The degree of sexual activity of male rabbits as affecting the seminal discharge and the nature of the offspring

Frank Alfred Hays

Iowa State College

Follow this and additional works at: https://lib.dr.iastate.edu/rtd

Part of the Physiology Commons, Veterinary Physiology Commons, and the Zoology Commons

Recommended Citation

Hays, Frank Alfred, "The degree of sexual activity of male rabbits as affecting the seminal discharge and the nature of the offspring" (1917). Retrospective Theses and Dissertations. 14759.
https://lib.dr.iastate.edu/rtd/14759

This Dissertation is brought to you for free and open access by the Iowa State University Capstones, Theses and Dissertations at Iowa State University Digital Repository. It has been accepted for inclusion in Retrospective Theses and Dissertations by an authorized administrator of Iowa State University Digital Repository. For more information, please contact digirep@iastate.edu.
INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps.

ProQuest Information and Learning
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA
800-521-0600

UMI®
NOTE TO USERS

This reproduction is the best copy available.

UMI
THE DEGREE OF SEXUAL ACTIVITY
OF MALE RABBITS AS AFFECTING THE SEMINAL
DISCHARGE AND THE NATURE OF THE OFFSPRING.

by

Frank Alfred Hays

A Dissertation Submitted to the Graduate
Faculty of the Iowa State College of
Agriculture and Mechanic Arts in
Partial Fulfillment of the
Requirements for the
Degree of Doctor
of Philosophy

No. 3 [Signature was redacted for privacy.]

1917
THE EFFECTS OF FREQUENCY OF COPULATION IN RABBITS ON THE PROPERTIES OF THE SEMINAL DISCHARGE.
# Table of Contents

## Part I

<table>
<thead>
<tr>
<th>Introduction</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Materials and Methods</td>
<td></td>
</tr>
<tr>
<td>Animals Used</td>
<td>2</td>
</tr>
<tr>
<td>Methods Employed</td>
<td>2-7</td>
</tr>
<tr>
<td>Manner in which the Males were put to service</td>
<td>3-6</td>
</tr>
<tr>
<td>Recovery of Semen</td>
<td>6-7</td>
</tr>
<tr>
<td>General Considerations</td>
<td></td>
</tr>
<tr>
<td>The male genitalia (Plate I)</td>
<td>7-9</td>
</tr>
<tr>
<td>Function of accessory glands</td>
<td>9-12</td>
</tr>
<tr>
<td>Effects of service on the nature of accessory fluids</td>
<td>12-14</td>
</tr>
<tr>
<td>Properties of normal semen</td>
<td>14-16</td>
</tr>
<tr>
<td>The act of coition</td>
<td>16-17</td>
</tr>
<tr>
<td>The effects of many copulations on the behavior of the males at different services</td>
<td>17-19</td>
</tr>
<tr>
<td>The average interval between copulations (Table)</td>
<td>20</td>
</tr>
<tr>
<td>Discussion of table</td>
<td>21-24</td>
</tr>
</tbody>
</table>

## Data and results

<table>
<thead>
<tr>
<th>The Effects of Heavy Service on the Properties of Semen</th>
<th>24-29</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume</td>
<td>24-27</td>
</tr>
<tr>
<td>Viscosity</td>
<td>27</td>
</tr>
<tr>
<td>Specific Gravity</td>
<td>28</td>
</tr>
<tr>
<td>Color</td>
<td>28</td>
</tr>
<tr>
<td>Reaction</td>
<td>28</td>
</tr>
<tr>
<td>Chemical Composition</td>
<td>28-29</td>
</tr>
<tr>
<td>Possible sources of error connected with properties of semen</td>
<td>29-31</td>
</tr>
</tbody>
</table>
II

The Number of Spermatozoa as affected by frequency of copulation

Table of sperm counts

Comments on counts of the table

Motility as Affected by Frequency of Copulation

Tables showing the rate of motion of rabbit sperm

Comments on the tables

Possible sources of error in motility studies

Longevity as Affected by Frequency of Copulation

Tables showing the length of life of rabbit sperm

Comments on tables

Possible sources of error in longevity studies

Certainty of Pregnancy as Affected by Frequency of copulation

The female genital--Plates II to VI

Table showing the percentage of pregnancies in the different service groups

Comments on the table

Possible sources of error as related to observations on the certainty of pregnancy

Size of Litters as Affected by the Number of Copulations Made

Table showing the average size of litters produced by males at different services

Comments on the table

Correlation table of number of services to size of litters

Comments on the correlation table

Possible sources of error related to factors operating to produce the size of litters observed

Summary

Bibliography
THE EFFECTS OF FREQUENCY OF COPULATION ON THE PROPERTIES OF THE SPERMINAL DISCHARGE.

Since the discovery of spermatozoa as an essential factor in fertilization, by Spallanzani in the last quarter of the eighteenth century, much speculation and some study have been directed to the properties of semen as something of an index to what the nature of the offspring derived therefrom might be. Since spermatozoa are the only contribution of the male that takes part in reproduction, a thorough knowledge of their physiological properties as related to the nature of offspring is therefore exceedingly important.

The properties of semen discharged by males when subjected to varying degrees of sexual activity has not been extensively studied. The popular idea has been that males in heavy sexual service discharge smaller amounts of semen than males doing only moderate service. Moreover, this semen is said to contain greatly reduced numbers of sperm cells which have a decreased rate of motility and greatly reduced vitality.

Marshall (1912, p.45) mentions excessive service on the part of the male as one of the important causes of sterility because of the greatly reduced numbers and low vitality of the spermatozoa discharged by such a male.

Harper (1914, p.25) notes that partial sterility in males may result from excessive copulation and he attributes this to the greatly reduced numbers of germ cells.
The object of the work here reported was to gain more definite and complete information on the properties of the seminal discharge from males used in normal breeding operations in comparison with the seminal discharge of the same males under different degrees of sexual use.

Materials and Methods.

1. Animals Used.

In work of the nature undertaken here it is necessary to employ animals that may be handled with ease, and that thrive well under the more or less artificial conditions of the laboratory. The common domestic rabbit, Lepus cuniculus, was chosen for the work and gave very satisfactory results. Three males were used, varied in weight from 2200 to 2850 grams and all were mature at the beginning of the experiment. The same three males were used throughout the experiment.

A stock of breeding females was also assembled for the experiment. None of these rabbits were pedigreed and there was a wide variation in color pattern. These rabbits were purchased from six different sources, they were all vigorous and healthy, and no inbreeding was practiced at any time in the experiment. The full stock of breeding females was twenty-two ranging in weight from 2467 grams for the smallest to 3235 grams for the largest, averaging about 3050 grams. No females were under eight months old at the start, and nearly all were mature at this time.


Since the plan outlined for carrying on this investigation was very different from the plans used by others who have investigated
this subject, it was necessary to devise most of the technique in
this laboratory to meet our conditions, profiting of course by methods
and suggestions of many others. There were two important questions
to settle before the work could progress; namely, 1, how best to
secure excessive copulation, and 2, how to recover the semen. After
methods were finally decided upon, it required no little patience
and considerable practice especially to recover the semen. Further,
the most suitable method for making counts of spermatozoa and for
measuring the physiological constitution or vitality of the spermatozoa
were matters that required considerable attention.

To make the results obtained of the highest practical value
it was considered best to allow the male to copulate according to his
desire and just as frequently as possible under natural conditions.
For this reason, artificial stimulation to ejaculation such as elec-
tricity, friction, etc. as have sometimes been used by others, were
never employed and only by natural copulation were the males "worked
out". In fact, it is possible that artificial stimulants to ejaculation
would not produce results with the rabbit.

(a) Manner in which males were put to service.

In order to provide females for the preliminary matings of
the males, special provisions were necessary. A single female rab-
bit will sometimes receive the male as many as fifteen times in the
space of two or three hours; but as a matter of fact, whenever a
series of matings were made it was necessary that two and preferably
three females be in heat at the same time. The number of females
in heat at the time of making any series of matings must be sufficient
to furnish a female that has not been previously served on that date
for each recovery of semen. Periods of oestrus in rabbits is 2 weeks
and the gestation period 31 days. To reduce the number of
females necessary to amply provide
for preliminary matings, the fallopian tubes were sectioned in a number of females of breeding age. These were allowed to recover from the operation and six such females were available for use at the time the experiment was well under way. More such operated females were added later until the number increased to sixteen.

Only such operated females were used for "working out" the males because all the normal breeding females were used in the progeny studies that follow.

Through this operation these females were apparently unchanged in sexual behavior, and for the most part retained the normal estrous cycle, which occurs every fifteen days when environment is kept favorable. Pregnancy was absolutely prevented by the operation and such animals were much more admirably suited to our purpose than normal females would have been had they been available in sufficient numbers. Normal females could not have been used satisfactorily for the recovery of semen at less than six week intervals for reason that blood and residues from the foetal nutrition would also be recovered with the semen if the female was used the day of kindling or the day following kindling, while these operated females were used whenever they would accept a service.

There was one notable exception to the rule in the behavior of the operated stock. There was one agouti female that could be induced to accept the service of the male at almost any time if the male was rather aggressive. This female apparently went through the regular estrous cycle, at which time she was much more ardent toward the male and would accept many more copulations than at other periods. A certain sooty female also showed some tendency to behave in the same way
but would accept services of the male at much more infrequent intervals than the first mentioned. Attempts to secure copulation with other operated females when not in heat were unsuccessful.

It was found difficult to use these females for more than a year because they displayed great progynacity and began to refuse the male, except at rare intervals. Post mortem examinations of some of the discarded females showed no apparent changes in their reproductive organs to account for their changed behavior toward the males. Many of them had become very fat and rather sluggish. A new lot of sixteen operated females was substituted at this point.

At this time all of the older females were discarded, except the agouti, which was used successfully for a period of sixteen months, when she suddenly died from an attack of Hemorrhagic Septicemia.

The twenty-two normal (i.e. not operated upon) breeding females were divided into groups I, II, and III. There were eight animals in the first group and seven in each of the other groups when the work began. Group I was assigned to male No. 1; group II, to male No. 3; and group III to male No. 4. It was the aim to mate these females to the male to whose group they belonged on the first, fifth, tenth, fifteenth, and twentieth copulation; and later to transfer females to another group in order to check one male against another on the same female. These different types of matings were distributed so as to avoid grouping the advanced services any closer together at one time than at another. This was for the purpose of avoiding seasonal variations. As a matter of fact, however, advanced services were relatively more frequent in the latter part of the experiment. Each female was bred every six weeks or two months, depending on whether or not she came in
heat while her litter was but two weeks old and were suckling or after they had been weaned.

The plan was to mate the male with operated females until they had made four, nine, fourteen, or nineteen copulations and then to allow them to couple with the breeding females; after which the males were immediately removed and the time required to make the requisite number of copulations recorded. The male was always kept in his cage and the female was only placed in the cage at intervals as frequent as he would serve her and again immediately removed and kept away until the male would couple again.

(b) Recovery of semen.

All semen was removed from the vagina of the female by means of a small rubber catheter. The catheter was first moistened in Ringer's solution and then carefully inserted into the vagina to a distance of from twelve to fifteen centimeters, depending on the size of the female. A glass pipette was inserted into the outer end of the catheter and then by applying suction and slowly removing the catheter, as much as .5 c.c. of semen was sometimes recovered. The average recovery was about .2 to .3 c.c.

During the earlier stages of the work, a female was simply bred once to a male for the recovery of semen without previously attempting to remove any uterine or vaginal secretions which might be present. As the work progressed, it was considered advisable to remove as much as possible of these secretions from the female in the manner described above before breeding her. By this latter treatment as much as four tenths of a cubic centimeter of secretions were often recovered from the vagina, and semen drawn there-
The male urinogenital ducts and related structures, viewed from the lateral surface. After Ranther: a., anal aperture; bu., bulbourethral gland; c.c., corpus cavernosum; d.d., ductus deferens; g.a., anal (rectal) gland; g.i., inguinal gland; g.p., glans penis; par., paraprostatic glands; pr., prostate; r., rectum; ur., ureter; u.v., urethra (membranous portion); v.s., seminal vesicle; u.u., urinary bladder.
after was much less diluted and represented more nearly the un-
changed discharge of the male. It would appear that the rather
large amount of female secretion would have greatly increased the
volume of fluid recovered from the female in the early stages of
the work. This was not the case, due probably to the withdrawal
of this female secretion into the uterus during copulation by
the sucking action of this organ.

DATA AND RESULTS

(a) The male genitalia.

In order to better understand the structure of the male
reproductive organs of the rabbit, a dissection was made of a
sexually mature male about five months of age and weighing about
2200 grams. Study in this specimen was confined mainly to the
accessory glands and to the passageway of the sperm to the exterior.
The testicles may be drawn at will through the external
inguinal ring. They are elongated oval structures about five
centimeters long, 1.3 centimeters in diameter in the middle por-
tion, and 1.0 centimeter at each end. Their tissue is rather
spongy and a milky fluid may be squeezed from their tubules.

The epididymis is much thickened and lies at the anterior
dend of the testicle. This structure narrows down to a small cord
along the testicle and the ductus deferens leaves at the post-
erior end. Considerable of a milky fluid is present in the
epididymis.

A ductus deferens passes from each testicle to the
urethra. These tubes are about 15 centimeters in diameter and
9.6 centimeters long. They communicate with the urethra each
by a minute opening. A short distance from the opening of these
tubules there is a somewhat increased diameter which continues to their outlet.

The vesicle seminalis is highly developed in the rabbit and presents somewhat the appearance of being divided at the anterior end. It is about 5.2 centimeters in length and about half as broad, tapering toward the posterior in general outline presenting a bladder-shaped appearance. The contents are strongly alkaline in reaction and have a milky appearance when examined very soon after death. This liquid coagulates on exposure to air to form a thick, transparent, jelly-like mass. The inner walls of the vesicle present a true secretory surface.

The prostate gland, situated on the posterior-dorsal surface of the seminal vesicle, is a whitish glandular mass. Its secretion is slightly viscous, nearly transparent in color, with no odor, and of a faint alkaline reaction. There are several minute openings from this gland into the urethra just posterior to the opening of the seminal vesicle. The prostatic fluid, according to Camus and Cley, contains a ferment, vesiculase, which coagulates the secretion of the vesicle seminalis, forming the bouchon vaginal in the cervix of the female. Camus and Cley, as well as others, are of the opinion that this solid mass checks any outward flow of semen in the vagina.

The bulbourethral or Cowper's glands are situated posterior to the glands already described on the urethra. There is a pair of inguinal glands, situated just anterior to the penis on the urethra. These glands are rather small and little is known.

Note sur quelques faits relatifs a l'enzyme prostatique (vesiculase) et sur la fonction des glandes vesicales, C.R.de Soc.de Biol., Vol.IV.(10th series), 1897.
of their secretion. The bladder opens into an urethra about six centimeters in length; the glans penis is fully one centimeter long when not erected. There is a slit-like opening about one-half centimeter long extending on the anterior-ventral side of the glans penis. The penis is attached to the pelvic girdle on either side, the corpus cavernosum is rather large, and the amount of erectile tissue is considerable considering the size of the animal.

Evidence goes to show that the spermatozoa are expelled from the epididymus during the act of coition. From the distance as determined by measuring of these parts, it appears that the force necessary to move the spermatozoa this distance must be very great.

Length of ductus deferens 9.6 centimeters and length from urethra to glans penis 8.0 centimeters. The total distance that sperm cells are moved in the male during coitus before expulsion would then be from 17 to 20 centimeters, depending on the size of the male.

**Function of Accessory Glands.**

Much difference of opinion has been expressed as to the function of the secretions of the male accessory glands. Carmus and Gleyë (1900, quoted by Ivanoff) and Steinachë (1900 quoted from Ivanoff p.96) consider the secretions of the accessory glands as necessary to successful fertilisation. The first authors mentioned removed the seminal vesicle from male guinea-pigs and found that they were sterile afterward, though they never lost their sexual desire. Steinach removed both the seminal vesicle and the prostate from white rats with the same results as reported by Carmus and Gley.
Lott, (1872, p.126) thinks that the accessory fluids are no longer of any value to the sperm cells after the latter have reached the uterine cavity.

Iwanoff, (1907, p.467) whose work on this question is conspicuously in the first rank, holds an entirely different view in the matter. He considers the fluids of the vesicle seminalis and prostate as simply exerting the part of a mechanical medium which aids the passage of the spermatozooa during ejaculation and he has apparently proved this point by successfully artificially inseminating many different kinds of animals with spermatic fluid taken direct from the exterupted testicle and thus free from the secretions of the prostate and seminal vesicle. He admits, however, that the vaginal plug, probably formed by coagulation of the secretion of the vesicle seminalis, may be a valuable aid to the sperm in enabling them to reach the ovum. Iwanoff is also inclined to look with disfavor upon the experimental work of Camus and Gley and Steinach because he thinks that it would be exceedingly difficult to perform operations of the character mentioned on small animals without injuring the tract to such an extent as to form an obstruction to the passage of the spermatozooa.

Such animals as dogs and cats that lack the seminal vesicle entirely, Iwanoff thinks furnish good evidence of the minor role of this gland in fertilization.

Iwanoff, (1907, p. 503) in summing up, expresses himself as follows on the role of the accessory glands: "ainsi, toute une serie d'expériences pratiques sur des animaux de grande taille et de petite taille, confirme que dans la fécondation le role des secretions des glandes génitales accessoires est avant tout..."
mechanique; ces secretions sont necessaires ici, en tout que milieu liquide sous lequel les spermatozoïdes ne pourraient pas parcouvrir rapidement le canal urinaire dans toute sa longueur, puis etre rejetees avec la force necessaire."

The function of Cowper's glands is not definitely known. They furnish more volume to the semen and probably do not perform as important a function as the prostate or seminal vesicle.

The accessory male glands are true secretory glands and are thought to be subject in a large degree to psychological stimuli such as govern in a large degree the activity of the salivary glands. There appears little definite data available on the modification of the products of the former as the effect of excessive function. A brief statement of the changes undergone by the salivary glands under continued functioning is here presented, because it is thought that the analogy may be valuable.

Howell (1903, p. 223) found that when salivary glands are stimulated after a period of rest, the percentage of organic material increased in accordance with the strength of the stimulus and much more rapidly than the percentage inorganic salts. After continued stimulation the proportion of organic material decreased to a very low percent, and to a much greater extent than the inorganic constituents.

Mathews (1915, p. 320) states that saliva is nothing other than transformed protoplasm, and that this accumulates during rest periods of the salivary glands to be broken down again by their functioning.

The conclusion may be drawn, therefore, from these observations that a considerable storage of reserve materials
takes place in such glands and that during function this reserve is used up. At the same time the character of the secretions themselves changes.

EXAMINATIONS ON THE NATURE OF ACCESSORY FLUIDS.

Examinations very often show that semen is largely made up of fluids from the accessory glands, and it appears that the glandular secretions tend to accumulate during rest and that this accumulation is largely excreted during copulation following these periods of rest, thereby greatly increasing the volume of the discharge. Since these glands must decrease in their activity as the number of copulations progresses, it is rather reasonable to conclude that as a mechanical medium the secretions of these glands becomes less effective as the number of copulations increases.

Volume of semen is subject to considerable variation in mammals. There can be no reasonable doubt that the volume in a large measure upon the volume of secretion furnished by the glands of the epididymis, as distinct from testis, the seminal vesicle, the prostate, and Cowper's glands. Since all of these glands are true secretory glands, the amount of their product would probably vary under different conditions, probably to a greater extent than the glands of internal secretion.

Semen studies on different animals seem to indicate that both volume and character of semen are changed by excessive functioning of the male genitalia.

Smith (1912, P. 649) states that the prostatic fluid precedes the spermatic in ejaculation and if stallions and bulls are used excessively from day to day, the fluid ejaculated is largely prostatic and infertile.
Lewis (1911, P. 30) was able to recover 65 cubic centimeters from a stallion on the first copulation of a test. This same stallion then made one copulation daily for nine days. On the ninth day only 5 cubic centimeters of semen was recovered. Again after the horse had rested nineteen days, 60 cubic centimeters of semen were recovered from a single copulation.

Lode (1891) on the other hand presents results in which the total volume recovered direct from a dog was 750 cubic millimeters on the first ejaculation and 1500 on the fourth ejaculation of the day. On a second observation, the volume of the first ejaculation was 300 cubic millimeters and 1500 on the fourth. He does not state the length of the interval between these ejaculations.

In a single observation reported by Lode in man, the volume of semen decreased from 3000 cubic millimeters on the first ejaculation to 2000 cubic millimeters on the second copulation of the day.

When a dog was allowed to rest for eight or ten days, Lode found that the total volume of semen was smaller than when the dog was made to ejaculate more regularly. Semen recovered after two or three days the dog had made four ejaculations in a single day, was less than the normal in amount and far above the normal in sperm content per cubic millimeter. The possibility exists that since all semen was obtained from the dog by stimulating ejaculation by friction, that the accessory glands were not stimulated to the same extent as would have been the case in normal coition and that consequently the semen volume obtained was less than might have been recovered after natural copulation.
Løde's results, reported on the volume of semen recovered from man are entirely comparable with the results obtained from rabbit males both for heavy service and copulation following a period of rest.

The amount of semen that may be recovered from a rabbit female after a single copulation of a male after different numbers of copulations that the male has made previously, varies considerably. The amount recovered has been found to vary from one or two drops to as much as two cubic centimeters. After a period of several days rest on the part of the male, a larger amount was recovered than when the male was in daily use. A study of the tables presented will reveal these facts.

(b) Properties of Normal Semen.

Normal semen, strictly speaking, means semen as it comes from the penis of the male before being mixed with the contents of the female genital tract. Concerning the rabbit, there is no method yet devised by which semen can be obtained direct from the male. The only possible means would be by the use of a membranous sac placed over the penis during coitus. This has been attempted with several males. Caeza of fowls were prepared in such a way as to make a thin flexible animal membrane that would fit over the penis. Thus far not a single service has been made by the rabbits under these conditions, since they refuse to serve the females at all. Consequently the semen used for these studies was recovered after a "natural" service and the term, normal semen, as used in this report will then mean the fluid recovered from the vagina of the female rabbit after the male has made but one service during that
particular twenty-four hour period. Such semen thus contains the testicular and epididymal secretions, secretions of the seminal vesicle, prostate secretions, and secretions of Cowper's glands from the male. These are probably mixed with uterine secretions, cervical secretions, vaginal secretions, lymph, epithelial cells, and leucocytes from the female.

Normal semen of the rabbit as recovered from the female just after the male has served her is milky white in color, with a faint odor that seems characteristic of the species. The odor probably comes from the secretion of the prostate gland.

A Text Book of Physiological Chemistry, Hamersten, (Mandel Translation, Page 590, 1911).

Semen has a slightly greater viscosity than cow's milk and an alkaline reaction.

Rabbit semen becomes more viscos on exposure to air, but does not exhibit this property to so marked an extent as does the semen from the boar or the bull.

In some cases, especially after a rest of a week or more, a male will ejaculate semen that is much more viscous and more ropy than is the case with males doing more regular breeding.

Different males seem to excrete semen with markedly different physical properties and the sperm content varies widely with individuals and at different times with the same individual. Iwanoff (1907, p. 506) finds that in domestic animals the quantity and the quality of the semen vary with the general health, the exercise, the nutrition, and the sexual use of the male. Semen as recovered from the services of different males varies greatly in the length of time that it will resist the attacks of dis-
integrating microorganisms. Undiluted semen deteriorates rather rapidly under laboratory conditions as it seems to furnish an excellent medium for the growth of putrefactive bacteria, which soon cause the liberation of the characteristic gases of protein decomposition. It is probable, as Reynolds (1916, p. 44) points out, that the nature of the vaginal secretions has much to do with this property of the semen. He found that in man the character of the vaginal secretion varies widely in women and has a very marked effect on the properties of semen. Our results seem to indicate that Reynolds' view is correct and that the secretions may vary considerably in character in the rabbit.

Lespinasse (1917, p. 347) likewise finds that the vaginal secretions in women are ordinarily acid and suggests that fertilization is more certain when the spermatozoa are placed in the uterus and not brought in contact with the vaginal fluids.

(c) The Act of Coition.

The act of coition is of very short duration in the rabbit. Only one or two seconds are required to perform the sexual act. The male generally mounts the female at once, when she is in heat; she will raise the vulva higher so as to make it readily accessible to the penis of the male. By a single thrust of the male the act is completed, and the force of the thrust varies greatly with different males.

Observations of Bischoff (1842) and Lott (1872) would seem to indicate that the force of the ejaculatory thrust has much to do with the spermatozoa reaching the ovum. The greater the force of the thrust, the farther the semen will be thrown into the vagina, and the less the distance the sperm cells will be required to cover to reach the ovum.
Male No. 4 never served a female with the same ardor as was characteristic of male 1 and 3. The breeding record of this male, presented farther on, shows how his percentage of pregnancies differs from those of the more ardent males 1 and 3. After making due allowance for the inferiority of the semen from No. 4, it still appears that if he had deposited his sperm cells farther toward the uterine, he might have stimulated the female genitalia to greater peristaltic activity, as Heape (1898) points out, and thus not only reduced the distance that the sperm must pass to reach the ovum, but also insured their more rapid transportation through the uterine body.

Male number 1 generally showed greater sexual activity than either of the other two males. For this reason he was used more extensively than the other two; he made more advanced services; and was a "super breeder" than the others.

Male No. 3 was more inclined to cling to the female during copulation, and has been observed in some cases to keep the penis in contact with the female for five seconds. This male also appeared to have more difficulty at times in getting an erection than the other males used, and for periods of a week or ten days at different intervals of the experiment he was unable to successfully serve a female though he showed a strong desire.

Male No. 4 was rarely as active in serving females as either No. 1 or No. 3. He appeared more indifferent, required more time between copulations, and showed much less thrust during coitus.

(a) The effects of many copulations on the behavior of males at different services.
Usually the first four or five copulations were made by the males with great ardor with intervals of from four to six minutes between services; after this there was a gradual decline in the activity of the male until he was very slow to serve, requiring sometimes as much as fifteen or twenty minutes rest between the more advanced services. The laboratory temperature, the amount of breeding done on previous days, the willingness of the female to accept the male, the number of times the same operated female had been covered by the male, and the number of copulations already made on the same day were all important factors in determining the length of time between copulations at any particular time.

In order to induce the males to make as many copulations in as short a time as possible, it was necessary to employ every means to induce him to serve often. Males will serve females much more frequently if they are not allowed to remain in the cage together. Because of this fact, the female was taken from the male's cage each time immediately after he had served her. Males were found to work better in their own cage and for this reason all breeding was done in the cage of the male. As large a number of operated females as would accept the male, usually two or three, was used for each male in order that he might not tire of them. At times it was found advantageous to put two or three females in the cage with the male at one time in order to arouse his sexual sense. Studies here carried on would seem to indicate that when the sex organs are in a high state of activity the male will show greater eagerness to copulate. This statement will be born out by a study of the number and activity of the sperm cells on days when many copulations were made within a short space of time.
Different writers have made much comment on the evil effects of heavy service on the physiological behavior of males. There are many cases in domestic animals where males showing indifference to females are supposed to have been used for breeding when too young or to have been used excessively.

Rabbit males that have been used for making a number of the tenth, fifteenth, or twenty services matings already described do show the effects of the strain placed upon them. This has been noted when two or three such matings have followed each other in the space of three or four days. At such times the males were unable to copulate, even though they made pronounced efforts. This was not universally true and this inability to serve sometimes appeared after two or three copulations had been made. In these latter cases the sexual desire appeared very strong, but there was an apparent failure of erection that prevented successful copulation. In most cases, however, males almost completely lost sexual desire at these periods. After a rest of some days such males apparently exhibit their normal physiological behavior.
Table No. I

The average Interval Between Copulations for All Mating
Made in the Experiment--Minutes--Together with Per cent of Pregnancies Resulting.

<table>
<thead>
<tr>
<th>Male No.</th>
<th>5th Service</th>
<th>10th Service</th>
<th>15th Service</th>
<th>20th Service</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of Time Freg.</td>
<td>%</td>
<td>No. of Time Freg.</td>
<td>%</td>
</tr>
<tr>
<td>1</td>
<td>7  10.7 85.71 12 17.3 58.33 9 11.3 55.55 10 21.5 60</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>11  17.8 61.82 8 16.2 75.00 4 14.3 50.00 1 12.0 0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>13  12.1 46.15 12 20.3 50.00 4 16.5 25.00 2 10.5 50.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weighted Average</td>
<td>13.8 67.74 18.1 59.37 13.2 47.06 19.08 53.85</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>31  32  17    13</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


Table I reveals the rather striking fact that there is on the average no marked increase in length of interval between copulations when males are making a large number of successive copulations in a period of a few hours. In other words, the sexual desire of the male does not diminish rapidly and in many instances the interval between the nineteenth and the twentieth copulation was no greater than between the first and the second copulation. The readiness with which the male copulates varies greatly from day to day and seems to depend upon a large number of factors which are beyond the control of the investigator.

The average interval between copulations in the fifth service group is 15.8 minutes; in the tenth service group, 18.1 minutes; in the fifteenth service group, 15.2 minutes; and in the twentieth service group, 19.08 minutes. The number of observations are 31, 32, 17 and 15 respectively for the different service groups. Just why there should be a shorter interval between successive copulations in the fifteenth service group than in the tenth service group is not clear. The small difference in the length of the interval between the tenth services and the twentieth services is difficult to explain. The data in regard to interval of service is rather unsatisfactory. There are two factors that may in part account for the variations in the interval between copulations; namely (1) the number of operated females available for preliminary matings and (2) the fact that the males became "practiced" by continued heavy service at irregular intervals so that they would perform a series of copulations in shorter intervals as the experiment progressed. But in addition to these factors the length of interval between copulations may depend upon the experimenter himself rather than upon the two factors above.
referred to. Copulations are generally made by the male very soon after the female is placed in his cage and if the male was slow to serve the next interval would probably be prolonged by the experimenter. In case the experimenter was busy in the study of the seminal discharge, the series of services would not be accomplished in as short a period as if all the attention had been given to crowding the male. This feature may be due in part to the fact that we have observed an increased sexual desire in the males after they have been used heavily for some months and also to the fact that more females were available for the preliminary matings at the time that the twentieth service matings were accomplished than when the matings of less frequency were made. As has already been pointed out, as a rule, the greater the number of operated females available for the preliminary matings, the more readily will the male copulate because he seems to prefer females with which he has not previously copulated on that day to those that he has served several times during a series of preliminary matings.

Considering the three males separately, we notice that there is considerable difference in the length of intervals shown between copulations. Male No. 1 served more frequently than the other two and also proved to be the surest breeder of the group. In the fifth service series the interval between copulations for No. 1 was shortest and the percentage pregnancies greatest, the same may be said for the fifteenth service series, but he did not serve as readily in the tenth service series as did No. 3. In the twentieth service series a comparison is unjust
because of the small amount of data on Nos. 3 and 4. No. 3 shows a consistent increase in length of interval for the first three series of the table together with a parallel decrease in the per cent of pregnancies. No. 4 shows a great increase in the length of the interval in the twenty service series as compared with the fifth service series and with this increase there is also an increase in the per cent of pregnancies. The increase on the fifteenth service ratings in length of interval and the decrease in per cent of pregnancies here is very consistent. The assumption seems to be justified, therefore, that the interval between services is a more variable factor than the per cent pregnancies resulting.

An important feature of table I is the relation of number of preliminary copulations to the certainty of pregnancy resulting. It will be noted that from 31 fifth service ratings 67.74 per cent of the females became pregnant and produced litters; from the 32 tenth service ratings 59.57 per cent of the females produced litters; from the 17 fifteenth service ratings 47.06 per cent of the females produced offspring; and from the 15 twentieth service ratings 53.85 per cent of the females reproduced. In other words there was 87.6 per cent as many litters resulting from the tenth service ratings as from the fifth service ratings; 79.3 per cent as many litters resulting from the fifteenth service ratings as from the tenth service ratings; and 14.4 per cent more litters from the twentieth service ratings as from the fifteenth service ratings.
The length of interval between preliminary copulations does not appear to be significant, but the number of copulations that a male rabbit is required to make before breeding a female does have an important bearing on his ability to successfully fertilize a female.

There seems to be a close relation between the sexual desire of the male and his ability to get offspring. This being the case, a natural male, will not ordinarily copulate twenty times unless his reproductive organs are discharging fertile semen.

(c) The effects of heavy service on the properties of semen.

1. Volume.

The volume of semen discharged by a male is undoubtedly considerably influenced by the amount of sexual service that the male is required to perform.

C. Ludwig (1891, quoted from Lode) writes: "Ein vermutlich, dass line oftcr Leisung des Sperma die Neubildung desselben beschleunigen."

Hathnagel (1891) according to Lode, views semen production as quoted below: "Für den Hoden hingegen sei man keineswegs gezwangen eine constante Secretionsarbeit zuzuschreiben."

Hathnagel (1891, quoted from Lode) in his own words expresses the following viewpoint: "Mancher weist vielmehr darauf hin, dass nur nach vorherigen Entleerungen eine stärkere Production stattfinde."
Lode (1892) found that with men the volume decreases considerably on the second copulation made in one day.

Iwanoff (1907, p. 434) found that the volume of semen ejected generally decreases in stallions with several copulations, but this decrease is variable. He reports one case of a stallion where on one day 33 c.c. was discharged at the first copulation, 30 c.c. on the second, and 28 c.c. on the third copulation. The day following 20 c.c. were discharged on the first copulation and 18 c.c. on the third. On the third day there were 50 c.c. on the first and 40 c.c. on the fourth copulation.

Lewis (1911) found that the volume of semen ejaculated by a stallion diminishes from day to day when he is continually making one or two copulations daily.

The volume of semen discharged by the male rabbit is necessarily very small; since the time of ejaculation is extremely short and the animal is small, this is necessarily the case. We have found it impossible, as has already been pointed out, to recover this semen free entirely from the secretions of the female genital tract. By using a catheter and applying suction it is possible to recover the bulk of the secretions in the vagina of the female before putting her to the male. By this means it has been possible to recover rabbit semen comparatively free from these secretions for study. Volumes thus recovered represent only a part of the actual contribution of the male, but there is no reason to think that the amounts recovered are not proportional to amounts deposited. By a comparison of volumes thus recovered, a marked decrease has been noted as the number of copulations increases.
Table II

Volume of Semen Recovered from Different Services, in cubic centimeters. (Composite of all Males).

<table>
<thead>
<tr>
<th>Date</th>
<th>1st</th>
<th>5th</th>
<th>10th</th>
<th>15th</th>
<th>20th</th>
</tr>
</thead>
<tbody>
<tr>
<td>1916</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7/6</td>
<td>.2</td>
<td>.2</td>
<td>.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7/7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7/11</td>
<td>.2</td>
<td>.2</td>
<td>.2</td>
<td>.15</td>
<td>.05</td>
</tr>
<tr>
<td>8/23</td>
<td>.5</td>
<td>.1</td>
<td>.35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9/16</td>
<td>.2</td>
<td>.1</td>
<td>.05</td>
<td>.05</td>
<td></td>
</tr>
<tr>
<td>10/22</td>
<td>.4</td>
<td>.5</td>
<td>.4</td>
<td>.4</td>
<td></td>
</tr>
<tr>
<td>11/9</td>
<td>.3</td>
<td>.3</td>
<td>.3</td>
<td>.3</td>
<td></td>
</tr>
<tr>
<td>11/14</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12/9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12/26</td>
<td>.2</td>
<td>.2</td>
<td>.2</td>
<td>.4</td>
<td>.4</td>
</tr>
<tr>
<td>12/26</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12/28</td>
<td>.1</td>
<td>.1</td>
<td>.15</td>
<td></td>
<td>.05</td>
</tr>
<tr>
<td>12/30</td>
<td>.1</td>
<td>.1</td>
<td>.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12/31</td>
<td>.2</td>
<td>.2</td>
<td>.2</td>
<td>.4</td>
<td>.4</td>
</tr>
<tr>
<td>1917</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/1</td>
<td>.2</td>
<td>.2</td>
<td>.2</td>
<td>.05</td>
<td>.05</td>
</tr>
<tr>
<td>1/10</td>
<td>.6</td>
<td>.6</td>
<td>.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/13</td>
<td>.8</td>
<td>.8</td>
<td>.05</td>
<td>.05</td>
<td>.05</td>
</tr>
<tr>
<td>1/14</td>
<td>.3</td>
<td>.3</td>
<td>.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/17</td>
<td>.5</td>
<td>.5</td>
<td>.13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/26</td>
<td>.2</td>
<td>.2</td>
<td>.15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/28</td>
<td>.2</td>
<td>.2</td>
<td>.15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ave.</td>
<td>.36</td>
<td>.30</td>
<td>.34</td>
<td>.16</td>
<td>.16</td>
</tr>
</tbody>
</table>

* After this date the female secretions were removed from the vagina of the female before copulation for the recovery of semen.
Table II shows that there is considerable range in the amount of semen recovered for study. A further striking feature of the data is the fact that the removal of the female secretions does not seem to affect the volume of semen recovered. The possible explanation has already been given. The average volume of semen recovered for the entire experiment is as follows:

From the first copulation, .38 c.c.; fifth copulation, .50 c.c.; tenth copulation, .54 c.c.; fifteenth copulation, .16 c.c.; and twentieth copulation, .16 c.c. Small volumes of semen are very difficult to recover and to measure accurately. An accurately graduated pipette was used for measuring the volume in most cases, but in cases where the volume was extremely small, the volume was calculated from the weight. Average results seem to indicate that by the time the male rabbit has ejaculated fifteen times there is a fairly constant and minimum production of semen. These observations show that heavy service does reduce the volume of semen and this tends to increase the certainty of the sperm being carried forward by peristaltic action of the female genitalia. This question has been considered more fully in another place.

2. Viscosity.

The viscosity of semen also decreases as the number of copulations increases. The secretions of the seminal vesicle and the prostate gland are very viscous. This decreased viscosity therefore furnishes good evidence that the quantity of the product of these glands is greatly reduced by continued functioning. The number of spermatozoa in the epididymal fluid also adds to the viscosity. The decrease in number of spermatozoa would then partly account for the reduced viscosity of semen from the fifth,
tenth, fifteenth, and twentieth copulations.

3. Specific Gravity.

Although the specific gravity has not been determined in samples of rabbit semen, it is probable that there is a reduction in the advanced services as Lode (1891) found with the dog. Here there was a decrease in specific gravity from 1.014 at the first ejaculation to 1.010 on the third ejaculation in a day.


Samples of semen from the first copulation were more opaque than samples which were obtained from advanced copulations. This is probably due in part to modifications in chemical composition somewhat similar to those reported by Lewis (1911, p. 37) for a stallion used at the rate of two services daily. In this case there was a decrease in total solids and especially the nitrogenous constituents from the more advanced services. The number of spermatозоа per unit volume undoubtedly has considerable to do with the color of the semen as Hammarsten (1911, P. 589) states in discussing human semen.

Semen of the rabbit continues to become more transparent with heavy sexual use and nearly loses its milky appearance when obtained from the fifteenth to the twentieth services.

5. Reaction.

All semen tested from the rabbit showed an alkaline reaction, and it appears doubtful if normal males of this species ever produce neutral or acid semen.

6. Chemical Composition.

Chemical composition was not determined in this exper-
iment. The quantities of the seminal material are so small as to make this work rather difficult and rather unreliable. There can be no reasonable doubt that the chemical composition varies both in organic and inorganic constituents, especially the former with the degree of sexual service. The organic constituents are probably the most variable as Stovtsov (1916, p. 208) shows in semen from man, dog and horse.

Possible Sources of Error.

Though care was taken in carrying on this investigation to avoid as far as possible any chances of error, never-the-less certain errors have probably crept into this work and it is deemed advisable to enumerate some of the more important of these possible sources of error in order that no unfair deductions may be drawn from the results to be reported.

1. Possible sources of error connected with properties of semen as obtained for this work.

(a) Males used excessively at irregular intervals, as has been the case in this experiment may discharge semen with very different properties than that discharged by males used regularly several times daily for long periods. This specially applies to males under practical breeding conditions where a number of females are to be bred daily through the breeding season. One of the important objects of this work was to so use the males as to cause them, if possible, to produce germ cells of low vital force. It is questionable if the procreative powers of the males would have been as severely taxed by several copulations each day as by ten, fifteen, or twenty copulations within the space of from two to five hours at irregular intervals with a few days intervening.
between each series of services.

Lewis (1911, p. 30) found that in chemical composition, number of spermatozoa, and longevity of spermatozoa that there was a noticeable fluctuation from day to day in a stallion making two services a day for six successive days. The same investigator (1911, p. 30) found that from seven to ten days were required by such a stallion to recuperate so that he would excrete normal semen.

The secretions of secretory glands that have been studied have been found to be modified by the continued functioning of the glands. Although this point has not been investigated in connection with the male accessory glands, certain analogies probably exist in their behavior and a brief consideration of the behavior of the salivary glands is here presented.

Howell (1902, p. 225) discovered that if salivary glands are stimulated after a period of rest, the percentage of organic material present in their secretions increases with the strength of the stimulus and much more rapidly than the inorganic salts. After continued stimulation the percentage of organic material decreases to a very low percent, and to a much greater extent than the inorganic constituents.

Matthews (1915, p. 320) states that saliva is nothing other than transformed protoplasm which accumulates during rest periods of the salivary glands to be broken down again to form their secretions while they are functioning. It would therefore seem safe to assume that the secretions of the male accessory glands change as these glands continue to function during excessive sexual use.
Had the object of this investigation been to study the effects of heavy service on males as judged by body weight, physiological behavior, etc., rather than the effects of heavy service on the offspring of these males, there would probably have been an advantage in allowing each male to make several copulations regularly each day because this would give the male a chance to adapt himself to such use and would have given his sexual organs an opportunity to adjust themselves to such conditions, and probably would have placed these males in about the same position as a stallion, required to make sexual service daily throughout the breeding season; whereas very irregular and excessive use of the males placed a much more severe strain upon his reproductive ability. In as much as the object was the study of the effect on glands and their secretions, it was deemed wise to follow the plan here pursued.

(b) The possibility exists that in the rabbit the secretions of the female genitalia may vary widely in character as Reynolds (1915) has shown in women, so as to considerably affect the properties of semen drawn from the female genitalia, thus making a comparison of semen from the same male but from different females somewhat unreliable. It should not be assumed that this variability in female secretion is great enough to obscure the results of heavy copulation on the part of the males.

2. The Number of Spermatozoa as Affected by Frequency of Copulation.

The most common method used for determining the breeding power of males, other than the actual breeding test is the numerical study of the sperm cell content of their semen. Writers on
artificial insemination in mammals have made this one of their chief points of discussion, and have not hesitated to emphasize the importance of a large number of active sperm cells for successful fertilization.

Lade, as already pointed out, has studied the properties of human semen and of dog semen and he attaches considerable importance to the sperm counts that he made.

Miles (1907, p. 279) states that it has been demonstrated experimentally that several sperm cells are necessary for complete impregnating of the egg of lower animals, but he considers it perhaps impossible to determine whether this is the case in the higher animals.

Iwanoff (1907) considers the presence of a large number of sperm cells as essential for successful results in artificial insemination. He also says that sexual excitement is a very potent factor in stimulating the frequent liberation of sperm cells into the epididymis, thus probably increasing the certainty of a larger number of eggs being fertilized.

Lewis, to whom reference has been frequently made, considers the number of sperm cells produced by the horse and the pig as a fairly good index to their breeding power, and considers the decrease in sperm content of semen from stallions which are used regularly at about the rate usually practiced by stallion owners, as an index of reduced reproductive power.

Detlofson (1914, Figs. 91-92) found that with hybrid guinea-pig males, the presence of a high percentage of normal active spermatogenesis in the fluid obtained from the epididymus was a very good indication of breeding power. He did find, how-
ever, that 10.2 per cent of such hybrid guinea-pig males were sterile though a copious supply of active sperm was present. He attributes these cases to the inability of the sperm cells from some hybrid animals to fertilize the eggs and not to a lack of active sperm cells.

Reynolds (1916, p. 1194) on the other hand, deems quantitative study of sperm an insufficient index of fertilizing power. In discussing the properties of the semen of man in relation to fertility, writes: 'By our studies have gone perhaps further than this (referring to Betlofen's work just quoted), tending to show that in estimating the fertility of a given male we must judge not only of the numerical frequency of the spermatoscoa and of the percentage of motility present but must further study carefully their vitality, both, as it is determined by their duration observations and still more importantly by the quality of the motility present.'

The number of sperm cells per cubic millimeter in semen of mammals has been known for a long time to vary greatly with the amount of sexual use that the male is put to. Lepinkassse (1917) agrees with Reynolds and follows the same general methods for studying the fertility of men.

Lede (1891) presents data that shows that the number of spermatoscoa per cubic millimeter in human semen decreases from 55,200 at the first copulation to 0 on the third copulation of that day. On a second observation, the number decreased from 56,800 on the first to 19,400 on the second copulation. With a dog, the number of sperm for the first ejaculation was 75,000 per cubic millimeter and fell to 5,000 on the fourth ejaculation.
With a second observation on the same dog, the numbers were 50,000 on the first ejaculation and 55,000 on the fourth. Still a third count at a later period showed 56,480 on the first and no sperm on the fourth ejaculation.

Iwanoff (1907, p. 494) in writing of a stallion, says that the number of sperm cells decreased greatly during the third and fourth copulations in a day but he does not give the numbers.

Lewis (1911) reports the number of sperm cells per cubic millimeter in the semen from a draft stallion as 151,750 at the first service and 5,840 on the ninth service made at the rate of one copulation daily for nine successive days. A grade stallion showed 66,500 sperm cells in semen from the third copulation made in two days and 25,000 in the twentieth copulation made at the rate of two copulations daily.

Since the total number of sperm cells actually ejaculated by a male during coitus depends upon the number of cubic millimeters of semen produced as well as on the number of cells in each millimeter, and since, as has been shown above the volume of semen decreases with repeated copulations, it is apparent that the total sperm number also decreases very rapidly.

Lewis (1911) found 8,365,750,000 as the total sperm number from the first ejaculation of a stallion and only 29,200,000 on the ninth copulation made in a period of eight days.

Lode (1891) found that a dog produced semen containing 56,250,000 sperm cells on the first ejaculation and 4,512,000 on
the fourth ejaculation of that day.

Later observations showed the number to decrease from 45,184,000 to 0. He found a man to ejaculate 153,000,000 sperm on the first ejaculation and no sperm on the third copulation of that day. A second observation showed 170,400,000 at first copulation and only 38,600,000 at second copulation.

Rabbit semen, like semen from other mammals, reported by different observers, shows great variability in sperm numbers per unit volume. Rather frequent copulation seems to stimulate the production of spermatozoa. When a male rabbit is not allowed to serve a female for a week or more, there is a great decrease to far below the normal number of sperm cells for the first ejaculation. The records show that Male No. 1 on June 28 ejaculated semen with 5,000 sperm cells per cubic millimeter, while semen from the second copulation made a few minutes later showed 70,000 sperm cells to the cubic millimeter. The semen from this same male on June 30 showed 220,000 sperm cells at the first copulation and 780,000 in semen from the fourth copulation made during the same afternoon.

Male No. 3 on September 26, ejaculated semen with 6,250 sperm cells to the cubic millimeter, while on the second service made a few minutes later the number rose to 26,560. Counts made from the first copulation of this male on December 21, failed to reveal any sperm cells, while from the fifth copulation made one hour later, there was recovered semen with 10,7000 spermatozoa per cubic millimeter. On the tenth copulation made two and three-fourths hours after the first there were no sperm cells found, and from the twelfth made three and one-half hours after the first semen showed 29,500 cells to the cubic millimeter. This simply shows what a variable factor
the number of sperm cells is as is apparent from an examination of the data obtained by many investigators.

It is interesting to note that male No. 3 made eight copulations in one hour on December 11 after which he refused to serve any more on that day. He was tried again on December 17 and made two copulations after which he was unable to serve again though he made a number of attempts, apparently an erection was impossible. December 21 or ten days from the date that he was apparently overworked, he was able to serve normally and accomplished twelve copulations in three and three-fourths hours. Observations and counts were made on this date and are reported above.

Observations made showing the number of spermatozoa to be far below normal on the first copulation after a period of rest following excessive service confirm the observations of Lodge (see tables of sperm number presented in this paper) on the dog. He attributes this to the resorption or destruction of the sperm, for he found an enormous increase in number to far above normal on the second day following a series of four ejaculations. This condition is apparently due to the increased stimulus to sperm production initiated by repeated copulations two or three days previous.
### Table III
Number of Sperm per Cubic Millimeter in Semen from Different Services.

**Male No. 3**

<table>
<thead>
<tr>
<th>Date</th>
<th>1st</th>
<th>5th</th>
<th>10th</th>
<th>15th</th>
<th>20th</th>
</tr>
</thead>
<tbody>
<tr>
<td>1915</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12/8</td>
<td>235,000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1916</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7/5</td>
<td>21,250</td>
<td>55,000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7/7</td>
<td>108,000</td>
<td>15,600</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7/11</td>
<td>279,000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9/26</td>
<td>6,250</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2d service</td>
<td>26,560</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10/22</td>
<td>7,290</td>
<td>45,740</td>
<td>26,250</td>
<td>10,410</td>
<td></td>
</tr>
<tr>
<td>11/6</td>
<td>66,700</td>
<td>16,300</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12/30</td>
<td>44,000</td>
<td>72,000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12/31</td>
<td>47,000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1917</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/24</td>
<td>26,000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Male No. 4**

<table>
<thead>
<tr>
<th>Date</th>
<th>1st</th>
<th>5th</th>
<th>10th</th>
<th>15th</th>
<th>20th</th>
</tr>
</thead>
<tbody>
<tr>
<td>1916</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6/27</td>
<td>230,000</td>
<td>50,000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6/29</td>
<td>5,000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7/6</td>
<td>191,400</td>
<td>117,500</td>
<td></td>
<td></td>
<td>557</td>
</tr>
<tr>
<td>9/20</td>
<td>8,330</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10/1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11/9</td>
<td>15,000</td>
<td>5,000</td>
<td>1,500</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12/21</td>
<td>10,786</td>
<td></td>
<td>29,500</td>
<td></td>
<td>10,700</td>
</tr>
<tr>
<td>1917</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/10</td>
<td>316,000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/19</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>107</td>
</tr>
</tbody>
</table>

**Male No. 1**

<table>
<thead>
<tr>
<th>Date</th>
<th>1st</th>
<th>5th</th>
<th>10th</th>
<th>15th</th>
<th>20th</th>
</tr>
</thead>
<tbody>
<tr>
<td>1915</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11/15</td>
<td>244,000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11/17</td>
<td>344,000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1916</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6/22</td>
<td>270,000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6/28</td>
<td>5,000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2d service</td>
<td>70,000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4th</td>
<td>780,000</td>
<td></td>
<td>25,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7/6</td>
<td>93,700</td>
<td>27,000</td>
<td>15,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8/9</td>
<td>1,250</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6/23</td>
<td>597,000</td>
<td>370,000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9/16</td>
<td>310,000</td>
<td>160,000</td>
<td>240,000</td>
<td>17,857</td>
<td></td>
</tr>
<tr>
<td>10/13</td>
<td>7,140</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11/14</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11/23</td>
<td>6,000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12/9</td>
<td>300</td>
<td>250</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12/22</td>
<td>62,000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12/26</td>
<td>72,000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Table III (Cont)

#### Male Eq. 1 (Continued)

<table>
<thead>
<tr>
<th>Year</th>
<th>Value</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1917</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/1</td>
<td>194,000</td>
<td>12,000</td>
</tr>
<tr>
<td>1/4</td>
<td>58,000</td>
<td></td>
</tr>
<tr>
<td>1/13</td>
<td></td>
<td>1,120</td>
</tr>
<tr>
<td>1/14</td>
<td>22,000</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>136,894</td>
<td>77,511</td>
</tr>
</tbody>
</table>
A striking feature of the data presented in Table III is the marked variability of sperm numbers produced by the same male at different periods. The minimum count for the first service was 3,000 spermatozoa per cubic millimeter, and the maximum count was 597,000. Male No. 4 that produced the minimum number so far recorded for the first service, had two days previously produced 230,000 spermatozoa to the cubic millimeter. This wide range of numbers appears to hold good for all three males and is difficult to explain in many instances. The minimum production by Male No. 4 may be explained, however, by the fact that two days previously he had made ten copulations and probably had not recovered his normal condition of sexual activity. Why there should be a production of sperm cells as large as 597,000 per cubic millimeter is difficult to explain, since this male had not copulated for thirteen days previously.

A study of Table III indicates that the maximum sperm cell production lies between the first and the 5th copulation or thereabouts. In no instance does the number of spermatozoa per unit volume decrease on the second copulation and generally always shows a marked increase. In a considerable number of observations there are larger numbers of spermatozoa to the unit volume on the fifth than on the first copulation. This can be accounted for in no other way than by assuming that a certain amount of sexual activity stimulates sperm production. There is a considerable decrease in numbers between the fifth and the tenth copulations, and only one observation showed larger numbers per unit volume on the tenth than on the fifth copula-
tion. Since this male had not copulated for sixteen days previous, it would appear that his testes had become somewhat inactive and that the stimulus of copulation to increase the sperm numbers extended further than is generally the case. There is always a marked falling off in numbers by the time the fifteenth copulation is reached. This decrease ranges from 75 to 95 per cent of the number at the tenth copulation, but no sample from the fifteenth service has been found to be absolutely sperm-free. There appears to be a rather important difference in numbers also between the fifteenth and twentieth copulations. This decrease is very significant because the numbers are becoming extremely small. One observation shows the complete absence of spermatogenesis on the twentieth copulation and in other observations the numbers were greatly reduced. These small numbers certainly would make the chances for the sperm reaching the ova much less certain.

As has already been mentioned, heavy service tends to stimulate the testes to greater activity. This is apparent as soon as the male has recovered the normal sexual state, which is usually about the third or fourth day after he has made a series of fifteen or twenty copulations. This is well illustrated by the counts made on samples from male No. 3 on July 3, 7 and 11, 1916, and male No. 1 on December 22 and 26. The counts made on successive copulations show how the sperm cell numbers increase up to somewhere around the fifth copulation. Counts made before the males had recovered their normal sexual state are shown in Table III. For example male No. 4 made ten copulations on June 27
Semen from the first copulation showed 230,000 sperm cells per cubic millimeter on this date. Two days later on June 29, a sample of semen recovered from the first copulation showed but 3,000 spermatozoa to the cubic millimeter.

The average number of sperm cells is presented in Table III including all males as determined from the different services. To simplify results the second service observations and all other observations below the fifth are grouped with the first service group. The decrease from the average of the first service column to the fifth service column is about 50 per cent; from the fifth to the tenth about 35 per cent; from the tenth to the fifteenth about 90 per cent; and from the fifteenth to the twentieth service group about 11 per cent. While such averages are only rough approximations, the fact is very apparent that between the tenth and the twentieth copulation there is a very great falling off in the sperm content of the semen.

To recapitulate, studies on the number of sperm cells per cubic millimeter reveal very great fluctuations. This agrees with the observations of other workers on the sperm cell numbers of other animals; and seems to indicate that the general state of vital force of the male organism acts as the governing agent of sperm production. Sexual service stimulates the production of semen with larger numbers of spermatozoa up to about the fifth copulation. Excessive copulation reduces the sperm numbers to a very low figure, and some three or four days are required for such a male to recover the ability to produce normal semen.
Possible sources of error in connection with spermatocoe counts.

(a). Spermatocoe exhibit a strong tendency to attach themselves to the vaginal and cervical walls. There is thus the possibility that the semen, when recovered from the vagina, contains fewer spermatocoe per unit volume than when it was deposited there by the male. This possible inaccuracy would be more pronounced when the number of spermatocoe is relatively small as in advanced services. This matter is altogether beyond the control of the investigator.

(b). The possibility exists that the secretions of the female genitalia may vary widely in amount so as to considerably influence the counts even though the secretions were removed as far as possible before the cohabitation. The influence of these secretions is certainly quite important in this connection.

(c). Considerable error is likely to occur in counts made with a haemocytometer. Errors from clumping were avoided by diluting the fresh semen with either twenty-four or forty-nine volumes of a two per cent solution of sodium carbonate, which is very effective in preventing clumping. The rule was to make several counts of one hundred squares to the slide and to average the results.
On the whole, studies on the male rabbit seem to indicate that a certain amount of sexual use tends to increase the number of sperm cells, that ten successive copulations has no marked effect, that fifteen copulations greatly reduces the sperm numbers, and that twenty successive copulations generally reduces the number of sperm cells to a very low figure and that semen from the twentieth copulation should be considered decidedly inferior as judged from its numerical sperm content.

3. Motility as affected by frequency of copulation.

Motility of the spermatosoea is considered very good evidence that a sample of semen possesses fertilizing power. Such modern workers as Iwanoff, Heps, Lewis, Betleisen, Reynolds and many others who are studying the question have referred to this matter; they consider that motility is a good index to fertilizing power.

Iwanoff (1907, p. 473) writes that the sperm cells may accumulate in the epididymus for several days without losing their motility and that for successful artificial insemination the motility of the semen is of considerable importance.

On the other hand Betleisen's (1914) work with guineas-pigs, already mentioned, shows that the presence of active spermatosoea in the semen does not always indicate that the male is a "sure breeder".

Reynolds (1916) after extensive studies on the question of sterility in the human subject does not consider the mere presence of active spermatosoea in the semen of a man as certain evidence of his power to reproduce. He considers that investigations, to be reliable, must take into account not only the power of endurance
of the sperm cells but also their quality of motion.

It is absolutely necessary for a sperm cell to come in contact with the ripe ovum for fertilization to take place, and the power of motility of sperm cells seems to furnish the only mechanism by which these male and female elements are brought together.

Lott (1872, P. 120 and 140) mentions seven possible factors that may aid the sperm in reaching the egg: 1. Force of ejaculation; 2. Motility of vagina and cervix; 3. Sperm sucked out of the vagina by sucking action of the uterus; 4. Sperm deposit in the cervix by the penis; 5. Sperm are carried by capillarity into the cervix and from there further on; 6. Through ciliary movement; and 7. By their own force of movement. He found that sperm taken from the epididymis of a dog move at the rate of .06 m.m. per second against a current under a glass slide.

Bischoff (1848, Pgs. 25 and 27) states that the sperm advance in the fallopian tubes to meet the egg upon its liberation. In regard to the rabbit, he says: "Wie fand ich auch hier Samenfaden nach dem Dierstech, vorher dagegen oft in verschiedenen Distanzen in Uterus und in den Hileiter vorgerückt." Bischoff (page 29) reports that Henle observed mammalian sperm to move forward in a straight line at the rate of .056 m.m. per second.

Caste(1869, quoted by Hensen) found that spermatozoa are deposited only in the vagina of the rabbit by the male and that 2-3/4 hours later they have advanced as far as the ovaries.

Esape (1898, P. 206) has observed that in the rabbit the semen is taken from the vagina into the uterus.
sucking action of the uterus, the os appearing to dip down into the semen lying on the floor of the vagina and to be again withdrawn with peristaltic contraction of the uterus.

In order to progress toward the ovaries it is necessary for the sperm cells to advance against the force of the currents set up in the opposite direction by the cilia of the fallopian tubes.

Kraft (1890, p. 216) observed that rabbit sperm swim rapidly against the currents set up in a physiological salt solution by the movements of the cilia on a small piece of mucous membrane from the uterus of a freshly-killed cow. He concludes that the current produced by the ciliary movement act purely as a stimulant to the sperm.

Roth (1893, p. 352) found that sperm move with great force against the currents under a cover-glass. He thinks that the cilia in the tract give the sperm the necessary direction, and at the same time prevent the passage of anything but very active sperm toward the ovaries.

Fertilization is thought to take place in the fallopian tubes of the rabbit, according to the observations of Bischoff and others. The fact that the semen is always deposited in the vagina by the male has already been pointed out. Taking these facts into account, it appears that the spermatozoa would be required to pass from the os to the upper end of the fallopian tubes to accomplish fertilization. This furnishes a mechanism whereby the most active sperm reach the ovum first and accomplish fertilization; the weak and slow-moving or immature sperm cells would be held back by the force of the cilia. Thus as Roth (1893)
points out, a mechanism is provided which prevents the weak and slow moving sperm from fertilizing any of the ova. If all of the spermatozoa are weakened or immature, the result should be very apparent in the breeding records of such males for the semen of such males would cause conception in only a small percentage of cases.

Keape (1905) in commenting on the fertilization of the rabbit, writes: "It is necessary then, in the case of the rabbit at any rate, that spermatozoa should be present in the fallopian tubes, and I find that as a rule they are to be found at the top of the uterus horn two hours after coxulation and close to the infundibulum, if not actually within its folds, four hours after copulation".

Henson (1875, p. 232) estimates that about 68 minutes are required by the sperm of the guinea pig to reach the ovum after copulation.

It seems very apparent from these observations that the motility possessed by sperm cells is of vital importance in bringing them quickly to the ovum. Iwanoff (1907, p. 494) found that in a stallion the rate of motility was largely dependent upon the amount of breeding the animal was doing. Beginning August 20, this stallion made two copulations; August 21, three copulations; August 22, three copulations and August 23, four copulations at intervals of two hours. Semen from the last three copulations showed very feeble motion.

The most extensive and suggestive and at the same time most recent work on motility of mammalian spermatozoa is that of Reynolds (1916). This investigator found that there are different categories of motion which sperm may exhibit. He reports that human spermatozoa show three normal types of motion: 1. Progressive vibrat-
ile, which consists of a rapid vibration of the after part of the flagellum propelling the sperm rapidly forward with the head moving practically in a straight line. This appears to be the type first shown by the most active sperm cells and by this form of motion they make greater forward progress than by any other type. 2. Progressive Vibratile is characterized by a lashing from side to side of the entire tail, causing the head and middle piece to sway back and forth through an arc of sometimes 90 degrees. This type of motion normally follows the first and is characterized by a loss of speed and the sperm seem to have the power to govern their position largely by the surroundings. 3. The third type succeeds the second and is distinguished by a bunting action of the head and a vibratile motion of the tail like that of the caudal fin of a fish. This type of motion is slower than the other two and the sperm is noticed to bunt into any debris or other material in the semen. Reynolds considers that this type enables the spermatozoa to penetrate the egg membrane and to fertilize the ovum.

These three normal types of motion succeed each other and unless the sperm cells are very vigorous they do not attain all these types, especially the third. Reynolds considers the third type the most important in that it may enable the sperm to bore its way through the egg membranes in the ovum.

In studying the motility of rabbit spermatozoa in this experiment an effort has been made not only to study the rate of progress of the cells but also the types of motility present. For the motility studies it is necessary to use a slide with a hollow chamber so that the sperm cells may not be impeded in their pro-
gross by the cover-glass. A haemocytometer was found best because the graduations made the linear distance covered by the sperm easily determined and there was an abundance of free space for the cells to move in. Fresh semen was diluted with nine volumes of Ringer's solution and motility determined at once. A drop of this diluted semen was placed in the counting chamber on the haemocytometer and the cover-glass placed over. A stop watch was used for recording the time required by a considerable number of spermatozoa showing type one of motion recorded. The number of spaces covered by the sperm cell was considerable and an effort was made to take the average rate of as many cells as possible in a short time. An average of the results was taken as the correct measure of motility and recorded together with the temperature of the liquid in which the sperm were moving. Ringer's solution was used for studying this progress, using a uniform dilution with 9 volumes of this solution. Unless there were a number of cells exhibiting type one of motion, motility was not recorded. All types of motion present were noted and their relative prevalence recorded.

Observations made on the rabbit semen agree rather closely with Reynolds as to the types of motion that may be present. A considerable number of examinations of semen from the tenth, fifteenth and twentieth services shows the absence of progressive vibratile motion or its very infrequent occurrence among the cells present. This condition was rarely met with in the first or fifth service as the tables show.
Many cells from these advanced services move in a small circle either clockwise or counterclockwise and show little or no progressive motion. Other types such as the pendulum-like swing—considered by Reynolds as abnormal—and the spiral motion by a rotary movement of the entire cell, are more often present in the semen from the advanced services.

The accessory ruff that Reynolds describes has also been observed in rabbit semen, more often in semen that has been kept in the laboratory for several hours or in semen from advanced services. This is a ruff around the middle piece like a collar. It may be all on one side or it may not take away from the symmetry of the cell.
Table IV (Cont)
Rate of Motion of Spermatozoa From Different Services; Millimeters Covered per Second; and Temperature at Each Observation.

**Male No. 3**

<table>
<thead>
<tr>
<th>Date</th>
<th>lst</th>
<th>5th</th>
<th>10th</th>
<th>15th</th>
<th>20th</th>
</tr>
</thead>
<tbody>
<tr>
<td>1916</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8/1</td>
<td>.043</td>
<td>.040</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8/18</td>
<td>.044</td>
<td>All Dead</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10/6</td>
<td>21</td>
<td>.026</td>
<td>21</td>
<td>.022</td>
<td></td>
</tr>
<tr>
<td>10/22</td>
<td>22</td>
<td>.021</td>
<td>22</td>
<td>.024</td>
<td>20</td>
</tr>
<tr>
<td>11/6</td>
<td>22</td>
<td>.029</td>
<td></td>
<td>22</td>
<td>.023</td>
</tr>
<tr>
<td>12/21</td>
<td>21</td>
<td>Whirling 21</td>
<td>.020</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12/30</td>
<td>19</td>
<td>.021</td>
<td>20</td>
<td>.021</td>
<td></td>
</tr>
</tbody>
</table>

**Male No. 4**

<table>
<thead>
<tr>
<th>Date</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1916</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10/1</td>
<td>No Sperm</td>
<td>22</td>
<td>.023</td>
<td>22</td>
<td>.009</td>
</tr>
<tr>
<td>10/15</td>
<td></td>
<td>20</td>
<td>.004</td>
<td>19</td>
<td>.016</td>
</tr>
<tr>
<td>10/31</td>
<td>16</td>
<td>.005</td>
<td></td>
<td>18</td>
<td>.003</td>
</tr>
<tr>
<td>11/9</td>
<td>16</td>
<td>.009</td>
<td></td>
<td>17</td>
<td>.012</td>
</tr>
<tr>
<td>12/23</td>
<td>19</td>
<td>.019</td>
<td></td>
<td>18</td>
<td>.008</td>
</tr>
<tr>
<td>1917</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Second*</td>
<td>21</td>
<td>.017</td>
<td>21</td>
<td>.022</td>
<td>20</td>
</tr>
</tbody>
</table>

*" Indicates very little motion.
**Observations made on semen from second copulation."
Table IV represents a compilation of our observations that were made at times when specimens of semen could be obtained from a series of services. Each observation represents a large amount of time and labor. Since the object in presenting this table is to show the relative rates of motion of spermatozoa from different copulations made by the same male, a large number of observations on first and second service specimens of semen are not included.

Table IV shows that there is considerable fluctuation in rate of motility of the spermatozoa in specimens of semen from the same service and that there is a tendency for the movement to be less in the advanced services at the same temperature. Variations in temperature of four or five degrees do not seem to have a very pronounced effect upon movements of the sperm cells. As a rule there is a slightly greater rate of motility in specimens of semen from the fifth than from the first copulation. This may be explained by the fact already pointed out that sperm that have been in the epididymis for some hours, as probably most of the sperm cells liberated on the first few copulations, have lost to a considerable degree the power of motility when compared with spermatozoa that have been freshly produced by the testicle. An examination of Table IV also shows that when the tenth copulation is reached there is a diminution in rate of motility and that specimens of semen from the fifteenth seem to show a still greater decrease in motility. The table does not show a constant decrease in motility and for this reason too much stress can not be laid upon the matter of decrease in the rate of motility due to excessive copulation until further experimental evidence is available.
The apparent decrease in rate of progressive motion certainly must have considerable significance in our breeding operations. The fact that several hours probably elapse between the time of coition and the time of fertilization has already been pointed out. This interval is due to two causes; first, in the female rabbit the ovum is not liberated for several hours after the beginning of the heat period, and; second sometime is required for the spermatozoa to advance to reach the fimbrial tubes where fertilization takes place even though the ova were present in the tubes at the time of copulation. If the sperm are greatly retarded in reaching the ovum there would certainly be a noticeable effect on the offspring record both as to size of litters and certainty of pregnancy. This will be discussed further when these two phases of the subject are considered.

Numerous examinations of specimens of semen from the early and from the advanced services show that as the number of copulations increases the number of spermatozoa showing the progressive type of motion becomes smaller. This is a most significant observation and certainly must be a change in the properties of spermatozoa that means much in breeding work. In fifteenth and twentieth copulations the number of sperm cells showing this type of motion is often exceedingly small thus indicating that there must be but a few spermatozoa that will ever reach the ovum in the tubes even though, in some cases, there may be several thousand spermatozoa present in each cubic millimeter of semen. The exact percentage of spermatozoa showing the progressive type of motion in fresh semen diluted with ten volumes of Ringer’s solution has not been recorded but observations show that the percentage falls
after the fifth copulation as the number of copulations increases, but we are of the opinion that this fact is of such importance as to be greatly emphasized in studying the effects of heavy service on the properties of semen.

3. Possible sources of error in motility studies.

(a) Variations in air temperature may have had a more marked effect upon the motility of spermatozoa in small quantities of semen than in the larger quantities recovered from the earlier services, because small volumes are more subject to temperature variations as well as to the effects of desiccation. The tables presented show that sperm cells are affected considerably by air temperature and this will partly explain why specimens of semen from the same service of the same male vary in longevity and motility.

(b) The presence of larger number of epithelial cells in semen from the advanced services may have put the spermatozoa to considerable disadvantage in their progressive forward motion as compared with spermatozoa in semen containing less of this "foreign" material. This material can not be removed from the semen used for study.

(c) Certain errors are made by the use of the stop watch for recording time since the time of starting and stopping must be considered. This error is not considered very great, however.

4. Longevity as affected by frequency of copulation.

The longevity of the sperm cells in a sample of semen undoubtedly has an important bearing on the fertilizing power of that semen. Unless the spermatozoa possess sufficient endurance
to retain their vital activity for several hours within the
female genitalia, they never take part in fertilization. Females
are generally bred when first showing signs of heat, and according
to Bischoff (1842, p. 141) the rabbit ova are not liberated for
nine or ten hours after copulation.

Espe (1905) expresses himself similarly on this point
and notes that from two to four hours are required after copulation
for the spermatosoma to reach the point in the fallopian tubes
where fertilization probably takes place.

Lewis (1911) found that fertilization in the hog may
be accomplished in some cases by sperm cells that have been in
the female genital tract for twenty-four hours, but never for a
longer time.

His work also shows that the longevity of horse sperm
decreases with the number of copulations.

Wright (p. 579) thinks that the superior vigor of Amer-
ican turkeys may be due in part to the fact that male birds are
allowed to run with the hens and thus to fertilize them more than
once during each egg-laying cycle; while in Britain hens are usually
taken to the male bird but once during each egg laying cycle.

Spermatosoma probably live longer within the female gen-
ital tract than in vitro under laboratory conditions. Prevost and
Dumas (1910, quoted from Marshall) found moving spermatosoma in
the genital tract of the female rabbit eight days after coition.

Summer's (1915) studies in mice seem to indicate that
spermatosoma may retain their fertilizing power for as much as
twenty-five days in the female genital tract.
Lewis (1911) investigated this question quite thoroughly in the pig and found that spermatozoa would not live longer than twenty-four hours in the genitalia of the sow and still retain the power to fertilize the ovum. Similar sperm lived from six to sixteen hours in vitro in the laboratory.

All the above observations confirm the view held that the longevity of spermatozoa is a factor of great importance to successful fertilization.

Results of rather extensive studies on the effects of frequency of copulation of rabbit males on the longevity of their spermatozoa, confirm the observations of Lewis on the stallion; namely, as a rule, the greater the number of services made by a male in a given period, the shorter will be the life of his spermatozoa.

All the observations were made by placing samples of rabbit semen from the first, fifth, tenth, fifteenth, and twentieth copulations in a period of not more than six hours, into small glass vials with cotton stoppers. These were all placed on the same shelf in the laboratory and were not exposed to direct sunlight, but were exposed to the varying temperatures of the room. There is much fluctuation in results from day to day, due in part to changes of air temperature, humidity, light, etc. Results obtained at the same period, however, are entirely comparable for different samples under observation at the same period. The percentage of sperm living was obtained by counting out a hundred or more cells just as they appeared in the field of observation on a freshly prepared slide, and these were distributed into the classes "living" and "dead"
depending on whether or not there was any motion present. As has been mentioned under motility semen was diluted 10 volumes with Ringer's solution, which does not reduce the longevity, in order to avoid the deleterious effects of desiccation.

Longevity was recorded in natural semen as well as in the diluted semen whenever the recovery was sufficiently large to supply amounts for dilution and for study in the undiluted state. In case the recovery of semen was small, it was all diluted and no observations attempted on the material in the natural state.

Table V. A.

Length of Life of Spermatids as Affected by Number of Services—Percentage Moving after Various Periods of Time have been Used. (All Semen Diluted with Ten Volumes of Ringer's Solution.)

<table>
<thead>
<tr>
<th>Male No. 1</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Date</td>
<td>1st</td>
<td>5th</td>
<td>10th</td>
<td>15th</td>
<td>20th</td>
<td></td>
</tr>
<tr>
<td>1916</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10/2</td>
<td>16</td>
<td>24</td>
<td>16</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11/14</td>
<td>23</td>
<td>20</td>
<td>20</td>
<td>0</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>10/23</td>
<td>16</td>
<td>55</td>
<td>16</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10/31</td>
<td>23</td>
<td>45</td>
<td>20</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12/9</td>
<td>23</td>
<td>93</td>
<td>2</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12/22</td>
<td>4</td>
<td>90</td>
<td>12</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12/26</td>
<td>6</td>
<td>60</td>
<td>16</td>
<td>5</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>20/15</td>
<td>28</td>
<td>1</td>
<td>8</td>
<td>5</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>1</td>
<td>44</td>
<td>4</td>
<td>35</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>12/18</td>
<td>75</td>
<td>44</td>
<td>8</td>
<td>35</td>
<td>12</td>
<td></td>
</tr>
</tbody>
</table>

Second Service

4 44
8 35
12 18
### Table V. A. (Cont.)

#### Male No. 1

| Date       | 1st | 2nd | 3rd | 4th | 5th | 6th | 7th | 8th | 9th | 10th | 11th | 12th | 13th | 14th | 15th | 16th | 17th | 18th | 19th | 20th |
|------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|------|------|------|------|------|------|------|------|------|
| 12/25      | 20  | 15  | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    |
| 1917       |     |     |     |     |     |     |     |     |     |      |      |      |      |      |      |      |      |      |      |
| 1/1        | 0   | 35  | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    |
| 1/3        | 0   | 35  | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    |
|           |     |     |     |     |     |     |     |     |     |      |      |      |      |      |      |      |      |      |      |      |
|           |     |     |     |     |     |     |     |     |     |      |      |      |      |      |      |      |      |      |      |      |

#### Male No. 3

<table>
<thead>
<tr>
<th>Date</th>
<th>1st</th>
<th>2nd</th>
<th>3rd</th>
<th>4th</th>
<th>5th</th>
<th>6th</th>
<th>7th</th>
<th>8th</th>
<th>9th</th>
<th>10th</th>
<th>11th</th>
<th>12th</th>
<th>13th</th>
<th>14th</th>
<th>15th</th>
<th>16th</th>
<th>17th</th>
<th>18th</th>
<th>19th</th>
<th>20th</th>
</tr>
</thead>
<tbody>
<tr>
<td>1916</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12/30</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>16</td>
<td>4</td>
<td>55</td>
<td>16</td>
<td>25</td>
<td>24</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>24</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
### Table V. A. (Cont.)

<table>
<thead>
<tr>
<th>Date</th>
<th>1st</th>
<th>5th</th>
<th>10th</th>
<th>15th</th>
<th>20th</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hrs.</td>
<td>Hrs.</td>
<td>Hrs.</td>
<td>Hrs.</td>
<td>Hrs.</td>
</tr>
<tr>
<td>1916</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10/1</td>
<td>30</td>
<td>72</td>
<td>30</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>10/15</td>
<td>28</td>
<td>0</td>
<td>28</td>
<td>75</td>
<td>26</td>
</tr>
<tr>
<td>11/9</td>
<td>20</td>
<td>75</td>
<td>All Dead</td>
<td>15</td>
<td>50</td>
</tr>
<tr>
<td>24</td>
<td>20</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12/28</td>
<td>16</td>
<td>5</td>
<td>16</td>
<td>15</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>0</td>
<td>20</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1917</td>
<td>Second</td>
<td>Service</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>71</td>
<td>0</td>
<td>77</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>35</td>
<td>2</td>
<td>30</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>12</td>
<td>43</td>
<td>2</td>
<td>12</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>16</td>
<td>42</td>
<td>13</td>
<td>0</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td>20</td>
<td>31</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>19</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table V. B.

**Longevity of Spermatozoa in Natural Season**

<table>
<thead>
<tr>
<th>Date</th>
<th>1st</th>
<th>5th</th>
<th>10th</th>
<th>15th</th>
<th>20th</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hrs.</td>
<td>Hrs.</td>
<td>Hrs.</td>
<td>Hrs.</td>
<td>Hrs.</td>
</tr>
<tr>
<td>1915</td>
<td>3</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12/8</td>
<td>5</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1916</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10/15</td>
<td>28</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10/14</td>
<td>28</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10/31</td>
<td>18</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>27</td>
<td>40</td>
<td>27</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1917</td>
<td>1/4</td>
<td>4</td>
<td>70</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>60</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>31</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>17</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table V. B. (Cont)

Male No. 3

<table>
<thead>
<tr>
<th>Date</th>
<th>1st Hrs.</th>
<th>5th Hrs.</th>
<th>10th Hrs.</th>
<th>15th Hrs.</th>
<th>20th Hrs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>9/26</td>
<td>20</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>10/16</td>
<td>28</td>
<td>30</td>
<td>24</td>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td>10/22</td>
<td>30</td>
<td>35</td>
<td>24</td>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td>11/5</td>
<td>24</td>
<td>24</td>
<td>24</td>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td>12/21</td>
<td>24</td>
<td>24</td>
<td>24</td>
<td>24</td>
<td>24</td>
</tr>
</tbody>
</table>

Male No. 4

<table>
<thead>
<tr>
<th>Date</th>
<th>1st</th>
<th>20 Hrs.</th>
<th>20 Hrs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>10/1</td>
<td>20</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10/15</td>
<td>24</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10/31</td>
<td>20</td>
<td>40</td>
<td>0</td>
</tr>
<tr>
<td>11/9</td>
<td>30</td>
<td>50</td>
<td>24</td>
</tr>
</tbody>
</table>

Table V shows that the length of time that spermatozoa retain the power of motion varies greatly and motility must be of considerable importance inasmuch as it may be a determining factor in the fertilizing power of the semen.

The average length of life of specimens of semen diluted with nine volumes of Ringer's solution depends upon the number of copulations that immediately precede the copulation from which the particular sample was obtained. From
first services the average duration of motility is 24 hours; for fifth services, 20 hours; for tenth services, 18 hours; for fifteenth services, 16 hours; and for twentieth services, 8 hours. These differences are certainly significant and cannot but mean that the fertilizing power of the semen tends to decrease as the number of copulations increases; and our results seem to indicate that the duration of motility is one of the most if not the most important index to fertilizing power.

In discussing the properties of semen the fact has been pointed out that spermatozoa tend to accumulate in the epididymus of the male from day to day. These sperm cells gradually lose their motility and samples of semen from a single copulation made after several days rest generally shows a larger percentage of dead or feebly moving spermatozoa than semen following several copulations. This has been accounted for by the removal of the stagnant seminal content from the epididymus. In table 7 the greater duration of motility in samples of semen from the second copulation as compared with the first is very apparent. The same is true when samples of first service semen are obtained on successive days; but when several days intervene the shorter duration of motion becomes very noticeable.

Table V. B, while not extensive, serves to show on the whole there is a shorter duration of motility of spermatozoa in samples of semen in the condition that it comes from the vagina of the female than when diluted. Another noticeable feature is the fact that there is relatively less difference in duration of
motility between advanced and first services than has been observed when dilutions of the samples were made with Ringer's solution. A possible explanation lies in the fact already pointed out that the stagnant residues of the epididymus and accessory glands are removed in the first few copulations. Moreover the deleterious products of the metabolism of the sperm are more highly concentrated than in the diluted semen. When the specimen of semen is diluted the toxic effect of these products is lessened by rather heavy dilutions.

Summarizing the observations on the duration of motility of spermatozoa, two important factors stand out: 1. There is a noticeable falling off in the rate of motility as the number of copulations increases. 2. Semen tends to stagnate when males do not copulate regularly from day to day consequently many inactive and short-lived sperm cells are often recovered in specimens of semen from the first few copulations.

4. Possible sources of error in longevity determinations.

(a) Results might have been more nearly comparable if all observations had been made under uniform temperature and moisture conditions. The temperature records in the tables show that the range in temperatures was from 9 degrees as the minimum to 30 degrees as the maximum. The average temperature was about 20.

(b) Comparison of results obtained from semen kept in vitro may be somewhat different from what would have been observed had the spermatozoa been studied within the genital tract of the female, and the less favorable conditions may have reduced the length of life of the sperm cells to an undue extent.
(c) The type of motion exhibited by spermatozoa (as already described) at different observations may have been a more correct index of their longevity within the female than the actual length of time that any motion may have been observed in vitro.

(d) Motion may not be the correct criterion of life, for many spermatozoa probably cease moving long before dying simply going into a motionless state; and varying temperature and moisture conditions may be in part responsible for this.

5. Certainty of Pregnancy as Influenced by Frequency of Copulation of Males.

A brief description of the genitalia as they appear in a specimen of adult female rabbit is here presented in order to give the reader a clearer understanding of the passageway of the sperm to reach the ova.

A transverse slit is continued dorsally on either side of the space between the rectum and clitoris and is two centimeters long and four to eight millimeters deep. It contains near its dorsal end an oval elevation in the anterior wall, lateral to the vagina. This oval elevation is three by five by two millimeters in size and is marked in the center by a pin-point duct. The mucous membrane of the slit shows white and brownish material, which is evidently inspissated sebum.

Beginning with the external opening, we find a thick muscular coat surrounding the vagina and clitoris. In the pelvic portion the wall is very thin and membranous and a well-developed plexus of veins (Bulbus vestibule) erectile tissue appears. The mucous membrane is thrown into longitudinal folds and probably contains some muscle fibers. At the union of the pelvic and abdominal
Plate II

Superficial Dissection of the Female Genitalia of the Rabbit—Lateral View. 1. Labia Majora. 2. Ischium. 3. Ilium. 4. Transverse Slit.

Plate III

Dorsal-Lateral Dissection of Genitalia of Female Rabbit
1. Ilium. 2. Pubis. 3. Ischium. 4. Symphysis Pelvis. 5. Labia Majora.

Plate IV

Deeper Dissection of Genitalia of Female Rabbit—Lateral View

Plate V


Plate VI

portions of the vagina, a well-developed fold, resembling hymen, was present. The abdominal portion of the vagina is very thick walled with the mucus membrane thrown into folds, mostly longitudinal.

The cervix projects into the vagina and is completely surrounded by an annular space, the fornix vaginalis. The muscular coat of the cervix is well developed. The mucus membrane is thrown into distinctly longitudinal folds so that the os uteri externum is surrounded by plicated folds of mucus membrane. The mucus membrane of the uterus is plicated; folds run in all directions but with a tendency toward longitudinal arrangement.

The following measurements of the tract were secured: From external slit to tip of pelvis; 5.5 cms.; membranous portion, 2.2 cms. long; abdominal portion, 6.0 cms. to fundus of pharynx vaginae; cervical canal, 1.4 cms.; uterine horn straightened, 14.6 cms.; Fallopian tube and fimbrice, 10.5 cms. Total length of tract from external opening to the fimbrice surrounding ovary, 38.2 cms.

A study of the female genitalia and a knowledge of where the male deposits the sperm cells during coitus, reveals the fact that a considerable distance must be covered by sperm in their passage to meet the ovum.

It has already been pointed out that in the rabbit semen is largely deposited by the male in the anterior portion of the vagina; and there is great improbability that any of the semen is deposited by the male in the cervix or the uterus, as Holt (1872, P. 128) has shown, that the sperm cells are then carried by the uterine horns by the sucking action of these parts. The sperm cells would then be required to pass practically the full length
of the fallopian tube by their own motion to reach the ova in the upper part of these tubes. This distance is from ten to twelve centimeters in the adult female rabbit.

Sperm cells travel forward in the field of observation on a hemocytometer at the rate of .05 millimeters per second. At this rate 33 to 40 minutes would be required by the sperm to reach the ova after they reach the posterior extremities of the fallopian tubes. This would be the interval in cases where the sperm had the highest rate of motion thus far observed in the rabbit. The observed rate of progress is generally slower, however. In the case observed with male No. 1 on November 14, where the rate of motion was .0003 millimeters per second from the twentieth copulation; 34.7 hours would be required by such sperm to reach the ova. Under laboratory conditions, however, no live sperm cells were observed in the semen secured after 26 hours. The records show that female No. 50 gave birth to two young from this service. One of these was dead at birth. The supposition is that a few of the most vigorous sperm from this copulation came in contact with the uterine membrane or in some other way escaped the catheter when the semen was drawn from the female and were able to progress to meet and fertilize two of the ova. Then, too, the rate of motion of these sperm within the tract where the temperature was about 37 degrees must certainly have been much greater than the rate observed under the microscope at a temperature of 15 degrees.

Lewis (1911) found that horse sperm move more rapidly at high temperatures than at low temperatures, but their length of life in the laboratory is reduced by the higher temperature. The same fact has been observed in the case of rabbit sperm.
There is another factor that probably causes a higher rate of motion of the sperm in the genital tract than that observed on the microscopic slide; namely the counter currents set up by the cilia in the tract. Both Roth (1893) and Kraft (1890, p. 216) are of the opinion that the movement of the cilia of the female tract stimulate the sperm cells to a higher rate of motion toward the ovaries. These observations enable us to conceive how sperm cells with reduced longevity and a slow rate of motion under laboratory conditions sometimes reach and fertilize some of the ova.

Sometimes two services of the male are considered more conducive to pregnancy than a single service. 1st. There may be two factors which might account for this. Heape (1893) attributes this to the greater stimulus produced to bring about the sucking action of the uterus, thus tending to carry the sperm into the uterus and farther toward the fallopian tubes. 2nd. Ivanoff (1907) noted that the sperm cells tend to become stale and less energetic after the male has not copulated for some days. It would appear that fresher and more energetic sperm would be liberated on the second copulation. Thus after a rest period two services are better than one—but if service is regular, this second factor would not be involved. Rusch (1915, p. 182) states that two services may sometimes be better than one to insure pregnancy, but gives no explanation.

Our observations on rabbit semen repeatedly show that sperm from the first copulation are fewer in number, less active, and show less endurance than those from the second copulation made a few minutes later. There is also a much higher percentage of dead spermatozoa in semen from the first copulation. It appears probable, therefore, that the advantage of two services over a single service lies rather in the nature of the sperm cells than in the number produced and added by the second copulation.
Table No. VI
Per Cent Pregnancies from Different Males Grouped by Services.

<table>
<thead>
<tr>
<th>Male No.</th>
<th>No. of Observations</th>
<th>No. of Pregnancies</th>
<th>No. of Observations</th>
<th>No. of Pregnancies</th>
<th>No. of Observations</th>
<th>No. of Pregnancies</th>
<th>No. of Observations</th>
<th>No. of Pregnancies</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>17</td>
<td>47.06</td>
<td>7</td>
<td>35.71</td>
<td>12</td>
<td>58.33</td>
<td>9</td>
<td>55.55</td>
</tr>
<tr>
<td>3</td>
<td>17</td>
<td>70.69</td>
<td>11</td>
<td>81.82</td>
<td>8</td>
<td>75.00</td>
<td>4</td>
<td>50.00</td>
</tr>
<tr>
<td>4</td>
<td>16</td>
<td>50.00</td>
<td>13</td>
<td>46.15</td>
<td>12</td>
<td>50.00</td>
<td>4</td>
<td>25.00</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>50.00</td>
<td>31</td>
<td>50.00</td>
<td>32</td>
<td>50.00</td>
<td>17</td>
<td>50.00</td>
</tr>
<tr>
<td>Weighted Average</td>
<td>56.01</td>
<td>67.74</td>
<td>59.37</td>
<td>47.06</td>
<td>53.85</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table VI presents the complete results of the experiment to show the relation of number of services previously made by males before serving females to the per cent of pregnancies resulting from such matings. The three males used are grouped separately and the weighted averages given are obtained by multiplying the per cent pregnancies from each male by the number of matings made by that male, adding the products and dividing by the total for each of the five service groups.

Considering the males separately, we note that with the exception of Male No. 4, there is a marked increase in the certainty of pregnancy in fifth service matings over first service matings. There is a falling off in the per cent pregnancies in the tenth service group as compared with the fifth service group with males No. 1 and 3 and the reverse with Male No. 4. The per cent of pregnancies is uniformly lower in the fifteenth service group than in the tenth service group. Comparing the twentieth service group with the fifteenth service group the somewhat meager data does not show a decrease on the twentieth. For the other two males the data is too meager to draw any conclusions on the twentieth service.

The weighted average points to the fact that first service matings are less certain than fifth and that from the fifth to the fifteenth service there is a gradual decrease in the certainty of pregnancy. There is no decrease between the fifteenth and twentieth services probably because the number of observations is less than for the first, fifth, or tenth service series. There are 96.1 per cent as many pregnancies in the twentieth service matings as compared with the first service matings, however, not a marked
decrease, which tends to emphasize the fact that males are not as certain to successfully impregnate females by a single copulation as by several copulations. This observation bears out the plausible conclusion that may be drawn from the studies of the properties of semen, especially from the fifth service as compared with the first service. Observations on semen from the first service almost uniformly show a larger percentage of dead spermatozoa, a lower rate of motility and a shorter duration of motility than in samples from the fifth copulation. This being the case, it is only reasonable to conclude that such semen would be less viable and the chances of spermatozoa reaching and successfully fertilizing the ova should be less in first service matings than in fifth service matings.

We again observe that somewhere around the fifteenth service there is an appreciable decrease in the percentage of pregnancies. The higher percentage of pregnancies in the twentieth service group, we think, should be attributed to experimental error until more extensive observations are available.

5. Possible sources of error as related to observations on the certainty of pregnancy.

(a) Force of ejaculation is generally considerably reduced by a number of copulations. This reduced force of ejaculation would mean that the spermatozoa would need to travel a greater distance to reach the ova. This may have been partly responsible for the reduced certainty of pregnancy as well as the decrease in the number of the male cells, the decreased motility and the reduced longevity being responsible.
(3) Possibly the character of the secretions of the female genitalia is a greater factor in the certainty of pregnancy than the properties of the spermatozoa studied here.

6. Size of litters as affected by the number of copulations made.

Two important factors are vitally concerned in determining the size of litters produced by multiparous animals. These are probably of equal importance and any other factors of minor consideration.

The first of these two factors is the number of ova liberated by the female. Investigators of this subject generally agree that the state of nutrition of the female at the time of ovulation determines in a large measure the number of ova that will be liberated at any heat period.

Marshall (1908, pgs. 138, 51) reports the effects of "flushing" ewes in Scotland for the years 1905, '06 and '07. The plan followed was to feed ewes on turnips, oats, maize, and other dry grains for about three weeks previous to breeding them. Results show that under this treatment flocks of ewes produced 200 per cent lamb crops; while flocks of the same breed which received no special feed produced only 150 to 160 per cent lamb crops.

Evward divided a flock of grade ewes into three groups based on their average gain at the breeding season. The table below shows the average number of lambs produced by the ewes of each group:

<table>
<thead>
<tr>
<th>14 Best Gainers</th>
<th>14 Medium Gainers</th>
<th>14 Light Gainers</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.8 Lambs</td>
<td>1.59</td>
<td>1.44</td>
</tr>
</tbody>
</table>
Evvard also grouped sows into two groups based on their average daily gain at breeding time. The fourteen best gainers farrowed 8.75 pigs per litter; the fourteen lightest gainers farrowed 7.5 pigs per litter.

The age of the rabbit mother probably has one effect upon the size of litters she will produce for King (1916 p. 285) finds this to be the case from extensive records on the albino rat.

Numerous other observations of stockmen and investigators might be cited confirming the same facts. It suffices to say that the state of the general metabolism of the female at breeding time in multiparous animals has much to do with the number of ova liberated, and consequently the size of the litters produced.

Turning our attention to the male, with which we are more vitally concerned, we find that under ordinary breeding conditions, as Marshall (1911, p. 45) states, that the spermatozoa are present in sufficient numbers to fertilize all ova liberated at any time unless the male is severely overtaxed, as to lower the number or vitality of his spermatozoa.

Wright, says that it is known that in poultry if cocks are used cessively, they will produce sperm cells that fertilize the eggs but that the embryos only begin to develop and then die.

Newport conceives the idea that more than one spermatozoon is necessary for successful fertilization of the ovum, and that one sperm cell may start the development of the embryo but will not induce all necessary changes for successful fertilization.
Harper (1914, p. 251) states that among males partial sterility is frequently due to excessive sexual use. He states that when the number of services during a day is increased, temporary sterility may result from too great a decrease in number of sperm cells.

Reynolds (1916) thinks that a large number of sperm cells is absolutely necessary before pregnancy is certain in women.

Other writers have tried to account for the large number of apparently useless spermatozoa liberated by males at each copulation, but without definite conclusions. It does appear, however, that these vast numbers of sperm cells are produced by the male for a purpose not now understood as a factor of safety.

Considering the mechanism of fertilization, and the vast number of sperm cells ordinarily ejaculated by a male at each copulation, it is difficult to explain why one male should have any greater influence on the size of litters resulting from his service than any other male so long as neither is depleted of spermatozoa.

Some breeders of swine and sheep hold the idea that certain boars and rams will get larger litters than others when mated to the same females. Marshall (1908, p. 150) writes of a Border Leicester ewe procured in 1895 that consistently bore twins or triplets, and in each of the last two years had four lambs. Her progeny have always produced twins or triplets. Marshall also writes of a Mr. Stephens who by breeding from
twin-bred rams and ewes for several years, noticeably increased the fertility of his flock of Hampshire sheep so that the lambing record became as high as 200 per cent. The increased production is here attributed solely to heredity.

Riotz and Roberts (1915, p. 482) after studying the question of twin production in American Shropshire sheep, from over 19,000 registered sheep, present data that appears to show that rams are a factor in determining the number of lambs that will be produced by ewes. Both males and females when born as singles produced 35.54% of twins; males born as singles on twin ewes produced 40.89% twins; males born as twins when mated on ewes born as singles produced 38.31% twins; and twins on twins produced 42.68% twins.

Wentworth (1916, Pgs. 1151-1154) studying fertility in swine, presents data which shows that boars have no influence on the size of litters and that the determining influence lies fully in the sows. He expresses his opinion on this point (Pg. 1150) by saying that boars probably have no influence on the size of litters.

Table VII A

<table>
<thead>
<tr>
<th>Tables showing the average size of litters produced from rabbit males at different services.</th>
<th>1st</th>
<th>5th</th>
<th>10th</th>
<th>15th</th>
<th>20th</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male No.</td>
<td>Lit.</td>
<td>Lit.</td>
<td>Lit.</td>
<td>Lit.</td>
<td>Lit.</td>
</tr>
<tr>
<td>No. of Size</td>
<td>Lit.</td>
<td>Lit.</td>
<td>Lit.</td>
<td>Lit.</td>
<td>Lit.</td>
</tr>
<tr>
<td>1</td>
<td>6</td>
<td>6.67</td>
<td>6</td>
<td>6.00</td>
<td>7</td>
</tr>
<tr>
<td>2</td>
<td>12</td>
<td>6.50</td>
<td>8</td>
<td>5.75</td>
<td>7</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>7.76</td>
<td>6</td>
<td>6.17</td>
<td>6</td>
</tr>
<tr>
<td>Average</td>
<td>26</td>
<td>6.93</td>
<td>20</td>
<td>6.95</td>
<td>20</td>
</tr>
</tbody>
</table>
In table VII A we have a presentation of our observations on the relation of number of services to size of litters resulting from such matings. There does not appear to be any consistent falling off in litter size as the number of previous copulations increases. The data obtained from Male No. 4 is the most consistent if we should wish to consider that heavy service tends to reduce the size of litters in multiparous animals. Here we are confronted by the fact that only one litter is recorded on the fifteenth service series and one in the twentieth service series. Male No. 1 shows the same average size of litters from the fifteenth and from the twentieth service matings and the same number of litters is considered in both instances. Male No. 1 produced one litter each with the following numbers of individuals in the fifteenth service series: 1, 2, 8, 9, and 10; while in the twentieth service series he produced three litters of two individuals, one of three and one of ten. This being the case, the probable error in average litter size is very much greater in the twentieth service series for this male than in the fifteenth service series. These somewhat meager data seem to indicate that if the number of litters sired by this male were greater there would be a considerable falling off in size of litters in the twentieth service series over the fifteenth service series.

Assuming the average size of litters as shown by Table VII A to be correct, we notice that there is an important decline in litter size between the 15th and the 20th service series; the litter size drops from 7.0 to 4.0 individuals.
This being the case, there seems to be some point between the fifteenth and the twentieth copulation at which the reproductive powers of the male rabbit are so severely taxed as to prevent his producing spermatosoa in sufficient numbers of equipped with the necessary vital force to successfully fertilize all ova liberated by the female.

Reference to Table III shows that although the number of spermatosoa is greatly reduced from the fifteenth to twentieth copulations, still it is scarcely conceivable that when several thousand sperm cells are liberated by the male at a single ejaculation even on the twentieth copulation, that the reduced numbers is sufficient to appreciably affect the size of litters if at all.
Table VII B.

The Correlation Between Number of Services and Size of Litters Resulting.

<table>
<thead>
<tr>
<th>Number of Services</th>
<th>5th</th>
<th>10th</th>
<th>15th</th>
<th>20th</th>
<th>fs</th>
<th>fscn</th>
<th>Ds</th>
<th>D^2s</th>
<th>fD^2s</th>
<th>( \leq ) P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ms = 6.48±0.098</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Size of Litters</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>In</td>
<td>26</td>
<td>26</td>
<td>20</td>
<td>20</td>
<td>6</td>
<td>518</td>
<td>8</td>
<td>136.85</td>
<td>507.92</td>
<td>-179.85</td>
</tr>
<tr>
<td>fIn</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>60</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>fscn</td>
<td>26</td>
<td>26</td>
<td>100</td>
<td>200</td>
<td>8</td>
<td>518</td>
<td>8</td>
<td>136.85</td>
<td>507.92</td>
<td>-179.85</td>
</tr>
<tr>
<td>( D_n )</td>
<td>36.8449</td>
<td>4.2849</td>
<td>8.5349</td>
<td>4.93</td>
<td>120</td>
<td>556</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( D_{n}^2 )</td>
<td>957.9674</td>
<td>35.6900</td>
<td>172.6900</td>
<td>200</td>
<td>120</td>
<td>556</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( fD_{n}^2 )</td>
<td>36.8449</td>
<td>4.2849</td>
<td>8.5349</td>
<td>4.93</td>
<td>120</td>
<td>556</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( fD_{n}^2 )</td>
<td>957.9674</td>
<td>35.6900</td>
<td>172.6900</td>
<td>200</td>
<td>120</td>
<td>556</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( r = -1.843±0.073 )</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table VII B shows that there is a very slight negative correlation between number of services and size of litters produced. As previously stated, the number of observations from fifteenth and twentieth service matings is too small to make the results entirely representative of what might reasonably be expected to occur if the experiment were repeated. The results would be more conclusive if the same number of litters were included in each of the five service groups of the table.

The probable error of the coefficient of correlation is very large. If we take the results as presented in the table to fairly represent general results, we will note that the probable error of the coefficient of correlation is so large—over one-third of the value—that an assumption of negative correlation between number of services and size of litters is unwarranted and independence may exist in some cases. Only after we have sufficient numbers of litters in all classes will we presume to draw any valuable conclusions in this phase of the subject.

Table IV shows that the rate of motion decreases considerably after about five copulations are made and continues to decrease as the number of copulations increase. It seems reasonable to assume that herein lies a partial explanation for the small litters resulting from twentieth service matings. Previously we pointed out that the spermatozoa must advance a considerable distance by their own motion, through the fallopian tubes in order to reach the ova. Since the sperm cells have such a reduced power of motion and but few show the necessary progressive type of motion to carry them forward when they come from the twentieth ejaculation, it is probable that only very few will ever survive to reach the point of fusion with the ova.
Again in Table V we have shown that the longevity of spermatozoa is strikingly decreased by heavy service. Moreover we have stated that the mechanism of fertilization in the rabbit is such that several hours must elapse between copulation and fertilization. Since the sperm cells from the twentieth copulation have been found to survive but a few hours under laboratory conditions, we have a sound basis for the conclusion that short life of the spermatozoa from the advanced services results in but very few ever surviving to take part in fertilization.

7. Possible sources of error related to factors operating to produce the size of litters observed.

(a) Reduced numbers, reduced motion, and decreased longevity of spermatozoa may not be the only cause of small litters, since it is conceivable that the changed secretions of the male accessory glands may have an effect upon the entrance of the male cell into the egg even after the two are in contact.

Summary.

1. The physical properties of rabbit semen such as volume, color, viscosity, and agglutinating properties are greatly influenced by heavy sexual service of the male.

2. The number of spermatozoa per cubic millimeter of semen fluctuates widely with the same male at different observations.

3. Sexual use seems to stimulate the production of spermatozoa in greater numbers up to about five copulations beyond which the reduction in numbers begins.
4. The number of sperm cells in a unit volume of semen is greater from the second copulation than from the first and greater from the fifth than from the first copulation.

5. The decrease in sperm numbers is rather marked after ten copulations are made but not before this.

6. Semen obtained after a male rabbit has been allowed to make twenty services as rapidly as he will copulate, usually in 2½ to 4 hours, is rarely, if ever, devoid of living spermatozoa.

7. Rabbit spermatozoa in semen drawn from the female and diluted with ten volumes of Ringer's solution exhibit three normal types of motion, usually succeeding each other, and at least two types that are probably abnormal.

8. Samples of semen recovered from first services as a rule show a higher percentage of inactive spermatozoa than do samples recovered from later copulations.

9. After five copulations have been made the rate of progressive forward motion of sperm cells generally decreases as the number of copulations increases and to a marked degree in samples from fifteenth and twentieth copulations.

10. Spermatozoa lose their motility much earlier in specimens of semen as it is drawn from the female rabbit, that may or may not have been relieved of the secretions of the female genitalia, than samples of the same material diluted with 10 volumes of Ringer's solution.

11. After more than five copulations there is a constant decrease in longevity of spermatozoa with advancing services.

12. Probably most of the sperm cells from the fifteenth and twentieth copulations do not retain the power of motion long enough to reach the ovum.
13. There is a smaller percentage of spermatozoa that exhibit the progressive type of motion in specimen of semen obtained from the advanced services.

14. After five copulations have been made the certainty of pregnancy is consistently decreased by continued copulation.

15. There is a very close relation between the rate of motion exhibited by spermatozoa and the certainty of pregnancy.

16. The longevity of spermatozoa is probably one of the most important factors in determining the certainty of pregnancy.

17. Deductions number (4), (15) and (16) indicate that two copulations are more likely to insure pregnancy than a single copulation.

18. Females bred to males after they have made four copulations are more likely to become pregnant than those bred on the first service.

19. Although there does not appear to be any absolute relation between size of litters and the number of copulations made by the sire; the reproductive powers of the male do seem to be greatly impaired by twenty copulations.

20. A certain amount of sexual use seems to be beneficial to the male from the standpoint of certainty of pregnancy.
Bibliography


2. Camus and Gley. La fonction des vésicules séminales et de la glande prostatique dans l'acte de la fécondation, (Quoted by Ilic Ivanoff), F. 95, 1903.


15. Lode, Alvis., Untersuchungen über die Zahlen- und Regenerations-
verhältnisse der Spermatozoen bei Einund Mensch, Pflüger's
Archiv., Vol. 50, 1891.
16. Lott, Gustav., Anatomie und Physiologie des Corvix Uteri,
Page 155, 1872.
Vol. 20, Pgs. 159-152, 1906.
Marshall, F.R.A., The Physiology of Reproduction, P. 134,
1910, N.Y.
24. Piets and Roberts, Degree of Resemblance of Parents and Off-
spring with Respect to Birth of Twins for Registered Shropshire
26. Roth, A., Über das Verhalten beweglicher Mikrovigoureisn in
stromender Flüssigkeit; Deutsche Medizinsche Wochenschrift,
19. P. 252, 1895.
27. Smith, F. Manual of Veterinary Physiology, P. 649, 1912,
Chicago.
28. Sumner, F.B., Notes on Superfetation and Deferred Fertiliza-
tion Among Mice, 1916.


II

INFLUENCE OF FREQUENCY OF COLONIZATION OF

BARKS ON THE NATURE OF SINTER CRUSTS.
# Table of Contents

## Part II

### Introduction

Materials and Methods

- Animals Used  3-5
- Records Kept  5
- Weighing and Measuring  5-10
- Methods of Interpreting Weights and Measurements  10-14

Data and Results

- Growth in Weight of Young as Related to Frequency of Copulation of Fire  14-19
  - Charts I to XIII  15
  - Discussion of Charts  19-20
- Coefficient of Variability of Individuals Within the Litter at Three Different Periods  21
  - Discussion of Coefficient of Variability  22-24
- Growth by Measurements as Related to Frequency of Copulation  24-26
  - Growth Charts of Body Measurement XIII to XX  24
  - Comments on Growth in Body Measurements  25-26
- Percentage of Mortality the First Five Days of Life and Between the Fifth and the Ninetieth Day of Life  27-29

Summary of Facts  30-31

Discussion  32-34

Acknowledgments  35

Bibliography  36
Introduction.

Too frequent copulation of males is often given as an important cause of weak and inferior offspring from the standpoint of growth and thriftiness. This idea seems to be rather universal, though evidence of such being the case is difficult to obtain. Wright (p. 206) states that in his poultry breeding operations he does not expect normal size or vigor in offspring from cocks used on too many hens. auch (1913, p. 182) expresses the almost universal belief in this matter, though he does not consider the idea well grounded when he writes: "Draucht man die Deckenrabte, wie übervant jeder namentliche Fachmännier, zu stark, so schädigt man nicht nur deren Befruchtung- und Be-
fruchtungsverzögerung, sondern auch die Qualität ihrer Nachzucht; deshalb wird die Statensål für wertvolle Vollbluttumpte auch nur auf 50--60 Stuck berechnen und die zu deckende Stute erst durch den Probierpurchased auf ihre Fähigkeit hin geprüft."

Wright (p. 161) states that cocks that are used on too many hens show the effect in that the eggs show signs of hatching but do not hatch because the embryos fail in many cases to reach full development. Day (1913, p. 819) expresses the belief that excessive use of the boar is likely to result in small weak litters of pigs.

Just why sperm cells that are produced by a male in heavy sexual service should produce inferior offspring when they take part in fertilization is not clear. Can it be possible that the genetic makeup of the spermatozoa is changed by heavy service? Is it not possible that any sperm cell
possessing life, however depleted and weak it may be, will carry into the egg a potentiality of full vigor? Or on the other hand, can we conceive of different degrees of vital force in a sperm cell? Since all of the activities of the animal body are so beautifully coordinated it would appear very rash to assume without conclusive evidence that males under natural breeding conditions would so derange any vital function, such as reproduction, by continuing to copulate after the reproductive system was producing an abnormal product. As we have pointed out in our first paper, there appears to be a relation between the number of services performed by the male and the fertilizing power of his semen, but as far as we have been able to measure, we are led to believe that only a slight change can be brought about by this treatment. Fuchs (1915, p. 182) states that in Oldenburg, stallions are often allowed to make from four to six or even eight services daily. He also states that bulls have been used on 400 cows a year, and that even poorly fed bulls will make from four to eight copulations daily, and this without bad effect.

Strictly speaking, "vitality" of individuals cannot be measured, for the vitality of any individual really means the sum total of life force within every living cell of the organism; vitality, as used in speaking of animals, may, however, in part be measured by the rate of growth in weight, the skeletal development, and the ability of the individual to live to a good age. But such factors as body weight and the others mentioned above are measurable. The purpose of this work has been to study the effects of heavy service of males on the nature of their offspring, as far as we could measure the effect.
- 3 -

Materials and Methods.

1. Animals Used.

The character of the animals used in this investigation has been discussed to some extent in the first paper of this series. Stocks of the European domestic rabbit, Lepus cuniculus, secured from six different breeders, were used and no inbreeding was practiced at any time. The weight and age of the females is an important factor in that it affects both the number in a litter and the individual weights of the offspring. Likewise the weight of the male probably is of much importance in affecting the weight of the young. The maturity of the male is a factor that should not be lost sight of because all three of the males used were fully mature and were in their prime of life—about two years old. The average weights of the males are as follows: No. 1, 2850 grams; No. 3, 2575 grams; and No. 4, 2800 grams.

Shy breeders sometimes occur in rabbits, but most of these females proved to be regular breeders. No. 25, however, was barren and was discarded; No. 12 kindled three times, the last time Aug. 5, 1916, after which time she appeared never to come in heat again and continually refused to copulate and was discarded; both No. 22 and No. 29 died after having given birth to but one litter upon which we secured data; and No. 18 died after she had given three litters to the experiment.

*Professor E.J. Vaughan has found that large type Poland-China swine produce larger litters than the small type.*
Age of the dam is an important factor as affecting the number in the litter and probably to some extent the weight of the individuals of the litter. For this reason the approximate ages of the breeding animals is here given in order that the reader may understand fully how much error may have been introduced through immaturity in the breeding females. One female kindled when six months old; two, when seven; four, when nine; one, at ten; and one, at eleven months old. The remainder of the females was fully mature, i.e. fifteen months old or over, at the time they gave birth to the first litter used in this experiment.

The fact should be noted that the three females that died during the experiment were all immature at the time they first kindled and that only one of them (No. 18) had more than one litter upon which we secured date. Two of the three litters produced by No. 18 are fifth service litters by male No. 3 and the other is a first service litter by the same male. Female No. 22 kindled a single litter from the fifth service by male No. 1; and female No. 29, one litter from the first service by male No. 4; Female No. 12 has contributed but two litters to the records; namely, a first and a tenth service litter both by male No. 1.

It may appear to the reader that considerable error, resulting from the use of those immature females, was overlooked in making up our records of growth; but this has not been overlooked; therefore, a brief consideration of the system of ratings used to overcome this error is not out of place here.

The system of matings was arranged so that each female was noted to at least two of the males and many to all three males different and each female produced litters from all services where possible.
Thus reducing parental variability to the males alone. By making the three breeding groups of females as nearly equal as possible in age and weight; by distributing the heavy service among the females in such a way as to secure all types of litters from both mature and immature females, and by making ratings at such a time as to secure all types of litters at the same season of the year, as far as possible, we hoped to overcome many possible sources of error. However as the experiment proceeded it was found impossible to apply these corrections and we are thus not justified in comparing litters in growth in body weight and in mean dimension and assuming that any consistent differences are due to the number of services performed by the males. We should not overlook the slightly better opportunities offered the fifteenth and twentieth service litters, a considerable proportion of which were born during the latter part of the experiment and were produced when the females were all mature.

2. Records Kept.

The following records were kept: Date of breeding, pedigree, date of the next probable heat period—fifteen days after breeding; actual date of kindling; number in litter; number born dead; sex of offspring; individual weight of offspring on day of birth and for each five days thereafter up to ninety days; head length and breadth through extremes of ilium, taken at the same time as the weights; date of weaning; color; and mortality record.

3. Weighing and Measuring.

Breeding records were kept for each female so that it was possible to weigh each litter on the actual day of birth.
At this time each litter was given a number which was the same as the number of the mating, and each individual was given an individual number and marked in such a way as to be easily distinguished from litter mates by color description or by clipping ears and tail. The individual weight records were kept each five days until the litter reached the age of ninety days. The desirability of securing all records to the full maturity of the progeny is very apparent. As the work was handled forty or fifty animals were often weighed and measured on a single day and with the other routine work of the experiment, entailed a very large amount of labor. Such extensive records were impossible for reasons that need not be discussed here. Weights were secured on a sensitive torsion balance and variations of .5 gram were recorded. Great errors may be introduced by a "fill" if the records are not made at the proper times; therefore the records were secured at about the same hour each day before feeding, which was done once daily. However there are certain errors in weight records which cannot be avoided by the experimenter. The general degree of health of the animals has much to do with fluctuations in weight as MacDowell (1914) found in growth studies of rabbits; nevertheless, as with other animals, weight seems to be the best available index of growth.

Two methods for studying the growth of the progeny produced were chosen; namely, growth in body weight and growth in body measurements. The first will be discussed here.

Body weight, according to Minot (1908, p. 67) represents the total mass of the living body while body measurements
are only partial indices of growth. That individuals show wide fluctuations in weight has been pointed out by MacDowell (1914, p. 191) in his studies on the rabbit. Although growing rabbits show marked variability in weight on different days, it was thought possible by the use of large numbers to secure growth curves that would fairly represent a race of rabbits kept under uniform conditions. There is a possibility that these growth curves would diverge more as the animals grew older, because MacDowell has shown that though most rabbits apparently make a normal growth to maturity, others fall much below the normal and do not reach the average weight in what is considered the normal period. But complete records were out of the question as indicated above. Even though this is the case it is very important to ascertain if this reputed inferiority of progeny which is supposed to result from the weaker sperm cells of the over-worked male is going to be apparent when his progeny are in the most active stage of growth, i.e. during the first ninety days of the rabbit's life. If progeny from the advanced services of males are more poorly equipped with the necessary something to enable them to make normal growth, would this not be apparent when the young rabbits are thrown upon their own resources as was done during the sixty days following weaning time that the records were kept?

Concerning the second method for studying growth; namely, by body measurements, it is important to discover whether body development follows space with body weight and to check one against the other. For this reason all litters born up to Aug. 9, 1916, (45 in number) were measured as well as weighed, at five
day intervals up to the age of ninety days.

A measure of head length was considered valuable as measurements of the skull have been found to be less variable than measurements of long bones. MacDowell (1914, p. 86) found this to be true in rabbits. Hatai (1908) observed the same thing in the albino rat, and Quetelet (1871) likewise found the same in man.

The head length as here reported was measured by the use of calipers and represents the distance obtained by placing the stationary arm down across the nose and mouth and allowing the standard of the calipers to extend sagitally upward parallel with the face. The movable arm was then brought down until it rested snugly on the top of the head between the ears. The lower arm of the instrument was then just beneath the inferior extremity of the premaxillae and the upper arm was just above the superior region of the occipital bone. There is very little flesh or tissue covering the bones in this region, about the only structures obscuring the bones are the skin and the hair coat. It is apparent, that a head measurement in this particular region approximates rather closely the actual skull size.

Since there is the possibility that some other body measurement would make an entirely different growth curve from that of the above described head measurement, it was considered desirable to secure one other measurement that could be taken with considerable accuracy on the live animals. Moreover, we wished to obtain a Mean Dimension from the average of two
measurements, therefore some easy body measurements were searched for. There is no little difficulty in securing external measurements of the body with accuracy, as the writer has learned from much experience on cattle and swine. A measure of the breadth between the extremes of the Ilium was thought to be as easily determined as any of the possible body measurements and would represent a dimension of breadth in contrast to head length, which might be considered a dimension of depth. The Ilium expense was therefore used as the second measurement.

Both measurements were taken just after weighing on the five day periods beginning at birth and continuing to the age of ninety days. Steel calipers were used with vernier graduated to hundredths of a centimeter. Three independent readings of each dimension were taken by removing the calipers and shifting the arm after each reading. Readings were put down just as read and care was taken to avoid any tendency on the part of the observer to modify readings to make them check with others. As a rule it was possible to obtain readings that varied less than .1 centimeter from each other. An average of the three readings was taken as the correct reading for each measurement.

Little difficulty was experienced in securing what was considered a correct reading on head length. This was not always true for the other dimension. Three factors probably enter to modify the reading: 1. the amount of "fill"; 2. the degree of fatness; 3. the position of the hind limbs.

The amount of food and water in the alimentary tract seems to bulge the walls of the abdomen to such an extent as to
often obscure the points of the Ilium and make their exact location difficult. The variability of the feeding habits of the rabbit is thus a factor of no little importance in connection with the measurement of iliac extremes.

Some individuals carry more fat over the Ilium bones than others. In fat individuals the points of the bones are often greatly obscured, especially in the younger rabbits. This condition is much more common among the smaller and better nourished individuals and may prevail to some extent throughout the period of observation.

There is considerable flexibility in the pelvic girdle before the symphysis pelvis becomes bony and firm as the animals approach maturity. The Ilium, Ischium, and Pubis are also distinct and more or less flexible in early life. This great flexibility causes the position of the hind limbs to be an important factor in modifying the position and the breadth of the extremes of the Ilium as determined by the calipers. In so far as possible an effort was made to have the animals sit with the limbs in the natural position while being measured. The hair was also clipped from this region of the body in order that it might not obscure the point of the bones.

4. Methods of interpreting weights and measurements.

In order to make the data for different sized litters comparable all weight and measurement records are reduced to an "individual mean" for each litter for each of the nineteen periods of observation. The individual mean for each litter was calculated by dividing the total weight or total measurement of each litter by the number of individuals for each of the nineteen periods.
From these individual means the series of cumulative growth graphs are constructed.

In attempting to compare the growth graphs of rabbits in the different service groups, a very perplexing problem arose as to how to best compare results in litters that vary so much in number of individuals. Our observations on the rabbit lead us to believe that the number of individuals born in a litter is an intensely important factor in influencing the weight of the young. This agrees with the observations of Kinat (1891, 211) on guinea-pigs. His results, based upon 351 observations, show that the average birth weight is 25.5 grams in litters of one, the weight gradually decreased with the increase in number of individuals to as low as 52.2 grams in litters of eight.

Another item that makes comparisons of litters in different service groups difficult is the fact that litters in the first and fifth service groups are likely to contain more individuals than those from the fifteenth and twentieth services. For this reason the individual mean of these advanced service litters was greater and they grew faster because of a more generous supply of milk from the mother. In this connection we find that King (1916, p. 51) discovered that in rats "body weight at birth indicates the probable capacity of the individual for subsequent growth". This being the case, small litters from the advanced service should grow more rapidly than the larger litters from the first and fifth service.

In order to make the litters in the different service groups comparable with each other, whatever their number; it was thought first that litters of different numbers of indivi-
duals could be standardized to a mean litter number. Jackson (1913, p. 17) in comparing the standard deviation of individual rats with the standard deviation of the entire race, reduced all individuals to a common basis by multiplying the body weight of each rat by a factor obtained by dividing the mean of the total population at a given age by the mean of the given litter. Since the object we have in view is not the study of individuals but the study of individual means of litters, this formula cannot be used. Further attempts were made to obtain a factor for reducing large and small litters to a comparable basis but so far with no success. Again, it was thought possible that the coefficient of correlation between number of individuals in the litter and average weight per individual might be made use of to reduce litters to a comparable basis, but without any satisfactory results. Finally, it was deemed best to compare litters of the same number of individuals. Accordingly the growth rate in the different service groups must be shown by a whole series of charts, the graphs on each chart representing a certain litter number. In each case the chart shows the number of litters which are lumped in each graph. Thus each chart gives a direct comparison of the growth rate of the five service groups; namely, first, fifth, tenth, fifteenth, and twentieth, the comparison being always between litters of the same number.

As a further measure of divergence in rate of growth between the service group, the coefficient of variability of weight for all litters in each of the five service groups is valuable presented at birth, at weaning time or thirty days, and at ninety days for purposes of study. The object here sought is to find out if there is a greater variability in any one of the service groups.
which might be expected if any of the service groups contain weak offspring. This study will also reveal if heavy service tends to produce a wide range of variability in birth weight or a wide range in the weights of individuals at the time that they are thrown upon their own resources at weaning time, and it will show further if the individuals tend to deviate more from the mean as they grow older. Deviations, if they are going to occur, might be expected to occur, more strikingly at these three periods than at any other time during the observations. This coefficient of variability was determined by the following formula:

\[
\text{Coefficient of Variability} = \frac{\left(\sum \text{Deviations of Litters from Mean}\right)^2 \times \text{frequency of Class}}{\text{Number of Individuals}} \times 100
\]

For Each Litter In Service Group

\[
= \frac{\text{Mean of Respective Litters}}{\text{Number of Litters in Service Group}}
\]

The above formula is used for the birth weights, the thirty day weights, and the ninety day weights.

The measurement data secured was combined into one general expression, the Mean Dimension. The advantage of using one expression to stand for body dimensions lies in the fact that we have a mathematical expression for the cross section of the animal. Graphs expressing cumulatively the percentage increase in head length and iliac extremes are found to cross between the thirty-fifth and fortieth day of postnatal development; but previous to this date and later up to the time of the conclusion of the observations at ninety days, the graphs bear a close relation to each other, therefore it was deemed correct to combine the two measurements to obtain the Mean Dimension.
The Mean Dimension was obtained by the following formula:

\[ \text{Mean Head Length} - \text{Mean Iliec Extremes} \]

Mean head length is the sum of the average of the three readings for each dimension in a litter divided by the number of individuals in the litter. By this method a mean dimension was secured for each litter for each of the nineteen periods of observation. Graphs presented on the measurement data are made up in exactly the same way as has been described for making the graphs for weight, comparing only litters of the same number of individuals. A grand average graph is likewise made up regardless of litter size because of the fact that it may be of some value. The graphs based upon identical litter size are considered absolutely reliable for purposes of comparing the offspring in the different service groups, but the grand average graph is subject to considerable error.

Data and Results.

1. Growth in weight of young as related to frequency of copulation of sire.

For studying the offspring with a view of determining if there is any relation between the number of services made by the males and the rate of growth there appears to be no better measure than body weight. Body weight measures the animals as a whole and should thus reveal any inherent weakness that retards their growth.

Below are presented charts I to XI taken from the composite weight records of the young of all three males. Each chart represents a single litter number at birth, all litters of one
Chart No. I.
Growth Curves in Service Groups,
Litter Size, 1.

5th    1
10th   1
15th   1

Chart No. II.
Growth Curves in Service Groups.
Litter Size, 2.

1st  1
5th  1
15th 1
20th 3
Chart No. IV.
Growth Curves in Service Groups.
Litter Size, 5.

1st
5th
10th
15th
20th
Chart No. V.
Growth Curves in Service Groups.
Litter Size, 6.

1st
5th
10th

1st — 7
5th — 6
10th — 3

Age In Days.
Chart No. VII.
Growth Curves in Service Groups,
Litter Size, 8.

1st — 5
5th — 3
10th — 4
15th — 1

Weight in Grams.

Age in Days.
Chart No. VIII.
Growth Curves in Service Groups
Litter Size, 9.

1st _______ 1
10th _______ 4
15th _______ 2
Chart No. IX.

Growth Curves in Service Groups.
Litter Size, 10.

- 1st = 3
- 5th = 1
- 15th = 1
- 20th = 1

Age In Days.
Litter Size, Ill.

Chart No. X.
Growth Curves in Service Groups.

5th
10th
Chart No. XI.
Growth Curves in Service Groups,
Litter Size, 12.

15th ___ 1
Chart XII.
Growth Curves in Service Groups.
Grand Average of all Litters.

<table>
<thead>
<tr>
<th>Place</th>
<th>Weight in Grams</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>26</td>
</tr>
<tr>
<td>5th</td>
<td>20</td>
</tr>
<tr>
<td>10th</td>
<td>20</td>
</tr>
<tr>
<td>15th</td>
<td>8</td>
</tr>
<tr>
<td>20th</td>
<td>6</td>
</tr>
</tbody>
</table>
in each of the five service groups being grouped together and the same grouping being followed for all litter number as described on page 15. Each service group is represented by a different color as is disclosed by the legend on the charts. The number of litters represented by each curve is given in each case.

Chart XII shows the weighted Grand Average for all five service groups. It was obtained by adding together the individual mean of each litter and dividing by the total number of litters at each observation period. We have already shown that such a chart comparing directly the growth ratio of the different service groups may not be made with justice because as will be shown later the litters in the advanced service groups are consistently smaller. A rough comparison of all progeny may be made in this way, however.

Charts I to XI inclusive present the results in a form that may be easily grasped by the reader. There are a few points revealed by a study of these graphs that require some explanation. The reader will naturally be perplexed by the observations that with but one exception; namely litters of three, Chart III, the twentieth service graph is above all other graphs, even here we note that the twentieth service graph is above the first service graph and falls below the fifth service graph at 65 days. The question at once arises as to the cause of the almost uniform heavier birth weight and more rapid growth of both the fifteenth and twentieth service litters above the lower service litters in the same size group. The results are in direct contrast to what according to the traditions of breeders would be expected. On their face they actually show that the heavier the service
of the male, the more growthy the offspring. It seems best to here consider the possible factors that may play a part in causing for the superiority of these advanced service litters over litters from the first, fifth, and tenth service litters.

During the production of the majority of the first and fifth service litters the breeding animals were housed in somewhat cramped quarters in the Chemistry building. Conditions there were not conducive to the most rapid growth of the young and were not as favorable for the breeding females because of small space and rather poor ventilation, and poor light. Furthermore the progeny were crowded into rather limited exercising pens and probably for this reason they did not develop at so rapid a rate as would have been the case under the more favorable quarters used later. The majority of the tenth service litters on the other hand and about half of the fifteenth service litters were produced while the stock was housed in the Experiment Station barn where the space was large, the ventilation good, and everything was conducive to health and thriftiness. In fact the quarters used at that time were practically as good as the present quarters in the New Animal Breeding Laboratory where the twentieth service litters were produced. The superior environment of the advanced service litters is no doubt partly responsible for the greater growth of the advanced service litters. The environment therefore fails to explain the superiority of the twentieth service litters over the fifteenth and the fifteenth service litters over the tenth service litters. Let us therefore seek a further explanation.

Parentage must be an important factor affecting the weight. As has been previously noted, the variability of the
female breeding stock is considerable, the range of weight was from 2500 to 3250 grams, averaging 3050 grams, but the females have been so distributed among the three breeding males as to make three groups of practically uniform weight and variability in size, never-the-less lack of uniform weights in the progeny is still partly due to variability of the female breeding stock. The size of the sire may also be a factor in controlling individual mean weight. The three sires used were quite variable in weight; their weights are as follows: No. 1, 2850 Gms.; No. 3, 2575 Gms.; and No. 4, 2225 Gms. in the ordinary breeding condition. Male No. 1 sired five of the six litters included in the twentieth service group. He being the largest of the three males, would be expected to sire the heaviest offspring at birth, and such offspring could be expected to keep ahead of the other classes of offspring at least for ninety days. This way of explaining the position of the twentieth service graph above the others is called in questions by Chart III. The graph of the 20th service litter lays below the others. This graph represents the growth of a single twentieth service litter also by Male No. 1 and out of the heaviest female in the breeding stock (No. 15). Therefore, the fact that this litter lays below fifth and tenth service litters on this chart cannot be explained as the result of small ancestry.

When we consider the fifteenth service group, we find that five litters were sired by Male No. 1, two by No. 5 and but one by No. 4. Again we should expect a more uniformly heavy progeny than if all males had contributed an equal number of litters to the data. Chart No. VIII shows the superiority of the tenth service group over the fifteenth service group up to the 55th day.
The female ancestry in both service groups were practically equal in weight. One of the fifteenth service litters represented on the chart was sired by Male No. 1, the other by No. 3. Two of the four litters combined in the tenth service graph were sired by No. 1 and two by No. 3. The smaller weights of the fifteenth service litters cannot for the above reasons be explained by male ancestry of different weights.

Concerning the graphs for the tenth, fifth, and first service litters, we note that as a rule the first service litters are inferior in weight to either the fifth or tenth service litters and that the tenth service litters are for the most part superior to the fifth service litters. As previously noted, less favorable environment and greater immaturity of some of the female animals are thought to be the chief factors entering here. The male ancestry is pretty uniformly distributed among the three males. Below we note that from the table just how the ancestry is distributed.

**Table No. I**

*Number of Litters Included in Graphs of Charts I to XII inc., and the Male Ancestry.*

<table>
<thead>
<tr>
<th>Male No.</th>
<th>1st</th>
<th>5th</th>
<th>10th</th>
<th>15th</th>
<th>20th</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>6</td>
<td>7</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>8</td>
<td>7</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>6</td>
<td>6</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Table I shows us that the three males are about equally distributed in the progeny groups from the fifth and tenth services. In the first service group, however, No. 3 has sired twice as many litters as No. 1 and 50% more than No. 4. Since Male No. 3
is a smaller animal than No. 1, we have here a partial explanation for the apparent inferiority of the first service litters over all others.

A word of explanation in regard to a few remarkable features of some of the charts, may be of value at this point. On chart No. III the depression in the fifth service graph at 60 days is due to a failure to obtain data on the heavier two of the litters making this graph. This particular litter was unintentionally overlooked for four weighings. On chart V, the drop in the tenth service graph at 65 days is due to the incomplete record on one litter at the time the graphs were constructed and this litter was made up of very heavy individuals.

Chart XII represents the grand average growth of all litters in the five service groups as explained on page 15. Each graph thus represents the individual mean for the combined litters in each service group. These composite service group graphs bear out the general deductions that we have made from a study of the graphs taken one by one comparing litters of a given number with each other in the five service groups. There is one outstanding objection to the use of such graphs as are shown on Chart XII; we have explained in our first paper that we believe there is rather an important negative correlation between number of services of the sire and the number in litters resulting. In other words, we think heavy service does reduce the size of litters, especially in the two most advanced service groups used here. If this is true, the greater supply of nutrients furnished by the mother in utero as well as the greater supply of milk available after birth will enable the advanced service litters to outstrip the other litters during the periods of observation in this
experiment. This condition would hold if all litters were equally fit genetically; and we have no evidence that any class of offspring is rendered less fit by heavy service of their sire.

To recapitulate, certain errors have been introduced into the growth studies in body weight chief among which are environmental factors, the age and weight of the dam, and the weight of the sire. These errors have been partially corrected, and we feel justified in concluding that there is no evidence in this data to show that the amount of service that the male has been required to perform in any way affects the rate of growth of his offspring in body weight for the first ninety days of their life.
Table No. II

2. Coefficient of Variability of Individuals Within the Litters at Three Different Periods.

<table>
<thead>
<tr>
<th>Age Days</th>
<th>No. of Litters</th>
<th>1st</th>
<th>5th</th>
<th>10th</th>
<th>15th</th>
<th>20th</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth</td>
<td>26</td>
<td>10.81±1.01</td>
<td>20</td>
<td>10.73±1.14</td>
<td>18</td>
<td>12.43±1.40</td>
</tr>
<tr>
<td>30</td>
<td>26</td>
<td>9.48±.88</td>
<td>17</td>
<td>8.27±.96</td>
<td>18</td>
<td>9.69±1.09</td>
</tr>
<tr>
<td>90</td>
<td>23</td>
<td>10.10±1.02</td>
<td>14</td>
<td>6.77±.86</td>
<td>16</td>
<td>6.71±.81</td>
</tr>
<tr>
<td>Average</td>
<td>10.13</td>
<td>8.59</td>
<td>9.61</td>
<td>8.75</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The coefficients of variability in table II were obtained in the following manner: The coefficient of variability for each litter in each of the five service groups was determined at birth, at thirty days, and at ninety days by the formula.

\[
\text{Coefficient of Variation} = \frac{\text{Standard Deviation}}{\text{Mean}}
\]

The mean of the litter was determined at birth, at thirty days, and at ninety days. The coefficients of variability for all first service litters at birth were then added together and this sum was divided by the number of litters concerned to secure the coefficient as given in Table II. Likewise the coefficients of variability for all first service litters at thirty days were added together and this sum divided by the number of litters concerned to obtain the coefficient as given in Table II. The process was used on the weights at ninety days to get the coefficient, and a similar procedure used on the weights in the other four service groups to obtain their respective coefficients. For the information of the reader the number of litters concerned in each case is presented in the table. MacDowell (1944, p. 44) shows in studies on weight of adult rabbits, that there is less variability within the litters than between individuals of different litters. For this reason and because we wish to compare progeny of different ancestry, the method of expressing the coefficient of variation of the populations as the average of the individual litter coefficients of that population is considered accurate.

Table II shows that the coefficient of variation in rabbits is less at birth than at any other time during our observations. This fact holds good in all service groups. While the coefficient on the average is small it serves to indicate that prenatal
nutrition must be subject to wide variations otherwise greater uniformity in weight at birth should be expected. The thirty day period is the weaning time for all of the litters studied in this experiment. We note from the table that the coefficient of variation falls below what it was at birth in all service groups. There is no evidence here of a percentage of "weak" offspring, for if such were the case we should expect the coefficient to increase when the animals were thrown into competition for nutrition during the first thirty days of postnatal life and even one inferior individual would alter the coefficient for the litter. At the ninety day period there is again a decrease in the coefficient of variation in all service groups, with the exception of the first service group. In the first service group there is a slight increase over the thirty day coefficient, but it is doubtful if this increase is at all significant.

Taking up a comparison of the coefficients for the different service groups, there appears to be slightly less variability in the offspring as the number of services increases. Since the probable error is rather large, this difference is no way significant. As has been previously stated, there is also a slight reduction in the number of the litter in the same direction. Our data shows us further that there is less variability in the smaller than in the larger litters. This fact affords us an explanation for the slight reduction in the coefficient of variation as the number of services increases. More data is needed to confirm this fact in the twentieth service group.
To recapitulate, the fact seems apparent from Table II that occasional genetically weak offspring do not occur in any one of the service groups more frequently than in any other service group. The table also shows us that for the first ninety days of postnatal growth there is a tendency for individuals of the same litter to approach nearer to a mean weight than was the case either at birth or at thirty days of age. Postnatal nutrition is thus more variable than either the nutrition furnished by the mother during the first thirty days after birth or the ordinary food supply furnished from thirty days to ninety days.
Chart No. XIV.
Mean Dimension in Service Groups,
Litter Size, 5.
1st: 2
5th: 2
10th: 2

Age in Days:

Mean Dimension—Centimeters.

Made by: H. L. H. M. W. C.
Chart No. XVI.
Mean Dimension in Service Groups,
Litter Size, 7.
1st — 5
5th — 1
10th — 1

Age in Days.
CALCULATION SHEET

Mean Dimension--Centimeters.

Chart Measured--In Service group.

Age in Days.
Chart No. XIX.
Mean Dimension in Service Groups,
Litter Size, 10:
1st  →  2
5th  →  1

Mean Dimension - Centimeters:

Age IN Days.
5. Growth by measurements as related to frequency of copulation.

Charts XIII to XX are presented to show the growth in the mean dimension as obtained on forty-five litters. The method of making measurement, and the determination of the mean dimension have been already explained. Each graph represents averages of the mean dimension for all litters of the same age in the respective service groups. The mean dimension for a litter is obtained by adding all head measurements to all measurements of ilial extremes and dividing the sum by the total number of readings included in the sum. The expression thus obtained is the average individual mean dimension for the respective litters and may be compared with the average individual weights used in the previous charts.

These charts of body development show that there is a maximum increase in the mean dimension up to about the twentieth day, after which there is a very noticeable flattening of the graphs. From about the twentieth day on to the end of the observations at ninety days the progressive increase in the mean dimension is about constant. The increase in the mean dimension is in marked contrast to the increase in body weight that has previously illustrated by charts I to XII. Body weight has been shown to make a rather constant increase up to the end of ninety days and this is well illustrated by the fact that the weight graphs show little if any tendency to flatten out.

Though the number of litters making up a mean dimension graph is in most cases small, they serve to illustrate the same principle as the weight graphs; namely, that the advanced service
as fully equal to the first or fifth service progeny
progeny at all times during the ninety days of the observation.

On charts where but a single litter makes up a graph we sometimes
note a rather sudden break in the graph. This, in our opinion,
is the result of error in measurement and for this reason the
graphs made up several litters will be less influenced by minor
errors and hence should be more representative of actual measure-
ments.

In chart XIII are presented grand average graphs made up
as the average of 21 first service litters, 15 second service litters,
8 tenth service litters, and one fifteenth service litter. Here
the coincidence of the first, fifth, and tenth service graphs is
very striking. This fact bears out our previous conclusions from
body weight studies that heavy sexual service of the male has no
of his
effect upon the growth in body weight/offspring. Our evidence in
studying the increase in the mean dimension does not show any
effect on the progeny from the heavy service of the male. The
fifteenth service graph is made up of but one litter of two
individuals sired by male No. 1 and out of an average sized female.
This litter being small and having as a sire the largest of the
males will probably account for their larger mean dimension.

Summarizing the results of the measurement studies,
we note that a very close proximity of the graphs for the different
services. This points very strikingly to the probably fact that
heavy service has no effect upon the growth of their offspring in
the length of head and in the breadth of ilial expanse.
4. Percentage Mortality in offspring the first five days of life and between the fifth and the ninetieth day of life.

Table No. III

Mortality in Service Groups.

<table>
<thead>
<tr>
<th>Service</th>
<th>1st</th>
<th>5th</th>
<th>10th</th>
<th>15th</th>
<th>20th</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Animals Born</td>
<td>180</td>
<td>119</td>
<td>139</td>
<td>56</td>
<td>24</td>
</tr>
<tr>
<td>No. Dying First Five Days</td>
<td>16</td>
<td>15</td>
<td>16</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>Per Cent Dying First Five Days</td>
<td>8.89</td>
<td>12.61</td>
<td>11.51</td>
<td>12.50</td>
<td>16.67</td>
</tr>
<tr>
<td>No. Dying Between 5 and 90 Days</td>
<td>21</td>
<td>56</td>
<td>17</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Percent Dying Between 5 and 90 Days</td>
<td>11.67</td>
<td>30.25</td>
<td>12.23</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

In Table III we have the progeny grouped by services and the number and the percentage mortality is given for each service group. Under the row marked "Number Dying First Five Days" are included all animals dead at birth as well as those that died during the first five days of life. The other row of the table includes only animals actually dying between the fifth and the ninetieth day of postnatal life.

The percentage of mortality during the first five days shows a slight increase as the number of services increases. Comparing the first service group with the twentieth service group, we note that the percentage mortality in the first five days is almost doubled in the latter. Since the environment has been more favorable for the twentieth service litters than for the first service litters, as previously pointed out, if we accept the results with the small number of twentieth service litters, there is an indication that twenty copulations by a male do in some way tend to reduce the percentage of his progeny that will survive the first five days of postnatal life. The table shows
practically the same percentage mortality during the first five days in both the fifth and the fifteenth service groups. The explanation for the somewhat high percentage of mortality in the fifth service group is that two litters were destroyed outright by the mother and a number of the other fifth service litters were born during extremely hot weather when the mortality was very high even among the older animals. The tenth service group also shows a higher death rate than the first service group. In all these cases the percentage of mortality during the first five days seems to depend to no little extent upon the number of services that the male is required to make.

The table shows that there is very little consistency between the mortality percentages as revealed in the first part of the table and between the percentages of deaths that occurred between five and ninety days? The first five days is a very critical time in the life of the young rabbit and very slight exposure may bring disaster. When this period is over the deaths usually result from bowel disorders or from septicaemia. Bowel disorders are most common during the very hot weather of summer in our stock and it is very unfortunate that a large number of the animals in the fifth service groups should have been so attacked. The data is incomplete for the two highest service groups. The tenth service progeny here again show a higher death rate than the first service litters and even though most of these tenth service litters were housed under more favorable conditions than were most of the first service litters. The very high percentage of death in the fifth service group may here be explained by the fact that a large number of the progeny in this group were born in early summer and were consequently housed
through a very hot summer. A large number of the young were lost during the hottest weather by attacks of various disorders. Observations now in progress will furnish us more complete data on the question of mortality; for the present, however, we are forced to admit that a higher death rate seems to be associated with litters from the more advanced services.

Unfortunately our records do not enable us to study the relation of sex to mortality. There is a possibility that the sex ratio may be affected by heavy service of the male. In the fifteenth service group the sex ratio of males to females is 21 to 35, and in the twentieth service group the ratio of males to females is 10-to-14. All of the animals dying in the fifteenth service group reported in table III were females; three of the four dying in the twentieth service group were females. Though the data in these two service groups is not extensive, we believe that there is a preponderance of females to males in the offspring of males at heavy service. It seems possible that the female offspring are slightly less vigorous than the males of the same litter, and are consequently less able to survive the critical period during the first few days of postnatal development. Our data does not seem to justify a conclusion that offspring from heavy service are genetically inferior to offspring from males at ordinary sexual service.
Summary.

1. Body weight of the rabbit is a measure of growth that is subject to considerable variations largely brought about by slight changes in the environment.

2. The rate of increase in body weight continues at a uniformly rapid rate for the first ninety days of the rabbit's life.

3. The factors that appear to govern the weight of the young at birth are age of mother, state of health of mother, weight of mother, weight of sire, character of food of mother, and number of individuals born in the litter.

4. The factors that govern the rate of postnatal growth of the young for the first ninety days are weight at birth, number in litter, milk supply furnished by the mother, and after weaning, the character of the food supplied to the young and general character of the quarters.

5. No inferiority in the offspring from the heavy service groups is revealed by comparing the body weights with those of the light service groups.

6. The average coefficient of variability in body weight at birth at thirty days, and at ninety days is no greater in the progeny in the heavy service groups than in the light service groups. Greater variability might be expected if the offspring are made genetically inferior by the inferiority of the male element in the advanced service groups.
7. Body development seems to progress at the maximum rate during the first twenty days of life, after which time there is a rather marked decline in the rate of increase in head length and breadth of ilial expansion.

8. No inferiority in the offspring from the advanced services is revealed from the study of body growth by measurement.

9. Offspring in the more advanced service groups show a higher percentage of mortality during the first five days of life than do the offspring in the light service groups.

10. A slightly greater mortality between five and ninety days may exist among the offspring in the heavy service groups.
Discussion

In the first paper we have shown that the amount of sexual service that the male performs has a very marked effect upon the physical properties of his spermatozoa; the whole basis of this part of the work is to discover if these effects are in any way made manifest in the offspring.

Growth in body weight must be assumed to be due to a complex of stimuli acting upon every living cell of the organism. If it were possible to modify the contribution of growth stimuli from the male germ cell by extreme sexual use of the male, and effect should be produced upon every cell of the body in his offspring, and a reduction of these stimuli would thus result in a decreased body growth. The sum total of the body increase in the offspring from the heavy service series is fully equal and even superior to the increase in the offspring in the light service groups. This apparent superiority has been attributed to various factors, largely environmental and none of which have any connection with the male reproductive cell. After these factors are corrected for, which we have found impossible to do, we believe that the rate of growth in body weight would be identical in all five service groups. But a study of body weight as reported here will only reveal the character of the total population and will not reveal the occurrence of an occasional inferior individual.

The coefficient of variability, on the other hand, is valuable in that it will reveal the occasional inferior individual in the litter. If only a part of the offspring in the heavy service groups are inferior as far as rate of growth is concerned,
there should be a greater coefficient of variability in the litters from heavy service than among the light service litters. No such evidence appears in our data and this fact we feel warrants the assumption that not even a part of the offspring in the heavy service group are more inferior as far as the ability to increase in body weight is concerned than the offspring in the light service groups.

Body measurements furnish us with further material for the study of the offspring in the different service groups. These data do not reveal any new facts to indicate any greater inferiority of offspring in any one of the five service groups. Here again the same modifying factors have been in operation that have affected the body weight data, and a correction, if possible, for these we think would show that the offspring in all five of the service groups are identical in body dimensions.

Concerning the question of rate of mortality in progeny from light and heavy service, we have some evidence that there is a higher death rate in the advanced service groups over that observed in the light service groups. Just why this should be true is rather difficult to account for, since we have shown that heavy does not affect the rate of growth either in body weight or in body measurements. A possible explanation lies in some evidence that we have that the percentage of female offspring is increased by heavy service of the male as shown on page 29. The weight offspring (Ehnot, Jackson, Zing) of female/male multiparious animals at birth
is slightly less than that of the males. If this is true for the rabbit it may render the females less able to compete with the male offspring for nourishment during their early life when food supply is of such vital importance in determining the survival of the young. The fact that the great majority of the offspring dying in early life have been females seems to warrant the assumption that females are actually less able to compete with the males during the early part of life. The data are too incomplete to show whether or not there is any higher rate of mortality in the advanced service groups than in the lighter service groups after the first five days of postnatal life. If this apparent inferiority of the offspring in the advanced service groups is due to a modified sex ratio in the advanced service groups and the consequent predominance of females, which we may assume under all ordinary conditions are less able to survive than males, it is apparent that no real inferiority exists but that the mortality is greater because the percentage of females is greater in the heavy service groups.

In conclusion, it may be noted, (1) that the methods used for measurement of the character of offspring from different degrees of sexual service of sires fail to show that any inferiority of the offspring can be induced by using a male excessively; (2) that the occurrence of weaker offspring in the advanced service groups in early life is only apparent and probably due to a preponderance of females that are less able to survive than males; (3) that the male in heavy sexual service contributes germ cells that are fully the equal of those contributed by a male in very moderate sexual service.
Acknowledgments.

The writer wishes to express his high appreciation to Dr. C. Lloyd-Jones for his constant cooperation and helpful advice, to Dr. E.S. Murphy for assistance in making a study of the male and female genitalia, to Professors G.N. Purpin and H.D. Hughes for furnishing quarters for this work for a time.
**Bibliography**