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**HISTOLOGICAL STUDIES ON THE OVARIES OF SOWS II\*.**  
**ON THE BEHAVIOR OF ARGYROPHIL FIBERS IN**  
**THE VARIOUS STRUCTURES OF OVARIES**

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INTRODUCTION

On the argyrophil fibers of mammalian ovaries there are publications of CLARK (sows and women, 1899), HÖRMANN (women, 1907), CORNER (sows and cows, 1920) and MUTO (women, rabbits, guinea-pigs, rats and dogs, 1929), and recently of DUKE (dogs, 1945; rabbits, 1947), YOSHIMURA (rabbits and women, 1952) and TOMITA (women, monkeys, cats, dogs and rabbits, 1953).

The "extravascular fluid pathway", which are composed of tissue spaces of reticular or spongy structures based on argyrophil fibers, have been first described and confirmed by KIHARA<sup>9,10</sup> and his co-workers since 1949.

If argyrophil fibers form the "extravascular fluid pathways" and play the important part in absorption or excretion of granular substances, it is necessary to review the distribution of these fibers in various organs. Therefore detailed observations on the distribution of these fibers in the ovaries of various animals are also required.

Investigations of argyrophil fibers in pig ovaries were performed only in corpora lutea of non-pregnancy by CLARK, who used tryptic digestion method and in corpora lutea of pregnancy at the fully-formed stage by CORNER, who used BIELSCHOWSKY's silver impregnation method.

In the present writer's previous paper an attempt was made to classify the process of the maturing of "normal vesicular follicles", the regressive process of "cystic atretic follicles", the development and the regressive process of "corpora lutea of non-pregnancy", the regressive process of "vascular bodies" and so on according to the histological findings of the 5 groups of structures found on ovarian surfaces of sows.

In the present paper an investigation is reported on the behavior of argyrophil fibers in these structures for the purpose of classification and explanation of the

\* The investigation was conducted for preparation of a thesis for the doctor's course in the Faculty of Veterinary Medicine, Hokkaido University on September 30, 1958.

development and the process of regression of these structures.

#### MATERIALS AND METHODS

Materials for this research were pairs of ovaries of 19 sows used in the writer's previous report, and of 15 other sows. Ages, number of repetitions of parturition and so on are not known exactly, because the materials were collected at the slaughter-house in Sapporo, Japan.

As a routine matter, the fresh materials were fixed and sectioned, and then hematoxylin-eosin staining and GÖMÖRI's silver impregnation method were applied. Findings of silver preparations were compared with findings of H.-E. preparations respectively.

The total number of specimens, which belonged to the 6 groups of histological structures based on H.-E. preparations, used in this study are indicated in table 1.

TABLE 1. *Total Number of Six Groups of Histological Structures Used in This Research*

STRUCTURES BASED ON H.-E. PREPARATION	NUMBER OF TYPES BASED ON H.-E. PREPARATION	TOTAL NUMBER OF SPECIMENS
Normal Vesicular Follicles	6	73
Cystic Atretic Follicles	12	244
Atretic Follicles with Hemorrhage	4	7
Corpora lutea of Non-pregnancy	10	169
Corpora lutea of Non-pregnancy with a Large Central Cavity	6	13
Vascular Bodies	3	20
Total	41	526

#### FINDINGS

##### I. Classification of Connective Tissue Fibers Found in Various Histological Structures of Ovaries

Fibers of the connective tissue found in various structures of ovaries were divided into 6 types according to various features in silver preparations, as follows.

**Fine black fibers:** Fibers of this type are very fine and jet-black in color, with very distinct outlines.

**Thin black fibers:** Outlines of these fibers stained jet-black are also very distinct as are the previous fibers, but the thickness of the present fibers is twice to thrice that of the fine black fiber.

**Fine pale purple fibers:** Characteristics of these fibers other than pale purple in color are almost the same as those of fine black fibers.

**Thin dark purple fibers:** These fibers with rather indistinct outline are similar to thin black fibers in thickness; they are packed tightly.

**Thick dark purple fibers:** The thickness of these fibers is twice or more of that of

the thin dark purple fiber, staining comparatively deeper at the periphery and paler at the center.

Thick red-purple fibers: The thickness of these fibers is similar to that of the thick dark purple fiber, or rather thicker. The outline of them is very indistinct. The outsides of the fibers are dark purple, and central part is red-purple to red in color.

II. Findings on Normal Vesicular Follicles (AEf.)

For convenience, normal vesicular follicles were classified into two types according to the behavior of the connective tissue fibers which appeared in their walls, as follows.

Type AEf. I: In the granulosa layer connective tissue fibers were not found at all. Theca interna made up only of thin black fibers, which formed large networks showing spongy appearance around theca interna cells. Such networks were closely packed throughout this theca, especially remarkable at the boundary with the granulosa. Also in this theca many fine fiber networks were found in capillary walls. Theca externa was composed of thin dark purple fibers, which ran parallel with the follicle wall and did not tend to form networks. These fibers of the theca externa are continuous to the thick red-purple ones of ovarian stroma. Even in this theca there were found both types of the fine and the thin, black fibers in the arteriole and venule wall as well as thin black fibers of the wall of capillaries.

Type AEf. II: The theca interna of this type was composed of networks similar to thin black fibers surrounding theca interna cells classified as type AEf. I. But the meshes of these networks were larger and more conspicuous than those of type AEf. I. And in this theca partial wedge-shaped folds penetrating into the granulosa layer, in which no fibrous elements were observed, were found at the coincidental portions with plica-like folds of H.-E. preparations: in these portions thin black fibers were seen forming conspicuous networks around the theca interna cells.

TABLE 2. *Relation between Typing Based on H.-E. Preparation and Typing Based on Silver Preparation of Normal Vesicular Follicles*

TYPES BASED ON H.-E. PREPARATION (F.)	TYPES BASED ON SILVER PREPARATION (AEf.)		TOTAL
	I	II	
I	17	.	17
II	15	.	15
III	11	.	11
IV	7	.	7
V	.	17	17
VI	.	6	6
Total	50	23	73

Relations between the typing based on H.-E. preparations and the typing based on silver preparations of normal vesicular follicles are set out in table 2.

The normal vesicular follicles of type F. I to IV in H.-E. preparations were placed in

one group (AEf. I), because there were no remarkable differences among those types (F. I to IV) in silver specimens. And the follicles of types F. V and F. VI were brought together in one group (AEf. II) for similar reason.

### III. Findings on Cystic Atretic Follicles (AA.)

Cystic atretic follicles were divided into 9 types according to various findings observed with silver preparations, as follows.

Type AA. I: Appearances of connective tissue fibers in the theca folliculi of this type were similar to those of type AEf. I. In the degenerated granulosa layer there were no fibrous elements.

Type AA. II: Although in this stage of atresia the granulosa layer had already fallen off, fibrous elements still did not appear there. The inner layer of the theca interna consisted of distinct networks of thin black fibers as in type AA. I. But in the outer layer many thin dark purple fibers appeared partially showing coarser networks. The theca externa was similar to that of type AA. I, but the thin dark purple fibers showed radial arrangement partially toward the follicle cavity.

Type AA. III: Follicles with the completely fallen off granulosa layer and with hypertrophied cells of the theca interna within the follicle cavity were classified in this type. In the inner layer of the theca interna there newly appeared thin dark purple fibers among thin black fibers of the networks around the theca interna cells. Therefore meshes of its part were rather coarser than in type AA. II. In this type the border layer between the theca interna and the granulosa layer consisted of two to three lines of thin black fibers which ran parallel with the follicle wall, forming a distinct membrane bordering the follicle cavity. In the outer layer of this theca, networks surrounding theca cells were composed mainly of thin dark purple fibers with only a few thin black ones. Therefore the meshes of this part were more coarse than those of type AA. II. In part some radial arrangement of thin dark purple fibers appeared, and networks of these fibers also showed a tendency to take an arrangement radial to the follicle wall.

Type AA. IV: In H.-E. preparations numerous hypertrophied cells of the theca interna were found within the follicle cavity, and thin "hyaline layers" were seen in parts of the innermost layer of the follicle wall. In silver preparations the greater part of the inner layer of the theca interna was composed of networks of thin dark purple fibers running parallel with the follicle wall, whilst in the outer layer there were seen many coarse networks composed of many thin dark purple fibers and a few thick dark purple ones, arranged radially. The parts corresponding to "hyaline layers" were found as pale purple stained, finely granulated structures. Furthermore this layer seemed to establish small, remarkable waved folds toward the follicle cavity. Moreover there were seen a few, short, fine black fibers which penetrated irregularly from those layers into the follicle cavity.

Type AA. V: In H.-E. preparations "yellow body-like tissues" appeared partially surrounding the follicle cavity, and "hyaline layers" in the innermost layer of the follicle wall became thickened. In silver preparations the most part of the inner layer of the theca interna was composed of coarse networks of thin dark purple fibers, which were arranged radially to the follicle wall. A few broken black fibers were found mingling

among the dark purple fibers of these networks. Around this layer dark purple fibers were also found arranged densely and radially, without network conformation. These fibers connected with dark purple fibers and red-purple ones of the ovarian stroma, which ran parallel with the follicle wall. The pale purple, fine granulated structures corresponding to "hyaline layers" were thicker than those of type AA. IV, making the large folds which projected into the follicle cavity. In the parts corresponding to "yellow body-like tissues" many fine black fibers were found to penetrate from the "hyaline layers" into the follicle cavity, forming networks of various sizes.

Type AA. VI: In H.-E. preparations well-developed "yellow body-like tissues" were seen in the follicle cavity. "Hyaline layers" were thick. In silver preparations the parts corresponding to "yellow body-like tissues" consisted of large networks of fine black fibers. The parts corresponding to "hyaline layers" showed dark purple, fine granulated appearance, surrounding a few, various-sized empty spaces. In the outer side of this layer there were narrow networks composed of dark purple fibers with radial arrangement.

Type AA. VII: The follicles of this type showed scar tissue-like structures. Many dark purple fibers in addition to black fibers were found in the follicle cavity, forming indistinct networks of various sizes. Dark purple, fine granulated structures corresponding to "hyaline layers" constituted the most part of the follicle wall. In the outer side of this structure there were seen very thin layers composed of narrow networks of dark purple fibers.

Type AA. VIII: The follicles of this type presented the appearance of typical scar tissue. The original follicle cavity was narrow and occupied with thin dark purple fibers in addition to a few black ones. The structures of the follicle wall were similar to those of type AA. VII, but in its outer side there appeared numerous thick dark purple fibers.

Type AA. IX: In H.-E. preparations a few or many degenerated granulosa cells were seen in the granulosa layer, but in the theca interna no change was found. In silver preparations the structures of the theca interna were very similar to those of type AA. II. But unlike those of type AA. II, a few small folds which were composed of networks of thin black fibers as those of the theca interna were seen to project from the theca interna into the granulosa layer. This findings were rather similar to those in type II of normal vesicular follicles.

Relations between the typing based on H.-E. preparations and the typing based on silver preparations of cystic atretic follicles are set out in table 3.

All of both type AF. I at the earliest stage in the atresia and type AF. II at a somewhat advanced stage, and the majority of type AF. III at more advanced stage were put together in type AA. I in this study. In fact, these atretic follicles at the early stage were difficult to distinguish from the normal vesicular follicles on the basis of behavior of argyrophil fibers. Some follicles out of type AF. III belonged to either type AA. II or AA. III. In these follicles thin dark purple fibers appeared among the fibers of the networks around the theca interna cells, and tended to increase gradually. Type AA. III were consolidated into type AF. IV which showed a distinct early stage of atresia with completely degenerated granulosa layer and types AF. V and AF. VI at the middle stages of atresia with hypertrophic theca interna cells in the follicle cavity. Some follicles which were comprised in types

TABLE 3. *Relation between Typing Based on H.-E. Preparation and Typing Based on Silver Preparation of Atretic Follicles*

TYPES BASED ON H.-E. PREPARATION (AF.)	TYPES BASED ON SILVER PREPARATION (AA.)									TOTAL
	I	II	III	IV	V	VI	VII	VIII	IX	
I	20	.	.	.	.	.	.	.	4	24
II	7	.	.	.	.	.	.	.	2	9
III	46	14	2	.	.	.	.	.	4	66
IV	.	.	14	.	.	.	.	.	.	14
V	.	.	21	.	.	.	.	.	.	21
VI	.	.	16	.	.	.	.	.	.	16
VII	.	.	.	24	.	.	.	.	.	24
VIII	.	.	.	.	5	.	.	.	.	5
IX	.	.	.	.	15	.	.	.	.	15
X	.	.	.	.	.	15	.	.	.	15
XI	.	.	.	.	.	.	23	.	.	23
XII	.	.	.	.	.	.	.	12	.	12
Total	73	14	53	24	20	15	23	12	10	244

AF. I to AF. III were put in the type AA. IX. Atretic follicles which comprised types AF. VII to AF. IX were placed in types AA. IV to AA. V for this research. They showed the following features: penetration and net-formation of fine black fibers in coincidental parts with "yellow body-like tissues", appearance and thickening of pale purple, fine granulated structures in coincidental parts with "hyaline layers" and radial arrangement of meshwork of dark purple fibers in coincidental parts with "radial layers." Follicles of types AF. X, XI and XII which showed to be scar tissue, belonged respectively to types AA. VI, VII and VIII. There were observed disappearance of coarse networks of dark purple fibers with marked thickening of pale purple, fine granulated structures in the follicle wall, and diminishing of black fibers and increasing of dark purple fibers in the follicle cavity.

#### IV. Findings on Atretic Follicles with Hemorrhage (Aa.)

These atretic follicles were divided into 4 types.

Type Aa. I: Follicles of this type were similar to type AA. I of cystic atretic follicles above described.

Type Aa. II: In parts corresponding to the "yellow body-like tissues", which developed partially in the follicle wall, fine black fibers ran in parallel around the follicle cavity, showing a slight tendency to form networks. The theca folliculi itself was composed of only thin black fibers forming distinct networks around theca cells. In some inner parts of the theca 2 to 3 lines of thin black fibers ran parallel along the follicle wall. In outer sides of the theca there was developed a large meshwork of thin dark purple fibers.

Type Aa. III: In H.-E. preparations erythrocytes filled out the follicle cavity, and the

granulosa layer disappeared entirely. Moreover, "yellow body-like tissues" and "hyaline layers" developed well in parts of the follicle wall. In parts corresponding with the "yellow body-like tissues" many fine black fibers were seen to arrange closely parallel to the follicle wall. Therefore the meshwork was small and narrow. The next layer of the theca folliculi was composed of thin dark purple fibers parallel to the follicle wall without forming networks. The parts corresponding with the "hyaline layers" showed pale purple, fine granulated structures.

Type Aa. IV: In H.-E. preparations this type was a scar tissue. The type was similar to type AA. VII of cystic atretic follicles.

Relations between the typing based on H.-E. preparations and the typing based on silver preparations of atretic follicles with hemorrhage are set out in table 4.

TABLE 4. *Relation between Typing Based on H.-E. Preparation and Typing Based on Silver Preparation of Atretic Follicles with Hemorrhage*

TYPES BASED ON H.-E. PREPARATION (aF.)	TYPES BASED ON SILVER PREPARATION (Aa.)				TOTAL
	I	II	III	IV	
I	1	.	.	.	1
II	.	2	.	.	2
III	.	.	3	.	3
IV	.	.	.	1	1
Total	1	2	3	1	7

Type aF. I in H.-E. preparations belonged to type Aa. I, whose findings were similar to those of type AA. I. Therefore the behavior of argyrophil fibers in an early stage of atretic follicles with hemorrhage could not be distinguished from the behavior of normal vesicular follicles or cystic atretic follicles at an early stage.

Types aF. II and aF. III belonged respectively to types Aa. II and Aa. III, and showed characteristic appearances in the follicles of these types; in spite of active penetration of fine black fibers into the follicle cavity, the connective tissue fibers in the follicle wall did not show marked changes.

Type aF. IV belonged to type Aa. IV, regarding which the findings were similar to those of type AA. VII. Therefore the behavior of argyrophil fibers at the last stage of atretic follicles with hemorrhage could not be distinguished from that of cystic atretic follicles in the last stage.

#### V. Findings on Corpora Lutea of Non-pregnancy (AL.)

Corpora lutea of this group were divided into 9 types in the present study.

Type AL. I: In silver preparations two kinds of lutein cell layers were differentiated more definitely than in H.-E. preparations. The outer lutein cell layers (outer layers) were shown as wedge-shaped folds of inconstant size. These layers were composed of distinct networks of thin black fibers, showing remarkable spongy appearance. In inner lutein cell layers (inner layers) there were found a few fine black fibers forming networks. However

at the peak of the folds of the outer layers numerous fine black fibers were seen, and in some parts these fibers were seen to stretch among loosely arranged cells of the innermost follicle wall up to just beneath the border line of the cavity. In the central core of the folds of outer layers there were a few thin dark purple fibers which were continuous to thin dark purple fibers and thick dark purple ones of the capsule. These fibers were continuous to thin dark purple fibers of the adventitia of arterioles seen under the capsule. In the outer layers of the corpora lutea, especially at the demarcations with the inner layers, there were observed fine networks of fine black fibers on the wall of dilated capillaries.

Type AL. II: In this type two lutein cell layers could still be distinguished easily. In outer layers the thin black fibers of the meshwork were more abundant than in inner layers. In the latter, however, fine black fibers were more abundant than in type AL. I, and especially in the demarcations with outer layers each inner lutein cell was nearly completely surrounded with these fibers. Therefore the borderline between the two lutein cell layers was slightly obscure, more so than in type AL. I. At parts of the inner surfaces of the inner layers there appeared thin layers of minute networks of fine black fibers. Moreover, from these layers short fine black fibers were seen to stretch into the central cavity. Numerous delicate networks of fine black fibers in capillary walls were found among the inner lutein cells throughout the inner layers.

Type AL. III: The outer layers were found as narrow but tall folds possessing distinct networks of fine black fibers. Also at the base of these folds similar networks were seen to group into a triangular mass around small blood vessels under the capsule. In inner layers, on the other hand, fine black fibers were numerous in comparison with their occurrence in type AL. II. Nearly all inner lutein cells lying near the demarcation with outer layers and a few inner lutein cells in the deeper layers were surrounded completely with these fibers. At the inner surfaces of inner layers the layers of minute and compact networks of fine black fibers were thicker than in type AL. II. From these innermost layers many black fibers were seen to stretch as far as the deep parts of the central cavity.

Type AL. IV: In this type a few networks around the outer lutein cells were still found distinctly adjacent to small blood vessels under the capsule. Except for such portions, it was difficult to distinguish networks surrounding outer lutein cells from those surrounding inner lutein cells throughout the entire wall of the corpora lutea. Namely, in this type throughout the wall of the corpora lutea there appeared nearly uniform, large networks which were composed of thin black fibers as well as fine black ones around lutein cells.

Type AL. V: In this type the networks enclosing lutein cells were smaller than in type AL. IV; they appeared to be coarse and angular, because many thin dark purple fibers appeared among the black fibers of networks. Moreover, meshes of many networks were filled with fine dark purple fibers or fine pale purple granular substances.

Type AL. VI: The networks around lutein cells were smaller than those of type AL. V. In this type the thin black fibers of the networks were almost fragmentary. And networks which were filled with fine dark purple fibers or fine pale purple granular substances increased in number more greatly than in type AL. V.

Type AL. VII: In this type thin dark purple fibers of the networks around lutein cells were markedly abundant; many of the fibers were fragmentary and crossed irregularly, while there were a few thin black fibers. Moreover, networks filled with fine pale purple granular substances were predominant in this type.

Type AL. VIII: The striking feature of this type was the appearance of the pale purple uniform homogeneous structures in a large parts of the wall of the corpus luteum. In this structure many fragmentary, dark purple fibers and thin black ones were mixed together. The wall of arterioles in the deep layers was displaced by structures which were the same as the surrounding connective tissue fibers.

Type AL. IX: The entire wall of this type was found as a mass of pale purple uniform structures with a few fragmentary fibers. Small arterioles in the deep layer and under the capsule could not be distinguished from the meshes of the networks around lutein cells. But comparatively large arterioles under the capsule appeared to be surrounded with a thick band of fine pale purple granular substances.

Relations between the typing based on H.-E. preparations and the typing based on silver preparations of corpora lutea of non-pregnancy are set out in table 5.

TABLE 5. *Relation between Typing Based on H.-E. Preparation and Typing Based on Silver Preparation of Corpora Lutea of Non-Pregnancy*

TYPES BASED ON H.-E. PREPARATION (CL.)	TYPES BASED ON SILVER PREPARATION (AL.)									TOTAL
	I	II	III	IV	V	VI	VII	VIII	IX	
I	6	.	.	.	.	.	.	.	.	6
II	.	11	.	.	.	.	.	.	.	11
III	.	.	10	.	.	.	.	.	.	10
IV	.	.	.	15	.	.	.	.	.	15
V	.	.	.	16	.	.	.	.	.	16
VI	.	.	.	.	4	.	.	.	.	4
VII	.	.	.	.	.	38	.	.	.	38
VIII	.	.	.	.	.	.	25	.	.	25
IX	.	.	.	.	.	.	.	17	.	17
X	.	.	.	.	.	.	.	.	27	27
Total	6	11	10	31	4	38	25	17	27	169

Findings based on silver preparations coincided entirely with the findings based on H.-E. preparations, except that types CL. IV and CL. V showed similar features in this research.

To sum up, in this research, discrimination of both kinds of lutein cell layers in corpora lutea of non-pregnancy at the stage of vascularization was easier and more distinct than in the findings based on H.-E. preparations. In the fully-formed corpora lutea each lutein cell was surrounded completely with fine black fibers. At the beginning stage of regression, the behavior of argyrophil fibers surrounding the lutein cells was almost like that at the fully-formed stage. At the middle stage, however, the chief changes were the appearance

and increasing of purple fibers among the black fibers of networks around lutein cells, thickening and breaking of individual fibers, and appearance and increasing of fine pale purple granular substances. At the last stage of regression the whole wall of the corpora lutea was displaced entirely by fine pale purple granular substances.

#### VI. Findings on Corpora Lutea of Non-Pregnancy with Large Central Cavity (Al.)

In respect to behavior of argyrophil fibers, the corpora lutea of this group coincided well with the above described corpora lutea of non-pregnancy so far as lutein cell layers are concerned.

The corpora of this group were divided into the same 6 types from Al. I to Al. VI as in H.-E. preparations (table 6). The behavior of the connective tissue fibers in the lutein cell layers corresponded to that of the next preceding group, as follows: Al. I to AL. I, Al. II to AL. II, Al. III to AL. III, Al. IV to AL. IV, Al. V to AL. VI and Al. VI to AL. VII. In the corpora of types Al. IV, V and VI, however, there was a boundary layer of fine black fibers in the periphery of the central cavity. Fine black fibers of this layer increased gradually in order of these types.

TABLE 6. *Relation between Typing Based on H.-E. Preparation and Based on Silver Preparation of Corpora Lutea of Non-Pregnancy with a Large Central Cavity*

TYPES BASED ON H.-E. PREPARATION (l.)	TYPES BASED ON SILVER PREPARATION (Al.)						TOTAL
	I	II	III	IV	V	VI	
I	2	.	.	.	.	.	2
II	.	2	.	.	.	.	2
III	.	.	5	.	.	.	5
IV	.	.	.	1	.	.	1
V	.	.	.	.	1	.	1
VI	.	.	.	.	.	2	2
Total	2	2	5	1	1	2	13

#### VII. Vascular Bodies (Ag.)

In this study vascular bodies were divided into 3 types.

Type Ag. I: In this type the behavior of argyrophil fibers in the whole wall was similar to that in type VII of corpora lutea of non-pregnancy. However, in these vascular bodies there was a tremendous quantity of various-sized blood vessels with distinct minute networks of fine black fibers in the tunica media.

Type Ag. II: The greater part of the wall of these vascular bodies was occupied by fine pale purple granular substances as in those of type VIII of corpora lutea of non-pregnancy. However, numerous arterioles in these vascular bodies maintained minute networks in the tunica media, except for a few cases in which fine granular structures occurred in the tunica media.

Type Ag. III: The whole wall of vascular bodies of this type was occupied with various-sized arterioles with distinct minute networks of the tunica media. Among these arterioles thin dark purple fibers appeared.

Relations between the typing based on H.-E. preparations and the typing based on silver preparations of vascular bodies are set out in table 7.

TABLE 7. *Relation between Typing Based on H.-E. Preparation and Typing Based on Silver Preparation of Vascular Bodies*

TYPES BASED ON H.-E. PREPARATION (g.)	TYPES BASED ON SILVER PREPARATION (Ag.)			TOTAL
	I	II	III	
I	5	•	•	5
II	•	7	•	7
III	•	•	8	8
Total	5	7	8	20

To summarize, vascular bodies showed also specific pictures in behavior of argyrophil fibers.

#### DISCUSSION

For the purpose of studying the development and the regressive processes of the 6 groups of structures of pig ovaries on the basis of H.-E. preparations, the behavior of connective tissue fibers in them was observed by the use of GÖMÖRI's silver impregnation method.

Hitherto it has been reported that argyrophil fibers which were treated with silver method stained jet-black, their outline was clear-cut, and their tendency to form networks was strong. Therefore all the black fibers of the present writer may correspond to so-called "argyrophil fibers."

Generally, it has been said that collagenous fibers were thicker in comparison with argyrophil fibers and stained pale purple to brick-red, and that their outline was obscure. WOLFE and co-workers, who used the GÖMÖRI method for observations of fibrous connective tissues in the walls of the uterus, cervix and vagina of rats, discovered a variety of characteristics. That is to say, two general types of collagenous fibers were observed: compact and diffuse. The arrangement of the fibrils in compact fibers was not generally uniform. Usually in the thinner compact fibers one side of a fiber often appeared denser than the other side. In the thicker compact fibers, narrow zones of greater density almost regularly were found on both sides. These denser margins took a deeper stain than did the rest of the fibers, appearing darker red in color.

The present writer's thin pale purple and dark purple fibers, which resembled

the thinner compact fibers of WOLFE et al., and thick dark purple and red-purple fibers, which resembled their thicker compact fibers, should be considered to represent so-called "collagenous fibers."

In this research normal vesicular follicles were divided into two types.

HÖRMANN'S first descriptions concerning networks of fine fibers around theca interna cells were recognized by RABL, CESA-BIANCHI and PLENK in women, and by MUTO in women, rabbits, rats and dogs. However, they did not differentiate the argyrophil and the collagenous fibers. SLAVIANSKI was the first to find the networks of reticular fibers among theca interna cells in women. Recent studies of YOSHIMURA in women and rabbits, and of TOMITA in women, monkeys, cats, dogs and rabbits substantiated the observations reported by SLAVIANSKI.

In the sow BENCKISER and CLARK also reported the same findings as SLAVIANSKI.

The findings of the present writer's type AEf. I resemble well those reported by BENCKISER and CLARK in sows and of YOSHIMURA and TOMITA in other animals. In the sow, the writer could not find networks of the wall of small blood vessels in theca interna, though YOSHIMURA found them in women and rabbits. TOMITA observed a few collagenous fibers in the theca interna in cats, but the present writer did not find them in sows.

Type AEf. II is considered to be mature follicles or ones just before ovulation. Until this time it has never been reported that, in mature follicles, small masses of networks of argyrophil fibers protrude from the theca interna into the granulosa layer. But the present writer proved such a finding concretely. Such a character seems to be significant concerning the behavior of argyrophil fibers in the wall of freshly-formed corpora lutea of non-pregnancy.

Cystic atretic follicles were divided into 9 types.

Changes of connective tissue fibers throughout the atretic processes of cystic type were described by YOSHIMURA in women and by TOMITA in rabbits, cats and monkeys. The present writer recognized that, in the sow, features of connective tissue fibers in cystic atretic follicles altered with the processes of atresia. Findings were slightly different from the findings of YOSHIMURA and TOMITA. In TOMITA'S cases argyrophil fibers had already penetrated radially from the theca interna into the follicle cavity at the first stage of atretic processes in rabbits. However, in sows the present writer could not observe such a thing. The majority of atretic follicles at this stage showed the same fibrous structures as in normal vesicular follicles. But at the more advanced stage, networks of theca interna became coarse and in conspicuous in company with appearance and increase of collagenous fibers. In sows the stage at which argyrophil fibers penetrated into follicle cavity corresponds with the stage at which yellow body-like tissue appeared in the inner side of the follicle wall. In atretic follicles at the last stage, which showed sear-

tissue, fine pale purple granular structures corresponding to small zigzag-shaped glass membrane were conspicuous, and transformation of argyrophil fibers into collagenous fibers was observed.

In some atretic follicles, which showed features of the first stage in H.-E. preparations, a few collagenous fibers appeared at the outer layer of theca interna, and small masses of networks of argyrophil fibers protruded partially into the granulosa layer. These follicles are thought to be mature follicles at the first stage of the process of atresia.

Atretic follicles with hemorrhage were divided into 4 types.

It seems there has been no description of connective tissue fibers in this sort of atretic follicles. In the earliest stage in the atretic process of this group the behavior of connective tissue fibers was similar to that of atretic follicles of cystic type. In the middle stage of this group in comparison with the same stage of atretic follicles of cystic type, in spite of active ingrowings and increases of argyrophil fibers into the follicle cavity, conspicuous changes of connective tissue fibers occurred only in parts of the follicle wall. In the last stage the findings of atretic follicles of this group were also almost like those of atretic follicles of cystic type.

The process of changes of connective tissue fibers in the follicle wall of this group is thought to differ somewhat from the changes in atretic follicles of cystic type in the middle stage of atresia.

Corpora lutea of non-pregnancy were divided into 9 types.

Changes of connective tissue fibers in the walls during the formation and the regression of corpora lutea of non-pregnancy in sows have been described only by CLARK.

The present writer's findings on corpora lutea of non-pregnancy just after ovulation (AL. I) were quite different from the findings on just ruptured follicles reported by CLARK.

Configuration of connective tissue fibers in the writer's type AL. I, especially in inner lutein cell layers which were derived from the granulosa layer of mature vesicular follicles was similar rather to the results of ONO and YOSHIMURA in women.

In type AL. I a few argyrophil fibers were seen to have penetrated into the inner lutein cell layer. Therefore these corpora were considered to be at somewhat advanced stage in comparison to the proliferation stage of ONO.

Behavior of argyrophil fibers in inner lutein cell layer of type AL. I, II and III corresponded well to that of latticed fibers of the granulosa layer in the vascularization stage of ONO.

YOSHIMURA stated that in corpora lutea of women at the 18th day after

menstruation the theca interna, which formed the central core of folds of granulosa layers, were made up of typical reticular fibers which connected together in meshwork.

In the writer's observation at the first stage of vascularization (AL. I) the tall and broad plica-like folds of the outer lutein cell layer, which derived from the theca interna of mature follicles, were composed of a meshwork of argyrophil fibers, which were denser and showed more clear sponge-like structures in comparison with those of the theca interna of mature follicles.

Such folds showed themselves gradually as narrow layers of meshwork arranged in 2 to 3 lines (AL. II and III), and finally as small masses of networks around the small blood vessels under the capsule (AL. IV).

As to these findings, it is considered to be difficult to distinguish the networks on the inner lutein cell layer from networks of the innermost layer of the outer lutein cell layer, according to the formation of new networks surrounding the inner lutein cells in demarcation between the two layers and according to the increasing of argyrophil fibers in the meshwork.

The present writer's such view differs from that of ONO or YOSHIMURA who stated that the theca interna reduced and disappeared gradually with the development of yellow bodies. In the case of sows, the present writer would confirm CORNER'S dualistic theory in which inner lutein cells as well as the outer lutein cells constitute yellow bodies.

In fully-formed yellow bodies there is agreement in the opinion that argyrophil fibers completely enclose each lutein cell in differences of species of animals and in spite of theories on the origin of lutein cell.

The behavior of connective tissue fibers in fully-formed corpora lutea of non-pregnancy seems similar to that of corpora of pregnancy reported by CORNER. In the present research, however, small masses of networks surrounding theca lutein cells were found around small blood vessels under the capsule, and in deeper portions of the lutein cell layer the networks surrounding the theca lutein cells did not differ from the networks surrounding the granulosa lutein cells.

It has been stated by YOSHIMURA in women, MUTO in guinea-pigs and TOMITA in cats that the behavior of connective tissue fibers in the first stage of retrogression of the corpora lutea was similar to that of the bloom stage.

In sows the present writer's type AL. IV, which was thought to be corpora lutea of the earliest stage of regressive process, was also similar to fully-formed corpora lutea from findings of silver preparations.

In corpora lutea which were thought to be in developing stage by CLARK, a few degenerative lutein cells have already appeared, and by his digestive method it was proved that reticular fibers completely surround each lutein cell. CLARK'S

findings were rather similar to the characters of the present writer's type AL. IV.

The changes of connective tissue fibers during the process of regression of the corpora lutea of non-pregnancy were observed by CLARK.

The present writer's findings of types AL. V and AL. VI, which were considered to represent the middle stage in regression, seem to correspond to those of CLARK's "first stage of regression." CLARK treated them as the corpora fibrosa with characteristics of the disappearance of the lutein cells and progressive increase of connective tissue fibers.

Especially in type AL. VI, of connective tissue fiber surrounding each lutein cell, thick collagenous fibers, which may correspond to "coarse fibers" in CLARK's observations, were predominant, while argyrophil fibers were comparatively fewer and broken. Therefore these networks showed some large spongy structures. And in this type the meshwork which was filled out with fine pale purple fibers or fine pale purple granular structures seems to correspond to the "collapsed meshwork" of CLARK.

In this research corpora lutea of types AL. VIII and AL. IX, which were recognized to be at the last stage of regression, were seemed to be almost like those of the hyaline bodies at the "second stage of regression" of CLARK. The parts which appeared hyaline in H.-E. preparations showed fine pale purple granular structures, in which remnants of broken black or dark purple fibers were still found.

The present writer distinguished type AL. VII from types AL. VI and AL. VIII. Namely, in this type a small quantity of network which was filled out with pale purple fine fibers or fine granular structures was found throughout the lutein cell layers.

Corpora lutea of non-pregnancy with a large central cavity were divided into 6 types.

In silver preparations the process of the changes of the connective tissue fibers in the wall was almost like that seen in corpora lutea of non-pregnancy.

In this research "vascular bodies" were divided into 3 types.

Hitherto so far as known, there have been presented no descriptions on argyrophil fibers of "vascular bodies" in literature.

Although the process of the changes of connective tissue fibers of networks around lutein cells during the regressive process of "vascular bodies" was similar to that seen in corpora lutea of non-pregnancy, in these bodies argyrophil fibers of the wall of blood vessels, especially those of arterioles were seen in more advanced stages than in corpora lutea of non-pregnancy.

Above all the present writer could set up the grades of regression of "vascular bodies" to some extent from this research. This fact is very important for the consideration of their character.

## SUMMARY

The result of this research may be summarized as follows:

1) Connective tissue fibers appearing in the above described 6 groups of structures of pig ovaries were observed with the use of GÖMÖRI's silver impregnation method.

2) Normal vesicular follicles were divided into 2 types.

3) Normal vesicular follicles in the process of maturation could not be distinguished by the findings of silver preparations: fibrous elements are not found in the granulosa layer. The theca interna consists of only argyrophil fibers, which form a meshwork. The theca externa is composed of collagenous fibers, which run to parallel to the follicle wall and do not tend to form a meshwork.

4) In mature follicles and in just before ovulation the behavior of the connective tissue fibers in the walls is similar to that of follicles in maturing process. But in these follicles small masses of similar networks to these in the theca interna protrude from the inner side of the theca interna into the granulosa layer, showing plica-like configuration.

5) Cystic atretic follicles were divided into 9 types.

6) At the earliest stage of the regressive process the behavior of connective tissue fibers is almost like that in normal vesicular follicles. At the more advanced and the middle stages in a meshwork of argyrophil fibers of theca interna, collagenous fibers appear and increase gradually. Then after the appearance and gradual development of the yellow body-like tissues in the periphery of the follicle cavity, fine argyrophil fibers penetrate into these tissues from the theca interna, and gradually form a fine meshwork. Accompanied by the appearance and increasing of hyaline layers in the inner side of the theca interna, fine granular substances come into sight and increase in quantity. Moreover, with the appearance of radial layers in the outer wall of atretic follicles, collagenous fibers of these parts increase and form large networks in radial arrangement. At the last stage, in the scar tissue, the small zigzag folds composed of fine granular substances are found in parts corresponding to so-called glass membrane. In the outer side of this scar, radially arranged networks begin to decrease and argyrophil fibers which filled out the original follicle cavity show gradual transformation into collagenous fibers.

7) In this research there were found some follicles which were recognized to be undergoing change into the regressive process after maturation.

8) Atretic follicles with hemorrhage were divided into 4 types in this research.

9) In this research these follicles at the early stage of regression were found

to be similar to the normal vesicular follicles, and those at the last stage were also like in character to the last stage of cystic atretic follicles. At the middle stage, however, slight differences were found in the connective tissue fibers of the wall, in spite of the active penetrating and increasing of the argyrophil fibers into the follicle cavity.

10) Corpora lutea of non-pregnancy were divided into 9 types.

11) At the vascularization stage fine argyrophil fibers penetrate actively from theca lutein cell layers into granulosa lutein cell layers, and form networks enclosing 2 or more granulosa lutein cells in one of the same networks. At the bloom stage these fibers encircle completely each lutein cell. At the earliest stage of regression the condition of argyrophil fibers is similar to that of the bloom stage. But at a later stage collagenous fibers appear and increase gradually around lutein cells contrary to the diminishing of argyrophil fibers. And fine pale purple fibers or fine pale purple granular structures come into sight and develop gradually in the parts which are undergoing hyaline degeneration. At the last stage the whole wall of a corpus luteum becomes occupied by fine pale purple granular substances, in which a few broken argyrophil and swollen collagenous fibers are scattered.

12) On the derivation of lutein cells, the present writer in this research has confirmed definitely the dualistic theory of CORNER.

13) Corpora lutea of non-pregnancy with a large central cavity were divided into 6 types.

14) In this research the behavior of the connective tissue fibers in the wall was found to be similar to that of corpora lutea of non-pregnancy.

15) "Vascular bodies" were divided into 3 types.

16) In the regressive process the connective tissue fibers show similar processes of change as in corpora lutea of non-pregnancy. In this research, however, argyrophil fibers in the wall of blood vessels, especially arterioles are maintained in the far advanced stage, showing the characteristics of the present writer's so-called "vascular bodies."

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## EXPLANATION OF PLATES

## PLATE I.

Normal Vesicular Follicles (AEf.) and Cystic Atretic Follicles (AA.),  
GÖMÖRI's stain, × 350.

- |                       |                       |
|-----------------------|-----------------------|
| Fig. 1. Type AEf. I.  | Fig. 4. Type AA. III. |
| Fig. 2. Type AEf. II. | Fig. 5. Type AA. IV.  |
| Fig. 3. Type AA. II.  | Fig. 6. Type AA. V.   |

## PLATE II.

Cystic Atretic Follicles (AA.) and Atrétic Follicles with Hemorrhage (Aa.),  
GÖMÖRI's stain, × 350.

- |                        |                        |
|------------------------|------------------------|
| Fig. 7. Type AA. VI.   | Fig. 10. Type AA. IX.  |
| Fig. 8. Type AA. VII.  | Fig. 11. Type Aa. II.  |
| Fig. 9. Type AA. VIII. | Fig. 12. Type Aa. III. |

## PLATE III.

Corpora lutea of Non-pregnancy (AL.), GÖMÖRI's stain.

- |                       |       |
|-----------------------|-------|
| Fig. 13. Type AL. I.  | × 70. |
| Fig. 14. Type AL. II. | × 70. |

Fig. 15.	Type AL. III.	× 70.
Fig. 16.	Type AL. IV.	× 350.
Fig. 17.	Type AL. V.	× 350.
Fig. 18.	Type AL. VI.	× 350.

PLATE IV.

Corpora Lutea of Non-pregnancy (AL.), Corpus Luteum of Non-pregnancy with a Large Central Cavity (Al.) and Vascular Bodies (Ag.), GÖMÖRI's stain.

Fig. 19.	Type AL. VII.	× 350.
Fig. 20.	Type AL. VIII.	× 350.
Fig. 21.	Type AL. IX.	× 350.
Fig. 22.	Type Al. IV.	× 70.
Fig. 23.	Type Ag. I.	× 70.
Fig. 24.	Type Ag. II.	× 70.
Fig. 25.	Type Ag. III.	× 70.







