THE MORPHOLOGY OF THE LYMPHATICS OF THE RABBIT OVARY,  
AND THEIR POSSIBLE RELATION TO OVULATION

By

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THE MORPHOLOGY OF THE LYMPHATICS OF THE RABBIT OVARY,
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Introduction

The number and size of the lymph vessels in the rabbit ovary appear to be disproportionate to the size of the organ. From a cursory examination of the ovary, it is difficult to see why such a relatively small organ should require such an extensive drainage system.

The author was unable to find references to detailed work concerned with the lymphatics of the rabbit ovary, but 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 the 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.

The purpose of this investigation was to ascertain whether the lymphatics were subservient to some function of the ovary other than drainage. During the preliminary histological studies it was very difficult to distinguish between small veins and lymph vessels, and because of this fact, it became necessary to study the veins, and also the arteries, in order to be able to distinguish lymph vessels from blood vessels.
This paper presents evidence for the existence of direct connections between the blood vessels and lymph vessels in the rabbit ovary. So far as the author is aware, such connections have not previously been described.
Materials and Methods

Twenty-six adult, virgin, female rabbits were used in this investigation, and serial sections were made of forty-six ovaries. The sections were cut 12μ in thickness and were stained with Harris' hematoxylin and erythrosin or eosin.

In a number of animals the ovarian artery was injected with India ink. The ovaries of these animals were sectioned and stained as above. The India ink used for injection of the blood vessels was not colloidal Higgin's India ink. A suspension of carbon particles was made by rubbing up dried India ink in rabbit serum. The 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 although minute, 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 Culiner (1944).

Corrosion preparations were made of the veins and arteries by injecting the vessels with vinyl acetate and dissolving the tissues in hydrochloric acid. In addition, the vessels in relation with the ovary were studied in the living animal whenever the ovaries were removed.
The Arteries

The morphological studies presented in this paper were made largely from histological sections of rabbit ovaries, supplemented by corrosion preparations and studies made from living rabbits. 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. The medulla is made up of loose stromal tissue which supports a few arteries and many large veins; but the greater part of the medulla is occupied by large, anastomosing lymph vessels which give to the medulla a spongy, cavernous appearance. (Fig. 4, L.V.)

The arteries outside the ovary were studied in the living animal, but a clearer picture of the arrangement of these vessels was obtained from the corrosion preparations. The ovarian artery arises from the dorsal aorta about 3 cm. posterior to the renal artery, and midway along its course it gives rise to a branch vessel which supplies the uterus. Close to the ovary an additional branch is given off which supplies the oviduct. Before entering the ovary the artery
is thrown into one or two loose coils and then divides into two limbs. Both limbs penetrate the ovary, one limb passing toward the posterior end, and the other limb running along the margin of the ovary toward the anterior end. (Fig. 1, OA,U,Q) Within the ovary the posterior branch gives rise to three or four large, tightly coiled vessels, and these in turn give rise to a number of smaller coiled vessels. The anterior branch which runs along the margin of the ovary is also coiled, but the degree of coiling was found to vary in different ovaries. This artery also gives off smaller coiled arteries along its length. It was not possible to see the connections which the smaller coiled arteries made, because the vinyl acetate did not penetrate beyond them.

Because of the incompleteness of the injection, further studies on the small arteries were made from histological preparations. These studies revealed that the small coiled arteries seen in the corrosion preparations continue as straight arteries through the medulla to the cortex. These straight arteries give off only occasional branches to supply the interstitial tissue and medulla. In the region of the cortex the straight arteries break up into a capillary network, but in those regions of the cortex where follicles are developing, the straight arteries give off branches which extend around the follicles. These arteries penetrate the theca and form a rich capillary plexus.
The Veins

Gross studies of the venous system of the ovary were made from the living animal and from corrosion preparations. For ease of presentation the veins will be considered as arising from the vena cava, and terminating in the ovary.

The ovarian vein arises from the vena cava and parallels the ovarian artery in the mesovarium. Along its course a branch is given off to the uterus, and close to the ovary the vein divides into a number of short vessels which enter the ovary. (Fig. 2, A,B) Inside the ovary these vessels branch, some of the branches extending into the medulla, others merging to form a large irregular vein which extends along the thin edge of the ovary, and gives off large branches to the medulla. (Fig. 2, C) The medullary veins are relatively large vessels; some of them measure 260μ in diameter. It was also observed that the walls of these vessels are very thin and that they lack the characteristic tunics; in fact, the walls of even the largest veins are composed entirely of a single layer of endothelial cells. No muscle tissue or fibrous tissue is present. In stained sections the medullary veins are the most prominent vessels. These numerous, large vessels, full of corpuscles, suggest that they might function, in part, as reservoirs.
In the region of the cortex the medullary veins give off a number of branches, some of which extend around the follicles (Fig. 3) and become continuous with the capillaries in the thecae which originated from the arteries. Other branches form a capillary network in the cortical tissue between the follicles. The diameter of the capillaries in the thecae varies with the number of corpuscles which they contain. In those capillaries which are devoid of corpuscles, the capillaries are partially collapsed, but during the ovulation period, many of the capillaries become greatly engorged with blood.
The Lymphatics

The medulla is made up of loose stromal tissue which supports the large veins and also many large lymph vessels. (Fig. 4) The lymph vessels anastomose and interlace to give the medulla a spongy appearance. These vessels have relatively large diameters, and some of them measure 250\(\mu\) in diameter. Many of them contain a homogeneous substance which stains with eosin. The larger medullary lymph vessels were traced to the thin edge of the ovary, and they 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, it was observed that 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 the developing follicles. (Fig. 5) The larger vessels in the cortex receive smaller vessels which have two different origins. Some of these vessels arise in the usual manner in the tissue spaces; but other vessels are directly connected with the blood capillaries. Connections between lymph vessels and capillaries were seen in the thecal regions of developing follicles. However, the close similarity in structure of enlarged capillaries and lymph vessels
that were adjacent to each other in the thecae made it
difficult to distinguish between these two kinds of vessels.
Identification was often made more difficult because por-
tions of the enlarged blood capillaries were often devoid
of corpuscles, while in other portions the lumen was ob-
literated. The similarity between these two kinds of ves-
sels made it difficult to ascertain whether an empty vessel
was a lymph vessel connected to a blood capillary, or a
portion of a blood capillary devoid of corpuscles. Iden-
tification of the two kinds of vessels was still further
complicated by the fact that blood corpuscles were present
in small numbers in the lymph vessels. This finding was
disconcerting, and in order to establish the identity of
the vessels which contained the corpuscles, a number of
them were traced to their connections with definite lymph
vessels in the medulla. The corpuscles were not numerous
in the small lymph vessels, and were absent from most of
them. However, in the large lymph vessels the corpuscles
were more numerous, particularly in the bends of the ves-
sels, where they were often clumped together.

Definite connections between blood capillaries and
lymph capillaries were seen in only a few instances, be-
cause both types of vessels were often collapsed. Where
the connections were seen, the lumens of the blood capil-
laries were reduced in diameter, and the narrowed portions
of these capillaries were seen to become continuous with
lymph vessels. (Figs. 6 and 7) No sharp line of demarcation could be seen between blood capillaries and lymph capillaries. However, corpuscles from the blood capillaries were not dispersed along the length of the connecting vessels, but were massed in the regions where the connecting vessels originated. Beyond the constrictions, on the lymphatic side, only occasional corpuscles were seen, but the narrow parts of these vessels frequently contained a homogeneous substance which stained intensely with eosin.

These observations are illustrated in Figs. 6 and 7. The spaces marked "L" are parts of a continuous lymph vessel; the other vessels containing corpuscles are parts of a continuous blood vessel. A connection between the blood vessel and the lymph vessel is indicated. Fig. 7 is a camera lucida drawing of a region in Fig. 6 but under a higher magnification.

These studies indicate that the corpuscles cannot pass freely from the blood capillaries to the lymph capillaries, although occasionally a few corpuscles do pass over; these escaped corpuscles collect in the large lymph vessels in the medulla, and form the masses of corpuscles previously described. Krogh (1922) described a phenomenon in blood capillaries which he called plasma skimming. In this process, free passage of plasma is permitted through a constricted vessel, but only a few corpuscles are able to pass. It is suggested that plasma skimming occurs
between blood capillaries and lymph capillaries in the rabbit ovary, and that the acidophilic substance seen in the smaller lymph capillaries is plasma which has recently passed over.

As far as the writer is aware the blood vascular system and the lymphatic system are separate in all the tissues and organs of vertebrate animals. In an examination of the literature over the past fifteen years no evidence to the contrary has been found. The presence of connections between the blood vascular system and the lymphatic system in the rabbit ovary is, therefore, a very unusual condition. Since the findings in this investigation are not in accord with the accepted view, and since the few connections observed might be ascribed to misinterpretation, an effort was made to obtain additional evidence by injecting the blood system with India ink.

A suspension of carbon particles was injected into the ovarian artery, and the ovaries were fixed, sectioned, and stained in the usual manner. Examination of these sections revealed that not only the arteries and veins, but also the lymph vessels contained carbon particles, although more carbon was present in the veins than in the lymph vessels. The large medullary lymph vessels were traced to their exit from the ovary and carbon particles were seen throughout their length. In addition, carbon was seen in the large lymph vessels which were outside the
ovary in the mesovarium. Very small carbon particles were present in the smaller lymph vessels in the thecae. It was generally observed that the carbon particles in the lymph vessels were considerably smaller than those in the capillaries and veins. It is possible that this difference in size of the particles bears some relation to plasma skimming previously described. Since the injections were made through the artery, the presence of carbon in the lymph vessels lends strong support to the view that the blood vascular system and lymphatic system are directly connected in the rabbit ovary.
Physiology

An attempt was made to find out, if possible, the functional significance of the connections between blood capillaries and lymphatics. The rabbit is an unusual animal in that ovulation is not spontaneous, but occurs approximately ten hours after copulation. In this investigation, ovaries were examined in the living animal at intervals following copulation, and these ovaries were removed and fixed for sectioning. The sections showed that the capillaries of these ovaries were greatly engorged with corpuscles as compared to capillaries seen in sections of unstimulated ovaries. The engorgement was seen in all ovaries that were removed one hour or more following copulation. The capillaries had expanded considerably, and they were tightly packed with corpuscles. In most of the stimulated ovaries relatively large extravasations of corpuscles were seen in the tissue, apparently from ruptured capillaries. Corpuscles were also tightly packed in the large veins and leucocytes were numerous in these vessels, in some instances lined up along their walls. The expanded, engorged capillaries, the areas of extravasated blood, and the increase in the number of leucocytes in the veins indicate a condition of stasis in the capillaries and veins of stimulated ovaries.

Observations on living ovaries corroborate those
previously made by Walton and Hammond (1928). These investigators noted that following the stimulus of copulation the follicles begin to swell and that they exhibit their greatest increase in size during the final one or two hours of the ovulation period. In addition, we observed that during the ovulation period the entire ovary increased in size and towards the end of the period 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.

The routine procedure in removing ovaries for sectioning was to clamp off all ovarian vessels in the mesovarium with a locking hemostat. When a 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 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, and in some instances blood escaped from them. More follicles could be caused to rupture when the time of clamping off was close to the expected time of
ovulation; however, only those follicles which had already undergone some enlargement were affected when the clamp was applied.

Although ova could not be seen in the fluid escaping from ruptured follicles, sections clearly demonstrated that ovulation had occurred. In some sections expelled ova were seen in the coagulum which was adherent to the surface of the ovary.
Discussion

The writer believes that the arrangement of the lymph vessels in the medulla and the fact that they are connected to the blood system bear a definite relation to the process of ovulation. The loose, sponge-like medullary tissue has an appearance which is very suggestive of erectile tissue. Following copulation the lymph vessels become distended as a result of plasma being diverted into them; the medullary tissue becomes turgid, and the swollen tissue presses against the bases of the follicles. 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, and have large lymph vessels extending around their surfaces, as illustrated in Fig. 5. Elevation of the follicles by pressure from below would account for the formation of the avascular areas at the free surfaces of the follicles known as the rupture areas or stigmata. Markee and Hinsey (1936) state that during the pre-ovulation period this area thins out, and the avascular zone bulges an hour or so before it finally ruptures. Mature follicles are enveloped by a plexus of capillaries, but if the follicles were pushed up from below by turgescence of the medullary tissue, the capillaries at the free surface of the follicle would be compressed by the
restraining tunica at the surface, forcing the blood out of the vessels in that region, resulting in avascular area which would become progressively weaker as a result of being deprived of blood. Furthermore, if the pressure exerted on the follicles by the turgescent medullary tissue were maintained, it is conceivable that the follicles would be gradually pushed further out. This process of gradual elevation of the follicles and the accompanying expansion of the follicles by infiltration of lymph (described below) would tend to stretch the already weakened, avascular free surface (stigma) of the follicle, and finally cause it to rupture.

Another factor which contributes to ovulation is the accumulation of lymph in the ovary during the ovulation period. The accumulation of fluid in tissues is due to an increase in the permeability of the capillaries. Drinker (1942) and others have shown that the tone and permeability of the endothelial walls is related to the oxygen supply; when this is deficient, dilation of the capillaries occurs, and their walls become more permeable. It has been shown that following copulation, the capillaries in the ovary dilate and become engorged with corpuscles, indicating a condition of stasis. As shown in sections, in some instances the capillary walls break down, and blood was extravasated into the tissues. This change in the walls of the capillaries is apparent one hour after copulation,
and persists throughout the ovulation period. It seems probable that this change in the tone of the capillaries in the ovary may be caused by a hormone which is released as a result of the stimulus of copulation.

Since lymph is formed by filtration of plasma through the capillary walls, a change in the capillaries such as that described above, would result in a considerable increase in the formation of lymph. We have shown that connections exist between the blood system and the lymph system in the rabbit ovary and that the connections are such that it is chiefly plasma which passes over into the lymph vessels. In an ovary in which the blood vessels are not congested, the blood flows freely through the capillaries, but when the capillaries are congested and the flow of blood is impeded, plasma is forced over into the lymph vessels. Since these vessels are connected to the blood vessels, the plasma in them would be under some pressure. Under these conditions the drainage of fluid from the ovary by the lymph vessels would be considerably retarded, and since lymph is continually being formed from the blood capillaries it collects in the tissue spaces causing edema and an increase in the size of the ovary. Furthermore, the swelling of the ovary would probably cause the coiled arteries, which are enmeshed in the tissue, to become partly uncoiled. This straightening of the arteries would increase the pressure beyond the coils, forcing even more plasma
through the capillaries into the lymph vessels, and increasing the rate of filtration from the blood capillaries. The lymph which accumulates in the ovary throughout the ovulation period is subjected to increasing pressure as the tissues become stretched. The follicles are regions of least resistance, and they serve as reservoirs for lymph which is continually being driven into them from the tissues. As a result the follicles enlarge progressively, chiefly by stretching their free surfaces which are unrestrained regions in comparison with the deeper parts of the follicle. This process of lymph formation and its drainage into the follicles continues throughout the ovulation period and is climaxed by rupture of the follicles at their weakened surfaces, when they can hold no more lymph. This theory of ovulation depends upon two phenomena which occur concomitantly. The first of these is the excessive formation of lymph and the second is a turgescent condition of the medullary tissue which occurs because the blood vessels and lymph vessels are directly connected. Only a few connections were demonstrated histologically, but corroborative evidence was obtained by injecting the blood system. The connecting vessel illustrated in Figs. 6 and 7 is apparently large enough to allow corpuscles to pass through, and it is difficult to account for the scarcity of corpuscles in the lymph vessels. The size of the lumens of the few open connecting vessels which were seen may not have been
characteristic of the majority of these vessels; possibly their lumens are usually constricted to a size so small that virtually all corpuscles are held back, while the plasma is skimmed into the lymphatics, and were therefore difficult to demonstrate histologically.

Although the only observed connections between the two systems were on the venous side of the capillary network, it is possible that there were also connections on the arterial side. This would still further facilitate passage of plasma into the lymph system. An improved injection method might reveal such connections, and further work along these lines is contemplated.

The account that we have given concerning the mechanism of ovulation is partly supported by the fact that we were able to accelerate ovulation by thirty or forty minutes when the blood vessels and lymph vessels were clamped off simultaneously; ovulation was not only accelerated but the follicles attained a size two or three times greater than those which rupture when ovulation occurs normally. It is possible though, that this exceptional enlargement occurred because some follicles were weakened more than others during the process of enlargement and the lymph which caused the marked distension of a few follicles, would have in the normal course of events, been distributed over perhaps half a dozen follicles. We were never able to cause the enlargement and rupture of more than four
follicles at a time and more often only three follicles ruptured. It is, however, a matter of conjecture how many follicles would have ruptured normally.

We are unable to give a wholly satisfactory explanation for the sudden shift of lymph from the general ovarian tissue into the follicles when the clamp is applied. We have tried to show that during the ovulation period lymph is forming faster than it can be removed, and as a result of tension exerted on the lymph by the stretched tissues lymph is continually being forced into the follicles. Those follicles which are of optimum size and are situated close to the surface of the ovary expand as the lymph accumulates in them. The pressure of the blood supplies the energy for filtration of plasma through the capillary walls, and if the pressure of lymph in the tissues should build up to a value equal to the filtration pressure in the capillaries, filtration of plasma would cease. On the other hand, if the pressure of lymph in the tissues never comes into equilibrium with the filtration pressure, then the ovary would continue to expand and finally rupture at its weakest region.

When the blood vessels and lymph vessels are clamped off lymph ceases to form but the stored energy present in the stretched tissues forces lymph into the swollen, weakened follicles causing them to enlarge very rapidly and to rupture. It is disturbing though, that in the normal
course of events an additional thirty or forty minutes are required before the pressure in the ovary builds up to a value sufficient to rupture the follicles. However, there is reason to believe that in an elastic body, such as the ovary, pressure changes are not immediately transmitted to all regions. Thus, if the elastic arteries force their contained blood over into the large, thin-walled veins when the clamp is applied, there would be a local increase in volume and pressure in the region of the large veins, and an interval of time might elapse before the pressure was equalized throughout the ovary. A sudden increase in pressure might precipitate the crisis in the follicles by suddenly stretching and further weakening their surfaces. The weakened follicles would then be unable to oppose the pressure exerted on them by the lymph, and, as a result, lymph would be rapidly driven into the follicles causing them to swell and rupture. In this connection it is perhaps noteworthy to mention that the follicles did not begin to swell immediately the clamp was applied. There was a latent period of about fifteen seconds before there was any marked increase in size, but as soon as any enlargement was noticeable the increase in size was then rapid and striking.

The findings here reported have suggested a number of problems which we intend to investigate. At the moment we are studying the effects on the ovary of clamping the
artery, vein, and lymphatics separately and in combination. There are, however, some technical difficulties in connection with the lymphatics which must be overcome before this work can be completed and evaluated.
Summary

It has been shown:

1. That one hour following copulation, the capillaries become dilated and engorged with blood, and this condition persists throughout the ovulation period.

2. That during the ovulation period, the ovary becomes hyperemic and increases in size.

3. That the blood system and the lymph system are directly connected.

4. That ovulation could be accelerated by thirty or forty minutes by clamping off all the vessels connected to the ovary.

A theory of ovulation has been proposed based upon the above observations.
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Figure 1

Drawing of arterial supply of the ovary. Made from vinyl acetate corrosion preparation. About 12X. Broken line indicates outline of ovary.

OA Ovarian artery
U Branch to uterus
O Branch to oviduct
FIGURE 2
Figure 2

Drawing of venous supply of the rabbit ovary, made from vinyl acetate corrosion preparation. About 12X. Broken line indicates outline of the ovary.

A Ovarian vein
B Veins entering ovary
C Large irregular vein extending along thin edge of the ovary
FIGURE 3
Figure 3

Schematic drawing of a few medullary veins and their branches to the follicles. Composite drawing made from a series of camera lucida sketches of the vessels in several ovaries. About 50X.

M Medullary veins
F Follicles
FIGURE 4
Figure 4
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  The edge of the ovary
EDGE  which is continuous with the mesovarium that supports all vessels entering and leaving the ovary
V     Vein
LV    Lymph vessel
FIGURE 5
Schematic drawing showing a few of the lymph vessels and veins in the medulla and their relation to a follicle. Drawing was made from a series of camera lucida sketches. About 45X.
FIGURE 6
Figure 6

Freehand drawing of a connection between a blood vessel and a lymph vessel.

L Parts of a continuous lymph vessel
V Parts of a continuous blood vessel
C Connection between blood vessel and lymph vessel
FOL Portion of a follicle
Figure 7

Camera lucida drawing of the region in Figure 5 showing the connection between the blood vessel and the lymph vessel. About 540X.

FOL Follicle
LV Lymph vessel
V Vein
C Connection between blood vessel and lymph vessel