THE EFFECT OF LIGHT ON OVARIAN ACTIVITY IN THE RABBIT

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(With Two Text-Figures.)

INTRODUCTION.

Seasonal periodicity is one of the most striking phenomena in the physiology of reproduction. This annual rhythm is most marked in wild animals but is found more or less subdued in domestic species. Thus the wild rabbit, the vole and the wild fowl exhibit a distinct breeding season. The domestic rabbit, while capable of breeding throughout the year, under the best conditions, shows a slight seasonal variation in reproductive activity. Similarly, the domestic fowl passes through an annual cycle of egg production which can be correlated with the seasons (Whetham, 1933).

The results of recent experiments suggest that an important part of the mechanism which controls this seasonal cycle is variation in the daily exposure to light. Voles and ferrets have been brought into full oestrum during their normal anoestrous periods by increasing their daily exposure to light (Baker and Ranson, 1932; Bissonnette, 1932). Likewise light has been shown to stimulate effectively ovarian activity in the domestic fowl at its low-ebb period.

The reactivity of the wild mammals to light, as well as the stimulation of egg production in the domestic fowl, suggests that this factor may modify the sex cycle of the domestic mammals. If such a modification occurred, possibly a method of controlling the breeding season and litter size of domestic species could be evolved. It is of importance, therefore, to extend our knowledge to as many types as possible, and more especially to those mammals which, like the domestic species, show but slight seasonal periodicity. The rabbit was chosen for the present research partly because the various phases of its reproductive activity have been much studied in this laboratory, and partly because the rabbit is of importance both economically and as laboratory material.

SEASONAL PERIODICITY IN THE RABBIT.

Seasonal periodicity in the rabbit has been noted by several writers. Marshall (1922) states that in the wild rabbit in England the breeding season lasts from February to May, but may extend longer. In the domesticated breeds it sometimes lasts nearly the whole year if the circumstances be favourable in regard to warmth and food supply. He quotes Heape's opinion that 5–6 months is the usual duration

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in the domesticated rabbit and that, if oestrus is experienced in winter, it may occur independently of the possibility of pregnancy. Hammond (1921) states that the wild rabbit has a breeding season which lasts from about February to September, but that it depends to some extent upon the weather. In tame rabbits the season can be prolonged by controlling temperature and feeding. In wild rabbits he found that the number of eggs shed increased from a mean of 4·2 in January to 6·7 in April. In tame rabbits he found the highest number of eggs was shed in March and the lowest in February and September. In a more recent publication, Hammond and Marshall (1925, p. 31), state that, given suitable conditions of warmth and good food, domestic rabbits will breed at all times of the year, but from data collected in the laboratory they found that a higher percentage of females refused to mate during the months from September to March, while there were few refusals from April to July. Lush (1925) in Wisconsin found that the domesticated rabbit bred at all times of the year, but from figures collected over a number of years births were most frequent in spring and late winter. Manresa (1933) also states that from March to July is the most favourable season for rabbits to breed in Wisconsin. The least favourable time is in summer from July to September.

It will be noted that in regard to the wild rabbit there is general agreement that seasonal periodicity is more marked than in the domesticated rabbit. It is also to be noted that earlier writers (*e.g.* Heape and Marshall) are more emphatic in regard to seasonal periodicity than later writers. It is possible that this reflects a gradual modification with domestication due to improvements in management and nutrition, or through selection to a change of genetic constitution.

With the exception of Hammond, who took egg production, foetal atrophy and percentage refusals to mate as criteria of seasonal activity, little attempt at quantitative analysis has been made. If only the crude birth-rate in a colony is used as a criterion of seasonal variation, little exact information of a physiological nature is obtainable, since the birth-rate at any given time may be influenced by such factors as the numbers of rabbits in the colony, the average age of the does within the colony, the fertility of the males, the incidence of disease, and so on. Only where a particular function is specified and other factors either corrected or maintained relatively constant can valuable information be derived. In Fig. 1 are shown graphs illustrating seasonal sexual activity from records kept in Cambridge over the last 6 years. In order to avoid an apparent discontinuity between the first and last months of the year, the graphs are plotted in duplicate as though extending over a period of 25 months. Graphs A and B (Walton's data) are derived from records of animals bought in the open market and mainly used for experiments on artificial insemination. They represent the results of normal control matings, each rabbit being mated with two males of known fertility, and only those matings were abstracted which were both preceded and followed by normal pregnancies. Thus the possible influence of the male, and the possible influence of age and the incidence of sterility of the female are largely eliminated. A total of 480 matings or an average of 40 matings per month uniformly spread throughout the year was obtained. The graphs show that there is little or no significant seasonal variation either in per-

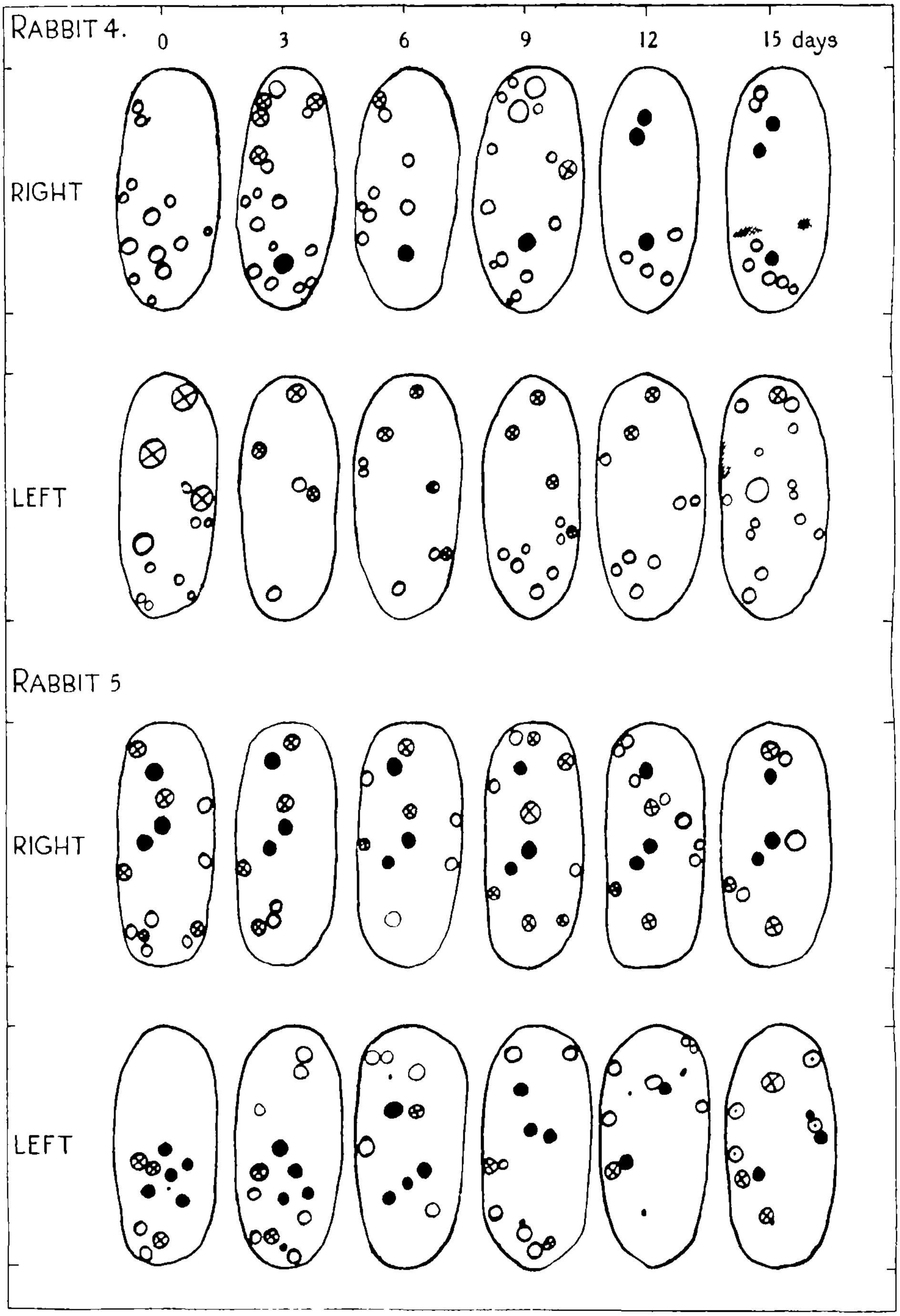

Fig. 1. Seasonal sexual activity in the rabbit.

○ Follicle
● Blood follicle
⊕ Corpus luteum
⊙ Ovulated follicle 10 hours after mating
///// Adhesion to fallopian tube

centage of fertile mating or in average size of litter. Graphs G and D and a similar pair E and F are derived from observations made by Dr Hammond on two inbred strains (C and E). Graphs D and F record the number of corpora lutea present in the ovaries, and give a more direct measure of ovarian activity than the number of young born, since the latter may be affected by foetal atrophy. A total of about ninety animals in each strain were available. Slight seasonal variation may be present, but from the small numbers available its significance is uncertain.

Owing to the great diversity of conditions, with respect to light, under which rabbits are maintained in good breeding condition, one might suppose that light was at most a minor factor influencing sexual activity. On the other hand, conditions might be such that a significant difference in regard to light, acting on some one component of sexual activity, might be obscured by interaction of other modifying factors. It is possible also that an animal would become conditioned to a given light ration but retain its reactivity to marked changes in amount. Hence, although a marked seasonal periodicity, corresponding to seasonal changes in length of daylight, might be taken as presumptive evidence of light sensitivity, absence of a seasonal periodicity does not necessarily imply that the animal is not sensitive to changes in the light ration. It was hoped that carefully controlled experiments and the use of extreme conditions of light and dark an effect might be demonstrable.

CRITERION OF EFFECT.

The initial phase of the problem necessarily involved obtaining a criterion of ovarian activity that could be used quantitatively.

Experimenters studying the effect of light on the domestic fowl have used egg-laying records as a criterion. This suggested the possibility that the number of mature follicles in the oestrous rabbit would serve as a suitable indicator.

Ten mature does were used in this preliminary study. They were kept in individual roomy cages and fed a stock diet of crushed oats, hay, and roots or cabbage when in season. The animal house was moderately well lighted, the light being mainly diffuse. The conditions were such that the colony bred readily.

We first attempted to determine whether the number of follicles was constant under the same conditions over a period of time. A bilateral laparotomy was performed, the ovaries exposed and a detailed plasticine model of the gonads constructed. The position and size of the major follicles, corpora lutea and blood follicles were indicated, and the relative distance between follicles was accurately recorded by the use of sterile dividers. A second observation on the ovaries was made from 4 to 7 days later in each case, and in two instances a third operation was performed. Each time the same method of recording the observations was used. Only four animals were used in this series, for it became apparent that the method of recording the data was too slow and cumbersome to be continued in a larger group.

Six rabbits were used in the second series and freehand drawings were substituted for the plasticine models. Six bilateral laparotomies were performed on each rabbit, the operations being spaced 3 days apart. Drawings were made without reference to any preceding records, and two of us made independent drawings so as to minimise personal errors. Mature follicles, corpora lutea, and blood follicles were recorded as before. The drawings of two of these cases are shown in Fig. 2. They are, in several instances, reconstructions made from two or more originals. This was necessary because follicles which lay near the ovarian mesentery on each side could

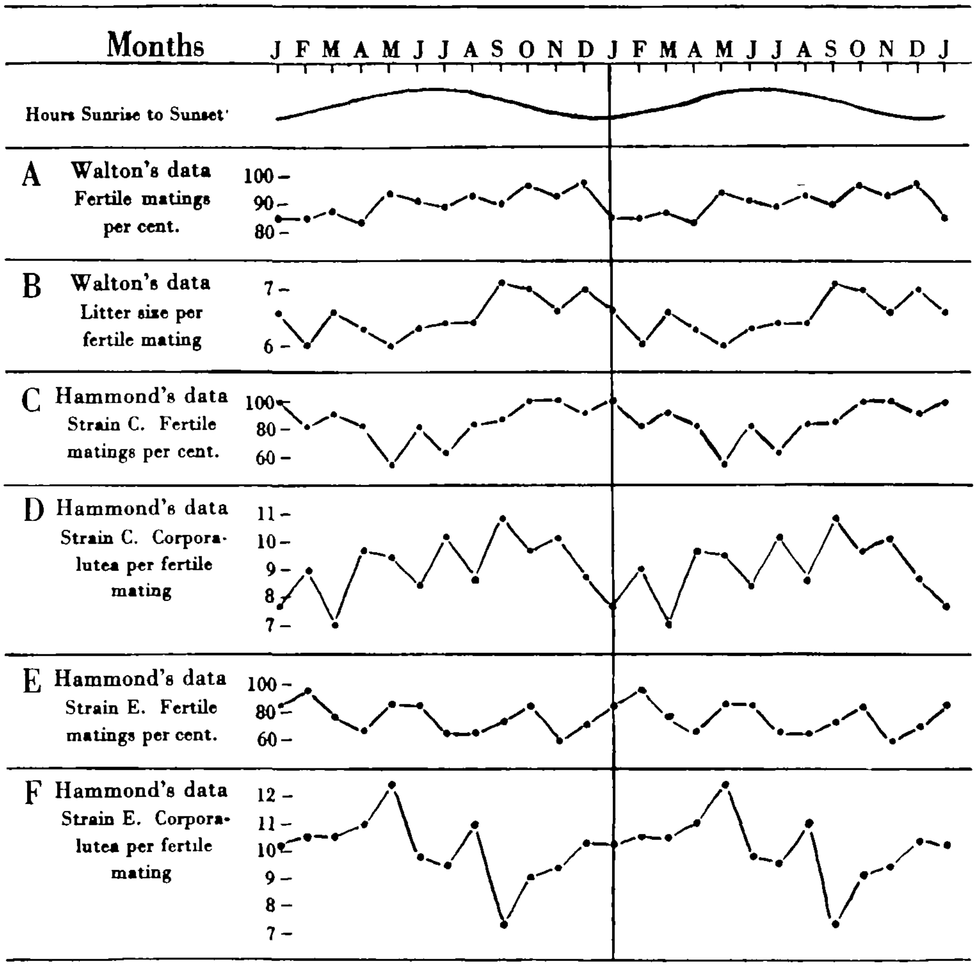

Fig. 2.

not be drawn from one point of view. All of the figures shown are, therefore, somewhat flattened so that the entire ovarian surface is represented.

The presence of blood follicles was found to be advantageous in that they served as landmarks which made it easier to place the relative positions of the other follicles. Old corpora lutea served much the same purpose but not as well, for their colour is very nearly that of the ovary, hence there was much greater variation in their recorded position and number.

In each case, following both recording methods, a consistent lack of agreement between successive drawings of mature follicles was found. In no case did a mature follicle persist through the experimental period of 15 days and it usually disappeared in from 3 to 6 days. This was not caused by injury during the operation, for in one or two cases when injury did occur, the damaged follicle was visible 3 days later as a reddish spot on the surface of the ovary. Neither is this phenomenon ascribable to ill health, for the animals did not suffer loss in body weight and in two cases, when they were mated at the close of the experiment, ovulation and fertilisation occurred. Viable young were found in one at autopsy, and the other delivered normally a litter of one. The other cases were mated and autopsied the following day.

Since these observations showed that the follicles of the rabbit are transitory structures, appearing and disappearing within the space of a few days, and since it is not easy to distinguish between fully mature and medium-sized follicles, it was clear that simple enumeration of follicles would not be a very satisfactory measure of ovarian activity. On the other hand, it was known from previous studies (Walton and Hammond, 1928) that the newly ruptured follicle was easily distinguished and also that the number of follicles which rupture and ultimately form corpora lutea is relatively constant for each animal, while reflecting fairly accurately the level of reproductive activity as conditioned by extraneous factors, such as age or nutrition. The number of newly ruptured follicles present in the ovary at a biopsy performed 24 hours after mating was therefore adopted as our criteria of ovarian activity.

METHODS.

Twelve adult does, some of them pregnant, were obtained from a dealer and were kept in the dimly lighted stock colony some weeks before the beginning of the experiment. The experimental arrangement consisted of two blocks of six wooden cages with wire-netting fronts. One block of cages was turned away from all sources of light and covered with a double thickness of heavy black cloth. This arrangement effectively excluded all light. The other block of cages was left open, facing a white surface on which were mounted six 60-watt bulbs, one directly opposite each cage and placed so that all parts of the cage were brightly illuminated. The distance between the bulbs and the back of the cages was from 3 to 4 feet. The darkened cages were much darker than any in the ordinary animal colony, and the lighted rabbits were exposed to illumination considerably greater. During the experiments on the effect of daily increasing and decreasing exposure to light both cages faced a white reflecting surface fitted with lights as described above and could be covered with heavy black curtains. The lights were turned on and off automatically by a time switch. When the lights went off the curtain was thrown over the cage as soon afterwards as possible, usually within half an hour. The cages were so arranged that the light from one set did not shine on the other. A record of the maximum and minimum temperatures of the two cages was kept and showed no difference in the average temperature of the two, but a slightly greater variation in the lighted cages.

The experimental animals were first mated to sterile (vasectomised) males, and then divided equally between the lighted and darkened cages, where they were subject either to continuous darkness or light for 30 days. This interval would allow for the termination of pseudo-pregnancy (18–20 days) resulting from the sterile matings, and would give sufficient time (10–12 days) for the growth and maturation of new follicles under the influence of the experimental lighting. On the termination of the period the rabbits were again mated to sterile males. In practice, with the exception of the first six matings, each female was mated twice with one or two males, then returned to her cage and an hour later the matings were repeated. Mating was observed in all cases. The duplication of matings was adopted as an extra precaution against failure to ovulate. In the first batch of six does matings were only made once, and on biopsy it was found that two had not ovulated. Gregory (1932) has shown that repeated matings do not influence the number of follicles ovulating, so that this factor may be neglected. On the day following the matings, *i.e.* 24 hours later, a laparotomy was performed and the number of ovulated follicles determined from drawings made as already described and also from direct counts. Practically no discrepancy in individual drawings or counts was noted. After the operation the rabbits which had been subjected to light were now replaced in the dark cages, and the rabbits from the dark cages replaced in the light, and this treatment continued for a period of 30 days.

At the close of this second month the sterile matings and laparotomies were performed as before, but the animals were returned to the same cages and kept under the same conditions of lighting for 6 days after the operation. Beginning with the seventh day the daily light exposure was increased or decreased by 1 hour. This process was continued for 24 days, so that the animals starting with continuous darkness were receiving light 24 hours per day at the close of the month, while those that began with continuous light experienced a daily decrease of 1 hour until continuous darkness was attained. They were then mated, a laparotomy performed, and the number of ovulations determined. The animals were now returned to their cages and kept either in continuously light or dark for 6 days, followed by a period of 24 days, during which the daily exposure to light was either increased or decreased by 1 hour per day. At the close of this period the usual examination was made. During the succeeding 30 days the animals were kept in continuously lighted or darkened cages as they had been in the earlier experiments. The total number of animals subjected to treatment is small but the greater variables are controlled. Each light condition is imposed on each animal in turn, thus avoiding individual variation, and each treatment is applied simultaneously to half the animals so that age or seasonal changes would cancel out.

The experiments on the influence of gradually lengthening or shortening the daily exposure to light were made because such a treatment more nearly simulates natural seasonal conditions. Data available on the influence of light on ovarian activity in the fowl (Whetham, 1933) indicate that it is the progressive increase of the daily light ration which acts as the stimulus to gonadal activity and not the total amount of light.

RESULTS.

The data are organised in Table I. The two groups A and B may be conveniently examined by the method of Analysis of Variance, taking out the different

Table I. *Effect of light on ovulation in the rabbit.*

No. of doe	1st treatment	No. of ovulations (May)	2nd treatment	No. of ovulations (June)	3rd treatment	No. of ovulations (July)	4th treatment	No. of ovulations (August)	5th treatment	No. of ovulations (September)	Totals (Animals)
A 73	Continuous dark	0	Continuous light	7	Decreasing light	12	Increasing light	10	Continuous light	12	41
A 71		9		10		11		11		9	50
A 69		8		9		8		8		8	41
A 63		11		13		15		15		11	65
A 68		8		12		10		10		8	48
A 70		0		8		11		11		10	40
Totals (light treatments)		36		59		67		65		58	
B 64	Continuous light	0	Continuous dark	5	Increasing light	7	Decreasing light	6	Continuous dark	8	26
B 65		8		8		9		8		5	38
B 66		9		9		7		6		9	40
B 67		8		10		10		7		0	35
B 72		3		4		8		8		6	29
B 74		7		5		6		6		7	31
Totals (light treatments)		35		41		47		41		35	

light treatments on the one hand, which may also include a slight seasonal effect, and the differences between the animals on the other. Table I is then as follows:

Analysis of variance.

Variance due to		Degrees of freedom	Sum of squares	Mean square
A	Light treatment	4	101·67	25·42 $Z=0{\cdot}7340$
	Animals	5	90·70	18·14 $Z=0{\cdot}5656$
	Error	20	117·13	5·856
	Total	29	309·50	—
	Between A and B	1	123·27	123·27
B	Light treatment	4	16·80	4·20
	Animals	5	29·37	5·87
	Error	20	130·80	6·540
	Total	29	176·97	—

It is clear that there are significant differences between light treatments (and animals) in Series A but not in B. In the absence of any hypothesis to account for this difference between the two series we cannot attach much importance to the results of the one series alone. In regard to the two series taken together we see that the errors are not significantly different in the two series, so that we may take for an estimate of variance the mean 6·198, based on 40 degrees of freedom. Thus the standard error of the mean of 12 results is 0·72, of 18, 0·59 and of 30, 0·45. These standard errors are appended to the averages for the light treatments shown in Table II.

Table II.

Continuous light	8·44	S.D. 0·59 diff. + 2·22 S.D. diff. 0·83.
Continuous dark	6·22	
Increasing light	9·33	S.D. 0·72 diff. + 0·33 S.D. diff. 1·02.
Decreasing light	9·00	
Continuous or increasing light	8·80	S.D. 0·45 diff. + 1·47 S.D. diff. 0·63.
Continuous or increasing dark	7·33	

These results show that there is a slight difference in favour of light treatment and that this difference is formally significant with respect to continuous treatment and to combined continuous and increasing treatment. The calculations however include the four cases, three in the continuous dark treatments and one in the continuous light treatments, in which does failed to ovulate. Now although failure to ovulate might be regarded as a concomitant of sexual inactivity there is complete lack of continuity between those does which ovulate and those which do not. If we regard failure to ovulate as a separate eventuality, we find that the distribution in the two series is as follows: Continuously light 1/18 and continuously dark 3/18 showing a difference which is obviously not significant. It would seem therefore more appropriate if these cases were not included in the computations of the means. Leaving them out we get the following values.

Continuous light	8·94	Diff. 1·47 [S.D. 0·83].
Continuous dark	7·47	
Continuous or increasing light	9·10 9·10	Diff. 0·95 [S.D. 0·63].
Continuous or increasing dark	8·15	

Assuming that the standard deviations would not be much affected by omission of the non-ovulating cases we see that this reduces the differences below the level of significance.

All we can deduce from the results is that under the conditions of experimentation does when subjected to light treatment produced slightly more ovulations than when subject to dark. But that as the difference was largely confined to one group of animals and as the differences were much reduced when does which failed to ovulate were omitted from the calculations it is doubtful whether the differences are to be regarded as due to light, to chance variation, or to some uncontrolled and unknown factors.

It may not be an entirely fortuitous coincidence that Series A shows both a significant effect of light treatment and also a significant difference between animals. In Series B the average number of follicles per doe is less than in A, and it may be that some factor is limiting follicular development or ovulation so that neither the full individual capacity nor the capacity to respond to light is attained. In Series A the average number of follicles per doe is higher and there is much closer individual constancy.

The results do not indicate any significant difference of effect between those receiving constant light or dark and those subject to continuously increasing light or dark.

Records were kept of the time elapsing between the matings and the date when the does plucked fur to construct a "pseudo-pregnant nest." Dr Hammond reports that normally this occurs 16–20 days after a sterile mating and is closely correlated with the decline of the corpus luteum as indicated by the ability to ovulate, in response to a mating. The construction of a nest at the close of pseudo-pregnancy marks the end of the period of corpus luteum activity. Nest building is, unfortunately, not indulged in by all rabbits, so the data obtained are too meagre to be susceptible of statistical analysis. The length of approximately one-half of the pseudo-pregnancies was determined. No difference in the length of life of the corpus luteum in lighted and darkened animals could be detected.

DISCUSSION.

A. *The length of life of the mature Graafian follicle.*

The unlooked-for discovery of the relatively short life of the mature ovarian follicle in the rabbit brings to light a wholly new concept of ovarian function in those animals with persistent oestrus. It has generally been understood that: "The ovarian cycle (in the Rabbit) consists in the maturation of Graafian follicles at the beginning of the breeding season and persistence of mature follicles until copulation takes place or until the breeding season ends" (Parkes, 1929, p. 55). The persistence of mature follicles in those animals typified by the rabbit has been in striking contrast to the short life of mature ovarian follicles in other rodents. The possibility that the condition of continuous oestrus in the rabbit was not necessarily correlated with the persistence of individual follicles throughout the breeding season was first noted by Hammond in 1925. This author states that mature follicles may either "(1) persist in the ovary for at any rate thirty-six days, or that (2) series after series of follicles ripen and atrophy, these series overlapping so that heat and fertility are continuous." Hammond is inclined to support the first possibility which has been the accepted one.

The short life of the mature follicle in our series, we believe, represents the condition existing in the normal oestrous doe. It is not the result of debility occasioned by the numerous operations. The post-experimental fertility of the animals is an adequate criterion of their normality.

The fundamental basis for this cycle of follicular growth and decline is obscure but we believe that it is essentially a follicular mechanism. Possibly a senescence or decreasing reactivity to extra-gonadal influence, such as the anterior pituitary, may account for the atrophy of these follicles, for if the basis for the fluctuation lay outside the ovary the cycle of the individual follicles would coincide as in the spontaneously ovulating mammals.

The conception of the transitory nature of the follicle may render necessary some modification of the generally accepted explanation of the blood follicle. Blood follicles are said to form if the male is withheld from the doe for a long time (Hammond and Marshall, 1925, p. 81). In the absence of ovulation the persisting follicle was supposed ultimately to break down internally to form the blood follicle. In some of our rabbits we observed the formation of blood follicles within a month of mating and ovulation; prolonged persistence is therefore not essential. It is possible that the formation of a blood follicle is alternative to the normal follicular decline.

B. *Effect of light on ovarian activity.*

It was our aim to study not only the influence of light on the desire for coitus and on fertility but also the potential fecundity as indicated by the number of ovulated follicles. It is reasonable to suppose that under slightly unfavourable conditions the number of ovulated follicles would decrease before a total absence of ovulation would occur or the doe go into anoestrum. The number of ovulations is certainly a delicate enough criterion to detect any major fluctuations in ovarian activity. It is obvious from the data presented that light has no great effect under the conditions of the experiment.

As has been pointed out earlier, the intensity of light and darkness to which the animals were subjected is far greater than any to which they are normally exposed in the stock colony which has excellent breeding conditions. The length of the exposure, about 1 month, to the experimental treatment may be regarded as too short; certainly seasonal conditions of increasing and decreasing daily light exposure were not closely imitated. However, the speed with which mature follicles can be formed is very great. Asdell (1924) and Walton and Hammond (1928) found that mature follicles were produced within 48 hours following unilateral ovariotomy or ablation of ripe follicles. However, in all probability we are not dealing with simply the potential reactivity of the ovary but with an intermediary, the anterior hypophysis. The mechanism involved in the effect of light on ovarian function in the ferret and vole is not clear but there is little doubt that it is an indirect one. It may be that due to the slowness of the reaction of the intermediary to light no ovarian reaction was obtained. However, in two series a month of continuous light or darkness was added to a month of either increasing or decreasing light. In these instances we have a cumulation of 2 months' experimental treatment. In neither experiment was the ovulation number affected as much as by even a single month of continuous light or darkness.

CONCLUSIONS.

1. The mature Graafian follicle does not persist indefinitely in the oestrous rabbit but is rather short-lived, so that during oestrus numbers of follicles appear and disappear, the series overlapping so that at any one time there are approximately the same number of mature follicles on the surface of the ovary and heat and potential fertility are continuous.

2. Mating response, ovulation and pseudo-pregnancy proceed normally in rabbits subjected to periods of 30 days' intense illumination or almost total darkness. The number of ovulations was slightly higher in those subjected to light. This is perhaps sufficient to indicate that ovarian activity is not altogether insensitive to light but when a small number of does which failed to ovulate was omitted from the data the difference was considerably reduced and ceased to be statistically significant.

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REFERENCES.

ASDELL, S. A. (1924). *Brit. Journ. Exp. Biol.* **1**, 473.
BAKER, J. R. AND RANSON, R. M. (1932). *Proc. Roy. Soc.* B, **110**, 313.
BISSONNETTE, T. H. (1932). *Proc. Roy. Soc.* B, **110**, 322.
GREGORY, P. W. (1932). *Journ. Exp. Zool.* **62**, 271.
HAMMOND, J. (1921). *Journ. Agric. Sci.* **11**, 337.
HAMMOND, J. AND MARSHALL, F. H. A. (1925). *Reproduction in the Rabbit.* Edinburgh.
LUSH, J. L. (1925). *Journ. Agric. Research,* **30**, 893.
MANRESA, M. (1933). *Philippine Journ. Sci.* **51**, 323.
MARSHALL, F. H. A. (1922). *The Physiology of Reproduction.* London.
PARKES, A. S. (1929). *The Internal Secretions of the Ovary.* London.
WALTON, A. AND HAMMOND, J. (1928). *Brit. Journ. Exp. Biol.* **6**, 190.
WHETHAM, E. O. (1933). *Journ. Agric. Sci.* **23**, 383.

Note. While this paper was in preparation observations on the growth and regression of follicles in the oestrous rabbit, which are in substantial agreement with our own, have been published by Hill and White (1933). *Journ. Physiol.* **80**, 174.