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THE EXISTENCE OF A TYPICAL OESTROUS CYCLE IN THE GUINEA-PIG—WITH A STUDY OF ITS HISTOLOGICAL AND PHYSIOLOGICAL CHANGES

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ONE TEXT FIGURE AND NINE PLATES

1. INTRODUCTION

The existence of a more or less regular and definite oestrous cycle has been recognized in a number of mammals, particularly among the different classes of primates, carnivores, ungulates and insectivores. Yet very little is actually known or understood regarding the oestrous cycles and heat periods of a great many other very common mammals. Strangely enough, our knowledge of the sexual rhythm in the guinea-pig is much confused and not properly understood despite the great number of breeding experiments and the several studies of the sexual conditions which have been performed on this animal.

While conducting an extensive breeding experiment with guinea-pigs for the past several years it has become more and more desirable to know their exact oestrous periods. 1 A careful study of the existing literature bearing on this subject serves merely to produce uncertainty and confusion regarding their

1 Throughout this paper we have used the terminology proposed by Heape, Quar. Jour. Mic. Sc., vol. 44, 1900, and adopted by Marshall and others. Anoestrous period or anoestrus, period of rest in the female; prooestrus, the first part of the sexual season; oestrus or oestrus, especial period of desire in the female; metoestrus, the short period when the activity of the generative system subsides and the normal condition is resumed in case conception did not occur; dioestrus, the short period of rest which in some mammals lasts only a few days. Such a short cycle as we shall describe in the guinea-pig consisting of four periods the prooestrus, oestrus, metoestrus and dioestrus is known as a dioestrous cycle.
ovulation times and heat seasons. The reason for such a lack of knowledge is that these small rodents do not reveal in a very evident manner the existence of their typical sexual rhythm as do many mammals of other classes.

The guinea-pig never, or only in rare cases, shows an external flow from the vagina, and there is no easily noticeable change in the appearance of the external genital organs during the different periods of sexual activity. The only expression generally observed of the sexual condition or heat period in the female is her willingness to accept the male, and this sign is, of course, only manifested when a male is present and a copulation takes place. The copulation then brings about the disturbing factor of pregnancy and the observation of the return of the heat period is prevented. The practical difficulties in observing successful copulation in these animals makes the study of their sexual conditions still more difficult.

Marshall ('10), in a recent summary has stated the case as follows:

It is difficult to determine the length of the prooestrums and oestrus in rodents, since the external changes which characterize these conditions are comparatively slight. Heape says that the prooestrus in the rabbit lasts, probably, from one to four days. At this time the vulva tends to become swollen and purple in color, but there is no external bleeding. The same may be said of the rat and the guinea-pig; but, in the experience of the writer, it is generally impossible to detect the prooestrous condition in either of these animals with absolute certainty.

It must be recalled here that Marshall has devoted a great deal of study to this subject.

The difficulty in observing signs of heat in the guinea-pig has led a numbers of workers during the past fifty years to a study of the ovaries in order to establish the ovulation cycle. The results of such studies, as we shall point out beyond, are inaccurate and confusing in all cases.

Recognizing the above state of affairs, we determined to ascertain whether by a more minute examination of the genital organs of the female it might not be possible to observe an oestrous cycle. In order to examine the vagina thoroughly we have in-
DIOESTROUS CYCLE IN THE GUINEA-PIG

introduced a small nasal speculum which facilitates a clear view of the interior and a smear is made of any fluid that may be present.

A microscopic study of these vaginal fluids, to be described in the following pages, has shown that the guinea-pig possesses a perfectly regular and typical dioestrous cycle. And further, the surprising fact that the composition of the fluids is exactly comparable to the menstrual fluid taken from so high a mammal as the monkey. Heape, (9'99), states that the menstrual fluid of the monkey contains a mucous secretion of the uterine glands, blood corpuscles, particles of stroma and epithelium from the uterus and the vagina and leucocytes. All of these elements are present in the fluid from the vagina of the guinea-pig during heat though the relative amounts differ from those in the monkey and the fluid is rarely sufficiently abundant to be recognized on the vulva.

The great advantage of this simple method of examination for the study of the oestrous cycle in these mammals which show no external signs of heat is evident, and we trust that the method may prove useful to those who find it necessary or desirable to know accurately the sexual periods in animals used for experimental breeding.

Having begun a study of the vaginal smears from guinea-pigs we have been led to a more complete consideration of the uterine changes which alter the composition of these smears, and finally to an investigation of the changes in the ovary and the process of ovulation and corpus luteum formation which accompany the activities on the part of the uterus. The present contribution comprises the results of these investigations.

2. CONSIDERATION OF THE LITERATURE ON OVULATION IN THE GUINEA-PIG

It has been recognized for more than half a century that the guinea-pig comes into heat very quickly after giving birth to a litter of young. This period immediately following parturition has been the starting point for the great majority of studies on the sexual behavior of this animal and it has been demonstrated
frequently by such studies that ovulation takes place a few hours after parturition, the female accepting the male at that time. These facts are generally admitted but the most varied opinions prevail regarding the times of the subsequent ovulations, when conception does not occur soon after parturition.

The question whether ovulation in the guinea-pig is spontaneous or dependent upon copulation has often been raised by various workers. The majority are of the opinion that ovulation is, or may be, spontaneous although influenced by copulation, and that there is no definite regularity or typical periodicity in the ovulation cycles.

Bischoff, was one of the oldest advocates of the theory of spontaneous ovulation. In a special paper devoted to the study of this problem in 1844, and later in a study of the development of the guinea-pig ('52), he defended the view that the guinea-pig, like all other mammals, has a spontaneous ovulation. Bischoff states that the mature eggs reach the oviducts through the rupture of the greatly distended Graafian follicles during the first twenty-four hours following parturition. This fact, he points out, had previously been observed and was generally accepted by the earlier investigators with the exception of Schulz, 1829, who failed to recognize a heat period before the fifteenth day after parturition, and sometimes even to the forty-ninth day.

According to Bischoff copulation takes place within three hours after parturition. He agrees with the earlier statements of Aldrordandi, Legullois, Fraser and Schultz regarding the length of gestation, or period of pregnancy, as being about nine weeks, which is very nearly correct, sixty-two days being the normal length of time. He held that the return of the heat period did not follow any regular periodicity: "Wenn die Befruchtung unmittelbar nach der Geburt verhindert wird, so scheint die Wiederkehr der Brunst an keine ganz bestimmte zeit geknüpft zu sein, sondern von Umständen der Individualität, des Alters, der Jahreszeit, der Fütterung, etc., abzuhängen." In four cases in which the females were prevented from copulating for some time after they gave birth to young a copulation occurred 40, 50, 51 and 51 days after the birth.
Reichert ('61), confirmed the observations of Bischoff regarding the existence of a heat condition and an ovulation process shortly after parturition—Reichert found many fertilized eggs in the oviducts 18, 19, 20 and 22 hours after parturition which showed by their condition that copulation must have taken place many hours before. His opinion is that the Graafian follicles rupture about twelve to fourteen hours after copulation.

Many recent authors have incorrectly stated Reichert's position and assert that he claimed ovulation in the guinea-pig not to be spontaneous but to depend upon copulation. This is due to a misinterpretation of Riechert's ideas, originated by Bischoff in his second paper, 1870, which is chiefly an answer to Reichert's arguments. No doubt many of the incorrect notions regarding Reichert's position have resulted from authors reading this paper by Bischoff without referring to Reichert's own paper for his exact position.

Reichert explains his position very clearly as follows:

Es wäre wünschenswerth die Zeit genau angeben zu können, in welcher das Ei nach der Begattung aus dem Graaf'schen Follikel ausgestossen wird um die Einwirkung der Begattung auf das Austreten der Eichen bemessen zu können. Es ist zwar zu keiner Zeit auch nur wahrscheinlich gewesen, dass das bis zu den Eierstöcken vordringende Sperma irgend wie direkt die Lösung der Eichen oder richtiger das Bersten der Graaf'schen Follikel bewirken könne. Es ist ferner die bei anderen Thieren bekannte Tatsache, dass reife, selbst eingekapselte Eier auch ohne vorausgegangene Begattung gelöst werden durch Bischoff's Versuche auch für die Säugethiere ausser Zweifel gesetzt. Das Bersten aber der Graaf'schen Follikel erfolgt unter vermehrtem Zudrang des Blutes zu denselben und in Folge der starken Vergrösserung ihres Inhaltes, des gallerartigen Fluidums und auch der Zellen der Membrana granulosa, sowie des Discus proliferus; das Eichen selbst vergrössert sich in der Brunstzeit wenig oder vielleicht gar nicht; dasselbe löset sich nicht, es wird, so zu sagen, von der Mutter ausgestossen. Daraus geht ferner hervor, dass die Begattung mit ihren aufregenden Wirkungen auf das Mutterthier, insbesondere auf den Zudrang des Blutes nach den geschlechtstheilen, einen sehr wesentlichen Antheil an Bersten des Graaf'schen Follikels und so also an der Befreiung des Eichens haben kann und haben muss.

This quotation shows that Reichert did not deny the existence of a spontaneous ovulation, but claimed that copulation had an important influence on the process of breaking the Graafian
follicle. He also admits that the existence of a spontaneous ovulation is proven for mammals by the experiments of Bischoff. The difference between the opinions of Reichert and Bischoff is not that the one denies and the other admits the existence of a spontaneous ovulation, but that the one believes copulation to exert an important influence over ovulation, while the other holds that such an influence, if it exists at all, is not really great. Leo Loeb ('11), who has studied the problem of ovulation in the guinea-pig very recently, still claims that copulation exerts an influence over the time of ovulation. That Bischoff also finally thought that there might be an influence on ovulation as a result of copulation is shown by the following remark from his second paper:

Sie meinen nur, es gäbe doch auch noch Erscheinungen, welche zeigen dass die Männchen und die Begattung auch einen Einfluss darauf ausüben. Wenn dieser Einwurf so gehalten wird, dass er (nämlich Reichert) zugesteht, die Erscheinung an und für sich ist vollkommen unabhängig von dem Männchen, dieses aber kann doch förderlich darauf einwirken, so wird dadurch nicht mehr gesagt, als wenn man sagen würde, eine gute Ernährung, günstige Verhältnisse der Temperatur und des Klimas haben ebenfalls einen Einfluss auf die Reifung und Lösung der Eier, und diese vielleicht einen noch größeren als die Gegenwart des Männchens und die Paarung. Und wirklich steht auch gar Nichts entgegen, dem Männchen in diesem Sinne einen Einfluss einzuräumen.

Hensen ('76), also recorded that in the guinea-pig a copulation takes place shortly (about one hour) after parturition and six to ten hours later an ovulation follows. In cases where this first ovulation was not followed by pregnancy he recorded another ovulation 17, 18, 35 and 37 days later in the different cases. The duration of pregnancy he found to be 66 days—This along with Bischoff's record of an ovulation 43 and 44 days after parturition made it difficult to admit that the guinea-pig had regular periodical ovulations every eighteenth day. Hensen, therefore, believed that the guinea-pig probably did not have a sharply expressed periodicity—"Es scheint also die Brunstzeit der Meerschweinchen nicht scharf periodisch zu sein."

Rein ('83), again reports the existence of a condition of heat in the guinea-pig within twenty-four hours after parturition.
Regarding the occurrence of further heat periods Rein failed to observe any regular periodicity. "Im Eintreten der Brunst habe Ich keine Periodicität bei den Versuchstieren bemerkt."

The foregoing studies are chiefly of historic interest yet they show that these earlier workers recognized the occurrence of ovulation shortly after parturition and were uncertain or confused regarding the time or periodicity of subsequent ovulations. Little of definite value has ever appeared in the literature to further clear up the last point. We may now briefly consider the more recent contributions which bear on the subjects of ovulation and oestrous in the guinea-pig.

Rubaschkin ('05), gives a detailed description of the sexual conditions in the guinea-pig. He also recognized, as did the earlier observers, that a condition of heat followed shortly after parturition. In almost all females killed a few hours (up to fifty hours) after the birth of a litter an ovulation had occurred. He never observed ovulation as early as five hours after parturition though he found fertilized eggs in the oviducts as early as fifteen and seventeen hours after. Copulation occurs directly after having given birth to young but for later heat periods Rubaschkin was unable to demonstrate any regular periodicity. "Es ist mir nicht gelungen, eine bestimmte Frist für das Auftreten der Brunst festzustellen."

He did observe, however, that in some animals ten to twelve days after having given birth to young the entrance of the vagina showed some signs of heat activity. "Öffnung der Vagina und Röthung der Vaginalöffnung." He claimed that heat ceased to recur after the month of October, at least when the animals were kept in a cold place. The duration of pregnancy was reported by Rubaschkin in three cases to be ten weeks.

Rubaschkin thus failed to recognize the regular oestrous cycles in these animals and also states the gestation period somewhat too long.

Königstein in 1907 recorded the results of observations made on eighteen rats, one guinea-pig and five rabbits. He states that in the rodents heat occurs immediately after giving birth
to the young and lasts for twenty-four hours. Copulation only takes place during heat and if pregnancy fails to occur at the period just after parturition the next heat periods follow after intervals of three to four weeks.

Königstein also examined sections of the genital tract giving some important histological descriptions based chiefly on the rat—we shall return to a consideration of these observations in connection with our findings on the guinea-pig.

Bouin and Ancel ('10), are of the opinion that guinea-pigs do not have a spontaneous ovulation, the process being dependent upon copulation. However, these workers seem to have reached this opinion from observations made on rabbits which were the chief objects of their study. Despite the striking classification which they make of animals having a spontaneous ovulation (monkeys, dogs, horses, cows) and those not having spontaneous ovulation (rabbits, guinea-pigs, cats) they admit that rare exceptions are possible and that in any animal an ovulation might occur independently of a copulation.

C'est là un fait général, mais soumis à des exceptions rares. Il peut arriver que des animaux à ovulation non spontanée opèrent la déchirure de leurs follicules mûrs en l'absence de tout rapprochement sexuel. Nous-même et M. Villemain avons constaté le fait chez le Lapin. M. Mulon vient également de l'observer chez le cobaye.

During the past several years Leo Loeb ('11 a, b) has contributed extensive and valuable studies bearing upon the sexual cycles in guinea-pigs, considering in particular the function and importance of the corpus luteum. Loeb examined a great number of ovaries at different periods, beginning with the time of the first copulation after parturition and concludes, as Rubaschkin 1905 and others had previously done, that the cyclic changes in the ovary take place independently of copulation. Loeb thought that the ovulations followed no exact and regular periodicity in all cases. The periodicity differed among the individuals and was influenced by certain external factors, particularly copulation. To quote:

The exact time at which the new ovulation occurs varies however somewhat in different animals, ovulation occurring earlier in some ani-
mals than in others. In some cases it can be hastened through certain external factors, especially copulation, but in the large majority of cases it occurs sooner or later even without a preceding copulation.

He holds that eight days after ovulation large follicles are present in the ovary but sometimes ovulation may not occur for twenty or twenty-four days.

The 'sexual period,' period between two ovulations, according to Loeb lasts usually twenty to twenty-five days instead of being about two weeks, the time necessary for mature follicles to appear. This delay in ovulation in spite of the presence of mature follicles within eleven to thirteen days, he believes is due to a mechanism in the ovary which prolongs the cycle, the corpus luteum begins this mechanism. The corpus luteum degenerates after a period of growth lasting from seventeen to twenty days and thus ovulation occurs about once in three weeks. We shall show beyond by a demonstration of the oestrus cycles, that Loeb's deductions drawn from studies of the histology of the ovary are incorrect and, therefore, cannot be employed for determining the ovulation cycles in these animals.

Loeb further finds that when the corpus luteum is cut out immediately after an ovulation, the next ovulation occurs soon after mature follicles are developed—about thirteen to fifteen days. Under these conditions the normal sexual cycle is re-established—but even here his periods are not exact being somewhat shorter than are actually normal.

The very varied time results obtained by Loeb may be given as follows: First, no ovulation has been found under normal conditions before the fifteenth day after the last copulation. Second, in a group of thirty-eight guinea-pigs killed fourteen days and eighteen hours and nineteen days and fifteen hours after the last copulation—one had ovulated about the sixteenth day, another the eighteenth and another at the nineteenth day, while the remaining thirty-five had not yet ovulated. Third, in a lot of twenty-two guinea-pigs, twenty to twenty-six days after the last copulation, one had supposedly ovulated at the eighteenth day, four at the nineteenth day, one at the nineteenth to twentieth day, one at the twenty-third and one at the
twenty-fifth day and a half, while fourteen had not yet ovulated. Fourth, in a lot of six animals killed twenty-six to thirty-four days after the last heat period or copulation, only one had already ovulated.

A recapitulation of these results may be stated thus: under fifteen days no ovulation; sixteenth day, one; eighteenth day, two; nineteenth day, five; nineteenth to twentieth day, one; over fourteen days and eighteen hours and nineteen days and fifteen hours, thirty-five; twenty-third day, one; twenty-five and a half days, one; over twenty to twenty-six days, fourteen; twenty-sixth to thirty-fourth day, one; over twenty-six to thirty-four days, five. These figures as Loeb points out do not show any regularity in the occurrence of the ovulation process and, as we shall show beyond, they demonstrate how difficult or almost futile it is to attempt to solve the sexual cycles of an animal by a simple study of the ovarian conditions found on killing the animals at different periods. To anticipate slightly, the figures above show that Loeb entirely failed to discover the presence of a definitely regular periodicity in the ovulation process of the guinea-pig. Thus his examinations though much more thorough were as ineffective as those of the previous workers.

In 1913, Lams gave an instructive review of this problem. He again confirmed the long known fact that a heat period followed parturition in the guinea-pig. The copulation was found to take place within two to four hours after the delivery while ovulation occurred from twelve to seventeen hours after. Thus copulation generally preceded ovulation without being its cause. Lams gives no data on the occurrence of later ovulations but devotes himself to a detailed account of fertilization and the early development of the egg.

A consideration of the sum total of these various observations compels the admission that the opinions concerning the oestrous cycles in the guinea-pig are highly confused and totally unsatisfactory for application in exact breeding experiments. The one fact which presents itself was established by the earliest workers and confirmed by all subsequent studies—that is, that a period of heat follows within the first few hours after partur-
tion. In the literature only Schulz ('29), according to Bischoff ('52), denies this fact.

No typical rhythm has been established so far for the subsequent ovulations in the guinea-pig. All observers who have examined a number of ovulations found great differences in the supposed periods of time intervening between two ovulations as we have reviewed in detail above. The numbers give no evidence of a regular periodicity in the ovulation process but on the contrary would lead one to believe that the greatest irregularity in time intervals was the rule.

On the other hand, really no observations exist to show anything like the occurrence of periodic changes in the uterus and vagina accompanying the return of the heat periods. Such a thing as a regular oestrous or preoestrous flow is completely undiscovered in these animals.

Königstein ('07), has examined sections of the uterus and vagina of a guinea-pig, and Blair-Bell ('08), has drawn comparisons giving many interesting observations, but they failed entirely, or made no attempt, to observe the regular reappearence of a definite order of changes in either the uterus or vagina of this animal.

3. OBSERVATIONS ON THE LIVING ANIMALS

During the past six years we have been using guinea-pigs in an extensive breeding experiment and it has become more and more evident as our work goes on that the existing notions of the ovulation periods in these animals are of no practical value, or are practically incorrect. In a number of the experiments it became important to know accurately when the females ‘came into heat’ and when ovulation took place. We had concluded, from numerous observations as well as theoretically, that the female guinea-pig very probably had a definitely regular and periodic sexual cycle if it could be worked out exactly. On account of the need of this exact information, we have studied the oestrous cycle in these animals during the past eighteen months.

Most other attempts at a solution of this problem have centered in a study of the ovary which necessitated either its removal
by operation or the killing of the animal. In either case the procedure brought to a conclusion the observation or experiments on the ovulation cycles in that specimen. Recognizing, on the other hand, that no thorough investigation of the uterus and vagina in the living female had been made, it occurred to us that possibly oestrous changes might take place even though they are so feebly expressed as not to be noticeable on casual observation. The absence of an apparent oestrous or prooestrous flow from the vagina of the guinea-pig has, as before mentioned, no doubt been the chief reason for the general lack of knowledge of the oestrous cycle. It was therefore determined to make a minute examination of the contents of the vaginae of a number of females every day for a long period of time, to ascertain whether a feeble flow might exist although insufficient in quantity to be noticed at the vaginal orifice or vulva.

The observations were made by using a small nasal speculum which was introduced into the vagina and the arms opened apart by means of the thumb screw. The speculum permits an examination of the entire surface of the vaginal canal. In this way the vaginae of a number of virgin females have been examined daily and smears made from the substances that happened to be present in the lumen.

By the use of such a simple method, it was readily determined after examining the first lot of animals for a few months that a definite sexual period occurs lasting for about twenty-four hours and returning with a striking regularity every fifteen or sixteen days. During this twenty-four hour period the vagina contains an abundant fluid which is for about the first half of the time of a mucous consistency. The vaginal fluid then changes into a thick and cheese-like substance which finally becomes slowly liquified and serous. This thin fluid exists for a few hours and then disappears. Occasionally toward the end of the process a slight trace of blood may be present giving the fluid a bloody red appearance, otherwise it is milk-white or cream-color.

According to the changes in appearance and consistency of the vaginal fluid, one may distinguish four different stages. The
first stage having a mucous secretion, a second stage the cheese-
like secretion, a third stage with the fluid becoming serous and a
fourth stage, not always recognized, during which a bloody dis-
charge is present. The duration of these several stages is sub-
ject in the different animals to individual variations. The first
stage, however, is generally longest and lasts from six to twelve
hours or even more and during this time there is a gradually in-
creasing quantity of the mucous secretion which at its height is
very abundant and fills the entire lumen of the vagina. The
second stage is shorter, lasting from two to four hours, and
passes gradually over into the third stage which lasts from four
to six hours. The fourth stage is the shortest, only about one
to two hours long, and for this reason it is often missed in exam-
ining the animals during the periods. It is also possible, as
mentioned above, that the fourth stage may not typically exist
in all individuals and the quantity of blood present is very dif-
ferent in the different specimens. The succession in which these
stages follow one another is remarkably definite. We have
never observed any change in the typical sequence of the stages
and the time consumed by the entire process is generally as
stated about twenty-four hours.

A macroscopical examination of the uterus and vagina during
this period of sexual activity shows the entire genital tract to be
congested. The vessels to the ovary, uterus and vagina are
large and conspicuous, the uterine horns and the vagina are
slightly swollen and inflamed. However, as soon as this short
period of activity is over, the congestion disappears and the
uterus and vagina take again their normal pale aspect. At the
same time the vaginal fluid diminishes and the vagina, especially
during the first week after this sexual activity, is as clean as
possible showing none of the secretion. The external vaginal
orifice, which during the period of activity is more or less open
actually showing in a few cases a little fluid or some blood, closes
and becomes less accessible after the period.

During the second week following oestrus a little mucous dis-
charge begins to appear in the vagina and increases progressively
indicating that the new period of activity is nearer and nearer
approaching. The orifice of the vagina is sometimes open during this stage and thus explains why this sign, which was observed before, does not make it possible to detect the actual time of the regular oestrous activity. Rubaschkin has observed the opening of the vagina ten to twelve days after parturition, but this period of time is certainly too short to indicate the return of heat. We agree with Rubaschkin in stating that during the ovulation the vagina is open, but we do not admit that the opposite is also true, that the opening of the vagina indicates unmistakably the return of the ovulation process.

4. MICROSCOPIC STRUCTURE AND CHANGES OF THE VAGINAL FLUID

A microscopical examination of the smears prepared from the vaginal fluid taken at the several stages separated above shows decidedly typical differences. The cellular character of a smear made at a given stage differs from the cellular make-up of all other stages. The relative numbers of various cell types in the fluid at different stages are so definite that one with a little experience may diagnose the exact sexual stage of the animal concerned solely by an examination of the smear.

A photomicrograph from a smear of the vaginal content during the first stage of mucous secretion is shown by figure 1. This mucous fluid is seen to contain an abundant mass of cells which, as shown in the figure, are of a squamous type with very small pyecotic nuclei sometimes broken into pieces. The cell protoplasm is also greatly degenerated having only a weak affinity for the plasma stains and exhibits a reticular structure. These cells derived from the wall of the vagina (fig. 17) characterize by their presence and great superiority in numbers this first stage. There are, however, to be seen particularly toward the end of the first stage a certain number of elongate, cornified cells without nuclei, which are desquamated from the more external portions of the vagina. These cells contrast in appearance with the first type cells since in smears stained with haematoxylin and eosin they present a decidedly red color, while the abundant first type cells are almost grey. The red cells rather
serve to indicate an intermediate period between the first and second stages or periods of the flow, and may really be found during both stages but particularly at the end of the first and beginning of the second stage. In addition to these two kinds of cells other types may also be found in a first stage smear but they are never present in such abundance nor are they so typical as the two just mentioned. All of the cells float freely in the mucus without assuming any definite arrangement.

During the second stage the vaginal fluid is filled with enormous numbers of cells which cause the cheese-like consistency of the discharge at this time. These cells illustrated by the photomicrographs, figures 2, 3 and 4 at three different magnifications, are derived from the upper portions of the vagina with a few from the uterus and they maintain to a higher degree the original or healthy architecture of an epithelial cell. The nuclei are fairly well preserved showing only slight signs of degeneration. The protoplasm has not greatly deteriorated and gives a good staining reaction thus differing from the grey-staining first stage cells. The cells are present in innumerable quantities forming the thick cheesy substance while the mucous secretion diminishes more and more until it almost disappears. This stage is of short duration.

The third stage begins with the liquefaction of the cheesy mass. A microscopical examination shows that the cells of the second stage become less and less numerous, while a great number of polymorphonuclear leucocytes appear among them (figs. 5 and 6). When the end of this process is reached almost every one of the cells has become isolated from others of its kind and lies in the midst of a number of leucocytes. The apparent action or effect of the leucocytes is to dissolve or digest the desquamated epithelial cells and this dissolving effect is not only noticeable on cells surrounded by the leucocytes but in some cases the leucocytes dissolve their way into the interior of the cell-bodies (figs. 7 and 8). These appearances are not due, as might possibly be supposed, to the cells having devoured the leucocytes. This destructive influence of the leucocytes begins, as will be described later, before the desquamated epithelial cells have fallen
away from the wall of the uterus and vagina (figs. 15, 16 and 17). But it probably continues also after the cells are free in the lumen of the vagina. The dissolving power of the leucocytes, which probably causes the liquefaction of the cheesy mass of epithelial cells is shown very well when leucocytes are seen within a cell and the nucleus is beginning to dissolve. The nucleus is apparently digested and dissolved by coming in contact with the leucocyte without being at all engulfed or enclosed within the smaller body of the leucocyte.

As the third stage approaches its end the material within the vagina is a thin fluid containing a great number of leucocytes as well as many epithelial cells of the second stage some of which contain leucocytes within their bodies. Such leucocyte containing cells are strikingly typical of the third stage. The leucocytes within these cells as would be expected very soon show signs of degeneration never staining so clearly as the free outside ones.

The fourth stage shows the same condition as the preceding but often at this time a slight hemorrhage takes place, though this does not always occur. A microscopical examination of the hemorrhagic fluid shows in addition to the great number of red blood corpuscles, a large number of leucocytes and also desquamated cells of the second stage, some of which are penetrated by leucocytes (fig. 9). Sometimes red blood corpuscles are enclosed within the bodies of the leucocytes and digested, this is probably a truly phagocytic action and not entirely the same as their dissolving effect on the neighboring epithelial cells within the fluid.

The presence of the leucocytes is not alone confined to the heat period but an abundant quantity of them is also to be found in the lumen of the vagina during the dioestrum. The only time that leucocytes are absent from the vaginal lumen is during the first and second stage described above at the beginning of the oestrus. Throughout the first week after heat the little fluid which exists in the vagina contains chiefly leucocytes and a few atypical desquamated cells. During the second week the number of epithelial cells increases more and more and among these
atypical cells there may exist isolated cells of the first or of the second stage type.

At the fourteenth and fifteenth day the number of first stage cells already described begins to increase gradually and the growing proportion of these cells indicates the approaching new period of heat.

5. THE OESTROUS RHYTHM

The periodical return of a typical flow showing the above described macroscopical and microscopical details, was found to be very regular in twenty-six virgin females examined during different seasons of the year. Table I shows the results of this examination. As this table indicates, all the females examined were virgin thus eliminating any chance of modification which might be due to the act of copulation. Their ages ranged between three and a half and fifteen and a half months during the time of examination. The female guinea-pig is sexually mature at about three months old. Almost every animal, as the table shows, was examined for a length of time covering several oestrus periods. In the sixty-seven periods examined altogether the vaginal flow returned regularly every fifteen to seventeen days with an average of 15.73 days interval between the beginning of periods.

This table contains nine oestrus periods for operated animals from which one ovary was removed. The operation was done to determine whether any decided alteration in the oestrus would result after the loss of one ovary. The animals 1080♀ and 1102♀ were semi-spayed during the time of examination given in the table. In the animal 1080♀ the first heat period following operation came at the sixteenth day after the last period but in the animal 1102♀ the first heat period following operation came on the fourteenth day after the last heat, a little earlier than it should come under normal conditions. The three heat periods following this came, however, very regularly every sixteenth day.

The animals 867♀, 923♀, and 1069♀ were semi-spayed a considerable time before the beginning of the examination and
# Table 1

Records of 26 Guinea-pigs Examined Daily for Their Oestrous Conditions

| Animal | Condition | Examine from ages | Doeestrus cycle in days | Total days | Jan | Feb | Mar | Apr | May | June | July | Aug | Sep | Oct | Nov | Dec | Remarks |
|--------|-----------|-------------------|-------------------------|------------|-----|-----|-----|-----|-----|------|------|------|-----|-----|-----|-----|-----|---------|
| 746♀ | virgin    | 4-6 months        | 15, 15                  | 2 30 15    | +   | +   |     |     |     |      |      |      |      |     |     |     |     |        |
| 753♀ |          | 4-5½              | 17, 16                  | 2 33 16    | +   | +   |     |     |     |      |      |      |      |     |     |     |     |        |
| 754♀ |          | 4-6               | 17, 16, 16              | 3 49 16    | +   | +   |     |     |     |      |      |      |      |     |     |     |     |        |
| 756♀ |          | 14-15½             | 16, 16, 16              | 3 48 16    | +   | +   |     |     |     |      |      |      |      |     |     |     |     |        |
| 765♀ |          | 4-5½              | 16, 15                  | 2 31 15    | +   |     |     |     |     |      |      |      |      |     |     |     |     |        |
| 768♀ |          | 4-5½              | 16, 17                | 2 32 16    | +   |     |     |     |     |      |      |      |      |     |     |     |     |        |
| 783♀ |          | 3½-5½             | 15, 15                  | 2 50 15    | +   |     |     |     |     |      |      |      |      |     |     |     |     |        |
| 786♀ |          | 3½-5½             | 16, 17, 15             | 3 45 16    | +   |     |     |     |     |      |      |      |      |     |     |     |     |        |
| 859♀ |          | 9-11              | 17, 16, 16, 17          | 4 66 165   | +   |     |     |     |     |      |      |      |      |     |     |     |     |        |
| 860♀ |          | 9-12              | 16, 16, 16, 16          | 4 64 16   | +   | +   |     |     |     |      |      |      |      |     |     |     |     |        |
| 867♀ |          | 11-12             | 16                  | 1 17 17    | +   |     |     |     |     |      |      |      |      |     |     |     |     |        |
| 923♀ |          | 11-12½            | 16, 17                | 2 17 17    | +   |     |     |     |     |      |      |      |      |     |     |     |     |        |
| 935♀ |          | 8-9½              | 16, 16, 16, 16, 15, 16 | 6 95 1532 | +   | +   |     |     |     |      |      |      |      |     |     |     |     |        |
| 1011♀ |         | 7-11              | 16, 17, 16, 16, 16, 16 | 7 113 1614 | +   | +   | +   |     |     |      |      |      |      |     |     |     |     |        |
| 1037♀ |         | 5-6               | 16                      | 1 16 16    |     |     |     |     |     |      |      |      |      |     |     |     |     |        |
| 1060♀ |         | 4½-5½             | 16                      | 1 16 16    |     |     |     |     |     |      |      |      |      |     |     |     |     |        |
| 1066♀ |         | 4-4½              | 16                      | 1 16 16    |     |     |     |     |     |      |      |      |      |     |     |     |     |        |
| 1069♀ |         | 8-9               | 16                      | 1 17 17    | +   |     |     |     |     |      |      |      |      |     |     |     |     |        |
| 1073♀ |         | 4-5               | 16                      | 2 32 16    | +   |     |     |     |     |      |      |      |      |     |     |     |     |        |
| 1074♀ |         | 4-5               | 16                      | 1 16 16    | +   |     |     |     |     |      |      |      |      |     |     |     |     |        |
| 1080♀ |         | 5-7               | 16, 16, 16, 16         | 4 64 16    | +   |     |     |     |     |      |      |      |      |     |     |     |     |        |
| 1086♀ |         | 4-4½              | 16                      | 1 16 16    | +   |     |     |     |     |      |      |      |      |     |     |     |     |        |
| 1089♀ |         | 4-5               | 16, 16                | 2 31 155   | +   |     |     |     |     |      |      |      |      |     |     |     |     |        |
| 1097♀ |         | 3½-4              | 16                      | 1 15 15    | +   |     |     |     |     |      |      |      |      |     |     |     |     |        |
| 1102♀ |         | 5-8               | 16, 16, 16, 16, 16, 16, 16 | 7 110 1571 | +   | +   |     |     |     |      |      |      |      |     |     |     |     |        |
| 1104♀ |         | 4-5               | 16, 16                | 2 32 16    | +   |     |     |     |     |      |      |      |      |     |     |     |     |        |
| Total  |         |                   |                        |            | 67 1054 1573 | 6 | 9 | 8 | 2 | 7 | 7 | 2 | 9 | 8 |      |        |

♀ Guinea pigs are sexually mature at about 5 months old
all of these showed an interval of seventeen days between the beginnings of the dioestrous cycles. The average of seven periods in animals with only one ovary is 16.57, this being much higher than the average of all the cases, which is 15.73 days. The average of the only two cases of first heat-periods after operation, on the other hand, is lower than the general average 15.0. The number of cases is, however, entirely insufficient to warrant a conclusion, though suggestive for further investigation. It is probable that when only one ovary exists, the period between ovulations is a little longer than under normal conditions. The two ovaries may alternate to a certain degree in their function or they may share the entire task in a less exhaustive way than one ovary is capable of doing. Semi-spayed females often have large litters which might indicate that the single ovary matured more follicles than would have been its share should the other ovary have been present.

Eliminating from the general table the results obtained by the examination of the semi-spayed animals, one finds an average of 15.65 days for the length of time from the beginning of one heat period to the beginning of the next in all normal cases. This we believe to be the length of the oestrus cycle of the guinea-pig under uniform conditions.

Table 1 further shows the months during which these observations were made. The animals were examined during early summer, fall, winter and spring and have shown at all seasons a perfect regularity in the return of the heat periods. Their oestrus cycle is certainly typically regular. The only months during which the animals were not examined are July, August and September. During the winter the guinea-pigs are kept in a fairly well regulated warm temperature running about 70° Fahrenheit on an average. It may be possible that in the wild state under natural conditions when the weather is cold and food somewhat scarce, the heat periods may cease for a season or become less frequent. Rubaschkin claimed that heat ceased to recur after October when guinea-pigs were kept in a cold place. But under the steadily favorable conditions in which the guinea-pigs here considered are kept, it is certain that
they are sexually active throughout the entire year with an astonishingly regular return of their oestrous flow and breeding reactions.

A more careful consideration of the figures obtained during the different months indicates, however, that there probably is a small difference in the length of the sexual cycles during the warm and the cold seasons.

The curve shown in figure A indicates graphically this slight fluctuation, operated animals are excluded. The lowest average 15.50 days, or the shortest oestrous cycles, was found in the month of October, while the highest 16.14 days is shown during January. The heavy line at 15.82 days indicates the mean between these two extremes. It is probably not without significance that the averages during the months December, January, February, March and April fall above the mean line, while the averages during the months of May, June and October are below the line. From the cases considered this indicates that the length of the oestrous cycle is probably a little shorter during the warm time of the year and a little longer during the cold weather. We must, however, admit that the number of considered cases, as given in table 1, is actually small and these slight seasonal variations may be more suggestive than demonstrative in importance, yet there is certainly a striking consistency in their arrangement.

6. CYCLICAL CHANGES IN THE UTERUS AND VAGINA

After having determined the regularity of the dioestrous cycle in a number of virgin females, they were killed at different stages of the oestrous period and their ovaries as well as pieces of the uterus and vagina were carefully examined and then fixed and preserved for microscopical study. The uterus and vagina must be fixed in certain fluids to avoid shrinkage and a tearing away of the epithelium from the wall. Bouin’s fixing fluid has proven most satisfactory for this purpose while the ovaries were generally fixed with Zenker’s fluid.

During the dioestrus or resting period the uterus is lined by a layer of cuboidal ciliated epithelium. Figure 10 shows a sec-
Fig. A Chart showing the average length in days of the guinea-pigs' dioestrous cycles during the different months of the year. The cycles are slightly longer during the colder months.
tion through the uterus at four and a half days after the last oestrus. At this time the epithelial cells present a normal and vigorous aspect. No loss or breaking down is to be noticed. A few leucocytes are occasionally seen among the cells of the stroma, but never in large numbers. Mitoses are not frequent at this time but they are to be seen now and then.

When the heat period begins, the epithelium loses its normal appearance (figs. 11, 12 and 13). The epithelial cells become tall and columnar and are filled with mucus which they begin to form in abundant quantity. The nuclei of the columnar cells appear closely pressed one against the other and are pressed into different levels in the various cells so as to give an appearance of several rows of nuclei. The epithelium thus takes on a pseudo-stratified arrangement. At the same time, a large number of leucocytes begin to migrate from the capillaries through the stroma and towards the epithelium. The stroma itself is congested and possesses a more profuse circulation than usual.

These appearances are to be seen in animals killed during the first phase of their period, that is, when the vagina contains an abundant mucous fluid filled with desquamated epithelial cells. A smear of this fluid is illustrated in figure 1.

As soon as the second phase of the vaginal fluid appears (figs. 2, 3 and 4), the uterus shows another aspect. The leucocytes are accumulating in large numbers below the epithelium, forming in some places a perfect wreath of leucocytes under the epithelium or actually a separate layer of cells (fig. 14). The stroma shows a more advanced degree of congestion.

During the third stage, smears figures 5 and 6, the leucocytes penetrate more and more into the epithelium some of them making their way into the lumen of the uterus by passing between the epithelial cells. Other leucocytes actually enter the epithelial cells and penetrate into their interior (fig. 15). A stage more advanced in appearance corresponding to a late third stage though from the same animal as figure 15, is shown in figure 16, where the entire epithelium is almost completely disintegrated. A great number of leucocytes has already penetrated the epithelium the cell structure of which has become largely destroyed.
Large vacuoles are to be seen between the epithelial cells, and these are probably produced by the dissolving power of the leucocytes. Under the destroyed epithelium haematomata are to be seen in several places, produced by the congestion of the peripheral capillaries in the stroma. A leucocytosis somewhat similar to the above has been described by Heape, Königstein, Blair-Bell and others in the uteri of several mammals.

The vagina of the guinea-pig also shows analogous conditions as illustrated in figure 17.

The broken down epithelium remains until the regeneration process begins. The reparation starts from the necks of the uterine glands which have remained intact during the entire process of destruction. A few leucocytes are to be seen between the epithelial cells of the uterine glands but this small number apparently passes through the epithelium into the duct without injuring the epithelial cells. The stage of reparation corresponds to the fourth stage, that is, to the period when blood is sometimes seen in the vaginal fluid, see smear figure 9. This is not difficult to explain since regeneration and the falling off of the degenerated epithelium take place at the same time. Regeneration of the uterine epithelium before the oestrous flow had ceased has been reported in other mammals.

After examining a number of specimens, one may get the impression that the new epithelium growing out from the neck of the glands tends to push off the old degenerate epithelium, as it becomes detached from the wall of the uterus. Figures 18 and 19 show this condition where the new and the old epithelium are still existing in close proximity, the one growing out from the gland, the other breaking away from the wall of the uterus. In figure 19, this condition is more advanced and one sees the old epithelium partly detached from the wall of the uterus. Generally the epithelium falls off still connected with pieces of the stroma, which also seems to be destroyed to some extent during every heat period. These masses of epithelial cells are commonly found in the vaginal fluid. When the epithelium falls away the haematomata are uncovered and the blood con-
tained in them passes into the lumen of the uterus. A similar bleeding may also occur into the lumen of the vagina.

The regeneration of the mucosa seems to take place very quickly. About six to ten hours after the above stage the new epithelium is already completely formed. The growth of the new and the falling off of the old epithelium seem to go hand in hand, so that no stage is to be found when the uterus is completely unlined by its epithelial layer. However, one may occasionally observe, during the above described fourth stage, limited naked regions from which the old epithelium has been detached before the new has formed.

The wall of the vagina undergoes somewhat the same destructive changes as the wall of the uterus except that the desquamation of the vaginal epithelium does not occur in cell clumps or groups at the end of the third stage. The vagina merely sheds its epithelial cells singly but in increasing numbers from the beginning of the heat period up to the third stage. The desquamation appears to proceed from near the entrance up into the inner portions of the vagina. The cells which appear during the first stage come from near the outer part of the vagina, while during the second stage the desquamated squamous cells are derived from the inner part of the vagina. This statement does not include the cornified cells from near the orifice, which are found as mentioned above, between the first and second stages. The vaginal epithelium is also invaded by the leucocytes. This migration is very vigorous during the third stage, about the same time as in the uterus. An innumerable mass of polymorphonuclear leucocytes migrate into the vaginal epithelium and actually enter its more superficial cells by penetrating into their cell bodies (fig. 17).

The beginning of the desquamation before the massive arrival of the leucocytes shows that the primary cause of the desquamation is not the presence of the leucocytes. But, on the contrary it is probably the presence of the altered and dying desquamated cells which induces the extensive migration of leucocytes to this epithelial surface. The large epithelial cells of the vagina photographed in figure 17 are the same cells which are
to be observed in the vaginal fluid during the third stage, see
smears figures 6, 7 and 8. A congestion of the capillaries of the
mucosa also takes place in the vagina, and slight hemorrhages
may occur as in the uterus, when the destruction of the stratified
epithelium chances to reach down to the tunica propria.

The leucocytes are chiefly attracted to that portion of the
epithelium covering the outfoldings into the lumen and this part
undergoes a greater destruction. In a similar way it is the epi-
thelium covering the prominent folds of the uterus which is
destroyed, while the ingrowths which form the uterine glands
are preserved and through regeneration from their necks furnish
the new material which is necessary for the restoration of the
lost epithelium.

During the dioestrum or rest period the desquamation of epi-
thelium from the vagina does not stop completely and the scant
vaginal fluid always contains some desquamated cells. At the
same time, and probably connected with the shedding process
the exodus of the leucocytes also continues though in a less
active way than during heat. The 'intermenstrual fluid' there-
fore always contains a considerable number of leucocytes.

7. THE OVARIAN CYCLE

A study of the ovaries fixed during different stages of the
oestrous cycle has shown that every change taking place in the
uterus and the vagina has its corresponding stage of change in
the ovary. At the beginning of the first stage the ovaries
possess large, ripe follicles, figures 20 and 21. The nuclei of the
eggs contained in the follicles are in a resting condition. The
theca folliculi shows the beginning of a slight congestion. As
the first stage advances this congestion becomes more and more
pronounced and by the beginning of the second stage it is highly
developed, figures 22 and 23. This extreme congestion of the
theca folliculi, which exist at about the same time as the con-
gestion stage in the uterus (cf. fig. 14) indicates that the follicle
is ready for rupture. Heape has pointed out that the rup-
ture of the follicle is due to this congestion and if the ovarian
blood supply be tied off follicles do not rupture. During this
time the nucleus of the egg is still in a resting condition.

The ripe follicles break at about the end of the second or the
beginning of the third stage. Figure 24 shows a follicle just
broken at the commencement of the third stage. It will be re-
called that at this time the active leucocytosis begins in the
uterus and the vagina, compare figures 15, 16 and 17. The
ovaries are not omitted from this active migration of the leuco-
cytes. A number of leucocytes are to be seen in the corpus
luteum during its early development, but great numbers of
leucocytes are to be found mainly in the atretic follicles, which
are now becoming the seat of regressive and degenerative pro-
cesses (fig. 25). The eggs in these disorganizing follicles show a
peculiar activity expressed by the formation of the maturation
spindle. Most of the eggs begin to degenerate before the forma-
tion of a polar body, though some of them succeed in completing
their maturation divisions. Figure 25 shows an egg within a
disintegrating follicle, the follicle containing a great number of
leucocytes. This egg possesses a well formed polar body in the
process of division. Kirkham has reported similar conditions
in the ovary of the mouse, he notices that eggs degenerate after
forming the first polar body and the second polar spindle, a con-
dition closely similar to that shown in our figure 25. The
outline of the polar body is clearly shown in the specimen.
The photograph is not ‘touched up.’

The chromatin of the nucleus is to be seen in the center of the
egg in figure 25. In all the cases observed, the eggs of the
atretic follicles degenerated, the nucleus breaking up into irregu-
lar pieces very soon after ovulation had taken place from the
ruptured follicles. We failed to find anything to indicate a
tendency toward parthenogenetic divisions in the many speci-
mens which we have examined as Leo Loeb reported for these
animals.

The ruptured follicles very quickly begin to undergo a reor-
ganization resulting in the formation of the corpora lutea. Even
during the third stage the corpus luteum is a well circumscribed
body beginning its differentiation by the ingrowth of the vascular
tissue of the theca folliculi into the hypertrophied follicular epithelium (fig. 26). This condition is more advanced during the fourth stage, when reparation begins in the uterus. Figures 27 and 28 illustrate two corpora lutea from the same ovary during the stage of uterine hemorrhage, the two are cut in different directions. The ingrowth of the vascular tissue toward the central cavity is apparent in these two figures. A well formed mature corpus luteum is shown in figure 29, taken from a section through the ovary of an animal about four and a half days after the heat period when the uterus was in a typical resting condition (fig. 10).

8. GENERAL CONSIDERATIONS

After a review of the above described facts there are several problems of general importance which may be profitably discussed in connection with them.

A fact of considerable significance is that the development and the degeneration of the uterine and vaginal mucosa corresponds very closely to the development and degeneration of the corpora lutea in the ovaries. At the time when the corpora lutea are highly developed and apparently active the mucosae of the uterus and vagina show a normally vigorous and healthy condition (cf. figs. 10 and 29). While on the other hand when the corpora lutea begin to degenerate during the second week after the 'heat period' the mucosae of the uterus and vagina also begin to show signs of degeneration and the process of desquamation slowly commences. At about two weeks after the last 'heat period,' when the wholesale destruction of the mucosa begins, the corpora lutea are almost completely degenerated.

The breaking of the Graafian follicles occurs during the oestrus as a result of a congestion which began in the theca folliculi at about the same time as the congestion of the stroma of the uterus and vagina. And finally when the regenerative growth of the uterine mucosa sets in, the ovaries then possess new corpora lutea in an active state of differentiation which were derived from these recently ruptured follicles.
These occurrences argue very decidedly against the theory advanced by Fraenkel ('03), and until recently supported by a number of other investigators. Fraenkel believed that the corpus luteum is the cause of the menstrual condition, producing through its secretion the destructive changes in the uterus and vagina. Such a supposition does not in any sense accord with the phenomena as they appear in the guinea-pig. If there is to be ascribed to the secretion of the corpus luteum an action upon the uterine and vaginal mucosae such an action is not of an injurious but of a protective nature. As we shall bring out further, the most plausible opinion of the action of the corpus luteum in the ovary itself, may also be interpreted as of a protective nature since it seems to prevent rupture of the Graafian follicles and the discharge of the ova. The facts obtained in the present investigation might not fully warrant the position that the corpus luteum really exerted an actively protective influence over the uterine mucosa, but they certainly in no sense suggest, and actually speak against, any injurious action on the mucosa by the secretion of the corpus luteum.

At the same time it is difficult to maintain that the absence of the protective action of the corpus luteum is the only or actual cause of the oestrous activity. The cause of oestrous is very probably more complex and the definitely regular rhythmical changes which take place in the uterus and vagina of the guinea-pig can not be fully explained as due alone to the degeneration of the corpus luteum. The absence of the luteal secretion possibly merely permits the uterine flow to occur as it seems also to permit the rupture of the ripe Graafian follicles. While the real mechanism determining the uterine reaction is a more complex factor and relatively independent, but affected in its expression by a close inter-relationship with the ovaries.

The various theories, however, which attempt to localize the cause of the uterine changes in the ovary are not in any case fully in accord with all the facts. It is of course true that the existence of the ovaries is necessary for the normal development and function of the uterus and vagina, and also that the removal of both ovaries leads to a disappearance of the typical oestrous
changes in the uterus and finally to a degeneration of this organ. Yet the complete removal of the ovaries does not always prevent the menstrual periodicity from expressing itself in an atypical but regular way for a considerable time afterwards (see Halban).

Our observations on three females from which both ovaries have been completely removed, show that such an operation does not fully abolish the return of the destructive menstrual changes as is generally claimed. But on the other hand, the absence of the ovaries promotes and prolongs the continuation of these destructive changes in such a way, that instead of a periodical menstruation, these spayed females have a long, continuous and atypical destruction of the uterine and vaginal mucosae, which leads finally to the degeneration of these organs. In some cases a distinct periodicity may be perceived, indicating that the rhythm of the menstrual activity may exist independently of the ovaries. The phenomenon that really is abolished and absent from the uterus after the removal of the ovaries is the return of any regenerative or reconstructive process which we believe is normally due to a secretion from the newly formed corpora lutea.

From such a view of these phenomena one may draw the following general conclusions: The oestrous changes in the uterus are regulated by two different factors, one direct and the other indirect. A secretion elaborated in the ovary apparently by the corpus luteum is necessary for the normal development and persistence of the uterine and vaginal mucosae. The absence of the secretion leads to regression and degeneration of the uterine tissue. Yet this control is not the entire explanation of menstruation. The regulation of this process and the return of definite changes in definite periods of time may possibly be due to the existence of a fixed mechanism somewhere outside the ovary. The rôle of the ovary and especially of the corpus luteum is not to produce but to permit and to stop the menstruation. Our conceptions correspond completely with the ideas of Halban, who has recognized the protective rôle of the ovaries upon the
uterus and the vagina and the existence of a separate causal factor of menstruation independent of the ovary.

Fraenkel's theory that the corpus luteum is an active factor producing menstruation does not correspond with our observations. Neither, on the other hand, does the assertions of Marshall and Runciman that "the corpora lutea evidently exert no influence on the occurrence of heat" seem to us justified. Marshall and Runciman ('14), have advocated the importance of the interstitial cells in considering the ovarian factor concerned in the recurrence of the oestrous cycle as opposed to any active effect of the corpora lutea. They point out the evident incorrectness of the old views that the ovaries and uterus are related by a nervous connection. Transplantation experiments have shown the fallacy of such a notion and have demonstrated the presence of an internal secretion from the transplanted ovarian mass. Marshall then in arguing against the importance of the corpus luteum uses Heape's ('97), observations which showed that in monkeys menstruation might take place in the absence of either ripe follicles or newly formed corpora lutea. This observation, it seems to us, does not in any way point towards the interstitial cells as being important. Nor does it argue against our view that the absence of the corpora lutea permits menstruation and that their presence exerts a protective influence over the uterine mucosa. Heape's observation is perfectly in accord with this and it is to be expected that corpora lutea should be either degenerate or absent when menstruation occurs.

Marshall and Runciman performed operation experiments on four bitches. At these operations they attempted to destroy the large Graafian follicles by pricking with a knife or needle. In the first fox terrier at least nine follicles were injured in this manner. But one who has operated on the dog's ovaries knows how difficult it would be to discover all of the ripe follicles and almost impossible to get those on the dorsal surface of the ovary which is often closely bound down and almost covered. Yet it is not necessary in this discussion to question the destruction of every ripening follicle since the photomicrographs, which the
authors publish, show that corpora lutea formed after the rupture of the follicles, and they state that the follicles artificially ruptured changed "into structures almost identical with normal corpora lutea"—except that development was not sufficient to fill the central cavity.

In the first two animals, which were their best experiments, since the time of the expected 'heat period' was fairly accurately known, the 'heat' came on about the time, or perhaps a little later, than it was expected and was not greatly influenced by the operation. This is just what we should expect on our supposition of the function of the corpora lutea. The dog is a mono-oestrous animal with a long anoestrous period and the destruction of Graafian follicles a few weeks before the oestrus was expected would have no bearing on the probable function of the corpora lutea in bringing on this period. The old corpora lutea resulting from the last ovulation were not disturbed and were probably just about degenerating and thus permitted the oestrus to occur very near the normal time. While the newly formed corpora lutea resulting from the operation were not sufficiently vigorous in their action to do more than slightly delay the menstruation.

Marshall and Runciman concluded that it is evident that the occurrence of 'heat' in the dog is not dependent upon corpora lutea, and that "The ovarian interstitial cells are possibly concerned in the process, but cyclical changes in the condition of these cells have not so far been observed in the dog's ovaries."

These conclusions and Marshall and Runciman's discussion are directed chiefly against Fraenkel's idea regarding the way in which the corpora lutea act; that is, the corpora lutea by their secretion perform an active function in bringing on the oestrous condition. We also disagree on the basis of the evidence furnished by the guinea-pigs with Fraenkel's views and for these animals at least such opinions are entirely incorrect. It seems to us, however, that Marshall and Runciman's experiments do not in any way argue against the position that the corpora lutea exert a protective influence over the uterine mucosa, nor that the absence or degeneration of the corpora lutea and the dis-
appearance of its secretion permits the uterine mucosa to undergo the degenerative changes typical of the 'heat period.'

Therefore, we must object to their conclusion that the occurrence of heat is not dependent upon corpora lutea—and further we are unable to believe that their experiments, or any other so far recorded, indicate that "the ovarian interstitial cells are possibly concerned in the process." The evidence to our minds does not in the least point in such a direction.

A most ingenious attempt at an explanation of menstruation and one of the first logical views regarding the function of the corpus luteum was advanced twenty years ago by Beard in his monograph on the 'Span of gestation and the cause of birth.' According to Beard "Menstruation is comparable to an abortion prior to a new ovulation, and it is an abortion of a decidua prepared for an egg which was given off subsequent to the preceding menstrual period, and which had escaped fertilization."

In the earlier mammals, Beard imagines that gestation extended over only one ovulation period or short dioestrum of Heape's terminology. Thus prior to each ovulation, a birth would take place provided pregnancy had ensued after the previous ovulation, and if not the ovulation would be preceded by an abortive birth act. In this connection it is interesting to recall the well known fact that in man and other mammals abortions occur with a far greater frequency at the times for regular menstrual periods than at other times. In the human the time of the first menstruation after conception is a most critical period, and the time when the third menstruation should occur is responsible for the great predominance of three month foetuses to be seen in most collections, and so on up to the tenth period when the normal birth takes place.

In the evolution of mammals Beard calls attention to the tendency to develop a longer gestation period and more fully developed offspring, but in all cases the length of the gestation period is a multiple of the primitive ovulation periods. A reminiscence of the earlier primitive conditions still exist in all of the polyoestrous mammals. The gestation period of the guinea-pig extends over four oestrous cycles making it about sixty-two days long.
During pregnancy in higher forms, according to Beard's scheme the corpus luteum exerts a protective function by preventing a new ovulation and an abortive birth. In non-pregnant females, however, this abortive process is not counteracted by the quickly degenerating corpus luteum spurium and the uterus undergoes the changes of menstruation and a new ovulation occurs. This ingenious theory aims to furnish an explanation of the periodically destructive changes occurring in the uterus and vagina of some mammals at the same time that the ovary is preparing to liberate its ova. And the chief virtue of the theory is that it points out the protective action of the ovary and especially of the corpora lutea on the uterine mucosa. Every menstruation process and every abortion reflex as well as every normal birth is the result of two different factors, one the condition produced by the absence of the luteal secretion and the other is the expression of a phylogenetically and physiologically fixed rhythmical tendency within the uterus itself.

Beard's conception of the corpus luteum as an organ preventing ovulation has been adopted and further developed by many later investigators, Prenant, Sandes and Skrobansky, Leo Loeb, Ruge, Pearl and Surface, Halban and Köhler and others. All of these investigators have added evidence in favor of Beard's corpus luteum theory partly by new observations and partly by experiments on the living animals.

To state Beard's ('98, p. 101) position in his own words:

The corpus luteum is probably a contrivance for the suppression or rendering abortive of ovulation during gestation. The commencing degeneration of this structure some little time before the end of the gestation (like its rapid atrophy where fertilization has not taken place) allows of preparation being made for a new ovulation.

We are indebted to Leo Loeb ('11), for first putting these conceptions of Beard to experimental test. And Loeb showed that pregnancy as such does not prevent ovulation if corpora lutea are extirpated from the ovaries. Loeb also destroyed the corpora lutea in non-pregnant guinea-pigs and later examined the ovaries after different periods of time. In forty-two females the corpora lutea were destroyed by cutting them out completely
with the following results: In one case the next ovulation had already occurred at the twelfth to thirteenth day (by the next ovulation is meant the ovulation following the last copulation) in one case at the thirteenth day, in five cases at the thirteenth to fourteenth day, in twelve cases at the fourteenth to fifteenth day, in four cases at the fifteenth to sixteenth day, in one case at the sixteenth to sixteenth day and a half, in one case at the sixteenth to seventeenth day, in one case after eighteen days, while in eight cases ovulation had not yet occurred at the time when the animals were killed.

Loeb also cauterized the corpora lutea in the ovaries of thirty-one guinea-pigs but the results, owing to the inferiority of this method, were not so satisfactory. The ovulation in some cases came at the fourteenth to fifteenth day, in other cases later. Loeb interpreted these experiments to indicate that the removal of the corpus luteum hastened the next ovulation. Such a conclusion is in no way actually contradicted by our observations, yet the experiments of Loeb are not completely satisfactory in the light of the present findings. Loeb thought the usual sexual period, or time between two ovulations, in the guinea-pig was very much longer, and much more variable than it actually is. On such a basis it seemed that the ovulation period in the animals he examined had been considerably reduced. But as the present study shows the normal oestrous cycle in the guinea-pig is from fifteen to seventeen days, usually about sixteen days with very insignificant variations. So that the periods recorded by Loeb, after the operations are actually just about of normal duration. He found the greatest number of cases to ovulate after a period of fourteen to fifteen days (12 such cases or 28.57 per cent) and considered this much shorter than the normal condition, where as a matter of fact such a period differs only insignificantly from what we find to be the regular length of the oestrous cycle.

When we also take into account his method of calculating the days between the last copulation and the next ovulation, and especially the fact that he figured the ovulation time by the condition and probable age of the newly formed corpora lutea
found in the ovaries examined, the slight variations are all very probably within the limits of error. We also believe that Loeb has been misled by the application of similar methods in calculating the normal sexual periods in these animals.

In order to test the influence of the removal of the corpora lutea on the following ovulation time, one must first definitely establish a normal ovulation period. Since this was not done we are forced to acknowledge that Loeb's experiments do not demonstrate the importance of the corpus luteum in regulating the ovulation process, though he must be credited for having definitely attacked the problem experimentally. Some doubt will also exist in the minds of those who have attempted the operation as to whether all of the corpora lutea are often to be removed from the ovary while it is in position in the abdomen.

We are not at all opposed to admitting the probability that the removal of the corpus luteum may shorten the usual sexual cycle. In fact such a discovery would accord with our notions of the function of the corpus luteum. We feel further that the present study has established the existence of a definite normal oestrous cycle and this knowledge makes the experimental analysis of the influence of the corpus luteum much more readily approached.

The knowledge of a typical and regular sexual cycle in the guinea-pigs as here demonstrated, paves the way for a better and more uniform understanding of the oestrous conditions prevailing in the different classes of mammals. All cases that have been studied with sufficient care give evidence at least of some rhythmical activity. The absence of external signs of oestrus in a great number of mammals, one of which was the guinea-pig, is the most evident cause of a lack of understanding of their sexual periodicity. It is to be hoped that the application of the simple method of examination of the vaginal fluid used in the present study may enable workers to readily obtain a clearer understanding of the sexual activities of other commonly used laboratory animals as well as mammals in general, since such information is of the greatest value in all exact experimental breeding.
The typical oestrous cycles are probably more regularly expressed among mammals living in a state of domestication, and consequently under steady environmental conditions, than among their relatives living in the wild, where the existence of great disturbing factors, especially variations in food and temperature conditions, may tend to modify their behavior. The evidence of such modification by these disturbing factors is the existence in most mammals of differences in their sexual behavior during the different seasons of the year. Such seasonal variations are frequently lost under uniform conditions of temperature and feeding as is the case with rabbits, and also with guinea-pigs if these show seasonal changes in their native wild.

It has been reported by some investigators, Rubaschkin and others, that guinea-pigs in captivity breed less frequently in winter than during the warmer months, though they may become pregnant at any season. Such results are probably due to a failure to keep the animals properly warm during winter.

Guinea-pigs under the uniform conditions of our experiments do not show any apparent changes in their sexual rhythm with the seasons, but as indicated on previous pages, it is probable that their sexual cycle is a little shorter during the summer than in winter, yet even this difference does not seem to be very definitely expressed.

9. SUMMARY

The above description of the details of the oestrous cycle in the guinea-pig may be briefly summarized as follows:

1. Guinea-pigs kept in a state of domestication and under steady environmental conditions possess a regular dioestrous cycle repeating itself in non-pregnant females about every sixteen days throughout the entire year with probably small and insignificant variations during the different seasons.

2. During each cycle typically corresponding changes are occurring in the vagina, the uterus, and the ovary; a given stage in one of these organs closely accompanying parallel stages in the other two.

3. Each period of sexual activity lasts about twenty-four hours and is characterized by the presence of a definite vaginal
fluid, which is not sufficiently abundant to be readily detected on the vulva but is easily observed by an examination of the interior of the vagina.

4. The composition of the vaginal fluid changes with the several stages of change occurring in the uterus and vagina.
   a. To begin with, during what we term the first stage, the fluid consists of an abundant mucous secretion containing great numbers of desquamated vaginal epithelial cells. At this time sections of the vagina show an active shedding or desquamation of its epithelial lining cells. The cells of the uterine epithelium are loaded with mucus, and an active migration of polymuclear leucocytes is taking place from the vessels of the vagina and uterus out into the stroma and towards the epithelial layer.
   b. During the second stage the contents of the vagina become thick and cheese-like on account of the great accumulation of desquamated epithelial cells. The walls of the uterus and vagina become congested and the migration of leucocytes becomes still more active.
   c. The leucocytes reach the epithelium and vigorously invade its cells and intercellular spaces during the third stage. These wandering cells become enclosed within and apparently dissolve the breaking-down dead cells of the epithelium. The vaginal fluid becomes thinner under the dissolving or digesting action of the leucocytes. The congestion in the uterus and vagina becomes still more pronounced giving rise to small blood masses or haematomata beneath the epithelium. The epithelium of the uterus is highly disorganized, vacuolized and richly invaded by the leucocytes, so that portions of it fall away en masse actually carrying with it in some cases cells of the stroma.
   d. The fourth stage is merely a continuation or result of the activities of the third. The falling away of the epithelial pieces and stroma cells permits the escape of the small haematomata or blood knots thus causing a slight bleeding into the lumen of the uterus and vagina. These traces of blood often give a redish aspect to the vaginal fluid. At this same stage a regeneration process begins from the necks of the uterine glands and also apparently from the epithelial infoldings in the vagina, so that
the lost epithelium becomes rapidly replaced almost before it has ceased falling away. If one may picture the epithelial surface of the uterus and vagina as consisting of innumerable prominences and depressions, it may be said that the destructive processes mentioned above are largely confined to the epithelium covering the prominences and that this epithelium is finally restored by regeneration from the epithelium lining the depressions, or in the case of the uterus from the epithelium of the uterine glands. The congestion with the diapedesis of corpuscles and the formation of the blood haematomata and the great accumulation of leucocytes all occur chiefly in the outpushed or protruding parts of the uterine wall.

The regeneration process in the guinea-pig is very short, lasting only a few hours, from six to twelve in all.

5. Ovulation seems to occur spontaneously during every heat period without exception. The rupture of the follicles with the consequent ovulation takes place about the end of the second stage or the beginning of the third; that is, during the presence of the thick cheese-like vaginal fluid.

6. During the dioestrus or intermenstrual period there is very little fluid to be found in the vagina. This scant fluid consists of mucus in which are some atypical squamous cells from the vaginal wall and many leucocytes. A number of the leucocytes are old but there are probably new ones arriving almost continuously from the wall of the vagina. The only time at which the vagina seems to be practically free of leucocytes is immediately before and during the first and second stages of the oestrous period described above.

7. A marked correlation exists between the oestrous changes in the uterus and the developmental cycle of the corpora lutea. When the corpora lutea are highly developed and apparently active the mucosae of the uterus and vagina show a normally vigorous and healthy condition. While, on the other hand, when the corpora lutea begin to degenerate during the second week after the 'heat period' the mucosae of the uterus and vagina also begin to show signs of degeneration and the process of desquamation slowly commences. At about two weeks after
the last 'heat period,' when the wholesale destruction of the mucosa begins, the corpora lutea are almost completely degenerated. The breaking of the Graafian follicles occurs during the oestrus as a result of a congestion which began in the theca folliculi at about the same time as the congestion of the stroma of the uterus and vagina. And finally when the regenerative growth of the uterine mucosa sets in, the ovaries then possess new corpora lutea, in an active state of differentiation, which were derived from the recently ruptured follicles.

It, therefore, might be imagined that the secretion from the corpora lutea exerts a protective influence over the uterus and vagina while the absence of this secretion permits the breaking down and degeneration of the uterine epithelium typical of the 'heat period.'
10. LITERATURE CITED

1852 Entwicklungsgeschichte des Meerschweinchens. Giessen.
1911 b The cyclic changes in the ovary of the guinea-pig. Jour. Morph., vol. 22.


SANDES AND SIKROBANSKY (Quoted from Oppenheim’s Handbuch der Biochemie III, 1, p. 377).


PLATE 1

EXPLANATION OF FIGURES

The figures in all of the plates are photomicrographs made by Mr. Wm. Dunn of the Photographic Department of Cornell Medical School.

1 Squamous epithelial cells contained in the vaginal fluid during the first stage of oestrus from animal 1089 ♀. The vaginal fluid at this time is mucous filled with abundant cells of this type.

2 Cells from the second stage vaginal fluid. The great majority are squamous epithelial cells from the wall of the vagina with a few uterine epithelial cells. From animal 1066 ♀.

3 and 4 Cells of the second stage more highly magnified from 1066 ♀.
PLATE 2

EXPLANATION OF FIGURES

5 A smear of the fluid during the third stage, from animal 1104 ♀. This shows the arrival of myriads of leucocytes among the epithelial cells in the vaginal fluid. Such an appearance is characteristic of the third stage.

6 A more highly magnified view of the same stage showing in clearer detail the cell structures.
PLATE 3

EXPLANATION OF FIGURES

7 and 8  Highly magnified epithelial cells containing many leucocytes within their cell-bodies. A condition typical of the third stage—also from 1104 ♀.

9  A smear showing the presence of red blood corpuscles in the vaginal fluid during the short period of hemorrhage, following the third stage. From animal 1099 ♀. rc, red corpuscles.
PLATE 4

EXPLANATION OF FIGURES

10 A section of the resting uterus during dioestrum, four and one-half days after oestrus, showing the normal cuboidal ciliated epithelium—animal 1074 ♀.

11, 12 and 13 Sections showing the condition of the uterine epithelium during its active secretion of mucus and the beginning of the leucocyte migration, from animal 1080 ♀ in which the oestrus was just commencing—leu, leucocytes. Note the contrast with figure 10. A corresponding smear of the vaginal fluid from the same animal just before it was killed is shown by figure 1.
PLATE 5

EXPLANATION OF FIGURES

14 A section illustrating the condition of the uterine epithelium and the accumulation of large numbers of leucocytes below the epithelium during the second stage of oestrus, from animal 1006 ♀. Corresponding smears of the vaginal fluid at this time are shown in figures 2, 3, and 4 from the same female.

15 and 16 Sections of the uterus during the third stage of oestrus showing the invasion of the epithelium by migrating leucocytes. The epithelium is partially destroyed and greatly vacuolized, as a result of the dissolving action of the leucocytes, but is still adherent to the underlying stroma which also contains leucocytes. leu, leucocytes. Both sections are from 1104 ♀ and corresponding smears of the vaginal fluid from this animal immediately before being killed are shown in figures 5, 6, 7 and 8.

17 A section of the wall of the vagina from the same animal, 1104 ♀, during, of course, the same stage. The vaginal mucosa is also invaded by leucocytes in a manner similar to that of the uterus, several epithelial cells are seen to contain leucocytes within their bodies. The epithelium here is being desquamated or thrown off while the uterine epithelium is seen to be disintegrating before being shed. leu, leucocytes.
PLATE 6

EXPLANATION OF FIGURES

18 A section of the uterus from animal 1099 ♀ during the fourth stage, the short period of slight hemorrhage. The beginning regeneration of new epithelium from the neck of a uterine gland is shown while simultaneously the breaking down of the old epithelium is still taking place, and other portions of this section show a loss of the old epithelium from the uterine wall. A smear of the vaginal fluid from the same animal just before killing is shown in figure 9.

19 A similar section from the uterus of another animal, 860 ♀, during the same stage. This shows better the falling off of the old epithelium and the simultaneous formation of new epithelium.
PLATE 7

EXPLANATION OF FIGURES

20 A section of ovary from animal 1089 ♀ killed during the first stage of oestrus. A ripe follicle is shown a few hours before congestion of the theca begins. A smear of the vaginal fluid from the same animal is seen in figure 1 and sections of the uterus in figures 11, 12 and 13.

21 A higher magnification of the ovum and follicular wall shown in figure 20.

22 A section of the ovary from 1066 ♀ killed during the second stage of oestrus. The theca folliculi surrounding the ripe follicle has become highly congested. br, blood vessels.

23 Shows at a higher magnification a clearer view of the congested condition of the follicle in figure 22, br, blood vessels. The nucleus of the ovum is in a resting condition. Corresponding vaginal smears from this animal 1066 ♀ just before being killed are illustrated in figures 2, 3 and 4, and a section through the uterus in figure 14. All of these figures illustrate commonly seen second stage conditions.
PLATE 8

EXPLANATION OF FIGURES

24 A section of the ovary from 1086 ♀ showing a follicle shortly after rupture. The congestion in the theca folliculi is evident. bv, blood vessels. This animal was killed at the end of the second stage or early beginning of the leucocytosis, the third stage.

25 A degenerating atretic follicle from the same ovary as figure 24, the cells of the cumulus oophorus are degenerating while the follicle is being invaded by leucocytes. The ovum shows the first polar body in process of division while the nucleus of the egg is represented by a small chromatic mass near the center.

26 An early corpus luteum from animal 1104 ♀ killed during the third stage. Near the corpus luteum is seen a degenerating atretic follicle invaded by leucocytes. Compare smears figures 5, 6, 7 and 8, and sections of uterus figures 15 and 16, and section of vagina figure 17, all from the same animal.

27 A somewhat older corpus luteum from 1099 ♀ killed during the hemorrhage stage. The vascularization of the corpus is apparent at the periphery and is growing toward the center. bv, blood vessels. Compare the smear in figure 9, and section of the uterus figure 18.
PLATE 9

EXPLANATION OF FIGURES

28 A higher magnification of another corpus luteum from the same ovary as figure 27. The ingrowth of the peripheral vessels is more apparent, \textit{br}, blood vessels.

29 A fully developed corpus luteum from animal 1074 ♀, killed four and one-half days after oestrus. The typical glandular structure is clearly shown, cords of cells surrounded by capillaries, \textit{cap}, capillaries. A section of the wall of the resting uterus from the same animal is given in figure 10.