

a considerable period of time. This has been done by Leo Loeb for the guinea-pig. For the rat there are no published observations except those by Kirkham and Burr (1913), from which it is to be inferred that the ovarian cycle has a length of twenty-one days.

Although further studies on the rat are being carried on by the senior author, it seems worth while at this time to present in outline the chief conclusions arrived at, reserving for a later paper a more complete presentation and discussion of evidence.

The most obvious and certain evidence of the occurrence of ovulation is the presence of eggs in the oviduct. It is chiefly upon this kind of evidence that the conclusions are based. There is also a further source of information concerning the ovarian cycle in the corpora lutea, formed in most cases from the ripe follicles which have discharged their eggs. The corpora lutea grow and undergo such changes before degenerating that there may be as many as 40 in one ovary, of which only the youngest and oldest can sometimes be identified with certainty. However, the newest corpora up to an age of about  $2\frac{1}{2}$  days can be distinguished from older ones. Such young corpora are always present when eggs are in the oviduct, and their absence when no eggs exist in the tubes is additional proof that ovulation either has not occurred (especially if the ovary contains large follicles), or took place several days before.

All of the 80 females used were isolated from males before their last litters were born, and thereafter were kept alone or with other females. Also their young were at once removed, usually before being suckled.

The ovaries and oviducts were sectioned, the position of the eggs (when present) in the oviduct was determined, and the condition of the corpora lutea noted. The animals were killed at intervals during 101 days after parturition, 67 of the 80 rats being taken during the first four 10-day periods as follows:

|    |    |    |       |    |      |
|----|----|----|-------|----|------|
| 1  | to | 9  | days, | 18 | rats |
| 10 | "  | 19 | "     | 15 | "    |
| 20 | "  | 29 | "     | 17 | "    |
| 30 | "  | 39 | "     | 12 | "    |
| 40 | "  | 42 | "     | 5  | "    |

making an almost complete series at one-day intervals. They are grouped at still closer intervals about the tenth, twentieth, thirtieth and fortieth days. The rest of the animals were killed only at about ten-day intervals from 50 to 101 days.

Unfertilized eggs pass through the oviduct in about three days, usually having degenerated by the end of that time, as determined by a study of 15 animals killed during the first four days post partum. Accordingly the distance traveled by the eggs in the oviduct is of importance and was taken into account in estimating the time of ovulation.

Of the 80 animals examined 49 revealed eggs in the oviduct. To these may be added 14 more in which it is permissible to estimate the time of ovulation. Summarized they are as follows:

| Ovulating after Parturition |                                   | Average         |
|-----------------------------|-----------------------------------|-----------------|
| Rats                        | Days                              |                 |
| 15                          | $\frac{3}{4}$ - 1                 | 11              |
| 11                          | $9\frac{1}{2}$ - 13               |                 |
| 1                           | $15\frac{1}{4}$                   |                 |
| 13                          | 19 - $23\frac{1}{2}$              | 20              |
| 1                           | $24\frac{1}{2}$                   |                 |
| 5                           | $27\frac{3}{4}$ - $34\frac{1}{2}$ | $30\frac{1}{4}$ |
| 5                           | 38 - $41\frac{1}{2}$              | $39\frac{1}{2}$ |
| 2                           | $49\frac{1}{2}$ - 50              | 50              |
| 2                           | $57\frac{1}{2}$ - $58\frac{1}{2}$ | 58              |
| 2                           | $67\frac{3}{4}$ - $70\frac{1}{4}$ | 69              |
| 2                           | 78 - 82                           | 80              |
| 2                           | 87 - $89\frac{1}{2}$              | 89              |
| 2                           | $97\frac{1}{4}$ - 101             | 99              |

Of the other 17 rats none had eggs in the oviduct, and the ovaries presented no evidence of recent ovulations. They were killed between the periods enumerated above.

The foregoing indicates that female rats when kept isolated from males ovulate on the average every 10 days.

J. A. LONG,  
JESSIE E. QUISNO

ZOOLOGICAL LABORATORY,  
UNIVERSITY OF CALIFORNIA

#### OVULATION IN MICE

It has been known since the time of Tafani (1889) that mice normally ovulate soon after giving birth to litters. According to Sobotta (1895) a second ovulation takes place in

nursing mothers on an average 21 days after parturition, a discovery he made use of in his study of maturation. There are no other printed records of spontaneous ovulations in addition to that coming immediately after parturition.

The following is a summary of the results, with respect to the occurrence of ovulation, of an investigation, still under way, of the ovarian cycle in mice. The study is being carried on in the same way as for rats outlined in the preceding article.

Sixty-two female mice of various coat colors were bred, allowed to have their litters when isolated from males, kept alone or with other females, and killed at intervals during a period of 91 days. Most (52) were killed during the first 56 days at intervals of about 2 days, except between 18 and 21 days, 34 and 38, and 50 and 56 days when the interval was a day or less. The rest of the animals were taken between 70 and 74½, and 87½ and 91 days.

The sections of the ovaries and oviducts were examined for eggs in the oviduct and for the youngest corpora lutea. In determining the time of ovulation the position of the eggs in the oviduct was considered; and the presence or absence of the youngest corpora lutea was used as a check.

The examination of these mice indicated that the second ovulation occurred at from 15 to 19 days following parturition, the third at about 35, the fifth at 69 to 72, and the sixth at 87 to 90. No ovulation was found at the expected fourth, perhaps because too few animals were killed at that time. But it is significant that of those animals killed at 70 to 74, and 87 to 91 days which fall within the expected later ovulation periods, 3 and 2 animals were found to have ovulated at the sixth and seventh periods respectively; also that none of the mice killed between the ovulation periods was found to have ovulated.

It thus appears that the normal ovulation period in mice recurs at about 17½ to 18 days.

J. A. LONG,  
H. P. SMITH

ZOOLOGICAL LABORATORY,  
UNIVERSITY OF CALIFORNIA

#### AGAR AGAR FOR BACTERIOLOGICAL USE

AGAR AGAR is used by so many, as a basis of nutrient media, that any suggestion as to how to select the most suitable grade is worthy of consideration.

Fellers<sup>1</sup> has recently published some bacteriological studies on agar agar. The same author<sup>2</sup> has also prepared a paper on the composition of agar agar and given methods for purifying commercial agar. No matter how easy the method proposed for the purification of a substance is, we have to select that which we intend to purify. One of the best ways of determining the stability of an organic substance is to find out how much it will be hydrolyzed under the conditions it is to be used. Hydrolysis is, generally, increased with temperature, and thus increased acidity at high temperatures is often due to greater hydrolysis at the high temperatures. If substances show an increased acidity at high temperatures, but when cooled back to normal temperatures return to the acidity they had before the heating, the high temperatures have not materially changed their composition. Some samples of agar agar have been known to develop a large increased permanent acidity due to autoclaving. It is evident that such samples should not be used for accurate work. The increased acidity due to autoclaving and due to titration made in hot solutions can be made use of in selecting agar agar for laboratory use.

The following described test has been found to designate the superiority of some samples of agar agar over others. Samples chosen by means of this test are always those which go completely in solution when heated with carbon dioxide free distilled water. Further media made with them have a lower acidity than media made with agars not so good by the test.

#### THE TEST

The test depends on the increase in acidity of water solutions of the agar due to autoclaving and to titrations made near 100° C.

<sup>1</sup> Fellers, *Soil Science*, Vol. II., No. 3, p. 255.

<sup>2</sup> Fellers, *Jour. Ind. and Eng. Chem.* (Article to appear soon.)