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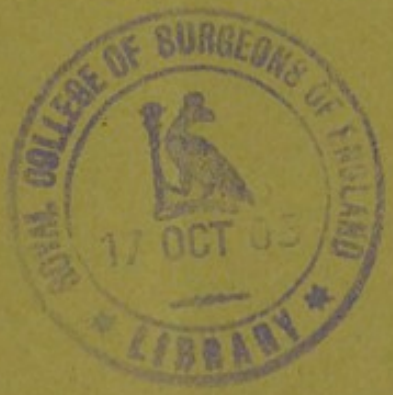

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Ovulation and Degeneration of Ova in the Rabbit.

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(Communicated by Adam Sedgwick, F.R.S. Received March 6,—Read April 6, 1905.)

It has long been held that ovulation invariably occurs in all animals at each period of œstrus.

I have already shown (Nos. 13, 14, 15) that this is not necessarily true for menstruating animals. In other polyœstrous animals also there seems reason to believe that when coition is prevented during the first few recurrent œstrous periods, ovulation is interfered with during the subsequent periods, for conception is then much more uncertain than it is if coition occurs when the sexual season first appears.

Moreover, among bats there is clear evidence that ovulation does not necessarily occur during œstrus (Nos. 2, 4, 5, 8), for the mature females are impregnated in the autumn and do not ovulate until later, probably the following spring; although the young females, born in the late spring, do not copulate until the spring of the following year at the time when ovulation also occurs.

With these facts before me I began, in 1894, investigations on the domestic rabbit. Over one hundred does were experimented on, and being kept in locked cages, of which I only had the key, certain errors so common with breeding experiments were avoided. The ovaries were preserved in various ways, and the histological results here given are determined from serial sections of which I have some 120 series.

It was found that the domestic rabbit does not ovulate until, approximately, 10 hours after copulation (*cf.* Nos. 1, 3). The doe rabbit only permits coition when undergoing œstrus, and if the male is withheld at that time the ripe ova in the ovary degenerate; they are not dehisced from the ovary. Neither stimulation of the vulva with electrodes, nor artificial insemination, nor subcutaneous injection of spermatozoa induced ovulation; moreover ovulation did not follow coition if from any cause a sufficient supply of blood to the ovaries was interfered with; while at the same time, provided this supply of blood was not interfered with, artificial stoppage of the progress of the spermatozoa from the vagina did not interfere with ovulation.

The Graafian Follicle and Ovum.—The follicle consists of a thick layer of epithelium, bounded on its outer edge by a basement membrane. Within, the ovum lies surrounded by its zona radiata. The structure is embedded in

the ovarian tissue, which consists essentially of a connective tissue network enclosing large parenchyma cells, through which blood vessels run. In the region of the follicle this tissue is somewhat modified. Close round the basement membrane I find a thin layer of parenchyma cells in a connective tissue network, the theca interna, and immediately outside that the connective tissue itself is specially developed into closely approximated fibrous bands with a few parenchyma cells in the interstices thereof—the theca externa (Nos. 23, 29, 30, 33).

The first sexual season begins in the domestic rabbit as a rule in February or March, but is dependent somewhat on the food given, on the warmth and shelter provided, and on the frequency with which they were allowed to produce young the previous year.

Prior to that time certain of the Graafian vesicles enlarge, a space containing liquor folliculi appears, and the ovum, surrounded by a thickened mass of epithelial cells (the discus proligerus) lies eccentrically but connected with various portions of the follicle wall by strands of this tissue. The remainder of the epithelium round the wall of the follicle is much reduced in thickness. The growth of those vesicles situated near the surface of the ovary causes them to project and form swellings on the surface; both the wall of the follicle and the tunic of the ovary is here very much attenuated, so much so that in some of them, when ripe, the structure is sufficiently transparent to allow of the ovum being seen within the vesicle. During proœstrum, the blood vessels which surround these follicles become more numerous, enlarged and congested, and such as run in the thin wall which projects on the surface of the ovary give these follicles the bright pink colour which is characteristic of them.

It is important to notice, however, that a brilliant suffused red colour does not denote a ripe Graafian vesicle but, as I will show below, a degenerate follicle which will not rupture; failure to distinguish between these two appearances has been the cause of much error.

Maturation of the Ovum.—Normally, immediately after copulation the ripe ovum in the swollen Graafian follicle is affected. The cells of the discus proligerus, which until now had closely invested the ovum, begin slowly to withdraw (*cf.* 19). During the growth of the ovum nutriment is supplied to it by the aid of these cells; as I have already shown (No. 12), protoplasmic processes from them are projected into the radiating canals of the zona radiata; now the cells withdraw radially and eventually remain attached to the zona only by these exceedingly fine strands. This process apparently occupies some hours. At the same time the ovum, bounded by its thin vitelline membrane, withdraws somewhat from the zona leaving a narrow space

between them. It appears obvious that thus the supply of ovarian nutriment is stopped. About nine hours after copulation maturation of the ovum takes place and two polar bodies are rapidly formed.

It is of interest to notice that maturation does not occur until after the supply of nutriment to the ovum is cut off, for in this particular it appears probable that the formation of polar bodies takes place under conditions of nutriment essentially different from those which prevail during segmentation.

Ovulation.—About 10 hours after copulation the Graafian vesicle ruptures through the attenuated wall which projects on the surface of the ovary. The ovum, entirely freed from the discus proligerus cells, is shot out into the infundibulum which now closely invests the ovary.

Once freed from the ovary the mature ovum is incapable of assimilating nutriment unless it be fertilised; if from any cause fertilisation is not effected the ovum quickly dies, although it is bathed in the nutrient material supplied by the maternal tissues; ova thus degenerating are, from time to time, to be seen in the fallopian tubes.

It is necessary then, in the case of rabbits 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 copulation and close to the infundibulum, if not actually within its folds, four hours after copulation.

In those animals in which the ovum does not undergo maturation in the ovary, the presence of spermatozoa at the top of the fallopian tube is not necessary. In the mouse, for instance (No. 29), when the ovum is dehisced from the ovary it is not free from discus cells, and the polar body may be formed during its passage down the fallopian tube.

Recently it has been shown (No. 23) that in the ferret ovulation does not take place without previous copulation, the same is also said of the pig and sheep (No. 11, *cf.* also, No. 22) and guinea-pig (No. 26), and I suspect it will also be found to be true for other animals. It is interesting to note that ovulation in the frog takes place only when a certain stage of maturation is reached (No. 24). The fact that maturation of the ovum does not occur until after copulation has taken place affects fundamentally the results of various experiments which have been made on artificial fertilisation of rabbit's ova (Nos. 10, 25, 28).

The Rupture of the Follicle and the Corpus Luteum.—The cause which induces the rupture of the Graafian vesicle is obscure. Immediately before rupture the wall of the distended follicle where it projects on the surface of the ovary is very thin, and is covered by a thin layer of the tunic of the ovary. The congested vessels which surround the follicle are present also between the follicle and the tunic of the ovary in this distended portion.

It has been argued that the follicle ruptures on account of the tension caused by the material secreted therein; but in the rabbit the follicles do not rupture unless copulation takes place and, as I will show below, the ripe follicles which do not rupture because copulation has not taken place are distended and vascular, so far as I can see, to the same extent as those which do rupture.

It has been suggested that the spermatozoa in the infundibulum and, as that organ is closely applied to the ovary, on the surface of the follicle, exercise influence; but as has been shown in the sheep, for instance (No. 22), ovulation may take place prior to copulation, and hence when no spermatozoa are present.

Again, it has been stated that the vessels surrounding the follicle burst and pour their blood into it, so causing increased pressure, which bursts the thin wall; but in the rabbit, at any rate, the blood is not poured in any considerable quantity into the follicles which burst while it floods those which do not burst.

The fact that in the domestic rabbit ovulation does not occur until after copulation has taken place, while in other animals it may occur prior to the sexual act, suggests the probability that in the former animal additional stimulus is necessary to induce the rupture of the follicle. Whether, as has been suggested (No. 27), the rupture is due to the stimulation of erectile tissue or not my experiments do not show, but the observations made on the nerves of the ovary render such explanation extremely probable (Nos. 9, 17, 18, 20, 21, and 34). All I can say is that the base of a discharged follicle appears to be pinched together, though I have been unable satisfactorily to demonstrate that the effect is produced by the contraction of the tissues which surround the follicle, and the actual cause of rupture I have been unable to discover.

The corpus luteum is formed by the ingrowth of cells surrounding the follicle together with the follicular epithelium; the ingrowth being at one time apparently a forcible rush before which the loosened epithelium is driven. The ingrowth takes place in the first instance in the region of the base of the follicle.

Degeneration of Ripe Follicles and the False Corpus Luteum.—When a doe has not been allowed access to the buck during œstrus, the ripe follicles which are present at that time do not burst, and the ripe ova contained therein do not undergo maturation.

The follicle is distended and projects on the surface of the ovary, its outer wall is thin and the whole structure is very vascular, precisely as is the case with the follicles which do rupture after copulation; but it does not rupture, instead, the surrounding congested vessels rupture and pour

their blood into the follicle itself, forming there a clot of blood, in the midst of which for many days the degenerating ovum may be seen.

This result causes the brilliant suffused red colour of degenerate ripe follicles at an early stage of the process; gradually the red colour is lost and results in a black patch which long persists, and which is reduced as time goes on by the absorption of the contents of the follicle.

The first rush of blood isolates the ovum and its discus proligerus, and subsequently washes away the rest of the epithelium from the walls of the follicle, disintegrates the theca interna, and permeates the meshes of the theca externa. The contents of such follicles are very brittle, and in sections are frequently lost, so that not infrequently this false corpus luteum appears as a cavity bounded by the theca externa (*cf.* No. 23).

The absorption of the contents is carried out mainly by in-growing parenchyma cells, though a few leucocytes are also similarly engaged, and as the blood clot disappears its place is taken by the normal ovarian tissue. Thus, the false and the true corpora lutea are markedly different structures and are readily distinguishable in sections.

Degeneration of Ova and Sterility.—If the buck is withheld from a doe during several consecutive periods of œstrus, most, if not all, the older and many younger follicles then undergo degeneration. The loss of ova from this cause is so great that frequently during the remainder of that breeding season, and sometimes apparently for one or more future seasons, the animal is sterile. This prevention for a time of the normal functions of the ovary results in more or less persistent sterility, a point of interest to students of the physiology of the generative system and not without economic importance.

Degeneration of Young Follicles: Variation and Nutrition.—But besides degeneration brought about in this manner, there are other causes which induce degeneration of ova. In many ovaries in which there are healthy ripening ova in healthy follicles, degenerative changes are to be seen in others, in some of which it is the follicle, in others the ovum, which first shows signs of disintegration. These are invariably younger follicles, and the cause of degeneration seems clearly to be associated with nutrition.

In cases where *follicles* degenerate in the neighbourhood of other healthy follicles, want of nutrition is strongly indicated, and it is not improbable that competition is at work and is responsible for the loss. Degeneration of the *ovum*, however, from the same cause, while it may be due to competition, may also be due either to want of vitality of the ovum or to want of the requisite quality of nutriment, in other words to inability of these ova to assimilate what is obviously sufficient for the needs of neighbouring

ova. In the latter case the constitutional capacity of the ova is concerned' and while their degeneration may be due to a defective supply of nutriment, it may, with no less probability, be inferred that failure to develop is due to their incapacity to utilise the nutriment which is supplied and on which other neighbouring ova flourish.

It is well known that domestic animals and cultivated plants are more prone to vary than are the same varieties or species in a wild state. De Vries (No. 32) expresses the view that by feeding up the mother-plant with manure the offspring are induced to exhibit a greater variation; that by rich or poor treatment of seed-plants greater variation can be produced than by selection of seeds; and that the influence of manures on the mother-plant is exercised on the seeds she produces. If this be so, any increased capacity on the part of the mother to assimilate nutriment of different qualities, or to manufacture such material, will enable her to produce more widely varying offspring.

I would suggest then that when young ova degenerate under the conditions above specified, there is great probability that in such cases the degeneration is due to peculiarities in the constitution of those ova, and that such ova require special facilities for development; that they give rise to, in fact, "sports," extreme cases of that variation which it is known domesticated animals are specially liable to produce. In view of De Vries' observations and the experience of many practical breeders and horticulturists, it would seem very important that the whole question of the effect of various kinds of nutriment upon the developing ovum and embryo should be investigated, for it is reasonable to expect that, given the requisite quality of nutriment, the power of producing variable offspring would be widely extended and the field for the study of variation correspondingly enlarged.

The Ovary as a Secretory Gland.—Evidence of the part the ovary takes in providing the ovum with nutriment demonstrates that it is a secretory gland, and it has been urged that ovarian secretion is responsible for much besides the growth of the ovum, that it governs, indeed, all activity of the other generative organs.

The experience of ovariotomists shows that excision of the ovaries has a marked effect on proœstrum (menstruation—human) and upon the severity of œstrus (mares), and it is claimed, though it appears to me this requires confirmation, that destruction of the corpora lutea prevents the gestation of the ova which were discharged from the pre-existing Graafian vesicles (*cf.* Nos. 6 and 7).

These facts would certainly indicate that if the force which controls the

activity of the whole generative system is not in the ovary, that organ is at least essential to the normal functions of the remainder of the system. In the same way excision of the generative glands affects, for instance, the growth of horns in the male, and exercises influence in various ways on other organs of the body of both sexes. Thus these organs are probably essential to the normal development of sexual characteristics.

There would seem to be little room for doubt that this is the case. But in all animals which have a special breeding season, the ovary has a quiescent period, so far as the development of the ova are concerned, varying in accordance with the length of the interbreeding seasons. For some time before the advent of the sexual season, however, with the probable exception of bats, the ovary exhibits activity, and is obviously engaged in transmitting nutriment to the ova, which now begin to develop. The commencement of this active season is marked by the increased vascularity of the gland. The fact that this ovarian activity precedes the sexual season is not unfavourable to the view that it exercises influence on the latter. It must be recollected, however, that monkeys, although they menstruate regularly each month throughout the year, have a subscribed breeding season, and their regular menstrual function goes on during the time when the ovary is quiescent. Moreover, as ovulation in the bat may take place many months after the sexual season is over, and the bat's ovary is certainly quiescent during those intervening months, it may be expected that the sexual season in bats occurs in the absence of ovarian activity. Finally, it is quite clear that ovulation and proœstrum are not necessarily coincident in many animals.

If these facts are true, it does not seem possible to accept the view that the stimulus which induces proœstrum and œstrus has its origin in the ovary.

There is another point to be considered, namely, that the advent of sexual activity may be hastened or delayed or, perhaps, prevented altogether; the severity of proœstrum and œstrus augmented or reduced; and the ratio of fertility influenced, by climatic conditions and food.

Similar conditions obtain and similar results follow in the male.

Generative Ferment and "Gonadin."—My belief is that the stimulus which primarily induces such activity is of extraneous origin; that it is due to a change in the constitution of the blood, brought about by climatic influences and food, which from the nature of its growth would seem to be always specially nutritious at this season; that it results in increased vitality throughout the body—clearly evidenced by the growth of horns, wattles, and other excrescences, by the growth of hair and plumage, and the accession of brilliant colouring to such epidermic growths or to the skin itself—and similarly affects the generative system.

The substance which causes this change in the constitution of the blood, which creates disturbance and results in activity, is probably of the nature of a ferment. It affects similarly both male and female animals, and may be considered as a special "generative ferment."*

As I have already shown, there is strong evidence that the increased activity of the generative glands, consequent on the presence of this "ferment," results in the secretion of material which exercises a profound effect upon the rest of the generative system and possibly upon other organs; for this secretion I will suggest the term "gonadin" should be used.

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* I originally suggested "œstrus toxin" as a name for this disturbing agent, but, as its influence is not confined to the female, and as there is no reason to assume the presence of poison, this term is misleading.

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