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# STUDIES ON THE RED LYMPHONODUS

## III. ABOUT THE ARGYROPHILIC FIBERS ON THE PERIPHERAL SINUS OF THE RED LYMPHONODUS IN GOATS

Norio KUDO

*Laboratory of Veterinary Anatomy, Faculty of  
Veterinary Medicine, Hokkaido University,  
Sapporo, Japan*

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### INTRODUCTORY

The general distribution and histological observations on the red lymphonodus in goat have not been recorded except for the studies of TESHIMA and of the present writer.

In the previous paper of this series the red lymphonodi were classified into four types according to the constructional features of the peripheral sinus of each specimen, and it was indicated that there are transitional forms between ordinary lymphonodi and red lymphonodi. In general, there were distinct differences observed for example, in ordinary lymphonodi, the sinuses were comparatively narrow, while in red lymphonodi there were seen spongy chambers and so-called subsinus-veins in many specimens having broad sinuses.

By such findings an important conception about the development of the peripheral sinus of red lymphonodus was suggested. However, so far as the writer knows no work has been done on this problem.

In the present paper the minute structures of argyrophilic fibers on the peripheral sinus of red lymphonodi are described in detail, and some views are discussed as to their developments.

### MATERIALS AND METHODS

The material used for this microscopic investigation was taken as fresh as possible from 23 goats, aged 3 days to 4 years old.

Five hundreds and fifteen red lymphonodi were found, and placed immediately in fixative fluid. They were subsequently treated in different ways.

The fixative fluids were formalin, SUZA's fluid, HEIDENHAIN's fluid etc. HEIDENHAIN's fluid was found to give the best results in the present investigations.

All the specimens were embedded in paraffin, and cut in serial sections of a thickness of 5~10  $\mu$ .

For staining methods use has been made of BÖHMER'S haematoxylin and eosin, VAN GIESON'S picroacid fuchsin, and GÖMÖRI'S silver nitrate impregnation method.

## RESULTS

### 1. Microscopic Structures of the Peripheral Sinus of the Red Lymphonodus

By observations of a number of preparations, numerous differences have been recognized between structures of peripheral sinuses in these and related organs. The peripheral sinuses of red lymphonodi and of ordinary lymphonodi differ markedly from one another, especially in the features of their argyrophilic fibers.

The peripheral sinuses of the red lymphonodi were found not to be uniform in structure, so it seems advisable to establish the typical forms of the peripheral sinus at first, and subsequently to note the points of difference between the histology of these types and that of other varieties.

In most specimens the peripheral sinus of the red lymphonodus, lying immediately beneath the capsula, was of irregular width varying about from 1/10 to 1/4 of the diameter of the nodus; it contains abundance of red blood corpuscles and other blood components.

Trabeculae seen in the peripheral sinus show no constant structures, and in some instances, even in large nodi, no definite trabeculae are present, but only trabecula-like tissues are seen in the form of offshoots from the capsula, which frequently communicate to form spongy work of connective tissue in the peripheral sinus. In other words trabeculae of this type do not reach to the medullary portion, but break up into individual filaments to make the spongy networks of the peripheral sinus.

The argyrophilic fibers in these trabecula-like tissues are compactly arranged, and as a rule these trabecula-like branches are surrounded by sinuses, and eventually end by breaking up into finer and finer strands, which terminate as the reticula of the sinuses. Strips of the branches impregnated with silver nitrate show the arrangement of the black fibers very beautifully.

The structure of the peripheral sinus is also variable in each specimen. In most cases the greater area, as seen in section, belongs to the spongy portion. This portion is limited externally by a capsula from which numerous fibrous processes radiate inwardly into the peripheral sinus and subdivide it giving a spongy appearance to it.

Fibrous connective tissues is the foundation of the whole peripheral sinus; it is most compact in the wall of chamber of spongy structure, consisting essentially of bundles of fibers stained by silver nitrate impregnation method.

These bundles were found to form a remarkably narrow network which sup-

ports the other tissue elements of the peripheral sinus.

In some specimens the writer differentiated these spongy area into external and internal portions, and made a sharp line of demarcation between two portions, because external sinuses are narrow-spaced and surrounded by well-developed connective tissue walls as if sinuses were situated within capsula tissue itself. In contrast to this the internal sinuses are widely-spaced and surrounded by small reticulated fibrous bundles. So the writer named the external portions "the Capsula-cavelets" and the internal portions "the Periphery-cavelets".

Directly in contact with the medullary side of the Periphery-cavelets, there are seen trabecula-like processes composed of the same elements as the chamber wall itself. In the innermost part of the peripheral sinus, the argyrophilic fibrils become denser, and the so-called subsinus-vein was detected there.

These items which have just been given above deal only with the general structures of peripheral sinuses.

Even within a single specimen the structure of peripheral sinus is not uniform but differs in parts. Therefore it is difficult to describe only a single type of structure, but the features of distinct portions can be mentioned. In the previous papers the writer characterized four types of peripheral sinus structures by the combinations of these features.

In this paper, moreover, the writer classified the red lymphonodi into five different types according to the state of the argyrophilic fibers on the peripheral sinus in each specimen and to the combinations of features observed in different portions of peripheral sinus (Figs. 1~5).

A more detailed account of these different five types follows.

Type I: The red lymphonodi of which peripheral sinuses exhibited so-called Capsula-cavelet in some portion.

Type II: The red lymphonodi of which peripheral sinuses have no Capsula-cavelet in any part, but the so-called Periphery-cavelet.

Type III: The red lymphonodi of which the peripheral sinuses display neither Capsula-cavelet nor Periphery-cavelet. Immediately beneath the capsula numerous trabecula-like projections pass from the capsula into the interior of the peripheral sinus.

Type IV: The red lymphonodi of which the peripheral sinuses are lacking Capsula-cavelet, the Periphery-cavelet and the connective tissue projection seen in type III, but the bundles of argyrophilic fibers appear like the wall of chamber of the sponge. In the area where the above features were not found the fine argyrophilic fibrils.

Type V: The red lymphonodi of which the peripheral sinuses of this type, like corresponding sinuses in an ordinary lymphonodus, are bridged across by fine argyrophilic fibrils only.

## 2. Breadth of the Peripheral Sinus

The peripheral sinuses are always appearing structures of the red lymphonodi, however, they vary in breadth with the size of the organ. In some sinuses, moreover, the breadth is more or less constant throughout the organ, but in the other specimens there is considerable variation in different parts, a variation that may be demonstrated even in a single section. Besides the form and size of the nodi there seem unknown factors which effect the degree of variation in the breadth of the peripheral sinuses.

To obtain the average degree of variations in breadth of the peripheral sinus, it might therefore be needful to consider the breadth of the sinus and the diameter of the nodus.

When nodi were approximately spherical and the breadth of the peripheral sinuses was comparatively constant, a single average diameter and breadth were taken; when nodi were flattened or elongated and the breadth of the sinuses was inconstant, three diameters and three breadth were recorded, and the averages of these measurements were taken.

The comparative breadth of peripheral sinuses in the microscopic section of red lymphonodi are indicated in table 1.

TABLE 1. *Comparison of Breadth of the Peripheral Sinus in Five Types of Red Lymphonodi*

RELATIVE BREADTH OF THE PERIPHERAL SINUS TO THE WHOLE NODULAR DIAMETER	TYPES OF PERIPHERAL SINUSES										TOTAL	
	I		II		III		IV		V		Cases	%
	Cases	%	Cases	%	Cases	%	Cases	%	Cases	%		
Over 20%	219	65.6	99	72.8	14	50.0	0	0	0	0	332	64.5
10~20%	99	29.6	34	25.0	11	39.3	2	66.7	1	7.1	147	28.5
Less than 10%	16	4.8	3	2.2	3	10.7	1	33.3	13	92.9	36	7.0
Total	334	100.0	136	100.0	28	100.0	3	100.0	14	100.0	515	100.0

The majority of the cases (332 cases in 515) have comparatively broad sinuses (over 20% of the nodular diameter), and only 36 cases in 515 showed a breadth less than 10% of the nodular diameter.

Case distributions in each of the five types of nodi showed more distinct tendencies.

In type I, 65.6% of cases have breadth over 20% of nodular diameters and only 4.8% of cases have breadth less than 10% of nodular diameters. This corresponds to average majority of total cases.

In type II, 72.8% of cases are over 20% of diameters, and only 2.2% of cases have breadth less than 10% of diameters. This showed the most distinct tendency of type characteristics.

In type III, 50% of cases are over 20% of diameters, and 10.7% of cases are

less than 10% of diameters. In this type the tendency seems to be somewhat weakened, but yet resembles the tendencies of types I and II.

In type IV, there occurred only three cases, so it seems difficult to define the tendency of this type, but considering the tendencies of above described three types and that of the next type, the fact that 66.7% of cases showed intermediate breadth (10~20% of diameter) seems not to be an accidental event.

In type V, cases are few also, but showed a very distinct tendency perfectly reverse to those of types I, II and III.

On studying above described results it will be noticed that the breadth of peripheral sinuses is least in type V nodi, greater in type IV, still greater in type III and reaches its maximum in types I and II. Types I, II and III differ from the other types in as much as the breadth of the peripheral sinus, that is in general the breadth in types I, II and III, is very broad. Although there is a certain amount of overlap between types II and III, the average breadth in the two groups is very different.

The peculiarities of the structure of the peripheral sinus already mentioned influence greatly the breadth presented by the prepared sections, and it seems that the appearance of the sinus in sections is more characteristic than breadth.

### 3. Quantity of Erythrocytes in the Peripheral Sinus

A considerable variety of cells take part in the formation of the peripheral sinus, however, in many cases the erythrocytes were the most conspicuous and abundant. It is considered that erythrocytes in the peripheral sinus have special relations to functions and developments and construction of the red lymphonodus.

In some specimens, the peripheral sinuses were completely blocked by a mass of erythrocytes, and the aggregated erythrocytes were crowded in the spongy chambers. However, both in different specimens and in different parts of the nodus there occurred many variations of features and amount of erythrocytes in

TABLE 2. *Comparison of the Quantity of Erythrocytes Included in the Peripheral Sinus of Red Lymphonodus Differentiated into Five Types*

QUANTITY OF ERYTHROCYTES	TYPES OF PERIPHERAL SINUS										TOTAL	
	I		II		III		IV		V		Cases	%
	Cases	%	Cases	%	Cases	%	Cases	%	Cases	%		
+++	191	57.2	90	66.2	14	50.0	0	0	0	0	295	57.3
++	68	20.4	29	21.3	2	7.1	0	0	0	0	99	19.2
+	66	19.8	14	10.3	9	32.2	1	33.3	6	42.9	96	18.6
-	9	2.6	3	2.2	3	10.7	2	66.7	8	57.1	25	4.9
Total	334	100.0	136	100.0	28	100.0	3	100.0	14	100.0	515	100.0

Note: Degrees of the quantity of erythrocytes are the same standard which the writer indicated in the previous paper.

the peripheral sinus.

In general, the different types of the nodi may be taken to show respectively a characteristic appearance of the quantity of erythrocytes in the peripheral sinus.

The results of these studies of the five different types are set out in table 2.

In only 25 cases out of 515 (4.9%) were there found no erythrocytes in the peripheral sinuses, and in a majority of cases 490 (95.1%) a larger or smaller number of erythrocytes were contained there.

The ratios of case occurrence of sinuses without erythrocytes are 2.7% and 2.2% in types I and II respectively.

In type III that ratio is a little higher (10.7%), and in types IV and V distinctly elevated to 66.7% and 57.1% respectively occupying by far more than the half of each of those types.

The ratios of case occurrence of sinuses which contain erythrocytes are 97.3% in type I, 97.8% in type II, 89.2% in type III, 33.3% in type IV and 42.9% in type V.

Considering the degrees of quantity of erythrocytes, it will be observed that in the two types of cases (Types I and II) the ratios of case occurrences of erythrocytes are very closely similar. Such resemblance is also seen between types IV and V.

In addition, from this table it is clear that there is a gradual diminution in the rate of quantity of the erythrocytes in turn from types I and II, to III, to IV and V.

In a few cases of types IV and V there is contained some quantity of erythrocytes somewhat corresponding to that seen in types I, II and III, but no cavelet structures was ever found in the peripheral sinuses.

Only such a comprehensive investigation would make it possible to correlate the variations, if such exists, or to determine the actual condition of the nodi in any particular specimen. A point of greater importance is the fact that the disappearance of the cavelet structure takes place at first on the innermost sinuses and gradually proceeds outwardly. The sinuses similar to those of an ordinary lymphonodus appear in the portion of the innermost cavelet, however, the blood in sinuses or cavelets gradually flows under from the periphery part inwardly. This fact suggested the absence of the cavelet structure at the inner portions of sinuses as well as the absence of the erythrocytes in the periphery portions of sinuses in some cases.

#### 4. Relation between the Five Types by Argyrophilic Fibrous Structures and Four Types by Cavelet Structures

In a previous paper the writer has proposed four types (A, B, C, D) of red lymphonodi based on the appearances of the peripheral sinus in the form of the cavelet structures.

However, in the present investigations, it seems that the great variation in

TABLE 3. *Relation of the Present Five Types with the Previous Four Types of the Peripheral Sinus of Red Lymphonodus*

PREVIOUS FOUR TYPES	PRESENT FIVE TYPES										TOTAL	
	I		II		III		IV		V		Cases	%
	Cases	%	Cases	%	Cases	%	Cases	%	Cases	%		
A	224	67.1	48	35.3	1	3.5	0	0	0	0	273	53.0
B	86	25.7	75	55.1	15	53.6	0	0	0	0	176	34.2
C	15	4.5	11	8.1	8	28.6	1	33.3	0	0	35	6.8
D	9	2.7	2	1.5	4	14.3	2	66.7	14	100.0	31	6.0
Total	334	100.0	136	100.0	28	100.0	3	100.0	14	100.0	515	100.0

Note: A, B, C and D indicate the four types that the writer classified in the previous paper.

the structure of red lymphonodi is due to the fact that many specimens of red lymphonodi have developed cavelet structures within the capsulae themselves such as the so-called Capsula-cavelet.

A study of the present five types of the red lymphonodi showed, as illustrated in table 3, that the definite sinus structures characteristic of each type as outlined above are found in all types of the previous investigations.

In type I a great majority of cases (67.1%) have the cavelet chambers, and a few cases, nine, belong to type D.

In types II and III almost a half of the cases (55.1%, 53.6%) of all nodi have the trabecula-like projections (type B), however, in type II occurrence of type A shows a considerably high ratio (35.3%), while in type III that ratio is comparatively low. In type III four cases out of 28 (14.3%) belong to type D, showing apparently high percentage.

Only three cases belong to type IV of the new classification, of which two belong to type D, only one case to type C.

All cases of type V (14) belong to the former type D.

Therefore, it seems very probable that the cavelet structures of the capsula were certain to occur only in specimens stainable by silver nitrate method. With regard to the cavelet chamber it may be pointed out that the wall of chamber was visible only in the so-called Periphery-cavelet in specimens stained with haematoxylin and eosin, and not in all of the cavelet.

#### DISCUSSION

During the past seventy years numerous contributions have appeared upon the subject of the red lymphonodus.

As regards the structure of the peripheral sinus of red lymphonodus, the opinions of the authors were not always agreed. It was thought that this part of this organ was very important in the essential function of this organ.

In the present studies it was cleared that the peripheral sinuses of red lymphonodi have not a few particular characteristics. The structure of the peripheral sinus of a red lymphonodus is somewhat complicated, and the peripheral sinuses are broken up by small trabeculae or very numerous fine strands of connective tissue. The sinuses are comparatively broader than those of ordinary lymphonodi, and in most specimens they were filled with a greater or smaller quantity of erythrocytes.

In this paper, the writer classifies the red lymphonodi into five varieties based upon the features of the peripheral sinus stained by silver nitrate impregnation method, which he terms "Types I, II, III, IV and V", placing the nodi in these five groups according to their distinct characters.

Between type V of the red lymphonodi and the ordinary lymphonodi it is not very easy to make distinguishment, but the peripheral sinus of the former is filled with blood corpuscles.

In the previous paper, the writer has mentioned that a considerable quantity of erythrocytes occurs in the peripheral sinus which was composed of chambers with thick walls. In the present, the writer described further details of the cavelet structures, differentiating them into two fundamental forms. The cavelet structure which seems to be developed within the capsula was termed the "Capsula-cavelet", and the cavelet structure located under the capsula was named the "Periphery-cavelet". The latter is more conspicuous, and is more varied in size and shape.

In many specimens there were seen the Capsula-cavelet and the ordinary lymph sinus structures simultaneously.

The problem as to the argyrophilic fibrils has received considerable attention in lymphatic tissue, but has not yet been thoroughly worked out in the peripheral sinus of red lymphonodus. In some specimens the argyrophilic fibrils were as abundant as those of an ordinary lymphonodus, but in others, especially in specimens having the cavelet structures, the peripheral sinuses did not wholly consist of argyrophilic fibrils.

The first record of the peripheral sinus of red lymphonodi was given by H. GIBBES (1884). He described the structures resembling ordinary lymphonodi, but having their sinuses full of blood instead of lymph. Many observers<sup>(1,2,4,8,11)</sup> have stated that blood, in large quantity, is found lying free in the peripheral sinuses of these nodi. Only very few specimens have occasionally exhibited blood in a free state in this portion, but in most specimens blood is almost invariably enclosed in the chambers of cavelet structure. In some cases, in the neighbourhood of the medullary portions quantities of blood were seen freely which might account for the view of previous observers.

Little attention has been paid to the cavelet part of the peripheral sinus. LEWIS's paper, which appeared in 1901, gave some detailed descriptions on this point, and he stated that there is never a complete peripheral sinus present; blood spaces are found only in certain restricted areas beneath the capsule. Also according to his paper, in addition to the sinuses both peripheral and central, other

minor sinuses occur in the capsule itself, and are most abundant in certain nodi with thick capsulae. He considered that the sinuses might be regarded as nothing more than a coarse capillary meshwork, or they might be looked upon as short irregular capillaries.

Here arises a question of much importance. Are the chambers of sponge continuous with the blood vessels? If so, the chambers and the blood vessels are probably of the same nature; or in other words, the spongy structures are nothing more than modified blood vessels. It is difficult to understand how the blood reaches the peripheral sinuses in red lymphonodi, unless some communication exists between sinuses and the blood vessels. Such an arterio-sinus communication is not yet proven by the writer, but for this problem a careful search will be continued.

The present writer was able to confirm LEWIS's report that certain spaces occurring in the capsule have been observed. The writer has seen that the peripheral sinus cavelet consists mainly of two distinct parts, the Capsula-cavelet and the Periphery-cavelet, and has considered that these two different parts were essentially different from one another.

As regards the breadth of the peripheral sinus and the quantity of erythrocytes included in the sinus, the writer can only state that he has found that there were gradual transformations to the ordinary lymphonodus in turns to the present types I to V.

Up to the present, a few descriptions have been offered about the development of the red lymphonodus. VINCENT and HARRISON considered that the red lymphonodi probably developed from ordinary lymphonodi. DRUMMOND described the development of the lymphatic system and the adenoid tissue of lymphonodi, and considered that in the earliest stages the development of the red lymphonodi may run parallel to that of the ordinary lymphonodi. LEWIS described that there was no direct evidence that red lymphonodi were developed in the individual from ordinary lymphonodi. Further remarks on this subject are offered below in discussing the development of the nodi. But the writer considers that there are very interesting facts in the present work in the description of the Capsula-cavelet.

The present study has also led to the conclusion that the appearances are identical in the red lymphonodi and the ordinary lymphonodi, however, there were gradual transitions from the red lymphonodi to the ordinary lymphonodi. So that no hard and fast line can be drawn marking off these structures from one another. Notwithstanding such a consideration the red lymphonodi are structures sui generis, because there are essential differences of structures between typical red lymphonodus and an ordinary lymphonodus.

By these facts the writer wishes to conclude that, the red lymphonodi and ordinary lymphonodi occur under different conditions respectively at first, and run parallel as to developments, but in the way of development in some of the red lymphonodi the characteristic so-called Capsula-and Periphery-cavelets transform into ordinary lymph sinus structure converting red lymphonodi themselves into

ordinary lymphonodi.

Among these differences between the red lymphonodus and the ordinary lymphonodus, the chief are the (usually) comparatively large size of the peripheral sinuses and the constant presence of erythrocytes in the so-called Capsula-cavelet or Periphery-cavelet.

#### SUMMARY

In this paper, points which the writer found to hold in the peripheral sinus of red lymphonodi in goats are somewhat as follows:

1. The peripheral sinuses of the red lymphonodi and the ordinary lymphonodi differ markedly from one another in their minute structures, yet these differences mainly resulted from the variable prominence of certain structural features present in all.

2. The writer classified the red lymphonodi into five types by state of appearance of peripheral sinus, applying the silver nitrate impregnation method.

3. In most portions of the peripheral sinus as in the present types I and II, the cavelet structures were detected. In type I these spongy chambers were found within the capsula itself, while in type II the chambers were beneath the capsula. Moreover, some intermediate features were detected between above mentioned types and the ordinary lymph sinus. These intermediate types were named III and IV. Some red lymphonodi having a peripheral sinus like the ordinary lymph sinus were decided to be type V.

4. As regards the breadth and the quantity of erythrocytes in the peripheral sinus, the writer found the fact that in the case of disappearance of the cavelet structure, the peripheral sinuses become increasingly narrow, and the quantity of erythrocytes gradually decreases.

5. The writer considered that in the earliest stage of the development of the red lymphonodus the cavelet structures appeared in the capsula itself, and when this stage was over these structures were combined so that the peripheral sinus had the same features as ordinary lymph sinus.

The present investigation was suggested by Prof. K. TAKAHATA, and carried out under his supervision; the writer takes this opportunity of thanking him for the generous advice and criticism. The writer is also very grateful to Prof. T. KIHARA, chief in the Laboratory of Anatomy at Kyoto University for his interest in the problem and his valued advice.

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## EXPLANATION OF PLATE

References common to Figs. 1~5.

C., Capsula.

P., Peripheral sinus.

M., Medullary portion.

C. c., Capsula-cavelet.

P. c., Periphery-cavelet.

- Fig. 1. Section of red lymphonodus of goat, showing Type I structure.  
GÖMÖRI's silver nitrate impregnation. × 215
- Fig. 2. Section of red lymphonodus of goat, showing Type II structure.  
GÖMÖRI's silver nitrate impregnation. × 215.
- Fig. 3. Section of red lymphonodus of goat, showing Type III structure.  
GÖMÖRI's silver nitrate impregnation. × 215.
- Fig. 4. Section of red lymphonodus of goat, showing Type IV structure.  
GÖMÖRI's silver nitrate impregnation. × 215.
- Fig. 5. Section of red lymphonodus of goat, showing Type V structure.  
GÖMÖRI's silver nitrate impregnation. × 215.

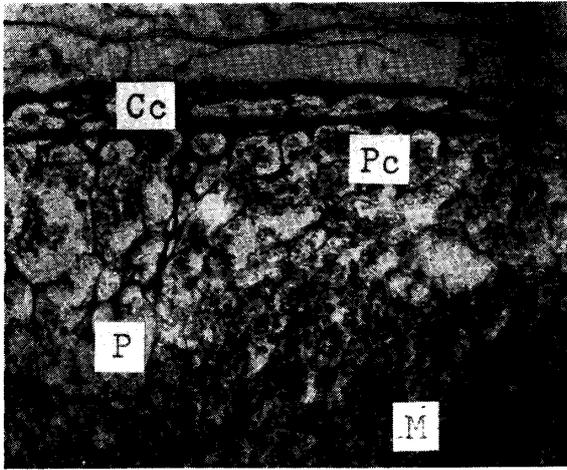


Fig. 1.

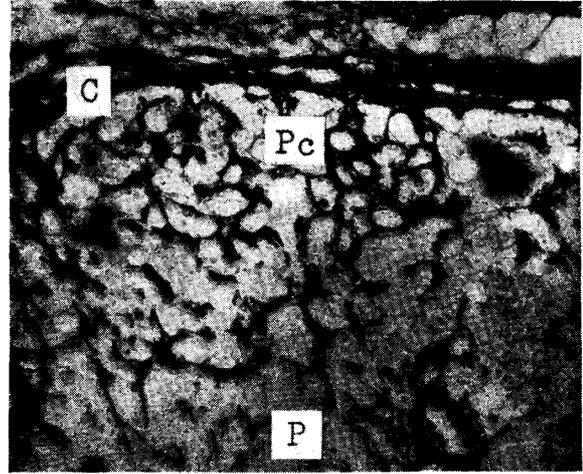


Fig. 2.

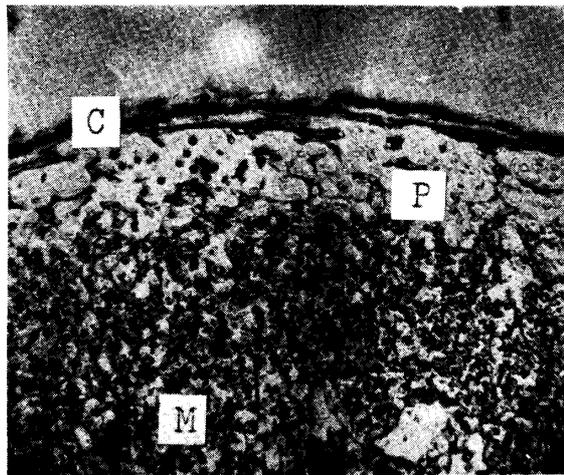


Fig. 3.

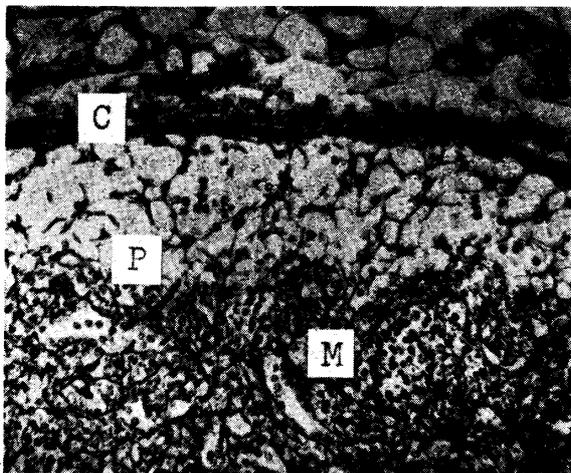


Fig. 4.

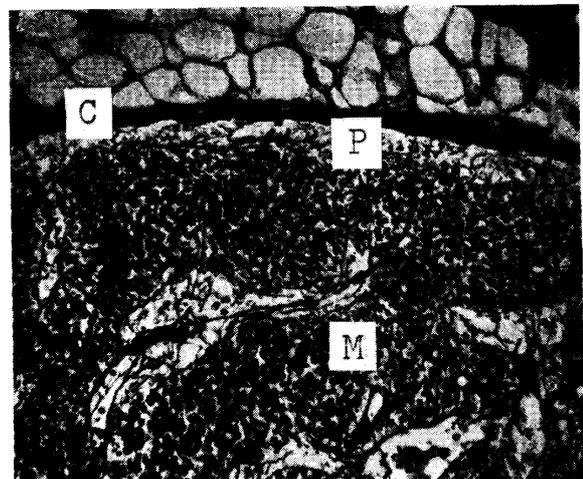


Fig. 5.