringed. The strands of chromatin are clumped. The capillary sinuses seem dilated and filled with blood. Evidently the ovaries have been injured by the operation.

Experiment 331-16 (spermophile 361). Captured May 4, 1916. Weight, 141 grams. Operated on May 12, 1916. The uterus was removed to the cervix.

Gross observations. The animal is pregnant, the placental swellings being 9 mm. in length.

Sacrificed May 20, 1916. Weight, 120 grams.

Microscopic observations of the ovaries. Right ovary: fixative, formalin zenker; stains, acid fuchsin and methyl green, and hematoxylin and eosin. The left ovary was lost. The right ovary shows no abnormality. The corpora lutea have gone on in their development. They are just entering the lipid stage.

Experiment 61-16 (spermophile 381) Captured May 20, 1916. Weight, 145.5 grams. Operated on May 22, 1916. The uterus was removed to the cervix.

Gross observations. The animal has given birth to young, probably a few days previously, as the uterus appears to be in an early stage of involution. There are good-sized corpora lutea in the ovaries.

Sacrificed October 20, 1916. Weight, 170 grams.

Gross observations. The ovaries are so small they are hard to find.

Microscopic observations of the ovaries. Right ovary: fixative, formalin zenker; stain, hematoxylin and eosin. Left ovary; fixative, acetic osmic bichromate; stain, acid fuchsin and methyl green. There is nothing abnormal about the ovaries. They are very similar to other ovaries removed at this time. The corpora lutea have disappeared normally.

Discussion and summary of results. Removal of the uterus has no apparent pathologic effect on the ovaries containing the corpora lutea of pregnancy. The latter pass through their normal cycle of development and regression. There seems to be a slight irregularity about the time at which the various changes in the life cycle come on; for instance, the corpora lutea in the ovaries of spermophile 348 seem to lose the red granules and take on the lipoid droplets sooner than normally. Then, in the ovaries of spermophile 349, the corpora lutea have retained their red granules longer than usual. However, this may not be significant since there is some irregularity about the cycle normally.

Series 3. Effects of the removal of both ovaries on the uterus of non-pregnant animals

Experiment 200-15 (spermophile 139). Captured in the spring of 1915. Weight, 130 grams. Operated on May 28, 1915. Both ovaries were removed.

Gross observations. The uterus is in a stage of early involution. Sacrificed April 22, 1916.

Gross and microscopic observations of the uterus. Fixative, formalin zenker. Stain, hematoxylin and eosin. The uterus is very small. It shows no signs of rut or having been in rut, either grossly or microscopically. This is very abnormal for this time of the year. A comparison of this uterus with a normal uterus in rut at this time of the year brings out the differences. (Compare figs. 1 and 3; also 2 and 4.) A comparison of the dimensions of the two uteri brings out the marked differences in size (table).

Microscopic observations of the uterus. Fixative, formalin zenker. Stain, hematoxylin and eosin. Cross-sections through the horns and body of the uterus show much fibrosis of all the layers. There is much less muscular and glandular tissue than in a normal inactive uterus (compare figs. 3 and 5, 4 and 6). One striking feature is the number of obliterated blood-vessels with hyalinized walls. The lumen of the uterus is closed and no mucous secretion is present. The cervix shows these same changes to an even greater degree than the rest of the organ.

Experiment 201-15 (spermophile 140). Captured in the spring of 1915. Weight, 101 grams. Operated on May 28, 1915. The ovaries were completely removed.

Gross observations. The uterus is in the condition of early involution.

Postoperative notes. This animal became very fat and went into a torpid condition at times. On July 24 it was changed to a cage with two other animals who killed it. The body was badly traumatized and specimens were not saved.

Experiment 202-15 (spermophile 141). Captured in the spring of 1915. Weight, 108 grams. Operated on May 28, 1915. Both ovaries were removed.

Gross observations. The uterus is undergoing involution.

Died September 21, 1915.

Gross and microscopic observations of the uterus. Fixative, formalin zenker. Stain, hematoxylin and eosin. This uterus appears very similar to the uterus of a normal animal killed the same day.

Experiment 218-15 (spermophile 142). Captured in the spring of 1915. Weight, 135 grams. Operated on June 3, 1915. Both ovaries were removed.

Gross observations. The uterus is undergoing involution.

Sacrificed November 10, 1915.

Gross and microscopic observations. Fixative, formalin zenker. Stain, hematoxylin and eosin. The uterus is very similar to the uteri of the controls.

Experiment 220-15 (spermophile 151). Captured in the spring of 1915. Operated on June 28, 1915. Both ovaries were removed.

Gross observations. The uterus has become completely involuted.

The uterus was removed September 28, 1915.

Gross and microscopic observations. Fixative, formalin zenker. Stain, hematoxylin and eosin. Compared with a normal control, this uterus shows no abnormalities.

Experiment 423-15 (spermophile 231). Captured in the spring of 1915. Weight, 200 grams. Operated on September 22, 1915. Both ovaries were removed.

Sacrificed March 18, 1916. Weight, 100 grams.

Gross and microscopic observations of the uterus. Fixative, formalin zenker. Stain, hematoxylin and eosin. This uterus shows the same changes as that of spermophile 139.

Experiment 424-15 (spermophile 232). Captured in the spring of 1915. Weight, 205 grams. Operated on September 22, 1915. Both ovaries were removed.

Gross observations. The uterus is normal.

Sacrificed May 8, 1916. Weight, 117 grams.

Gross and microscopic observations. Fixative, formalin zenker. Stain, hematoxylin and eosin. This uterus shows the same changes as that of spermophile 139. (Photograph of this uterus with a normal uterus of rut removed the same day (fig. 7). Table for measurements of these uteri).

Experiment 435-15 (spermophile 242.) Captured in the spring of 1915. Weight, 210 grams. Operated on September 28, 1916. Both ovaries were removed.

Sacrificed May 9, 1916. Weight, 155 grams. The same day a control animal, spermophile 233, which was captured the same time as spermophile 242, and had lived in the laboratory under the same conditions, was sacrificed also and the uteri of these two animals were photographed together (fig. 8).

Gross and microscopic observations. Fixative, formalin zenker. Stain, hematoxylin and eosin. The uterus of spermophile 242 shows all the changes noted in that of spermophile 139. (For dimensions of uterus see table.) The control uterus (table) does not show as marked enlargement as the uteri of animals brought in from the fields in the condition of rut, but aside from this variation in size, the uterus appears in every way like a typical one of rut.

Experiment 436-15 (spermophile 246). Captured in the spring of 1914. Weight, 95 grams. Operated on July 10, 1914. Both ovaries were completely removed.

Sacrificed September 29, 1915.

Gross and microscopic observations. Fixative, formalin zenker. Stain, hematoxylin and eosin. Compared with the uteri of the controls, this uterus shows atrophic changes of a similar nature to those of the uterus of spermophile 139, except that the atrophy must be even more marked to be noticed in a comparison with the controls of this time of the year.

Experiment 354-16 (spermophile 374). Captured May 20, 1916. Weight 128 grams. Operated on May 22, 1916. Both ovaries were removed.

Gross observations. The uterus is undergoing involution.

Sacrificed October 30, 1916. Weight, 120 grams. This uterus was photographed with that of a control animal and that of an animal ovariectomized and sacrificed on the same date—spermophile 378 (fig. 9).

Experiment 358-16 (spermophile 378). Captured May 18, 1916. Weight, 115 grams. Operated on May 22, 1916. Both ovaries were removed.

Sacrificed October 30, 1916. Weight, 135 grams. This uterus was photographed with that of spermophile 374 and a control.

Gross and microscopic observations. Some slight differences can be noted between the uteri of spermophiles 374 and 378 and their control. The uteri of the doubly ovariectomized spermophiles are both smaller than their control. This decrease in size is more noticeable in the cervix than in the rest of the uterus. The cervixes of these two uteri are much firmer also and contain very little mucus. It is true, the differences are only slight and are scarcely recognizable microscopically, especially after fixation. The main microscopic difference is a decrease in size of the blood-vessels in the uteri of the ovariectomized spermophiles. Perhaps this accounts for the fact that the uterus of the control has a healthier appearance grossly.

Discussion and summary of results. The uteri of animals doubly ovariectomized in the spring of the year show some changes over their controls by the fall of the same year. These are slight and are all of the nature of a functional atrophy. The cervix is affected the most. This atrophy increases so as to be quite noticeable by the fall of the next year. The very striking effect of double ovariectomy is the discontinuation of the changes in the uterus incident to the phenomena of rut.

Series 4. Effects of removal of both ovaries on the pregnant uterus

Experiment 293-16 (spermophile 328). Captured May 4, 1916. Weight, 119 grams. Operated on May 6, 1916. Both ovaries were removed.

Gross observations. The animal is in an early stage of pregnancy, the placental swellings being just visible grossly in the uterus.

Sacrificed May 20, 1916. Weight, 115 grams.

Gross observations. There are no signs of placental sites in the uterus. To be sure the animal had been pregnant, the ovaries were studied carefully. They contained early corpora lutea. Evidently the regression changes began in the uterus immediately after the removal of the ovaries.

Experiment 300-16 (spermophile 335). Captured in the spring of 1916. Weight, 101 grams. Operated on May 8, 1916. Both ovaries were removed.

Gross observations. The animal was in an early stage of pregnancy, the placental swellings being just large enough to be recognizable.

Died May 12, 1916.

Gross observations. The external wound is in bad condition, perhaps the fault of too much iodine. The inside of the abdominal cavity appears normal. The uterus is in good condition save on the ends where the blood supply has been injured. The congestion is much reduced. There are placental swellings, hard and dark red; five in the right horn and three in the left, very hard to see. The placentas and fetuses are apparently undergoing degeneration.

Experiment 302-16 (spermophile 337). Captured in the spring of 1916. Weight, 120 grams. Operated on May 8, 1916. Both ovaries were removed.

Gross observations. The animal is pregnant, the placental swellings being 6 mm. in diameter.

Sacrificed May 18, 1916. Weight, 142 grams.

Gross observations. The uterus is in a very unnatural condition. It appears dark red in color all over. (The color is not due to congestion as can be seen on microscopic section. Instead, it must be due to the presence of old clotted blood in the lumen.) There is one hard swelling still palpable. The pregnancy was interrupted and the placentas with the fetuses have been undergoing degenerative changes.

Experiment 303-16 (spermophile 338). Captured April 23, 1916. Weight, 221.5 grams. Operated on May 8, 1916. Both ovaries were removed.

Gross observations. The animal is pregnant, the placental swellings being of good size.

Sacrificed May 19, 1916. Weight, 140 grams.

Gross observations. The uterus is very dark-red colored, dead-looking and contains several dark-colored swellings. The blood supply to the uterus is intact as tested by Dr. Mann. It appears as though degeneration of the placentas with the fetuses has been going on.

Experiments 305-16 (spermophile 340). Captured May 6, 1916. Weight, 124 grams. Operated on May 8, 1916. Both ovaries were removed.

Gross observations. The animal is in an advanced condition of pregnancy, the placental swellings measuring 2.2 cm. in length and 1.3 cm. in breadth.

Died May 11, 1916.

Gross observations. The cause of death could not be determined. The uterus is very unhealthy appearing, dark red in color. The remaining placental swellings are of various sizes. They are very dark red and are hard. Apparently degeneration changes have been going on.

Experiment 307-16 (spermophile 342). Captured May 6, 1916. Weight, 142 grams. Operated on May 8, 1916. Both ovaries were removed.

Gross observations. The animal is pregnant, the placental swellings being 6.5 mm. in length. There are nine swellings in the left horn and three in the right.

Sacrificed May 20, 1916. Weight, 152 grams.

Gross observations. The uterus is very dark in color. There are some adhesions on the left end. In the left horn are four swellings, the first and fourth measuring 4 mm. in length and breadth, the second 4 mm. by 5 mm., and the third, 2 mm. x 2 mm. In the right horn are three swellings—the first measuring 4 mm. x 4 mm., the second

5 mm. x 5 mm., and the third, 3 mm. x 2 mm. Each swelling consists of a light-colored band surrounding a hard dark red mass. The same condition was found in all the other cases. Some of the placental swellings with the fetuses have disappeared entirely, the others are degenerating.

Experiment 333-16 (spermophile 362). Captured April 28, 1916. Weight, 168 grams. Operated on May 12, 1916. Both ovaries were removed.

Gross observations. The animal is in advanced pregnancy. The operation was difficult as there were so many large fetuses. No attempt was made to count the number for fear of disturbing them.

Sacrificed May 20, 1916. Weight, 125 grams.

Gross observations. The uterus appears about normal in color. There are three swellings in the left horn, 4 mm. by 5 mm., and ten in the right horn, same size, dark red and hard.

Experiment 355-16 (spermophile 375). Captured May 20, 1916. Weight, 143 grams. Operated on May 22, 1916. Both ovaries were removed.

Gross observations. The animal is in advanced pregnancy. The operation was performed with very little trauma, the fetuses not being removed from the abdominal cavity. The placental swellings were 1.5 cm. or more in length.

Sacrificed May 26, 1916. Weight, 130 grams.

Gross observations. The animal had aborted some time previously, perhaps twenty-four hours. There are seven placental sites in the right horn and two in the left, all 5 mm. x 6 mm. The uterus is normal in color. The placental swellings appear congested but not hard. They look very different from those previously described.

Experiment 357-16 (spermophile 377) Captured May 18, 1916. Weight, 116 grams. Operated on May 22, 1916. Both ovaries were removed.

Gross observations. The animal is in advanced pregnancy, the placental swellings measuring 2 cm. in length. The operation was performed with very little trauma, the fetuses not being removed from the abdominal cavity.

Sacrificed May 25, 1916. Weight, 110 grams.

Gross observations. The animal must have aborted shortly after the operation. There are three placental sites in the right horn and four in the left, 5 mm. by 4 mm.

Experiment 309-16 (spermophile 344). Captured May 6, 1916. Weight, 120 grams. Operated on May 8, 1916. Instead of removing both ovaries, the left ovary was removed and the right tube ligated on the opposite side.

Gross observations. The animal is pregnant, the placental swellings measuring 1.4 cm. in length. There are many of the latter in the left horn and only one in the right.

Sacrificed May 20, 1916. Weight, 120 grams.

Gross observations. The uterus appears healthy, a normal pale pink color, but it is very much swollen (table). There are three small palpable swellings in the left horn recognizable only from the increased size of the uterus at these points. Placental sites in the right horn not definitely established. Evidently the animal had aborted. The cause of the swollen condition of the uterus is not evident.

Microscopic observations of the ovaries. Left ovary: fixative, formalin zenker; stain, hematoxylin and eosin. Right ovary: fixative, formalin zenker; stain, hematoxylin and eosin. The left ovary removed at operation May 8, 1916, is normal. It contains eight corpora lutea, 0.8 mm. x 0.9 mm. in size. The right ovary obtained at autopsy May 20, 1916, is very interesting. It contains only one corpus luteum, 0.4 mm. x 0.5 mm. in size. This is in the final stage of its life cycle, the degenerative stage described in the first part of this work. The rest of the ovary is normal. Through its cortex are eight large follicles, apparently mature, not showing any atretic changes. One of these measures 0.8 mm. x 0.5 mm. There are many smaller growing follicles also, and quite a number of primordial ova. Almost in the middle of the ovary, just inside the cortex, is the one degenerated corpus luteum. It is full of large fatty degenerative vacuoles and enlarged blood-vessels and capillaries. The connective tissue has made great inroads. What made this corpus luteum degenerate is not evident. It will be shown later that after the removal of one ovary the other shows no pathologic effects. And even after removal of one ovary and a uterus containing fetuses, the other ovary with its corpora lutea shows no pathology. Evidently the ligation of the tube caused the corpus luteum in the right ovary to degenerate and abortion occurred, the same as on removal of both ovaries. This would make it appear as if the corpus luteum was the part of the ovary necessary to the development of the fetuses.

Discussion and summary of results. Ten animals were operated on to get results from the removal of both ovaries containing corpora lutea on the uterus containing fetuses. One of the animals died from too much ether. The others lived, but none of them came to term. Those operated on early in pregnancy did not abort. The placentas and fetuses degenerated. Those operated on when the pregnancy was well advanced, aborted. That this was not the effect of operative trauma will be shown by another series of experiments on the removal of one ovary in pregnant spermophiles.

Series 5. Effects of the removal of one ovary on the one remaining in non-pregnant spermophiles

Experiment 219-15 (spermophile 143). Captured in the spring of 1915. Operated on June 3, 1915. The left ovary was removed.

Gross observations. There are spots marking placental sites in the uterus.

Right ovary removed September 20, 1915.

Microscopic observations of the ovaries. Both ovaries: fixative, formalin zenker; stain, hematoxylin and eosin. The left ovary contains several large corpora lutea of the late lipoid stage. The right ovary contains no corpora lutea. It is a typical ovary of the fall of the year. There are many medium-sized growing follicles and many primordial ova. There is no demonstrable pathology. The corpora lutea must have disappeared normally.

Experiment 221-15 (spermophile 159). Captured in the spring of 1915. Operated on June 29, 1915. The left ovary was removed.

Gross observations. The uterus is normal and inactive.

The right ovary was removed August 5, 1915.

Microscopic observations of the ovaries. Both ovaries; fixative, formalin zenker; stain, hematoxylin and eosin. The left ovary contains six very large corpora lutea of the lipoid stage. The right ovary contains two small corpora lutea in the final stage of their life cycle, evidenced by their size, the large amount of fatty degeneration, the numerous large blood-vessels, and the great invasion of connective tissue. This ovary contains many medium-sized, ripening follicles, also many small follicles and primordial ova. No pathology is evident. It is a typical ovary of August 5. The results of these two successful experiments were so evidently negative that it seemed unnecessary to repeat them the next year.

Series 6. Effects of the removal of one ovary on the other ovary and the uterus in pregnant spermophiles

These experiments were controls for the experiments under series 4.

Experiment 352-16 (spermophile 372). Captured May 20, 1916. Weight, 148 grams. Operated on May 22, 1916. The left ovary was removed.

Gross observations. The animal is pregnant, the fetuses measuring 1.5 cm. in length. There are not many fetuses.

Sacrificed May 25, 1916. Weight, 135 grams.

Gross observations. All the fetuses are alive and no abnormalities are apparent. There are five fetuses in the right horn and two in the left. They measure 2.7 cm. by 1.5 cm.

Microscopic observations of the ovaries. Right ovary: fixative, formalin zenker; stains, hematoxylin and eosin, and Weigert's copper-chrome-hematoxylin. The left ovary was lost. The right ovary shows no abnormality. It contains five corpora lutea. They measure 0.8 mm. x 0.9 mm. in diameter and are in the early lipid stage, the condition which exists about the time of the birth of the fetuses. They still contain numbers of red granules, but the lipid droplets are very conspicuous in the cells. In fact, some cells are so full of the lipid that the red granules are in evidence only at the periphery of the cell.

Experiment 351-16 (spermophile 371). Captured May 20, 1916. Weight, 135.5 grams. Operated on May 22, 1916. The left ovary was removed.

Gross observations. The animal is pregnant, the fetuses measuring 2 cm. in length. The animal gave birth to young, May 31, 1916.

Sacrificed June 17, 1916. Weight, 130.2 grams.

Gross observations. The remaining ovary and uterus appear normal. There are three spots marking the placental site in the right horn and three in the left.

Microscopic observations of ovaries. Left ovary: fixative, formalin zenker, stains hematoxylin and eosin, and Weigert's copper-chrome-hematoxylin. Right ovary: fixative, acetic osmic bichromate; stain, acid fuchsin and methyl green. The left ovary contains three corpora lutea. These are in the late red-granule stage. The red granules are still quite numerous, but some cells contain lipid droplets about their periphery. The right ovary contains three corpora lutea of the lipid stage. It appears very similar to other ovaries containing luteal bodies of this stage. There is no demonstrable pathology in the ovary.

Experiment 359-16 (spermophile 379). Captured May 20, 1916. Weight, 154 grams. Operated on May 22, 1916. The left ovary was removed.

Gross observations. The animal is in advanced pregnancy. There was more trauma experienced in this operation than in the one performed on spermophile 377, in which both ovaries were removed. The animal gave birth to young on May 28, 1916, and kept them until June 8, 1916.

Sacrificed June 24, 1916. Weight, 136.5 grams.

Gross observations. The placental sites in the uterus are scarcely visible.

Microscopic observations of the ovaries. Both ovaries: fixative, formalin zenker; stain, hematoxylin and eosin. The left ovary contains six corpora lutea of the same stage as the left ovary of spermophile 371, the late red granule stage. There are a few lipid droplets located through the cells. The right ovary contains four corpora lutea of the lipid stage. There is nothing abnormal about it.

Discussion and summary of results. Three spermophiles were operated on in this series. The results were positive. The removal of one ovary during the second half of pregnancy does not affect in any way the normal development of the fetuses. They go on to term. There is no effect produced on the remaining ovary and the development of its corpora lutea.

Series 7. Effects on the remaining ovary of the removal of one ovary and the uterus in pregnant animals.

Experiment 273-16 (spermophile 308). Captured May 1, 1916. Operated on May 4, 1916. The right ovary and the uterus were removed.

Gross observations. Three or four placental swellings in the right horn of the uterus and three in the left about 4.5 mm. in length. Both ovaries show hemorrhagic areas which look like tiny hemorrhagic cysts. They are more noticeable in the right ovary.

Died May 11, 1916.

Gross observations. Absolutely no cause for death is apparent in the abdomen. The blood-vessels to the remaining ovary are very much congested.

Microscopic observations of the ovaries. Both ovaries: fixative, formalin zenker; stains, hematoxylin and eosin, acid fuchsin and methyl green, and Weigert's copper-chrome-hematoxylin. The right ovary contains five corpora lutea of a very early stage, measuring 0.7 mm. x 0.9 mm. The left ovary contains four corpora lutea, measuring 0.8 mm. x 0.5 mm. This organ shows no pathology except in the corpora lutea. These bodies show many degenerative changes. The cells have lost their red granules and their nuclei are undergoing chromatolysis. The connective tissue has increased greatly.

Experiment 290-16 (spermophile 325). Captured May 4, 1916. Weight, 111 grams. Operated on May 6, 1916. The right ovary and uterus were removed.

Gross observations. The animal is pregnant, the fetuses being just recognizable.

Sacrificed June 8, 1916.

Microscopic observations of the ovaries. Fixative, formalin zenker. Stain, acid fuchsin and methyl green. The right ovary contains five corpora lutea of a very early stage, about 0.5 mm. x 0.6 mm. in diameter. The luteal cells are filled with the red granules. There is a mass of blood in the center and practically no organization of connective tissue or blood-vessels. The left ovary contains six corpora lutea of the late red-granule stage. They have increased in size to 0.9 mm. x 1 mm. The normal development of the corpora lutea has apparently gone on in this ovary, as it compares very favorably with other ovaries of this date. There is no demonstrable pathology.

Experiment 279-16 (spermophile 314). Captured May 1, 1916. Weight, 100 grams. Operated on May 5, 1916. The left ovary and the uterus were removed.

Gross observations. It is impossible to recognize any fetuses in the uterus.

Sacrificed May 18, 1916. Weight, 112 grams.

Gross observations. The right ovary appears to contain corpora lutea. Everything in the abdomen is in excellent condition. There are no adhesions.

Microscopic observations of the ovaries. Right ovary: fixative, formalin zenker; stains, acid fuchsin and methyl green, Weigert's copper-chrome-hematoxylin, and hematoxylin and eosin. The left ovary was lost. The right ovary shows no pathology. It contains two corpora lutea which measure about 0.8 mm. x 0.8 mm. They seem to have reached their normal development for eighteen days. The number of red granules might have been slightly less than normal, but they are much more abundant still than the lipid droplets. There is a well-developed connective-tissue framework and network of blood-vessels throughout the luteal body. As there are only two corpora lutea in this ovary, several follicles have had room to mature. Two of these show atretic changes.

Experiment 292-16 (spermophile 327). Captured May 4, 1916. Weight, 130 grams. Operated on May 6, 1916. The right ovary and uterus were removed.

Gross observations. There are six swellings in the right horn of the uterus and four in the left, 8.5 mm. in length.

Sacrificed May 31, 1916. Weight, 170 grams.

Gross observations. The left ovary is found embedded in a mass of fat, but the blood supply to the ovary seems intact.

Microscopic observations of the ovaries. Right ovary; fixative, formalin zenker; stains, hematoxylin and eosin, Weigert's copper-chrome-hematoxylin. Left ovary: fixative, acetic osmic bichromate; stain, acid fuchsin and methyl green. The right ovary contains six corpora lutea, measuring 0.6 mm. x 0.7 mm. They are of the red-granule stage. There is no lipid. The mass of blood in the center is fairly well organized. The connective-tissue framework and network of capillaries are fairly well developed. The left ovary contains six corpora lutea which measure 0.7 mm. x 1.1 mm. in diameter. They have developed very normally, although not quite as rapidly as their controls. The red granules are still very numerous and there is scarcely any lipid present. The connective-tissue framework and the blood-vessel network are very well developed.

Experiment 295-16 (spermophile 330). Captured May 4, 1916. Weight, 116.5 grams. Operated on May 6, 1916. The right ovary and uterus were removed.

Gross observations. There are five swellings in each horn, all about 5.5 mm. in length.

Sacrificed May 26, 1916. Weight, 130 grams.

Microscopic observations of the ovaries. Left ovary: fixative, acetic osmic bichromate; stain, acid fuchsin and methyl green. The right ovary was lost. The left ovary contains five corpora lutea, measuring 0.7 mm. x 0.8 mm., and showing many degeneration changes. These are most apparent in the corpora lutea and the clumps of interstitial cells of the stroma. The latter cells appear swollen and their nuclei are undergoing chromatolysis. The cells of the corpora lutea are most degenerated in the center of the structure. Some cells seem to have disappeared entirely here, leaving holes. Many are undergoing fatty changes. Toward the outside of the structure there are cells which are still in fairly good condition, still containing red granules, though their nuclei have not taken the stains well, have indented margins and clumped chromatin strands.

Experiment 308-16 (spermophile 343). Captured May 6, 1916. Weight, 110 grams. Operated on May 8, 1916. The ovary and the uterus were removed.

Gross observations. The animal is pregnant. There are eight placental swellings in the left horn and one in the right, all 6.5 mm. in length.

Sacrificed May 31, 1916. Weight, 153 grams.

Microscopic observations of the ovaries. Right ovary: fixative, formalin zenker; stains, hematoxylin and eosin, Weigert's copper-chrome-hematoxylin, Mallory's connective-tissue stain. Left ovary: fixative, formalin zenker; stains, hematoxylin and eosin, Weigert's copper-chrome-hematoxylin. The right ovary contains one corpus luteum, measuring 0.8 mm. x 0.8 mm., and is of the red-granule stage. The left ovary shows no apparent pathology. The corpora lutea measure 1 cm. x 0.9 mm. and are of the early lipid stage. They have developed normally.

Discussion and summary of results. In four of these animals the remaining ovary with its corpora lutea was not at all affected by the operation. In two of the spermophiles operated on, the second ovary showed changes. In spermophile 330 the whole ovary was affected, and in spermophile 308, only the corpora lutea. There was evidently some injury to the blood supply.

Series 8. Studies on the production of the normal bursting of follicles and the formation of corpora lutea

Experiment 311-16 (spermophile 346). Captured in the spring of 1916. Weight 88 grams. This animal appeared to be in rut on May 7, 1916. She was placed with a male at 10:10 A.M. on this date and removed at 3 P.M. on May 8, 1916.

Sacrificed May 8, 1916.

Gross observations. The uterus shows no signs of pregnancy. The ovaries show very small hemorrhagic areas, the smallest noted so far. In all probability, the animal has ovulated during the time it was with the male.

Microscopic observations of the ovaries. Fixative, formalin zenker. Stain, hematoxylin and eosin. There are three corpora lutea in the right ovary and five in the left. They are in a very early stage of development, appearing just like other very early ones. There were a few mitotic figures, one very sure proof of their very recent development.

Experiment 323-16 (spermophile 357). Captured April 27, 1916. Weight, 125 grams. This animal was kept separate from the time of her capture until May 8, 1916. At 3 P.M. on May 8 a male was placed with her and kept there until 10:45 A.M. on May 10, 1916.

Sacrificed May 10, 1916, 10:45 A.M.

Gross observations. Gross evidences did not show that the animal had ovulated.

Microscopic observations of the ovaries. Fixative, formalin zenker. Stain, hematoxylin and eosin. Both ovaries showed beautiful early corpora lutea, three in the right ovary and four in the left. These could not have been more than twenty hours old.

Discussion and summary of results. The results obtained in these two experiments prove that ovulation follows on coitus during rut.

*Series 9. To determine if ovulation is followed by the formation of corpora lutea when fertilization is prevented by the resection of 1 cm. of the uterus. One ovary was removed, also, to determine absolutely whether the animal was pregnant or not.*²

Experiment 280-16 (spermophile 315). Captured May 3, 1916. Weight, 100 grams. Operated on May 5, 1916. The right ovary was removed and 1 cm. of the uterus just above the body was ligated and resected.

Gross observations. The animal is not pregnant. She was placed with a male on May 20, 1916, and remained with him until sacrificed.

Sacrificed June 5, 1916. Weight, 120 grams.

Gross observations. The remaining ovary appears to contain corpora lutea. There is a good deal of pus about the ligature on the uterus, with adhesions to the intestines.

Microscopic observations of the ovaries. Fixative, formalin zenker. Stains, hematoxylin and eosin and Weigert's copper-chrome-hematoxylin. There are no corpora lutea in the right ovary. Several

² This series of experiments is at present being continued. No conclusions can be drawn from the results obtained so far.

large mature follicles are present. There are six corpora lutea present in the left ovary. They are of a very early stage, and measure 0.6 mm. x 0.7 mm. or 0.7 mm. x 0.7 mm. The cells are full of granules. There is a core of blood in the center which is undergoing organization. It certainly appears as if ovulation had taken place shortly before the animal was sacrificed.

Comparative sizes of uteri

EXPERIMENT	SPERMOPHILE	DATE	LENGTH OF RIGHT HORN	LENGTH OF LEFT HORN	SIZE OF BODY OF UTERUS	SIZE OF CERVIX	WIDTH OF RIGHT HORN	WIDTH OF LEFT HORN
			<i>cm.</i>	<i>cm.</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>
20C-15	139	April 22, 1916	4.5	4.5	2.5	2.0	1.0	1.0
42C-15	232	May 8, 1916	5.6	5.6	4.0	1.8	0.75	0.75
42C-15	233	May 9, 1916	7.0	6.5	5.0	4.0	1.0	1.0
43C-15	242	May 9, 1916	6.3	6.3	3.0	2.0	0.7	0.7
24E-16	291	April 25, 1916	11.4	10.3	8.5	8.0	4.0	5.0
27E-16	298	April 29, 1916	7.2	7.0	9.0	8.0	3.0	3.0
37A-16	374	October 30, 1916	6.8	6.0	3.5	2.0	1.0	1.0
37C-16	378	October 30, 1916	6.3	6.3	4.5	2.5	1.0	1.0
67C-16	462	October 30, 1916	6.7	6.5	5.5	2.0	1.5	1.5
30C-16	344	May 20, 1916	6.8	7.2	8.0	8.0	5.0	5.0

SUMMARY AND DISCUSSION

The results obtained from the histologic and experimental investigations carried on may be summarized as follows:

1. In the spermophiles, ovulation occurs only once a year, during the rutting season in the early spring. Ovulation is dependent on the stimulus of coitus, for no corpora lutea were found in the ovaries of animals which were kept from the males.

2. The corpora lutea cannot be responsible for the phenomena of rut for they are not present in the ovaries at this time.

3. The corpora lutea develop and pass through their normal cycle after ovulation whether fertilization follows or not (foot-note 2, p. 159).

4. While the corpora lutea are present in the ovaries, especially during the two months following parturition, the process of developing and ripening the follicles is at a standstill.

5. If the uterus is removed after conception, the corpora lutea do not begin to degenerate, but pass through their normal cycle. No effects are noted in the ovaries.

6. Removal of the uterus at any time does not produce noticeable effects on the ovaries even after a year's time.

7. Double ovariectomy performed at any time during the period of pregnancy interrupts gestation. If the operation is performed after a little more than the first half of pregnancy, the placentas with the fetuses simply degenerate. The involution of the uterus in these cases is very abnormal. If both ovaries are removed late in pregnancy, the animal aborts and the uterus undergoes a much more normal involution. The removal of only one ovary does not interrupt the pregnancy.

8. Double ovariectomy at any time prevents the recurrence of the cyclic changes in the uterus and produces an atrophy of the organ scarcely noticeable within a year.

9. The corpora lutea apparently do not influence the development of the mammary gland. When the uterus was removed very soon after conception, before any signs of pregnancy could be noted grossly in the uterus, and before any development of the mammary glands could be noted grossly, the developing corpora lutea in the ovaries produced no development in the mammary glands. This would seem to substantiate the work of Lane-Clayton and Starling who attribute to the fertilized egg the stimulus for the development of the mammary glands.

10. The corpus luteum of the spermophile derives its elements from the follicle just as Sobotta states occurs in the mouse. The luteal cells are the transformed granulosa cells of the follicle. The connective tissue and vascular network are derived from the cells of the internal theca which spends itself entirely in their formation. The capsule of connective tissue surrounding the luteal structure is the same external theca which surrounded the follicle. The microscopic pictures of the corpora lutea in the succeeding stages of their development correspond also to Sobotta's descriptions. It may be well to emphasize the complexity of the vascular network throughout the luteal structure which, when it is completed, brings every luteal cell in intimate contact with the blood stream.

11. The life cycle of the corpus luteum is made up of three distinct phases. First, the phase characterized by the presence

of great numbers of red granules in the protoplasm of the luteal cells. This phase embraces a period dating from the bursting of the follicle and covering the entire period of pregnancy. From a point of time very shortly after the bursting, the protoplasm of the luteal cells shows these red granules which become more and more abundant until they seem to reach a crisis of abundance when the organization of the luteal structure is about perfected, which is not until the placental swellings have reached a length of about 8.5 cm. From this time on the granules seem very gradually to decrease in number in the cells until parturition, when there is a sudden considerable reduction in their number. Some are found in the cells, however, even as late as the fourth week after parturition. Second, the phase characterized by the presence of many lipoid droplets in the protoplasm of the luteal cells. This phase begins sometime before parturition and lasts for about six weeks afterward. About the fourteenth day of pregnancy when the placental swellings in the uterus measure 1.5 cm. to 2 cm. in length, the lipoid droplets usually begin to make their appearance at the periphery of the luteal cells next to the capsule of the corpus luteum. They increase in number until at the time of parturition, they are quite noticeable in the cells, being found scattered all through the protoplasm among the red granules. After parturition, there seems to be a more rapid increase in the number of lipoid droplets, which coincides with the sudden decrease in the number of red granules previously noted. With this increase in lipoid content the cells which, from the beginning, have been growing constantly larger, seem to begin to hypertrophy more rapidly. The luteal cells are largest and contain the greatest amount of lipoid about six weeks after parturition. In two more weeks practically all the lipoid has disappeared from the cells and they are beginning to show evidences of degeneration. Third, the phase of regression. This period begins about eight weeks after parturition and lasts for four weeks. By the last of August the corpora lutea have disappeared from the ovaries. This phase is characterized by a fatty degeneration of the luteal cells by an increased vascularization and a connective-tissue invasion.

From these results, the following conclusions were drawn as to the functions of the corpora lutea in the ovaries of spermophiles:

The corpora lutea fix the period of estrus by preventing the development and the ripening of follicles until the time for the next rutting season is at hand.

The corpus luteum is a gland with two internal secretions, both of which have specific effects on the uterus, one bringing about the changes incident to pregnancy and the other effecting the normal involution of the organ. The first internal secretion is represented in the luteal cells during the period of pregnancy by granules which are very similar in their location and staining reactions to the granules in the A cells of the islands of Langerhans, the glands of internal secretion of the pancreas, described by Bensley. The granules of the luteal cells, however, are much larger than those of the A cells, being very easily seen with high powers of the microscope. No mitochondrial granules or filaments could be observed, perhaps because of the abundance of the granulations in the protoplasm. These luteal cell granules are very much like other secretion granules described by various writers as occurring in the secreting serous cells of several glands of the body.

The majority of writers have agreed that there is no fatty product demonstrable in the corpus luteum of several species of animals and man in the very early stages. They all seem to have been of the same opinion that the activity of the ovarian gland of internal secretion begins with the appearance of the lipid droplets in the cells. These lipid droplets were considered by them to be the evidence of the secretory activity of the corpus luteum. Its period of activity would then begin when these droplets begin to appear in the cells, which time varies with different species, but in all seems to be about the time of the fixation of the blastocyst. This activity lasts, they consider, for varying periods in different species. In the rabbit, Cohn, Fraenkel, and Niskoubina consider that it lasts for nine or ten days, when regression sets in about the fifteenth day. Van der Stricht says that in the bat the lipid droplets are in much greater abundance during the second half of the period

of pregnancy and that regressive changes do not begin until the period of pregnancy is over. Miller says there is no neutral fat in the human corpus luteum until regression sets in at birth. Because the first-mentioned group of men found that double ovariectomy did not cause abortion in rabbits after the fifteenth day, and did so earlier than this, they considered this lipoid secretion related to changes in the uterus occurring between the fourth and fifteenth days after coitus, or between the time of the fixation of the blastocyst and the middle of the period of pregnancy.

Van der Stricht seems to have been the first to conceive of the presence of a secretion in the luteal cells prior to the appearance of the lipoid droplets which coexists with them for some time after their appearance. He judges of the presence of this secretion in the cells from its presence in the near-by intercellular spaces and lymphatics. The latter, according to van der Stricht, are the avenues of excretion of both the serous and the lipoid secretions.

In spermophiles, the lipoid product does not begin to appear in the luteal cells until the period of pregnancy is half over and is not very abundant until after birth. As far as these animals are concerned, then, the lipoid product is not the active substance of the corpus luteum which has specific effects on the uterus during pregnancy. This active substance is rather a secretion represented in the cells by the secretory granules previously noted, which are of a very different nature from the lipoid droplets.

The second internal secretion which is represented in the luteal cells by lipoid droplets and which formerly has been considered the secretion which is responsible for the changes occurring in the uterus incident to pregnancy, must be considered, as far as the spermophiles are concerned at least, as having another function. There seems to be some relationship in these animals between the period of greatest abundance of the lipoid product in the cells and the period of regression and atrophy in the uterus. The uterus of the spermophile atrophies very slowly, much more so than in animals that bear several

litters of young every year. The atrophy is not completed until six or seven weeks after parturition, about the time when the lipoid product reaches its crisis of abundance in the cells and begins to disappear. Another result which substantiates the theory that the lipoid secretion brings about the normal involution of the uterus in the very abnormal, even pathologic process which goes on in the uterus following the removal of both ovaries during all but the more advanced stages of pregnancy. During the first half of the period of pregnancy there is no lipoid in the corpora lutea, which, according to this theory, would account for the pathology in the uterus following double ovariectomy. If the ovariectomy is performed late in pregnancy after the lipoid droplets have become quite abundant in the luteal cells, the animal aborts and the uterus undergoes an involution more nearly like the normal, due to the specific effect of the lipoid secretion which is already present in the circulation. Mulon thought the lipoid of the corpus luteum had an antitoxic action toward the poisons elaborated in the development of the fetuses. It would seem more reasonable to suppose that it neutralizes the toxic products produced in normal involution, which would be only a part of its function as a specific agent in effecting this normal involution of the uterus.

It may be added, in closing, that the two luteal secretions are undoubtedly emptied into the blood stream in these animals. An observation of the elaborate capillary network of these structures could lead to no other conclusion. Lymphatic sinuses are demonstrable in the corpora lutea, but they are found only near the capsule in the proximity of the larger blood-vessels. There is no anatomical evidence for concluding that the secretions are carried away by the lymphatics.

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FIGURES

Fig. 1 (experiment 248-16). Fixative, formalin zenker; stain, hematoxylin and eosin. Cross-section through the body of a normal uterus of rut. $\times 12$.
 Fig. 2 (experiment 248-16). Fixative, formalin zenker; stain, hematoxylin and eosin. Cross-section through the horn of a normal uterus of rut. $\times 12$.
 Fig. 3 (experiment 200-15) Fixative, formalin zenker; stain, hematoxylin and eosin. Cross-section through the body of a uterus after the removal of both ovaries. $\times 40$.
 Fig. 4 (experiment 200-15). Fixative, formalin zenker; stain, hematoxylin and eosin. Cross-section through the horn of a uterus after the removal of both ovaries. $\times 40$.



Fig. 5 (experiment 443-15). Fixative, formalin zenker; stain, hematoxylin and eosin. Cross-section through the body of a normal inactive uterus of September 22, 1915. $\times 40$.

Fig. 6 (experiment 446-15). Fixative, formalin zenker; stain, hematoxylin and eosin. Cross-section through the horn of a normal inactive uterus of September 22, 1915. $\times 40$.

Fig. 10 (experiment 346-15). Fixative, formalin zenker; stain, brasilin and wasserblau. Cross-section of an ovary of the late summer. $\times 24$.

Fig. 11 (experiment 219-16). Fixative, formalin zenker; stain, hematoxylin and eosin. Cross-section of a normal ovary of early spring. $\times 20$.

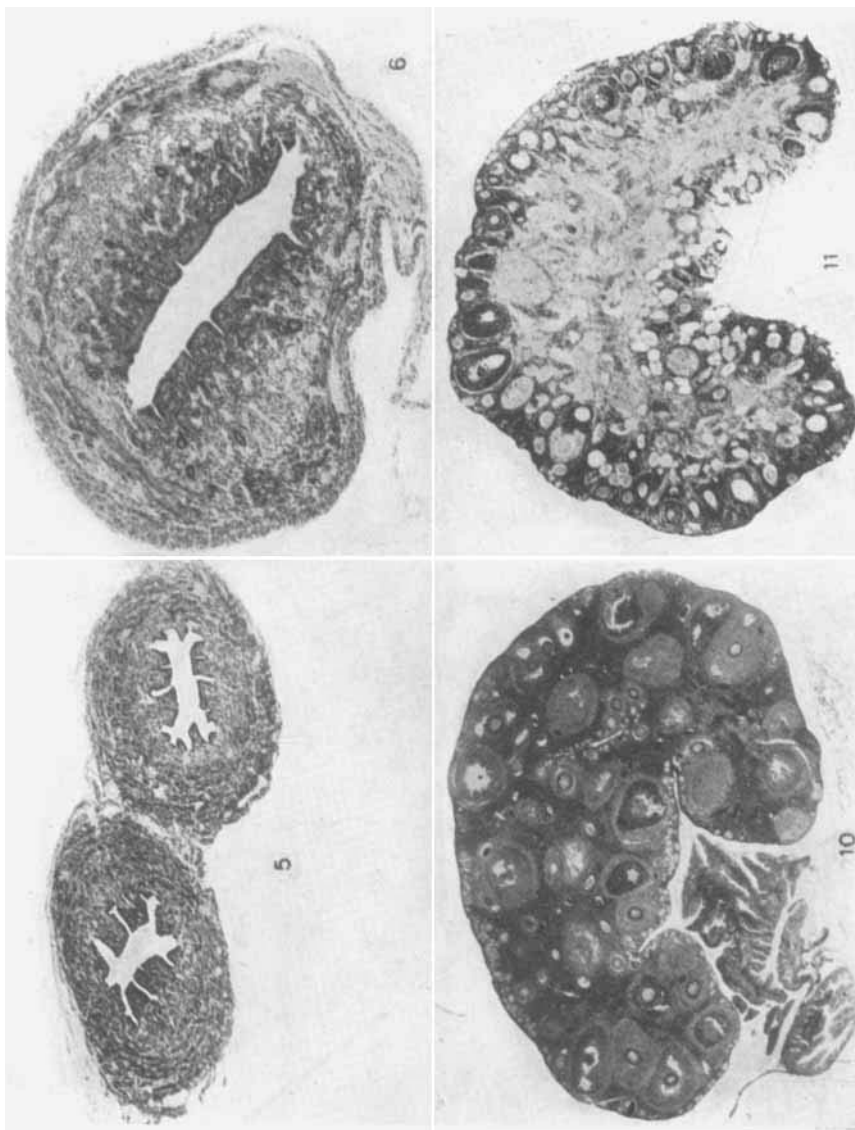
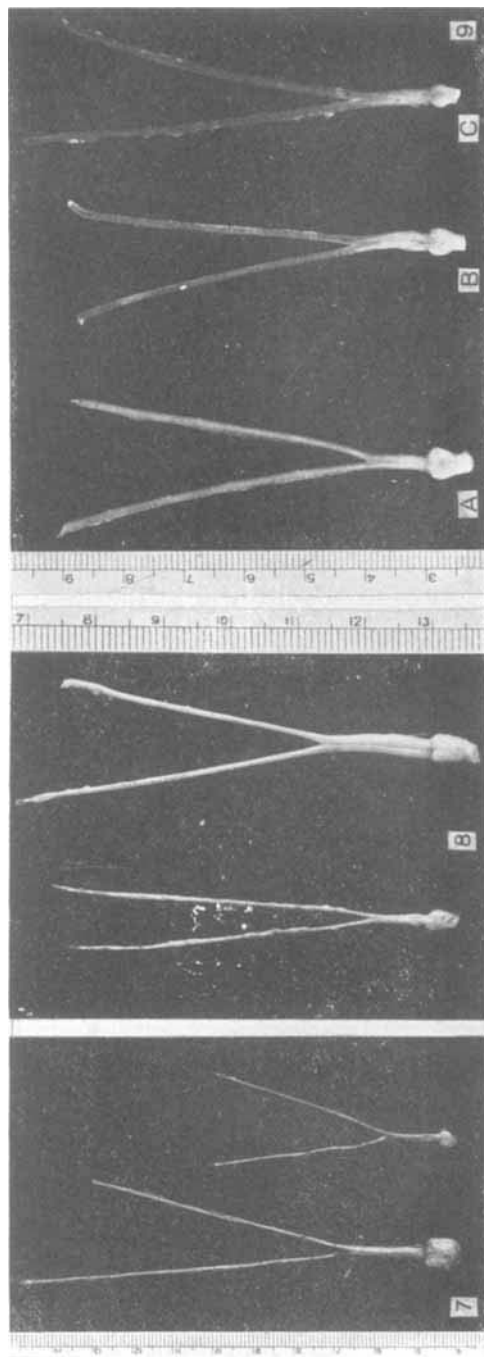


Fig. 7 (experiment 446-15). A uterus after the removal of both ovaries with a normal uterus of rut.

Fig. 8 (experiment 435-15). A uterus after the removal of both ovaries with a normal uterus of rut. Both were laboratory animals.

Fig. 9 (experiment 354-16). A is the uterus of a normal control. B and C are uteri of animals after double ovariectomy.



- Fig. 12 (experiment 246-16). Fixative, formalin zenker; stain, acid fuchsin and methyl green. Cross-section of an ovary of rut. $\times 20$.
- Fig. 13 (experiment 275-16). Fixative, formalin zenker; stain, hematoxylin and eosin. Cross-section of an ovary very early in pregnancy. $\times 20$.
- Fig. 14 (experiment 277-16). Fixative, formalin zenker; stain, hematoxylin and eosin. Cross-section of an ovary early in pregnancy after the corpora lutea have become completely organized. $\times 20$.
- Fig. 15 (experiment 444-16). Fixative, acetic osmic bichromate; stain, acid fuchsin and methyl green. Cross-section of an ovary about eight weeks after parturition. $\times 16$.

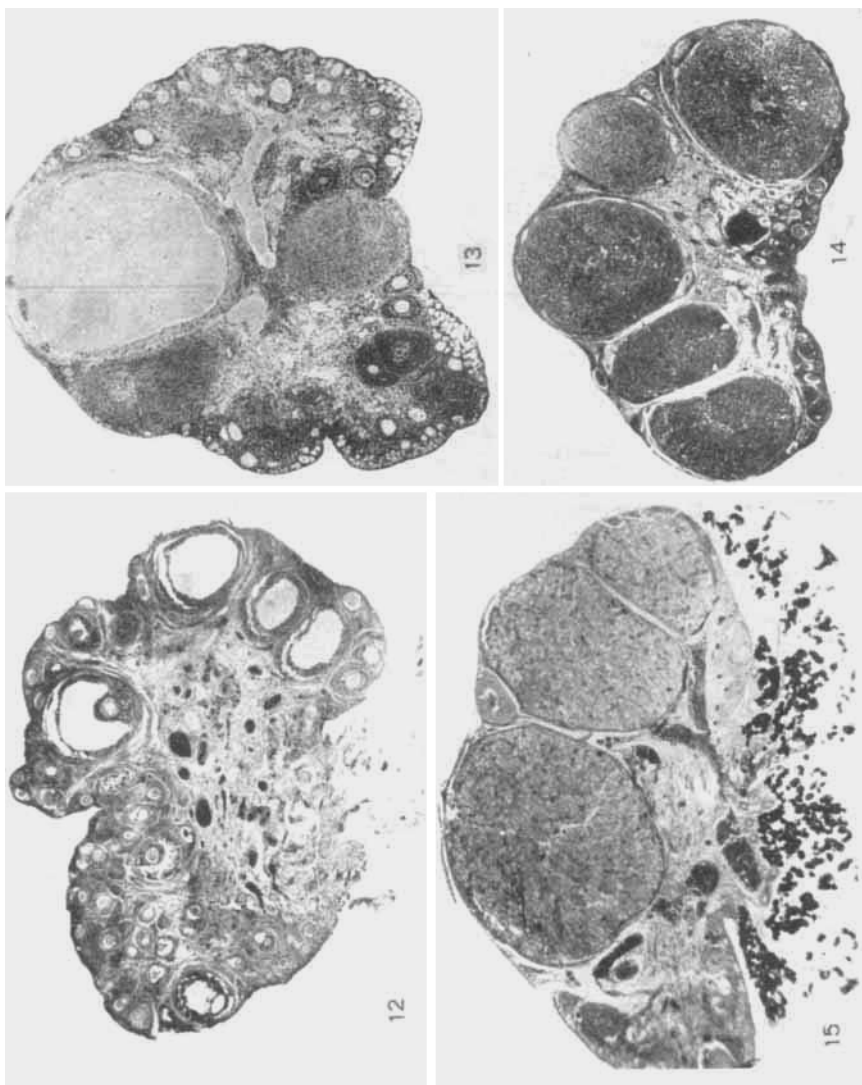


Fig. 16 (experiment 515-16). Fixative, formalin zenker; stain, hematoxylin and eosin. Cross-section of an ovary about twelve weeks after parturition. $\times 20$.

Fig. 17 (experiment 268-16). Fixative, acetic osmic bichromate; stain, acid fuchsin and methyl green. Cross-section of a very early corpus luteum. $\times 64$.

Fig. 18 (experiment 363-16). Fixative, acetic osmic bichromate; stain, acid fuchsin and methyl green. Cross-section of a corpus luteum at parturition $\times 64$.

Fig. 19 (experiment 413-16). Fixative, acetic osmic bichromate; stain, acid fuchsin and methyl green. Cross-section of a corpus luteum six weeks after parturition. $\times 64$.

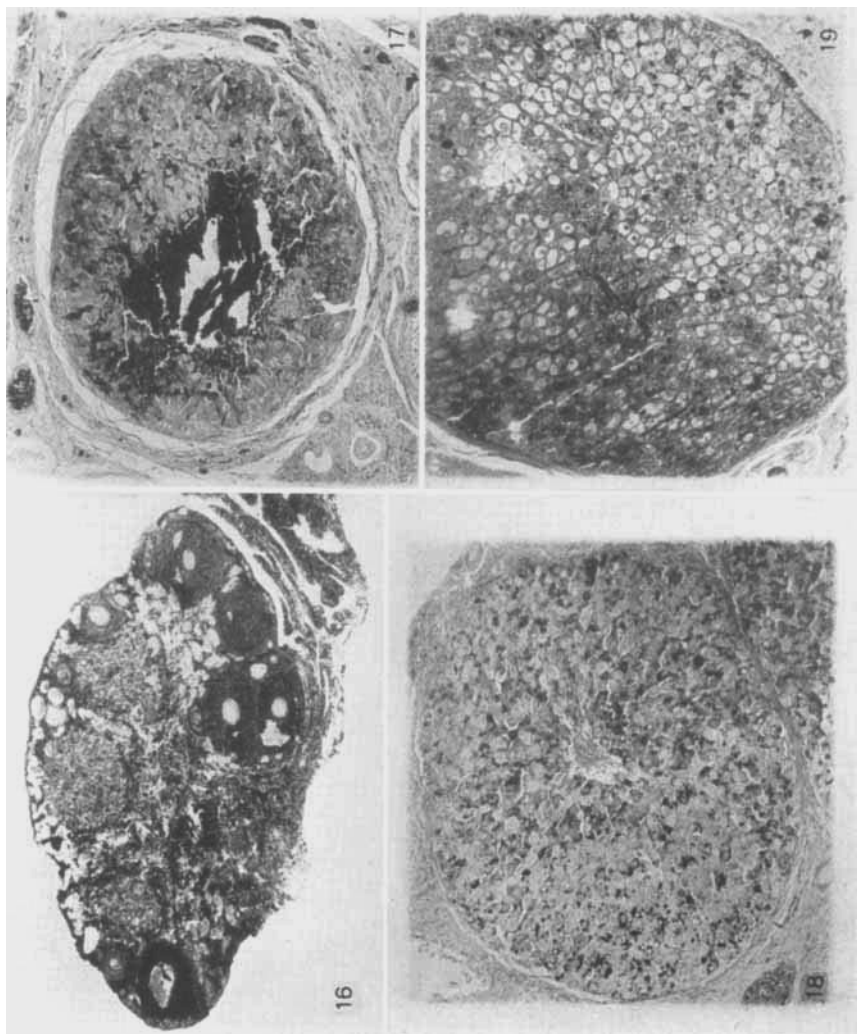
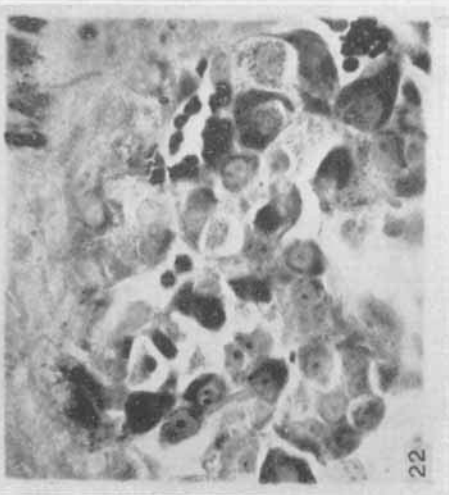
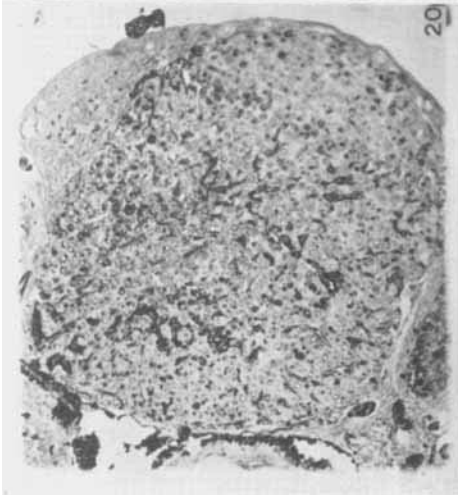
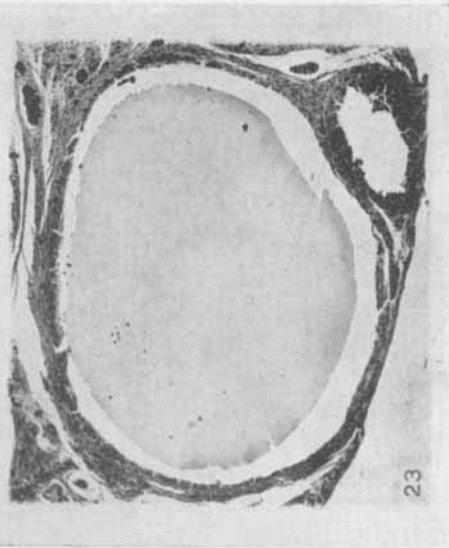
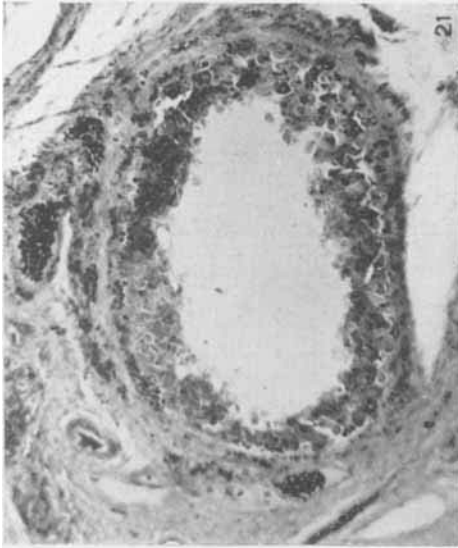


Fig. 20 (experiment 439-16). Fixative, acetic osmic bichromate; stain, acid fuchsin and methyl green. Cross-section of a corpus luteum about eight weeks after parturition. $\times 64$.

Fig. 21 (experiment 268-16). Fixative, acetic osmic bichromate; stain, acid fuchsin and methyl green. Cross-section of an abnormal corpus luteum. $\times 64$.

Fig. 22 (experiment 268-16). Fixative, acetic osmic bichromate; stain, acid fuchsin and methyl green. Section through the wall of an abnormal corpus luteum showing untransformed cells of the stratum granulosum of the follicle persisting among the early luteal cells. $\times 480$.

Fig. 23 (experiment 268-16). Fixative, acetic osmic bichromate; stain, acid fuchsin and methyl green. Cross-section of a corpus luteum cyst. $\times 64$.



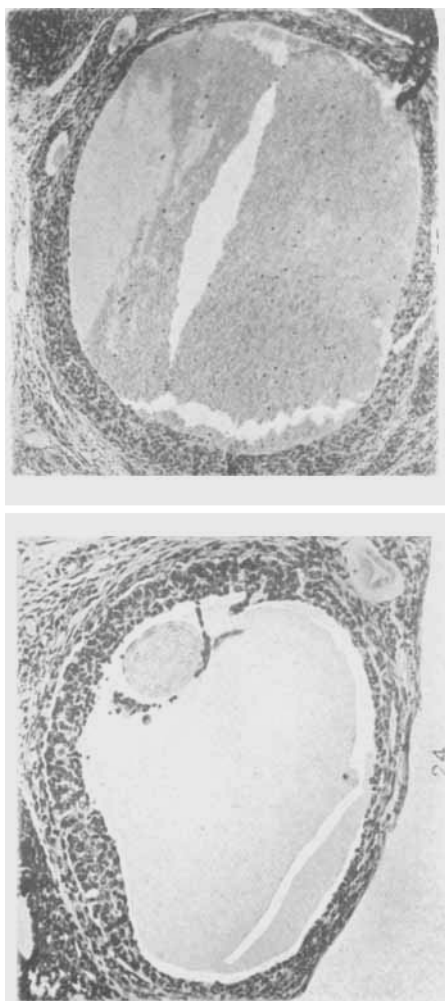
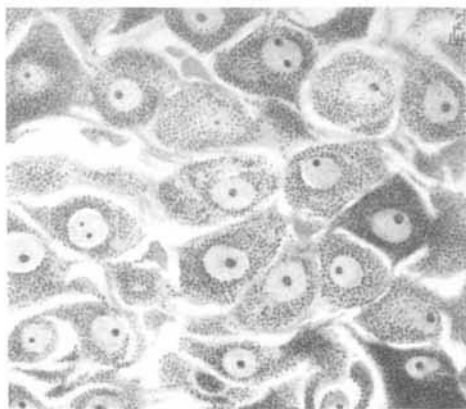


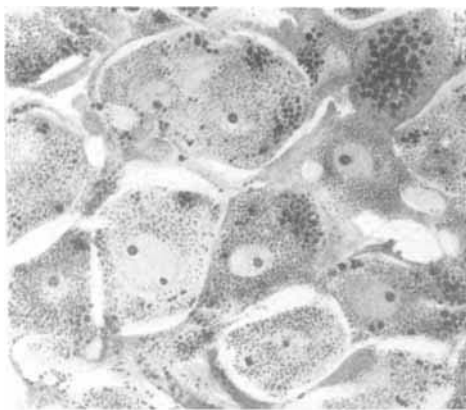
Fig. 24 (experiment 275-16). Fixative, formalin zenker; stain, hematoxylin and eosin. Cross-section of a corpus luteum with a retained ovum. $\times 64$.

Fig. 25 (experiment 293-16). Fixative, formalin zenker; stain, hematoxylin and eosin. Cross-section of a corpus luteum hemorrhagic cyst. $\times 64$.



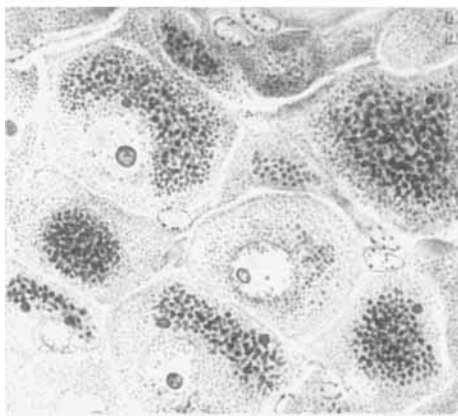
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Fig. 26 (experiment 268-16). Fixative, acetic osmic bichromate; stain, acid fuchsin and methyl green. Portion of very early corpus luteum. (Red-granule stage.) Note luteal cells with so many granules that their protoplasm appears a homogeneous red. Fibro-blasts are forming the connective-tissue framework $\times 960$.



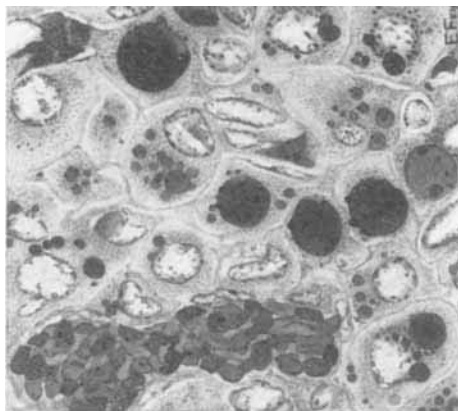
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Fig. 27 (experiment 363-16) Fixative, acetic osmic bichromate; stain, acid fuchsin and methyl green. Portion of a corpus luteum at parturition. Note the black lipid droplets at the periphery of the luteal cells. Compare their size with that of the red granules. There is a well-formed connective-tissue framework. $\times 960$.



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Fig. 28 (experiment 421-16). Fixative, acetic osmic bichromate; stain, acid fuchsin and methyl green. Portion of a corpus luteum six weeks after parturition (lipoid stage). Note the size of the cells, the active-looking nuclei, and the abundance of lipid droplets. $\times 960$.



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Fig. 29 (experiment 515-16). Fixative, acetic osmic bichromate; stain, acid fuchsin and methyl green. Portion of a corpus luteum about twelve weeks after parturition (regression stage). Note vascularization and fatty degeneration. The nuclei have taken the acid fuchsin stain which is an evidence of degeneration. $\times 960$.