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THE VASCULAR SYSTEM OF THE RABBIT OVARY
AND ITS RELATIONSHIP TO OVULATION

by

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A THESIS

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Approved
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THE VASCULAR SYSTEM OF THE RABBIT OVARY AND ITS RELATIONSHIP TO OVULATION

Introduction

Progress in endocrinology during the past twenty-five years has given a better understanding of the hormonal factors which are responsible for the cyclical changes in the ovary. However, the mechanism by which the follicles are caused to rupture has not been satisfactorily explained. Several causes have been postulated. Schochet (1915) concluded that rupture of the Graffian follicle was partly due to the digestion of the theca by a proteolytic enzyme. Guttmacher and Guttmacher (1921) suggested that involuntary muscle fibers in the thecae of the sow ovary may play some part in the ovulation process. Longley (1911) states that distension of the follicle by an accumulation of fluid causes rupture of the tunica albuginea and granulosa, but he does not state the source of the fluid. Brambell (1928) suggests a similar mechanism.

A number of workers have indicated that vascular changes are contributory to follicular rupture. Clark (1900) suggested that engorgement of the blood vessels of the ovary accompanied by increased arterial tension caused rupture of the follicles. Heape (1905) observed that ovulation in the rabbit did not follow coitus if the blood supply to the ovary was reduced. Keller (1943) attributes ovulation to increased internal pressure as a result of vascular congestion caused by contractile veins and smooth

muscle at the hilus of the ovary. He contends that these contractile structures bring about a sudden increase in pressure inside the ovary which frees the follicles from their connective tissue nets and allows them to rupture. Pearson (1944) suggests that ovulation in the shrew may be aided by engorgement of the blood vessels in the medulla. Landau (1938) drew similar conclusions from his work on the ovary of the insectivore *Hemicentetes*. In recent years, since the 'rediscovery' of the spiral arteries in the ovary (Reynolds, 1947 a,b, 1950), interest has been focused upon the local regulation of blood pressure within the ovary by the helicine arteries.

The writer believes that the lymph vessels must be considered in any mechanism which attempts to explain rupture of the follicle. Wislocki and Dempsey (1939) described the lymphatics in the ovary of the Rhesus monkey, and Andersen (1926) has given a detailed account of the development of the lymph vessels in the sow's ovary during the formation of corpora lutea. This investigator remarked upon the extensive network of lymph vessels, and she suggested that they might be concerned with carrying off endocrine products.

In this investigation studies were made to ascertain whether the blood system and the lymphatic system of the rabbit ovary are subservient to some function other than that of blood supply and drainage. The number and size of the lymph vessels in the rabbit ovary appear to be

disproportionate to the size of the organ, and if the function of the lymphatics is one of drainage it is difficult to see why such a relatively small organ as the rabbit ovary should require such an extensive drainage system.

Materials and Methods

One hundred and eleven young adult, virgin, female rabbits were used in this investigation. Sixty-two of these animals were used for experimental work, and the remainder were used for histological studies. Paraffin sections of fifty-nine ovaries from these animals were cut serially at 10 μ , and were stained with Harris's hematoxylin and counterstained with erythrosin or eosin.

In a number of animals Chinese ink was injected into the ovary through the artery, and these ovaries were sectioned and stained as above. The injection mass was prepared by rubbing up Chinese ink in distilled water to form a heavy suspension. Enough of this suspension was mixed with rabbit serum to give the desired density. The serum suspension was decanted several times to remove the larger particles. The preparation which was used contained carbon particles which were fairly uniform in size, and could be seen under the low power of the microscope.

The lymph vessels which emerge from the ovary were made visible by injecting Higgin's India ink directly into the substance of the ovary; the method of injection was that described by Culliner (1944).

Corrosion preparations of the veins and arteries were made by injecting the vessels with vinyl acetate and dissolving the tissues in hydrochloric acid. Three venous casts and five arterial casts were prepared.

The most instructive preparations were obtained by clamping the blood vessels of a congested ovary, fixing the ovary in formalin, and subsequently clearing it in methyl salicylate. These preparations were studied under the binocular microscope and they were especially good for studying the veins. For the most part, these vessels were filled with blood, the dark brown color of which, against the transparent ovarian tissue, gave a clear picture of the arrangement of the veins and small vessels.

Casts of the lymph vessels were made by injecting vinyl acetate directly into the substance of the ovary. This was a haphazard method, but after a number of trials five casts of parts of the lymphatic system were obtained. These injections were never completely satisfactory because a few blood vessels were also injected. However, the nature and arrangement of the lymph vessels made it possible to distinguish between the two kinds of vessels. In addition, the vessels in relation with the ovary were studied in the living animal whenever the ovaries were removed.

Histaminase determinations were made on stimulated and unstimulated ovaries by a procedure similar to that of Laskowski (1942) who measured the histaminase activity of crude extracts of cortex of fresh pig kidney. Due to the small amount of tissue available in the case of the rabbit ovaries it was not feasible to use extracts of them; instead, homogenates of the ovaries were used. The oxygen consumption was determined in the Warburg apparatus. The animals

were killed by a blow on the head, and the ovaries were removed, weighed, and homogenized as quickly as possible. The animals which were stimulated were killed nine hours and ten minutes after copulation. In all cases a ten percent homogenate was used. The reaction flasks contained 1 cc. of homogenate, 1 cc. of phosphate buffer at pH 7.4, and 1 cc. of histamine substrate. The histamine solution contained 1 mg. of histamine dihydrochloride (Eastman) per cc. of distilled water. The center well of each reaction flask contained 0.1 cc. of 20 percent KOH. The determinations were made in air at 38°C using a shaking rate of 110 oscillations per minute. For each run, a 1 cc. aliquot of the homogenate used was taken and dried for the determination of the dry weight of tissue used.

The Arteries and Veins of the Rabbit Ovary

The rabbit ovary is oval in shape and roughly triangular in cross section. It is reduced in thickness where it is attached to the mesovarium, and it is along this thin edge that the blood vessels and lymph vessels enter and leave. The substance of the ovary is divisible into a central medulla, which comes to the surface along the thin edge of the ovary, and a peripheral cortex, which invests all other portions of the medulla (Fig. 1). In the ovary of the young adult rabbit there is very little if any interstitial tissue, and the mature follicles, with their bases bordering the medulla, occupy the entire thickness of the enveloping cortical tissue. The medulla is made up of loose stromal tissue which supports the major arteries, veins, and lymphatics. The most numerous vessels in this region are the large, freely anastomosing lymph vessels which give the medulla a spongy, cavernous appearance. Much of the histological work which is described in this paper was submitted in an M.A. thesis (Burr, 1949).

The arteries in the ovaries of a number of different species of animals have an unusual spiral configuration. The only other organs in which coiled arteries have been observed are the uterus (Daron, 1936; Markee, 1940) and the testes (Harrison and Weiner, 1949). Clark (1900), Delson (1949), and Delson et al (1949) described coiled arteries in the human ovary and presented detailed figures of their

Figure 1

Camera lucida drawing of a portion of a section of a rabbit ovary. CORT., the deep staining cortical region, containing follicles in various stages of development. MED., the cavernous medullary tissue. THIN EDGE, the edge of the ovary. V, vein. LV, lymph vessel. X28

arrangement. Andersen (1926) described spiral arteries in the ovary of the sow, although she stated that they were not so numerous as those found in the human ovary. Basset (1943) observed that they were present in the ovary of the rat, and Nalbandov and James (1949) state that they are present in the chicken ovary. Reynolds (1947 a,b) has described the coiled arteries in the rabbit ovary, although his studies were chiefly concerned with the larger arteries and their causal relationship to the development of cystic follicles.

In rabbits which do not have large amounts of fat in the mesovarium, the ovarian arteries may be readily traced from their beginnings at the dorsal aorta to their entrance into the ovaries. Each ovarian artery arises from the dorsal aorta about three centimeters posterior to the renal artery. About midway between the dorsal aorta and the ovary each ovarian artery gives rise to a branch which supplies the uterus. Close to the ovary an additional, but smaller, branch supplies the oviduct. Before entering the ovary the artery is often thrown into one or more loose coils.

The vinyl acetate corrosion preparations were used for studies on the course and configurations of the larger arteries inside the ovary. These preparations showed that the ovarian artery enters the ovary near the posterior end and divides into two branches, a long branch which turns sharply anteriorly and a shorter branch which turns in a posterior direction. Both branches run along the thin edge of the

ovary (Figs. 2 and 3). Sometimes, in young adult animals, these vessels can be clearly seen in the living ovary. They have a definite spiral form and they diminish in size as they approach the ends of the ovary (Fig. 2). Both coiled and straight arteries arise abruptly from these vessels, with coiled arteries being the most numerous (Fig. 4). The straight arteries proceed directly through the medulla to the cortex, where they give rise to small vessels, some of which supply the follicles; however, most of the arteries in the cortex are derived from the large coiled arteries. These large coiled arteries are restricted to the medulla, although they give rise to a number of smaller coiled arteries which extend into the cortex.

The small coiled arteries give rise to straight vessels which penetrate the thecae of the follicles. However, the vinyl acetate did not penetrate the terminal portions of these straight vessels and their terminations in the follicles were studied in the serial sections. After penetrating the theca, the artery takes a direct course around the follicle, and in an optimum section it can often be seen over a considerable distance. These vessels are designated as arteries, although they have relatively large lumens and usually their walls consist of but a single layer of muscle cells. These arteries, along with the more prominent veins, form a distinct outer vascular wreath in the thecae. They give rise to precapillary vessels which immediately form a

Figure 2

Drawing of arterial supply of the ovary made from vinyl acetate corrosion preparation. Broken line indicates outline of the ovary. OA, ovarian artery. U, branch to uterus. O, branch to oviduct. X9

Figure 3

Drawing of the arterial supply of the ovary made from vinyl acetate corrosion preparation. AO, ovarian artery. X8

Figure 4

Two types of arteries which arise from large artery at thin edge of ovary. Composite drawing made from serial sections. F, follicle. T, portion of large artery at thin edge of ovary. X23

rich network of capillaries which lies against the basement membrane and completely envelops the follicle.

During the first half of the ovulation period these capillaries are more or less flattened against the basement membrane. They contain very little blood and their lumens are inconspicuous. Later in the period the capillaries become progressively more prominent, and close to the time of ovulation they become distended and engorged with corpuscles. At this time the expansion of the capillaries is so great that they have the appearance of sinusoids, and form a prominent vascular wreath bordering the basement membrane.

The veins in the ovary are unusual in that their walls lack the characteristic tunics; in fact, the walls of even the largest veins in the rabbit ovary are composed entirely of a single layer of endothelial cells. No muscle tissue or fibrous tissue is present; the walls of the veins are thickened by a thin layer of connective tissue where they emerge from the ovary. Since the histological structure of the veins closely resembles that of the capillaries, it is not possible to differentiate between the two types of vessels on the basis of their structure, the diameter of the vessels being the only distinguishing characteristic.

The network of capillaries which enmeshes the follicle connects with larger vessels which extend around the follicle in the theca and form part of the outer vascular wreath. These thecal vessels vary considerably in diameter, but in general they are relatively large and will be referred to as

veins. Close to the time of ovulation these veins become greatly engorged with corpuscles, and in stained preparations, they are the most prominent vessels in the theca. The thecal veins increase in diameter as they approach the bases of the follicles. In this region they merge and abruptly give rise to relatively large medullary veins. These large veins are the most prominent vessels seen in the stained sections. They are usually engorged with corpuscles and are the largest vessels in the ovary; some of them measure 260 μ in diameter. They are irregular in shape and often have pouch-like protuberances which give them a sacculated appearance. They were observed to be in intimate contact with large medullary lymphatics (to be described shortly), which also have walls that consist of a single endothelial layer.

The color and density of blood in the veins of whole ovaries cleared in methyl salicylate made it possible to see the medullary veins over their entire length, and it appears that drainage of the mature follicles is the primary purpose of these vessels. Each of the large veins takes its origin at the base or bases of one or two follicles and then proceeds on a direct course toward the thin edge of the ovary (Fig. 5 and 6). There are five or six of these vessels in the medulla of the ovary. They emerge from the ovary and, about one centimeter from it, they unite to form the ovarian vein which runs in the mesovarium to the vena cava.

Figure 5

Freehand drawing of principal veins in the ovary made from ovary cleared in methyl salicylate. Stippled structures, follicles in relation with veins. X10

Figure 6

Camera lucida drawing of a portion of one section 10 μ thick of a rabbit ovary. Solid structures, portions of a single large vein extending from a follicle to the thin edge of the ovary. Vessels drawn in outline only, lymph vessels. Stippled area, granulosa of follicle. Hatched area, cortex. X32

The Lymph Vessels

The most numerous vessels in the ovary are the lymph vessels. In the medulla they form an extensive network and give this region of the ovary a spongy appearance. The larger medullary lymph vessels were traced to the thin edge of the ovary and were seen to connect with a number of vessels which passed from the ovary into the mesovarium. These extra-ovarian vessels merge to form three larger vessels which enter the thoracic duct. Within the ovary, the large medullary vessels receive tributaries from the cortex. These cortical vessels vary considerably in their diameters and most of the larger vessels were seen in relation with developing follicles.

Small lymph vessels were numerous in the thecae and were in close association with the inner wreath of blood capillaries. These vessels connect with larger lymph vessels which lie in the outer vascular wreath. When these larger vessels were expanded and their lumens were evident, it was seen that they extended around the follicles for a considerable distance; however, in some sections these vessels were collapsed and they appeared as strands of endothelium. Sometimes expanded and collapsed portions of the same vessel could be seen in a single section. Andersen (1926) has suggested that the flattening of the thecal vessels is probably due to pressure exerted by the enlarging follicle. The corrosion preparations showed that these vessels form

an enveloping network in the theca. This network of thecal lymphatics connects with the medullary lymph vessels. In general the lymph vessels of the medulla are relatively large. Some of them measure 250 μ in diameter. They are irregular in shape and many of them have a bulbous appearance due to sacculations in their walls. They are the most abundant vessels in the medulla. Unlike the medullary veins the lymph vessels do not take a direct course to the edge of the ovary, but they intercommunicate with one another and form a labyrinth of vessels throughout the entire medulla (Fig. 7). It would appear that these capacious vessels could hold large amounts of fluid.

The sections showed that many of the lymph vessels contained a homogeneous substance which stained strongly with eosin. In addition, some of the vessels contained large numbers of erythrocytes; these cells were most numerous in the bends and pockets of the vessels.

The walls of even the largest medullary vessels consist of a single layer of endothelium, and valves, for the most part, are absent; although the lymph vessels outside the ovary have the typical valvular structure. In describing the veins it was mentioned that their walls were atypical, insofar that they consisted of a single layer of endothelium. In fact, the walls of the veins and lymph vessels are almost identical in structure; however, in general the endothelial cells of the veins are more numerous than those in the lymph vessels. This difference aided in

Figure 7

A small area of lymph vessels in the medulla of the ovary. Camera lucida drawing made from a vinyl acetate preparation. X52

distinguishing the two kinds of vessels. The sections showed that many of the lymph vessels were in close contact with the veins. In fact, the lumens of the two kinds of vessels were separated by only a double layer of endothelium. Not only were the veins paralleled by the lymph vessels, but often they were partly enveloped by bulbous expansions of them. Often the lymph vessels on either side of a vein were connected in such a way that portions of the vein were almost entirely ensheathed by lymph vessels (Figs 8 and 9).

Figure 8

A portion of a vein surrounded by numerous lymph vessels. Drawing reconstructed from a number of sketches made from serial sections of one ovary. Stippled vessel, vein. Hatched vessels, lymph vessels. X40

Figure 9

A series of camera lucida sketches of a portion of a vein (stippled) with a lymph vessel (outline only) in close relation with it. Drawings are in the same sequence as the serial sections. X80

Experimental

The rabbit was chosen for these studies because it belongs to a group of animals in which copulation is necessary to initiate ovulation (Heape, 1905; Marshall, 1904; Hammond and Walton, 1934; Foster, 1934; Greulich, 1934; Enders, 1939). Furthermore, it is a convenient laboratory animal, and in addition, it copulates readily, and ovulates approximately ten hours after copulation. Because of the relatively short time interval between copulation and ovulation, any influence that the blood vessels may have on ovulation could be referred to vessels already present in the ovary and not to the proliferation of new ones.

Observations on living ovaries

Observations on living ovaries in situ corroborate those previously made by Walton and Hammond (1928) and Hill, Allen, and Kramer (1935). These investigators noted that following copulation the follicles swell, and that during the final one or two hours of the ovulation period they exhibit their greatest increase in size and form avascular areas (stigmata) on their free surfaces. Markee and Hinsey (1936) state that prior to ovulation these areas thin out and the avascular zone bulges an hour or so before it finally ruptures. Parkes (1931) considers attenuation of the peripheral follicle tissues, which is caused by distension of the follicle, to be necessary for ovulation. In addition to the above observations we observed that the entire ovary

underwent progressive enlargement throughout the ovulation period, and that just prior to ovulation the ovaries were considerably swollen, somewhat translucent, and decidedly hyperemic. These changes are much more apparent in the ovaries of young adult animals which have never borne young, than they are in the ovaries of older animals, which are dense with interstitial tissue as a result of frequent breeding. It is suggested that the progressive swelling of the ovary is due to an increased rate of filtration of fluid through the capillaries into the tissues.

Sections of ovaries removed one hour or more following copulation showed that the capillaries were considerably distended and engorged with corpuscles. In most of these ovaries relatively large extravasations of corpuscles were seen in the tissue, apparently from ruptured capillaries; leucocytes were numerous in the veins, and in some instances they were lined up along the walls of these vessels. The expanded, engorged capillaries, the areas of extravasated blood, and the numerous leucocytes indicate an increase in permeability of the capillaries and a reduction in the blood flow. Ovaries from unstimulated animals showed no evidence of extravasation or stasis. The increased permeability of the ovarian blood vessels, and the increased production of fluid from them may be attributable to an increased production of estrogen. It is well known that during proestrus there is an increase in the fluid content of the uterine tissue of the rat, that the periovarial sacs, and the uteri

become distended with fluid at this time, and that there is a marked engorgement of the vascular system of the uterus (Turner, 1949; Astwood, 1938; Astwood, 1939; Carrol, 1945; Hechter et al, 1940).

Injection of carbon particles

Early in this investigation (Burr, 1949), in an attempt to distinguish between veins and lymph vessels, carbon particles were injected into the blood system. This injection method did not sharply differentiate the two types of vessels, since carbon passed into the lymph vessels as well as into the blood vessels. This was a frequent occurrence and we were led to the conclusion that the blood vessels and lymph vessels were directly connected by small vessels, and we found a few instances of what we believed to be morphological evidence for such connections. As the work progressed, and the injection technique improved, less and less carbon was found in the lymph vessels. In view of this we decided that the evidence for the connections was not conclusive. Clark and Clark (1937) studied adjacent veins and lymphatics in the ear of the rabbit by the transparent chamber technique. They observed the direct passage of erythrocytes from vein to lymph vessel during periods of inflammation. The passage of fluid was also demonstrated by injecting methylene blue into the blood system. It is now fairly certain, in view of Clarks' work, that in our experiments, the carbon particles passed directly through the walls of adjacent veins and lymphatics.

Occlusion of blood vessels

The routine procedure in removing ovaries for sectioning was to clamp off all ovarian vessels in the mesovarium with a locking hemostat. When the clamp was applied during the final hour of the ovulation period, the follicles rapidly increased in size and many of them ruptured. This change in the follicles occurred in about thirty seconds after the clamp was applied. As the follicles increased in size the swelling partially subsided in the remainder of the ovary. Follicles which were induced to rupture prematurely attained a size two or three times greater than follicles which rupture normally. In some instances, the follicles filled with blood which oozed from the rupture points. Sections of these ovaries revealed that ovulation had occurred, and in some sections the expelled ova were seen in the coagulum which was adherent to the surface of the ovary.

The observations made in connection with the routine procedure of clamping off the ovarian vessels when the ovaries were removed suggested that changes in blood pressure, and a rapid shift of fluid, might contribute to rupture of the follicles. Since changes in the follicles were brought about by simultaneous occlusion of the artery and vein, the effect of tying off these vessels separately was studied. In two living animals which were close to their ovulation time, the ovarian artery was tied off without any noticeable change in the ovary. In one of these animals the ovarian artery of one ovary was left intact and the vein of this

ovary was occluded. The effect on the ovary was almost immediate; in fact, in preparing the vein for ligation, it was momentarily flattened and a response was elicited before the ligature was actually applied. In this ovary four follicles increased to approximately double their size. In another series of experiments involving seven animals which were close to their ovulation time, the ovarian veins were tied off. The ovaries of all these animals underwent a change as soon as the ligature was applied; the follicles which had been stimulated increased considerably in size, and in five ovaries some of the follicles ruptured. In some animals conditions for these experiments were ideal because the follicles on the ovary were well spaced, and the artery and vein were sufficiently separated so that the vein could be tied off with very little manipulation. The changes in the follicles of these ovaries could be seen immediately, and could be referred directly to the occluded vessel. No changes could be elicited in the follicles of unstimulated ovaries by clamping or ligating the ovarian blood vessels.

The degree of response in the follicles following ligation of the ovarian vein was variable. The first noticeable change was seen in the network of capillaries which surround the follicles. These vessels underwent considerable enlargement and became engorged with blood. In some of the follicles which enlarged there was a concomitant rupture of the capillaries surrounding them; these follicles filled with blood and became dark red in color. In other follicles the

capillaries did not rupture, but, due to their dilation and engorgement with blood, a deep pink color was imparted to the follicles. In all instances in which the follicles ruptured, varying amounts of blood oozed from them.

Factors affecting blood pressure

An increase in blood pressure would augment the formation of fluid from permeable vessels. The expansion of the capillaries during the ovulatory period would result in a decrease in the rate of flow through them and there would be a concomitant rise in capillary blood pressure. It is also possible that the coiled arteries play some role in increasing the blood pressure locally. The coiling of the arteries allows a maximum of internal area of friction in a short lineal range and therefore brings about a greater reduction of blood pressure than would occur if the arteries were shorter. The coiled arteries are supported by, and imbedded in, the surrounding ovarian tissue, and as the ovary swells the helicine arteries would be stretched, i.e., their degree of coiling would be reduced, and possibly there would be some distortion. These changes could result in turbulence which would bring about a local increase in pressure and would augment the pressure in the capillary wreath surrounding the follicle. Reynolds (1950) suggests that the enlargement of cystic follicles is due to similar changes in the helicine arteries. We realize that an increase in capillary pressure due to vasodilation of the ovarian arteries cannot be ruled out, but experimental work indicates that such

changes in the arteries cannot be referred to a nervous mechanism (Cannon et al, 1929; Friedman, 1929; Hinsey and Markee, 1932; Hinsey, 1937; Little, 1951).

Since it was conjectured that uncoiling of the helicine arteries might possibly result in an increased pressure in the capillaries, an attempt was made to obtain morphological evidence for the uncoiling. The ovaries of a living stimulated animal were fixed in situ with hot Bouins solution just previous to the expected time of ovulation. It was hoped that this rapid fixation method would fix the arteries in a close approximation of their configuration in the living state. A modification of the technique of Daron (1936), who froze the endometrium and demonstrated the uncoiling of the arteries in this tissue, was also tried. Unfortunately sections of ovaries fixed by the above methods were vacuolated and shrunken and were unsatisfactory for study. However, it should be noted that Reynolds (1947 a,b) using a more indirect method of studying the larger arteries in the rabbit ovary by means of vinyl acetate corrosion preparations, observed that as follicle growth progresses after stimulation of the ovary with gonadotrophic substances, there is an increase in the distance between the coils of the arteries.

Increased capillary pressure can be induced by an obstruction to the flow of blood on the venous side. This effect was evident when the ovarian vein was tied off. The capillaries around the follicles became distended and some

of them even ruptured. In addition, the follicles increased in size and this is attributed in part to a sudden increase in the rate of filtration of fluid from the distended capillaries, and the infiltration of this fluid into the follicles.

Increased permeability, trypan blue

Experiments were undertaken in an attempt to demonstrate the increased permeability of the capillaries during the ovulatory period. Trypan blue was injected into the general circulation, and color changes in the ovary were noted. A series of trials showed that the optimum time for injection of the dye was one and one-half hours before the expected time of ovulation. The ovaries were examined thirty or forty minutes after injection. In the stimulated animals the ovaries had a distinct bluish cast, the enlarged follicles had a definite blue color, and the lymphatics emerging from the ovary were prominent and decidedly blue. The ovaries of unstimulated animals were only slightly colored; the follicles had the characteristic pink appearance, and only an occasional one was tinged with dye. The lymph vessels were inconspicuous and uncolored.

Histaminase activity

The edematous, hyperemic condition of the ovary during the ovulation period suggested that the hormonal stimulus resulting from copulation might release some substance from the blood or ovarian tissue which causes dilation and increased permeability of the capillaries. Histamine is a substance which is known to bring about these changes and

it was thought that it might be responsible for the changes which occur in the ovarian capillaries.

In comparison to other animals the blood of the rabbit contains a relatively high concentration of histamine; furthermore, it is curious that the capillaries of the rabbit are relatively insensitive to histamine (Barsoum and Gaddum, 1935). It has been demonstrated that the histamine in rabbit's blood is apparently held in the white cells, and is not free to produce its pharmacological effects until it is liberated from the cells (Code and Ing, 1937).

In 1930, Best and McHenry demonstrated the presence of a histamine-inactivating substance in the kidney and intestine of the dog. This substance has the properties of an enzyme and they suggested the name histaminase. Subsequently histaminase was found in the kidneys of the rabbit, dog, cat, ox, hog, horse, sheep, monkey, and human, in the lungs and intestine of the rat, and also in the blood and placenta of pregnant women (McHenry and Gavin, 1932; Rose et al, 1940; Anrep et al, 1947; Kapeller-Adler, 1944; Swanberg, 1948).

It was theorized that the capillaries of the unstimulated rabbit ovary might be relatively insensitive to histamine due to the action of histaminase in the ovary. Possibly the copulatory stimulus results in inhibition of histaminase activity and perhaps in an increased production of histamine. Experiments were undertaken to determine the histaminase activity of homogenates of ovaries from stimulated and unstimulated animals, using a substrate of histamine.

The activity was based on the oxygen uptake of the homogenates in air. The results of these experiments arranged in Table I indicate that the histaminase activity of unstimulated ovaries is approximately twice that of stimulated ovaries. The results of one run on stimulated ovaries is not included in the averages because the appearance of these ovaries left some doubt as to whether they had been stimulated. The uptake for these ovaries falls in between that of the average uptake of the stimulated and that of the unstimulated ovaries. These experiments indicate that there is a decided reduction in the histaminase activity of stimulated ovaries. Kapeller-Adler (1951) reports that the activity of purified histaminase preparations was reduced by fifty percent in the presence of chorionic gonadotrophic hormones. Quantitatively, this reduction closely parallels the reduced histaminase activity which we found in stimulated ovaries. The findings in our experiments would appear to be closely related to those of Kapeller-Adler when it is considered that the stimulus of copulation releases the gonadotrophins which initiate the ovulatory changes. These gonadotrophins are not present in unstimulated ovaries which exhibit a histaminase activity which is twice that of stimulated ovaries. If the inhibition of histaminase were accompanied by a release of histamine from the leucocytes passing through the ovary, or from the ovarian tissue itself, dilation and increased permeability of the capillaries could result. The results of these experiments indicate a marked

TABLE I

Histaminase Activity of Stimulated and Unstimulated Ovaries

Stimulated ovaries		Unstimulated ovaries	
Animal	QO ₂ *	Animal	QO ₂ *
1	0.53	6	1.41
2	0.64	7	1.02
3	0.85	8	1.39
4	0.54	9	1.34
	av.0.64		av.1.29

5 **	1.01
------	------

* QO₂= μ l.O₂ taken up per mg. dry weight of tissue per hour.

** The appearance of the ovaries from this animal was such that it was doubtful that they had been stimulated.

difference in the histaminase activity of stimulated and unstimulated ovaries; nevertheless, we recognize that relatively few experiments were done, and before this work could be properly evaluated more data is needed.

Discussion

There is a difference of opinion concerning the mechanism by which the follicles are induced to rupture, although it is generally agreed that accumulation of fluid under increasing tension is an important factor in causing rupture. It has been stated (Dawson and Friedgood, 1940; Walton and Hammond, 1927) that the follicle cells secrete the liquor folliculi, and it has also been suggested that the liquor is a transudate from the thecal blood vessels (Simon, 1904). Possibly it may have a dual origin, since it is stated that there is a primary and also a secondary liquor folliculi (Robinson, 1918). The primary liquor is formed during the early growth of the follicle and is more viscous than the secondary liquor which accumulates during the rapid growth of the follicle prior to ovulation. Umbaugh (1949) reports that immediately following rupture of the follicle in the cow there is an oozing of serous fluid which contains the ovum; this is followed by a viscous, mucoid substance. Similar findings have been reported in the ewe (McKenzie and Terrill, 1937). Follicles must attain a certain size in order to rupture and Walton and Hammond (1928) state that the enlargement must be fairly rapid, since the walls of the follicles can accommodate themselves to gradual accumulation of fluid, with the result that the follicles become unusually large and fail to rupture.

Usually rupture of the follicle is followed by the

formation of a corpus luteum. However, follicle rupture is not a necessary antecedent for corpus luteum formation. It is well known that injection of pituitary hormone will induce the formation of corpora lutea without rupture of the follicles (Friedman, 1929; Engle et al, 1929). Whether or not a follicle ruptures depends upon its size, the rapidity with which it enlarges as a result of the accumulation of liquor, and whether or not it is in a favorable position relative to the surface of the ovary. Those follicles which arise deep in the cortical tissue are so restrained by the surrounding tissue that they are unable to attain the size and position necessary for rupture. Nevertheless, even though they do not rupture, such follicles may transform into corpora lutea (Lipschütz, 1924).

The two phenomena most often observed in connection with ovulation are the engorgement of the ovarian blood vessels and the distension of the follicles due to the accumulation of fluid in them. The source of the follicular fluid has not been definitely established, but most workers agree that its formation is more rapid during the latter part of the ovulation period. It is at this time that the ovarian blood vessels are most heavily engorged with blood and we consider the secondary liquor folliculi to be a transudate from the blood as a result of an increased permeability of the blood vessels and an increase in pressure in them.

The histological studies on the rabbit ovary strongly suggest that the vasculature of the ovary is especially

adapted for promotion of ovarian swelling during periods of increased permeability. The veins have an atypical structure and are in intimate relation with the lymph vessels. The veins like the lymph vessels have walls which consist of only a single layer of endothelium. In addition, these veins are so closely apposed to the lymph vessels that there could be direct transfer of fluid from vein to lymph vessel. The numerous lymph vessels are so large, so labyrinthine, and so haphazard in their course that they appear to be better suited for confining and retaining large amounts of fluid rather than for providing a means of rapid drainage. It would seem, then, that this reservoir-like system of lymph vessels could, by retaining large amounts of fluid in the ovary, function as erectile tissue and contribute to the swelling of the ovary during periods when there is an increase in the permeability of the blood vessels. Lymphatic erectile tissue has been reported by Müller (1908), who states that erection in the drake is due to the secretion of lymph which fills the penis.

The capillaries surrounding the follicles appear to be ideally arranged to furnish progressively increased amounts of fluid to the follicles during the ovulatory period. These capillaries arise almost directly from arteries in the thecal layers, and are therefore very close to their source of arterial blood. Similarly, the capillary network of the follicle drains abruptly into large, thin-walled veins which are located at the base of the follicle. Thus there is a

compact system of arteries, capillaries, and veins devoted to the blood supply and drainage of a single unit, the follicle. This arrangement would minimize the amount of energy the blood usually loses due to friction when it flows between arteries and veins that are widely separated by an extensive capillary bed. When the arterial blood enters the rich network of irregularly shaped capillaries around the follicles, its velocity decreases and the blood pressure increases. Moreover, the large diameter of the veins which drain the follicles and extend into the medulla would tend to keep the pressure high in these vessels, resulting in a transudation of fluid from them. There would be an even further increase in pressure as the capillaries and veins around the follicles became progressively more distended, and as a result, more and more fluid would pass from them. That such a progressive dilation does occur is suggested by the greatly engorged condition of the capillaries just prior to ovulation. Sections of ovaries removed at this time show a ring of dilated capillaries between the theca interna and the limiting membrane of the granulosa. These capillaries were so engorged with corpuscles, and they pressed upon one another so closely that it was difficult to distinguish the limits of a single capillary and trace it from section to section.

The experimental results of this investigation suggest that rupture of the follicles in the rabbit ovary is in part caused by pressure changes within the ovary. These changes may be partially due to the uncoiling of the arteries, the

increased permeability and dilation of the capillaries and veins, and the stretching of the tissues as a result of the accumulation of fluid in the tissues and capacious lymph vessels. Presumably the fluid forms faster than the already filled lymph vessels can carry it off, causing distension of the ovary. The follicles could act as reservoirs and enlarge progressively as fluid accumulates in them. It might appear that the extensive system of lymph vessels present in the ovary would be capable of carrying off the lymph as fast as it is formed. However, Henry (1933) has shown that the rate of lymph flow in the rabbit's ear is low in proportion to the surface area of the lymph vessels. The heart and lungs of dogs have extensive lymph systems, but Drinker (1940) and Warren et al (1942) have shown that in these organs the drainage of lymph is slow. Menkin (1929, 1931) and Clark and Clark (1937) have demonstrated a decreased flow of lymph in areas of inflammation. It is possible that the large medullary veins might partially obstruct the flow of lymph from the ovary. The lymph vessels which are in such close relation with the veins would probably become partially occluded when the veins became engorged and distended with blood, since the pressure in the expanding blood vessels would be greater than that in the closely adherent lymph vessels. Partial occlusion of lymph vessels by blood vessels has been observed in vivo by Clark and Clark (1937) in the rabbit's ear. These workers state,

"Following periods of inflammation, veins and

capillaries which had undergone a change to a softer endothelial consistency often showed local widenings which persisted for days or even weeks. Such bulges in the vessel wall were relatively larger and persisted longer in veins which were accompanied by lymphatics and on the side next to a lymphatic than was the case in blood vessels or parts thereof not so situated. At times of active blood flow the wall of such a blood vessel indented the wall of the accompanying lymphatic, encroaching on its lumen,"

Because of the intimate relationship of veins and lymph vessels in the rabbit ovary, we believe that fluid passes directly from the veins to the lymph vessels, and further, that the rate of transudation increases if the vessels become more permeable. Increased amounts of fluid passing into the medullary lymph vessels would distend them and they would impart a quality of turgescence to the medullary region. The ovary of the young adult rabbit has only a small amount of interstitial tissue; and the mature follicles, which protrude slightly from the surface of the ovary, occupy the entire thickness of the enveloping cortical tissue, with their bases bordering on the medulla. It is possible that gradually increasing pressure, resulting from expansion of the turgescient medullary tissue, is partly responsible for the elevation of the follicles during the ovulation period. Elevation of the follicles by pressure from below, and the accompanying expansion of the follicles by infiltration of lymph, would account for the formation of the avascular areas (stigmata) at the free surfaces of the follicles. The capillaries at the free surface would be compressed by the

restraining tunica, and the resulting avascular areas would become progressively weaker due to being deprived of blood.

This work has led to the conclusion that the secondary liquor is a transudate from the blood, and that its formation is more rapid during the latter part of the ovulation period, and is responsible for the rapid expansion of the follicle and its ultimate rupture. Experiments with trypan blue indicate that fluid does pass from the blood into the follicles. This evidence alone is not conclusive, but it is supported by the experiments in which the ovarian vein was occluded. The resulting pronounced and rapid enlargement of the follicles could best be interpreted as being due to a shift of fluid into them. It is not felt that all the fluid which is driven into the follicles comes from the blood directly. When the vein is occluded there would be a rise in pressure in the veins and capillaries; theoretically this would cause an increase in the rate of production of lymph. However, lymph formation would soon cease if the blood were not circulating; furthermore, some thickening of the blood would occur depending upon the amount of fluid lost from the vessels. It was mentioned that the ovary enlarged progressively during the ovulation period. This effect is referred to the increased permeability of the veins and capillaries, an increased pressure in these vessels, and the apparent inability of the lymph vessels to drain the lymph as fast as it is formed. The lymph is subjected to increasing tension as the tissues of the ovary become stretched, and if the

pressure exerted on the lymph by the stretched tissue should build up to a value equal to the filtration pressure in the capillaries, filtration of fluid would cease. On the other hand, if the pressure exerted by the tissues never comes into equilibrium with the filtration pressure, then the ovary would continue to expand and finally rupture at its weakest region. The follicles are regions of least resistance and their free surfaces are unrestrained areas in comparison with the deeper parts of the follicle. The lymph, therefore, is forced into the follicles, and those follicles which are of favorable size, and which are situated close to the surface of the ovary, enlarge as the lymph is driven into them. The surface of the follicle has already become attenuated; the blood supply to this region has ceased; and further stretching culminates in rupture of the follicles.

Summary

Following the stimulus of copulation, the rabbit ovary becomes swollen, translucent, and hyperemic. This condition is most pronounced just prior to ovulation.

Experiments performed indicate that, as a result of increased capillary pressure and permeability, fluid collects in the ovary and causes it to swell. Distension of the follicles results when some of the fluid is forced into them.

The structure of the arteries, veins, capillaries, and lymphatics of the ovary, and their relationship to one another appear to be well adapted for promotion of swelling in the ovary during periods of increased permeability.

Rapid swelling and premature rupture of the follicles may be induced late in the ovulatory period by ligation of the ovarian vein.

Some evidence is presented for a hormone-enzyme relationship which may be responsible for the increased permeability of the ovarian blood vessels during the ovulatory period.

A theory is proposed for the mechanism of follicle rupture.

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