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ON THE HISTOLOGICAL STRUCTURE OF THE GONADS OF PANULIRUS ARGUS (LATR.)

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Many investigations were made on the reproduction system of Crustacea. Unfortunately, few researchers have tried to study the phases of the sexual cycle of the males as well as females.

In this paper we try to characterize the different stages of the development of the sexual glands of the spiny lobster *Panulirus argus* (Latr.). The results obtained must be taken only as a part of the theme, because we do not correlate the microscopic findings with the macroscopic aspects of the gonads. In spite of this, an objective appreciation of the cytological contents of the gonads is made.

MATERIAL AND METHODS

A total of 96 individuals were used in this study. Of these, 45 were males and 51 were females. All of them were caught in front of the County of Fortaleza (State of Ceará, Brazil), from August, 1963 to April, 1964.

For each individual we registered the total length and afterward we took off the gonads. Immediately after this, we put them into the fixative solution. For each male we measured the cephalothorax length and we calculated the total length through the regression equation presented by Paiva (1960).

As total length we considered the distance from the anterior margin of the cut between the rostral spines to the end of telson, taken in the symmetric plan over the dorsal part of the body, when individual is completely spread over a flat surface.

We used as fixative, for the testis, Bouin's picroformol solution, and for the ovaries, 10 percent formalin in sea water.

Fragments of the mid part of the gonads were embedded in paraffin, and sectioned at 7 micra. Routine sections were stained with Delafield's hematoxylin and eosin at 1 percent.

In each preparation we measured, at randon, 5 cells of each type of the germinal line, and we did countings of these cells in 5 microscopic fields.

We used for the measurements a microscope CARL ZEISS JENA, with micrometrical ocular 6 X, being the objetives for the preparations of testis 40/0.65 or 90/1.25, and for the preparations of ovaries 20/0.40, 40/0.65 or 90/1.25. In the countings, we used an ocular 7 X and the same objetives used in the measurements.

DESCRIPTION OF THE GERMINAL CELLS

In the description of the germinal cells, we based ourselves on criteria of topographical (histological) and cytological order, very much common to the scientific literature. To acknowledge the spermatozoa, we based ourselves on the descriptions of these cells for other genera of Decapoda presented by Labbé (1903).

Table I shows the mean diameters and ranges of variation of the germinal cells, determined after measurements made in the various histological preparations.

Spermatogenesis

Spermatogonia — cells relatively large, found in the periphery of the seminiferous tubules' walls, in contact with the basal membrane. Their diameter are generally around 23 micra. They posses voluminous nucleus, having 15 micra of diameter on the average. The nuclear membrane is very clear and the chromatic substance is evenly dispersed under the form of tender granules. They are extremely basophiles cells (figure 1).

Spermatocytes I — the primary spermatocytes measure 17.4 micra of diameter on the average. The nuclear substances are more or less condensed, forming chromatin masses. They are situated also at the seminiferous tubules' walls, a little more on the inside than the precedent form (figure 2).

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Spermatocytes II — the secondary spermatocytes differ from the elements I mainly by their minor size. They have about 10 micra of diameter. The nuclear substances are more condensed, showing thick chromatin masses. They have round nucleus, measuring about 5 micra of diameter (figure 3).

Spermatids — cells approximately spherical having about 6.1 micra of diameter. They present a little nucleus, also spherical, measuring 2 micra of diameter, approximately. The chromatic substance is densely distributed, and we can not distinguish the nuclear elements clearly. They are found on the margins of the seminiferous tubules' walls or sometimes inside their own lumen (figure 4).

Spermatozoa — they are found in the seminiferous tubules' lumen, gathered in great masses. They measure about 4 micra of diameter. The nucleus is round and keeps the same dimensions of the spermatids nucleus, about 2 micra of diameter. They have a very concentrated chromatin, so that in seems a unique block. The cytoplasm disappears almost completely and persists only as a halo which binds the nucleus irregularly, so that it looks sensibly eccentric (figure 4).

Ovogenesis

Ovogonia — short cells with diameters ranging from 15.6 to 23.4 micra. They have spherical nucleus, with well-defined shape and with blurred chromatic granules. They are always seen in groups from 6 to 8 cells (figure 5).

Ovocytes I — cells of irregular form, with round shape and diameters varying from 42.9 to 69.0 micra. They have voluminous round nucleus, enclosing an enormous nucleolus. The cytoplasm presents some granulations (figure 5).

Ovocytes II — they vary very much in their size, this being always over 78.9 micra, reaching about 174.0 micra. They differ from the ovocytes I, by their greater size, as well as by the disposition of the nuclear elements. They present, almost always, accessory nucleolus. The nucleus also differs as to the dimension, according to which ovocyte it belongs. In these last cells, they are always minor (figure 6).

Ovules — they show up enveloped by a very thin membrane, but perfectly differentiated. They have a well-marked form of a polyhedron of round borders. Round little nucleus in the middle of a cytoplasm with numerous vesicles (preparations embedded in paraffin) and granulations. Their diameter are variable, the greater reaching about 701.7 micra and the smaller 355.3 micra (figure 7).

DESCRIPTION OF THE GONAD STAGES

We consider for the male gonads three stages of evolution: stage I — underdeveloped stage, stage II — developing stage, and stage III — ripe stage.

For the female gonads are considered five stages, according to the classification used by Cummings (1961) for *Penaeus duorarum* Burkenroad. They are: stage I — underdeveloped stage, stage II — developing stage, stage III — nearly ripe stage, stage IV — ripe stage, and stage V — post-spawning stage.

In the testis:

Stage I — in this stage, the gonad presents a uniform aspect, with numerous spermatogonia situated in the seminiferous tubules' periphery, in contact with the basal membrane. Many of these spermatogonia are in division. We did not find elements in the seminiferous tubules' lumen (table II, figures 8 and 9).

Stage II — here, the number of spermatogonia in division shows up more clearly than in the stage I. We can observe numerous spermatocytes I, many of them also in division. We find parts of the seminiferous tubules' lumen totally occupied by these elements, furthermore, they appear associated with the spermatocytes II, which already appear in considerable quantity (table III, figures 10 and 11).

Stage III — this stage is characterized by the appearence of the spermatids and spermatozoa. These appear in the seminiferous tubules' lumen in great quantities. In this stage we find cuts of seminiferous tubules in which are stratified spermatogonia and spermatocytes, giving to the same a compact aspect. In the neighbourhood or mixing with them, there are tubules wich, in the cut, show a stratification from spermatogonium till spermatozoon (this in the lumen). This seems to indicate the existence of spermatogenetics waves (table IV, figures 12 and 13).

In the ovaries:

Stage I — the periovarian connective tissue sends septa that divide the organ in pseudo-loci of variable diameters. These pseudo-loci are found full of immature germinal cells, represented by ovogonia (table V, figures 14 and 15).

Stage II — in this stage the connective tissue disposition is regular enough and binds loci where are situated germinal cells. Here, besides the ovogonia, there also appear ovocytes I and II. Many of these elements are in division. In the ovocytes II, granules appear

which possibly represent nourishing elements. We have not yet noticed the ovules presence (table VI, figures 16 and 17).

Stage III — this stage is characterized by the appearence of ovules. Although younger cells exist, the greater frequency is of ovocytes II. which show up with basophile cytoplasm. At this time we can remark the four component elements of the female germinative line (table VII, figures 18 and 19).

Stage IV — the ovary has a uniform aspect, where predominate the mature ovules, with thick cytoplasm, less basophile than the ovocytes II and with a tendency for eosinophile, with numerous vacuoli and granulations. The young forms are rare (table VIII, figures 20 and 21).

Stage V — we observe numerous blanks which correspond to the plans in which were found the ovules before the spawning. In middle of the intersticial tissue persist many ovocytes I and II (table IX, figures 22 and 23).

GENERAL CONCLUSIONS

We have not remarked sudden transitions from a gonad stage to the other in both sexes. The remarked changes are always gradual.

The smaller sizes for sexual ripening were 19.0 centimeters for males and 21.0 centimeters for females (table X). Paiva & Costa (1963) found females in reproductive process from 16.8 centimeters of total length. We do not have any reference to smaller size of males in reproductive process in the scientific literature.

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TABLE I

Mean diameters and ranges of variation of the germinal cells of *Panulirus argus* (Latr.).

Germinal cells	Number of measurements (n)	Diameters in micra				
		maximum	minimum	arithmetic means (x)	standard deviations (s)	coefficients of variation (C.V.)
spermatogonia	225	24.9	21.6	23.2	4.1	17.8
spermatocytes I	165	19.9	14.9	17.4	5.5	31.6
spermatocytes II	165	13.3	8.3	10.7	1.7	15.5
spermatids	80	6.6	4.9	6.1	1.5	25.1
spermatozoa	80	4.9	3.3	4.1	0.6	15.3
ovogonia	120	23.4	15.6	22.8	6.0	26.3
ovocytes I	225	69.0	31.6	50.6	8.5	16.8
ovocytes II	185	173.7	78.9	124.9	11.8	9.5
ovules	180	701.7	355.3	537.9	15.2	2.8

TABLE II

Absolute frequencies of the germinal cells by microscopic field, in histological cuts of the testis of *Panulirus argus* (Latr.) in stage I — underdeveloped stage.

Germinal cells	Number of fields counted (n)	Germinal cells by microscopic field				
		maximum	minimum	arithmetic means (x)	standard deviations (s)	coefficients of variation (C.V.)
spermatogonia	75	549	398	476.0	13.5	2.8
spermatocytes I	—	—	—	—	—	—
spermatocytes II	—	—	—	—	—	—
spermatids	—	—	—	—	—	—
spermatozoa	—	—	—	—	—	—

TABLE III

Absolute frequencies of the germinal cells by microscopic field, in histological cuts of the testis of *Panulirus argus* (Latr.) in stage II — developing stage.

Germinal cells	Number of fields counted (n)	Germinal cells by microscopic field				
		maximum	minimum	arithmetic means ($\bar{x}$)	standard deviations (s)	coefficients of variation (C.V.)
spermatogonia	65	225	198	200.2	16.8	8.4
spermatocytes I	65	386	298	350.0	4.1	1.2
spermatocytes II	52	350	195	270.0	3.5	1.3
spermatids	—	—	—	—	—	—
spermatozoa	—	—	—	—	—	—

TABLE IV

Absolute frequencies of the germinal cells by microscopic field, in histological cuts of the testis of *Panulirus argus* (Latr.) in stage III — ripe stage.

Germinal cells	Number of fields counted (n)	Germinal cells by microscopic field				
		minimum	minimum	arithmetic means ($\bar{x}$)	standard deviations (s)	coefficients of variation (C.V.)
spermatogonia	70	90	70	87.5	8.2	9.3
spermatocytes I	85	495	309	414.0	9.5	2.3
spermatocytes II	85	450	342	391.6	6.8	1.7
spermatids	80	505	395	412.5	4.5	1.1
spermatozoa	80	500	415	455.3	8.7	1.9

TABLE V

Absolute frequencies of the germinal cells by microscopic field, in histological cuts of the ovary of *Panulirus argus* (Latr.) in stage I — underdeveloped stage.

Germinal cells	Number of fields counted (n)	Germinal cells by microscopic field				
		maximum	minimum	arithmetic means ($\bar{x}$)	standard deviations (s)	coefficients of variation (C.V.)
ovogonia	25	14	9	11.1	2.7	24.6
ovocytes I	—	—	—	—	—	—
ovocytes II	—	—	—	—	—	—
ovules	—	—	—	—	—	—

TABLE VI

Absolute frequencies of the germinal cells by microscopic field, in histological cuts of the ovary of *Panulirus argus* (Latr.) in stage II — developing stage.

Germinal cells	Number of fields counted (n)	Germinal cells by microscopic field				
		maximum	minimum	arithmetic means ($\bar{x}$)	standard deviations (s)	coefficients of variation (C.V.)
ovogonia	30	8	6	6.9	1.4	23.0
ovocytes I	30	11	4	6.2	2.6	43.5
ovocytes II	30	5	1	3.3	1.4	42.7
ovules	—	—	—	—	—	—

TABLE VII

Absolute frequencies of the germinal cells by microscopic field, in histological cuts of the ovary of *Panulirus argus* (Latr.) in stage III — nearly ripe stage.

Germinal cells	Number of fields counted (n)	Germinal cells by microscopic field				
		maximum	minimum	arithmetic means (x)	standard deviations (s)	coefficients of variation (C.V.)
ovogonia	35	6	3	4.1	1.2	30.2
ovocytes I	45	7	1	3.9	1.8	45.2
ovocytes II	45	5	3	4.0	1.1	27.7
ovules	45	7	2	3.9	1.7	42.7

TABLE VIII

Absolute frequencies of the germinal cells by microscopic field, in histological cuts of the ovary of *Panulirus argus* (Latr.) in stage IV — ripe stage.

Germinal cells	Number of fields counted (n)	Germinal cells by microscopic field				
		máximum	minimum	arithmetic means (x)	standard deviations (s)	coefficients of variation (C.V.)
ovogonia	15	7	3	4.8	2.1	42.2
ovocytes I	85	4	1	1.7	1.1	24.7
ovocytes II	80	5	1	3.2	1.6	53.6
ovules	135	4	2	3.4	1.1	32.7

TABLE IX

Absolute frequencies of the germinal cells by microscopic field, in histological cuts of the ovary of *Panulirus argus* (Latr.) in stage V — post-spawning stage.

Germinal cells	Number of fields counted (n)	Germinal cells by microscopic field				
		maximum	minimum	arithmetic means (x)	standard deviations (s)	coefficients of variation (C.V.)
ovogonia	12	5	2	3.2	2.8	87.8
ovocytes I	25	3	1	1.9	1.8	90.5
ovocytes II	30	6	1	2.8	2.7	90.3
ovules	30	2	1	1.5	1.9	95.5

TABLE X

Total lengths and corresponding gonad stages of individuals of *Panulirus argus* (Latr.).

Total lengths (cm)	Males				Females					
	individuals	gonad stages			individuals	gonad stages				
		I	II	III		I	II	III	IV	V
17	3	2	1	—	3	3	—	—	—	—
18	1	1	—	—	2	2	—	—	—	—
19	8	6	1	1	2	—	2	—	—	—
20	10	2	2	6	2	—	2	—	—	—
21	9	4	4	1	4	—	—	1	—	3
22	4	—	2	2	6	—	—	3	3	—
23	1	—	—	1	7	—	—	1	5	1
24	3	—	1	2	10	—	—	1	9	—
25	—	—	—	—	6	—	—	1	5	—
26	—	—	—	—	1	—	—	1	—	—
27	—	—	—	—	2	—	—	1	1	—
28	—	—	—	—	1	—	—	—	1	—
29	—	—	—	—	2	—	—	—	2	—

CAPTIONS

Figure 1 — Seminiferous tubules of **Panulirus argus** (Latr.), showing the spermatogonia (X 400).

Figure 2 — Spermatocytes I (X 400) of **Panulirus argus** (Latr.).

Figure 3 — Spermatocytes II (X 400) of **Panulirus argus** (Latr.).

Figure 4 — Spermatids and spermatozoa (X 900) of **Panulirus argus** (Latr.).

Figure 5 — Ovogonia (at left) and ovocytes I (at right) (X 400) of **Panulirus argus** (Latr.).

Figure 6 — Ovocytes II (X 400) of **Panulirus argus** (Latr.).

Figure 7 — Ovules (X 400) of **Panulirus argus** (Latr.).

Figure 8 — General aspect of a testis of **Panulirus argus** (Latr.) in stage I — underdeveloped stage. We can see in a transversal cut of the organ, numerous spermatogonia located at the seminiferous tubules' walls. Camera lucida drawing (X 100).

Figure 9 — Histological cut microphotography (X 400) showing stage I — underdeveloped stage of a testis of **Panulirus argus** (Latr.).

Figure 10 — General aspect of a testis of **Panulirus argus** (Latr.) in stage II — developing stage. We can see seminiferous tubules in full spermatogenetic activity. We can also see, numerous spermatocytes I and II, besides some spermatogonia. Camera lucida drawing (X 100).

Figure 11 — Histological cut microphotography (X 100) showing stage II — developing stage of a testis of **Panulirus argus** (Latr.).

Figure 12 — General aspect of a testis of **Panulirus argus** (Latr.) in stage III — ripe stage. We can see the seminiferous tubules with large clumps of spermatozoa in its lumen. These are surrounded by spermatids and numerous spermatocytes I and II. We can also see, some rare spermatogonia. Camera lucida drawing (X 100).

Figure 13 — Histological cut microphotography (X 100) showing stage III — ripe stage of a testis of **Panulirus argus** (Latr.).

Figure 14 — General aspect of an ovary of **Panulirus argus** (Latr.) in stage I — underdeveloped stage. We can see in a longitudinal cut of the organ, the connective tissue forming the pseudo-loci, where the ovogonia are situated. Camera lucida drawing (X 100).

Figure 15 — Histological cut microphotography (X 100) showing stage I — underdeveloped stage of an ovary of **Panulirus argus** (Latr.).

Figure 16 — General aspect of an ovary of **Panulirus argus** (Latr.) in stage II — developing stage. We can see in a transversal cut of the organ, ovocytes I and II in which we notice the large nucleus with well-marked nucleolus. Camera lucida drawing (X 100).

Figure 17 — Histological cut microphotography (X 100) showing stage II — developing stage of an ovary of **Panulirus argus** (Latr.).

Figure 18 — General aspect of an ovary of **Panulirus argus** (Latr.) in stage III — nearly ripe stage. We can see in a transversal cut of the organ many ovules associated with the ovocytes I and II, and some ovogonia in short number which are still seen. Camera lucida drawing (X 100).

Figure 19 — Histological cut microphotography (X 100) showing stage III — nearly ripe stage of an ovary of **Panulirus argus** (Latr.).

Figure 20 — General aspect of an ovary of **Panulirus argus** (Latr.) in stage IV — ripe stage. We can see the ovules distributed more or less uniformly, the immature cells remaining scattered and in a short number. Camera lucida drawing (X 100).

Figure 21 — Histological cut microphotography (X 100) showing stage IV — ripe stage of an ovary of **Panulirus argus** (Latr.).

Figure 22 — General aspect of an ovary of **Panulirus argus** (Latr.) in stage V — post-spawning stage. We can see some blanks which correspond to the places in which were situated the ovules before the spawning. We can also see few ovocytes I and II. Camera lucida drawing (X 100).

Figure 23 — Histological cut microphotography (X 100) showing stage V — post-spawning stage of an ovary of **Panulirus argus** (Latr.).

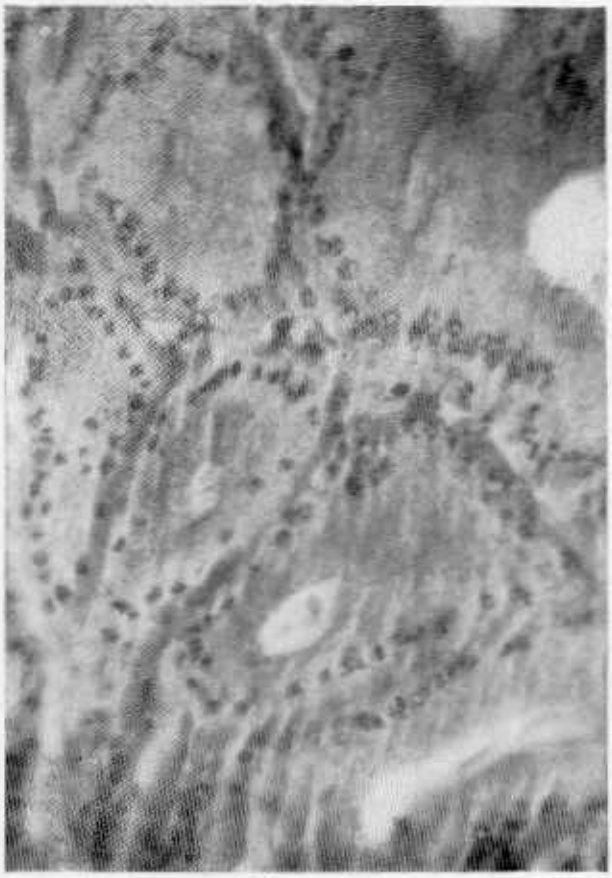
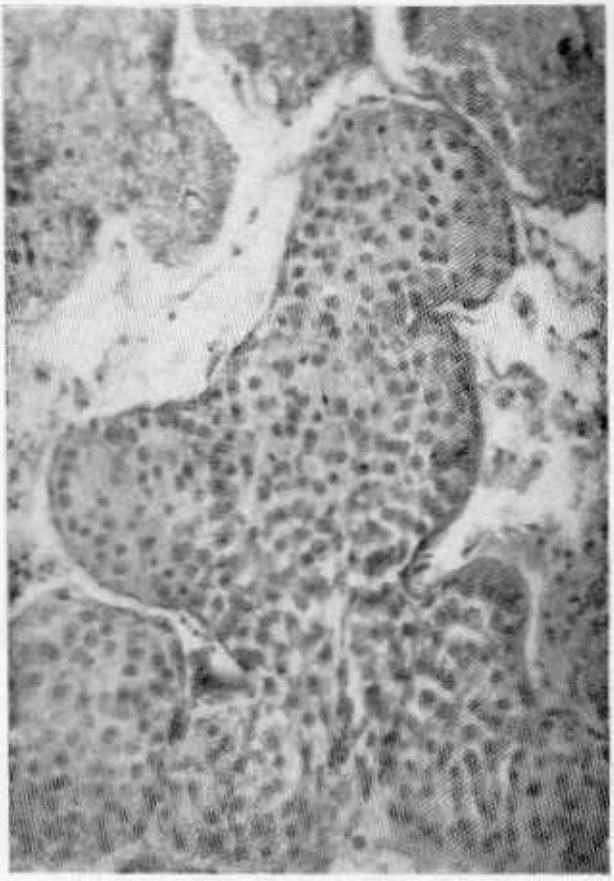


Figure 1



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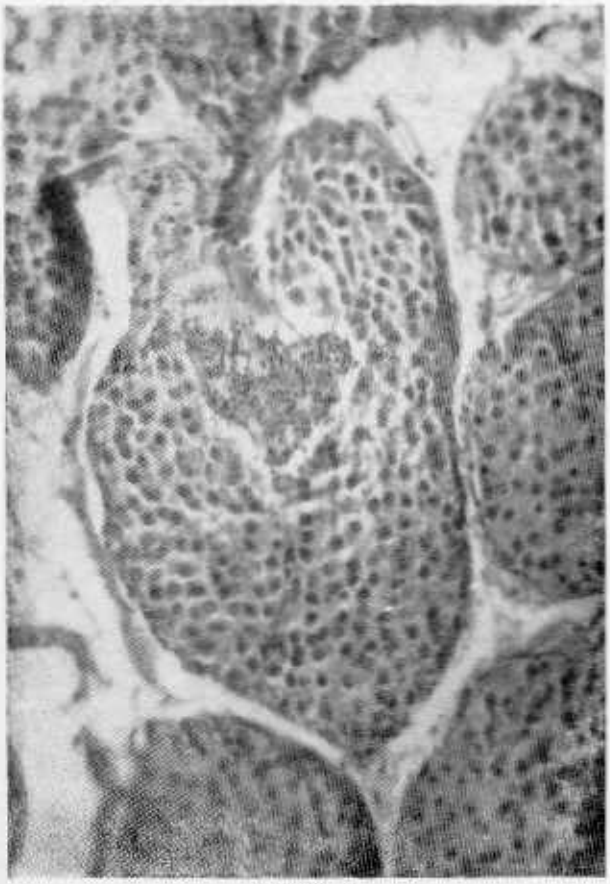

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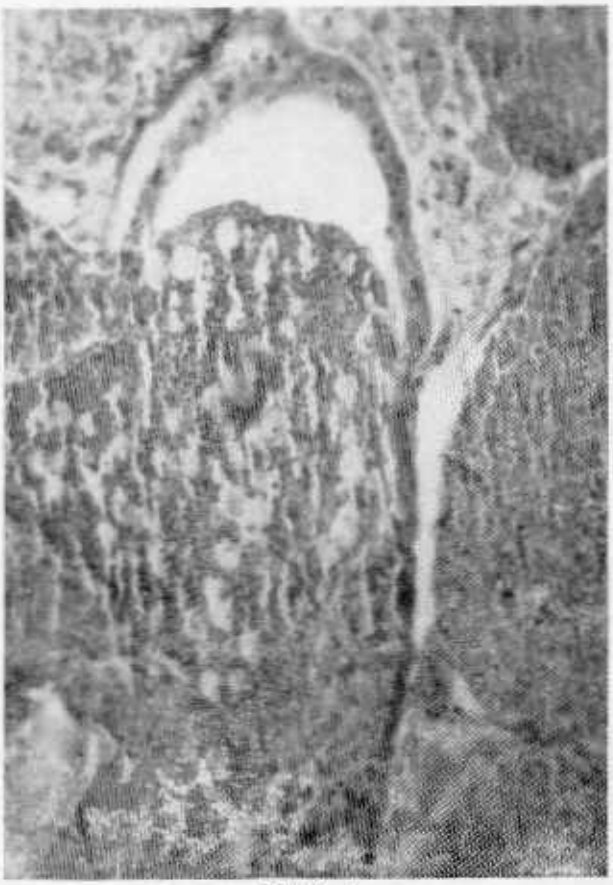

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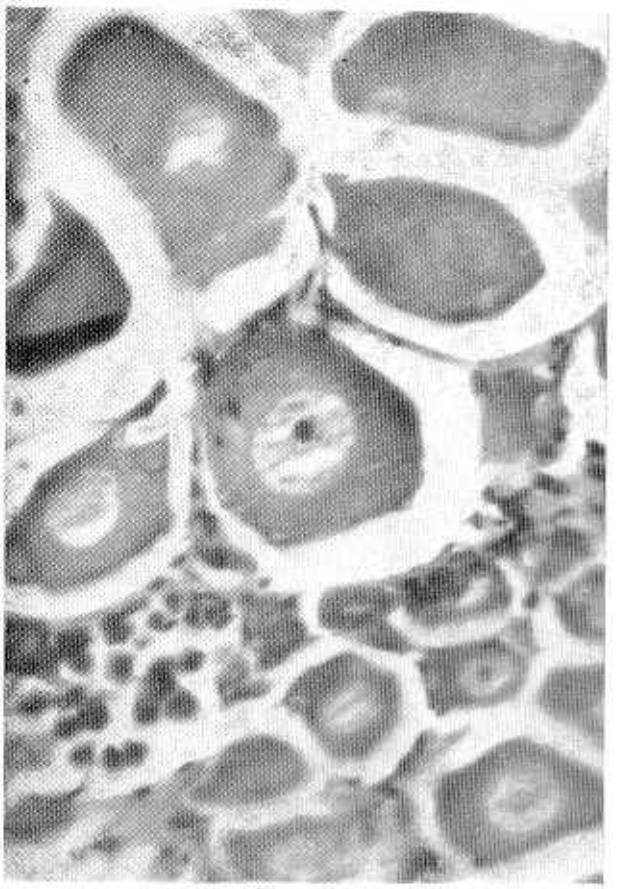
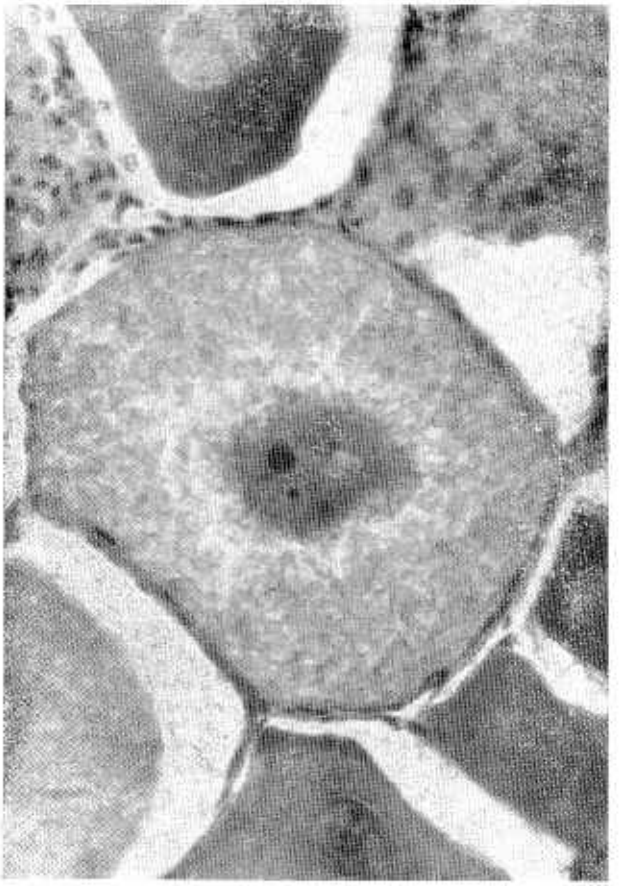


Figure 5

Figure 6



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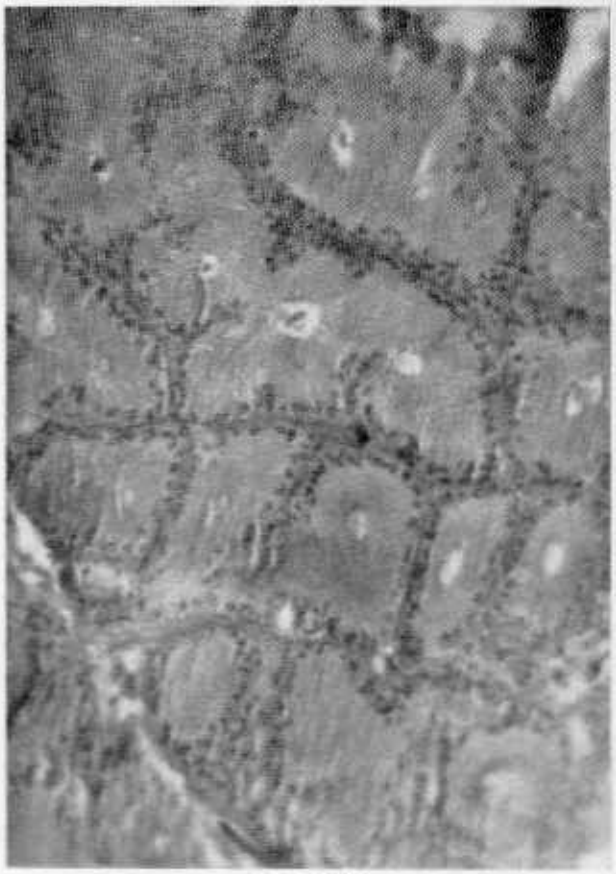

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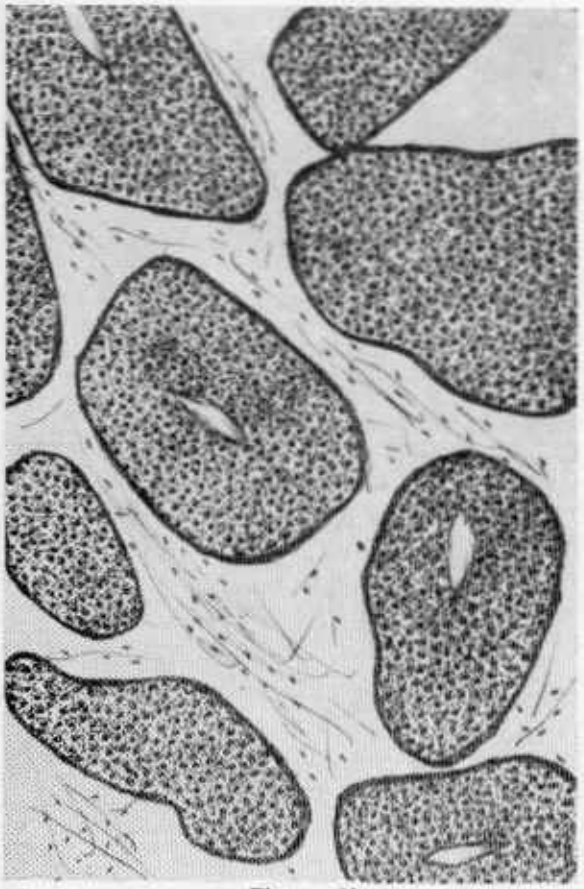

Figure 10

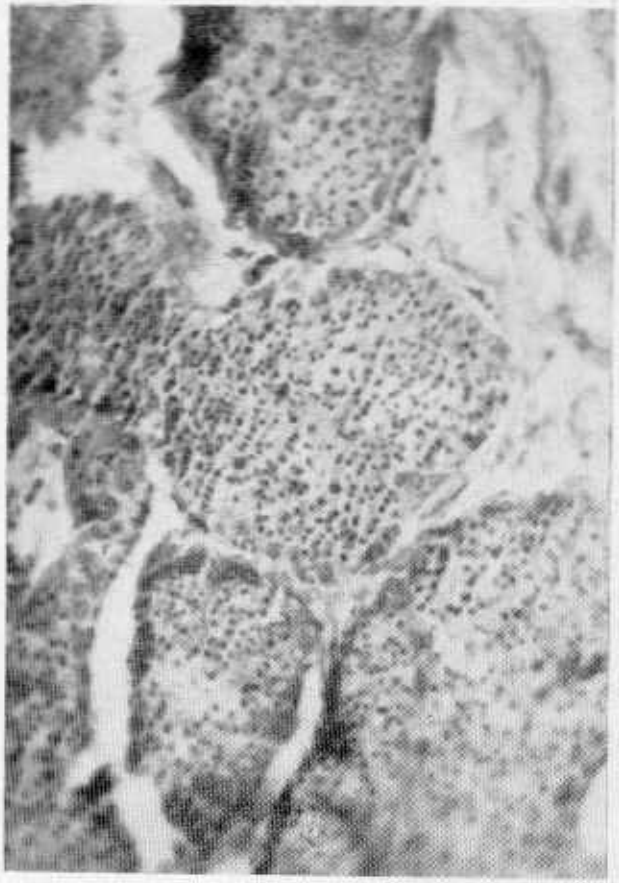

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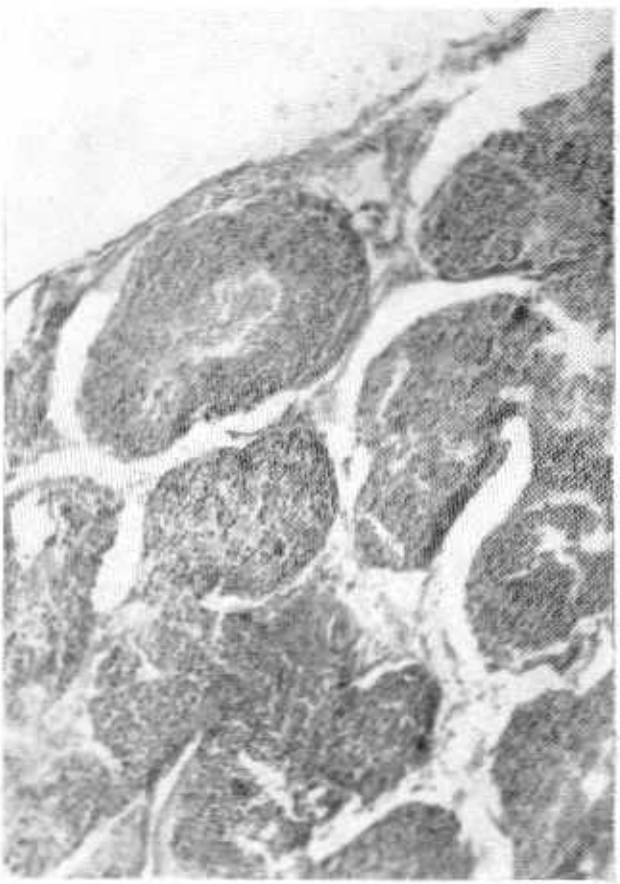
Figure 12



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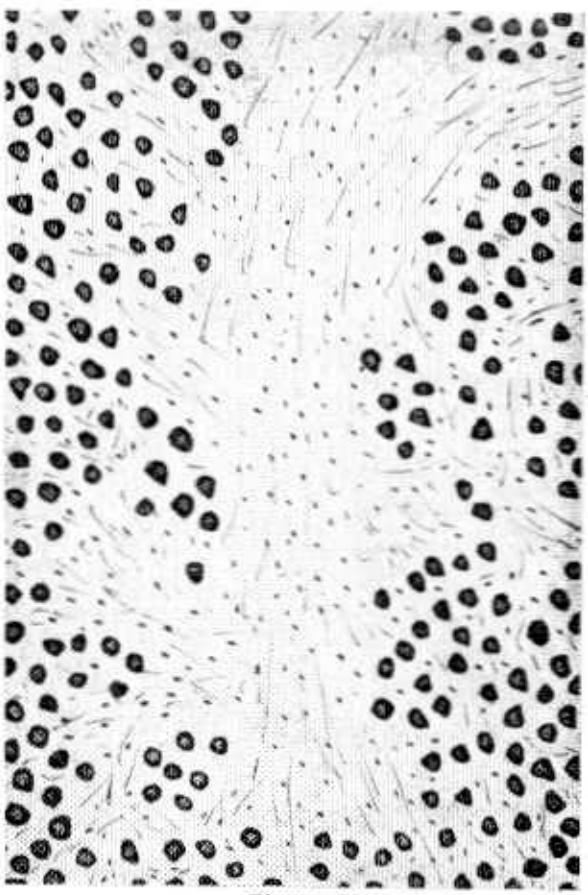

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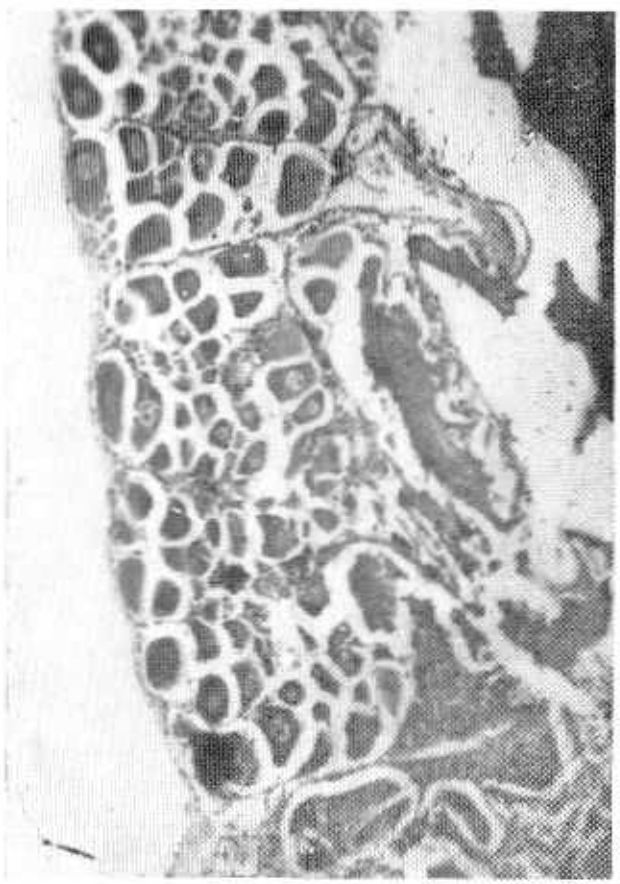

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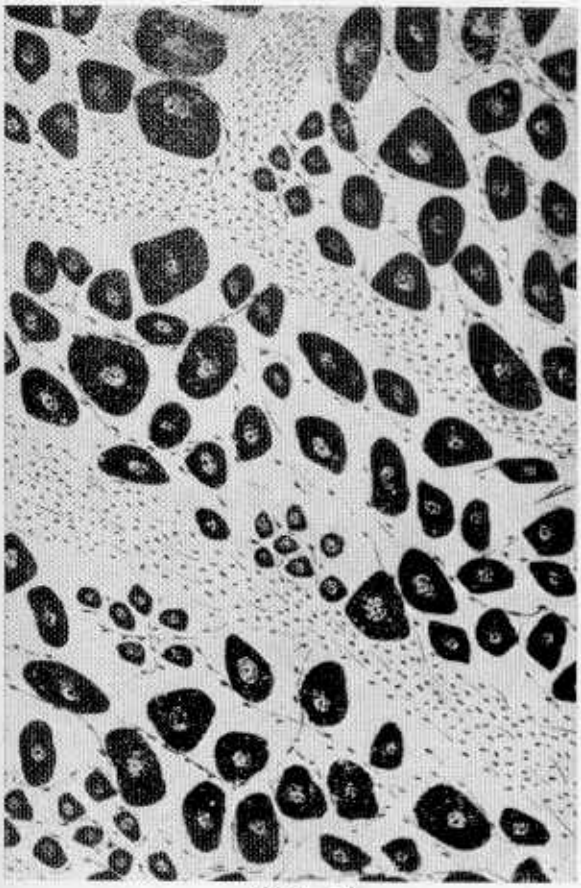

Figure 16

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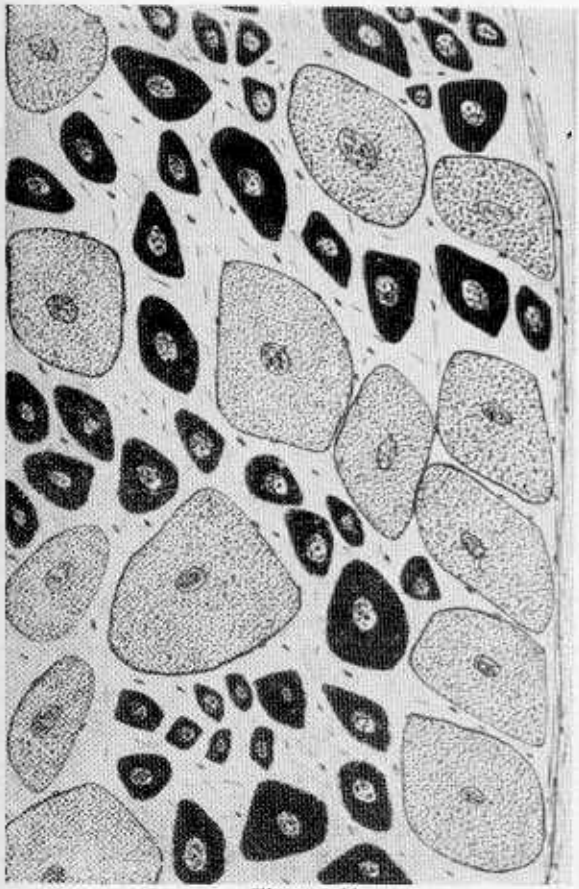

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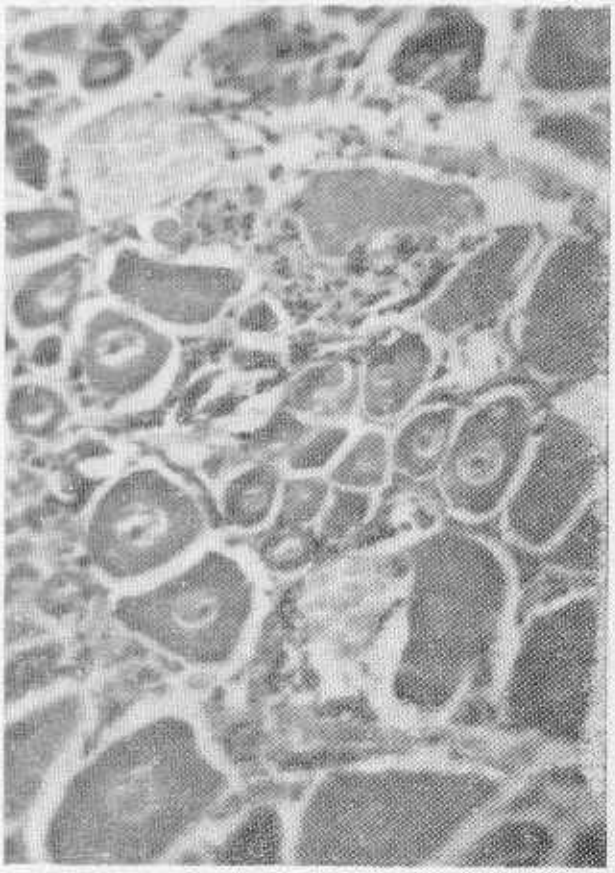

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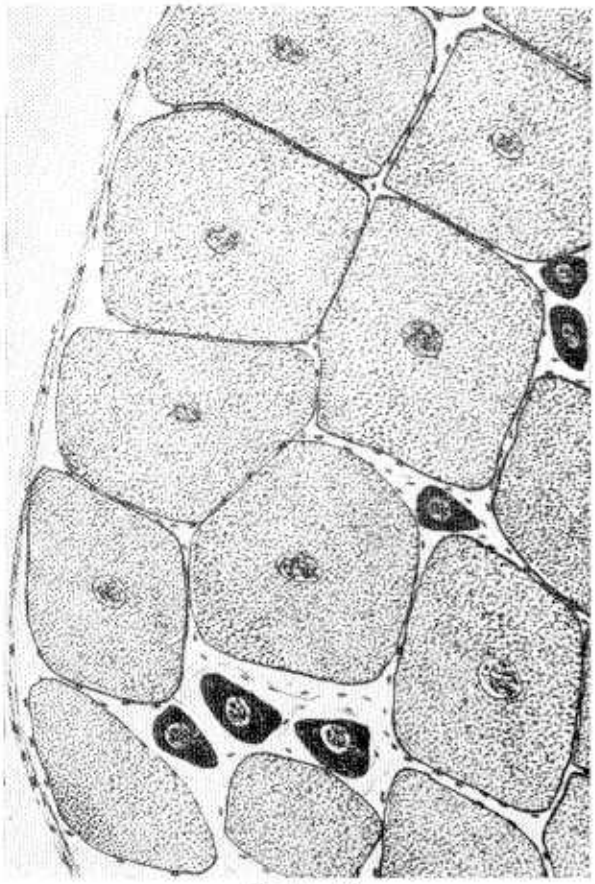

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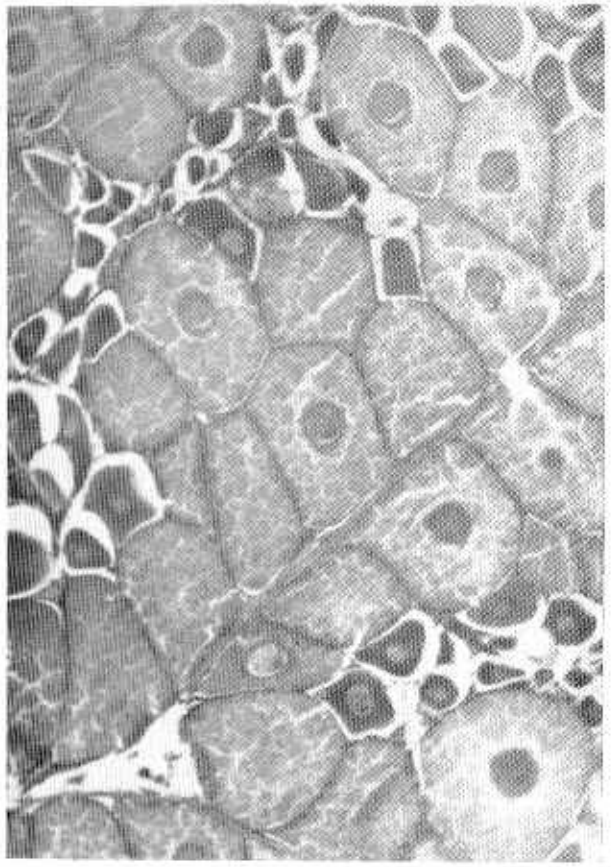

Figure 21

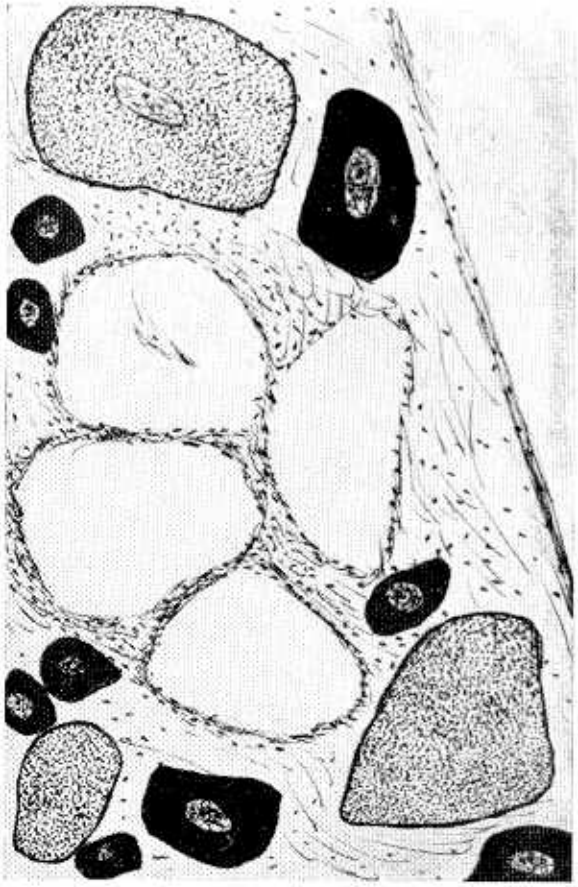

Figure 22

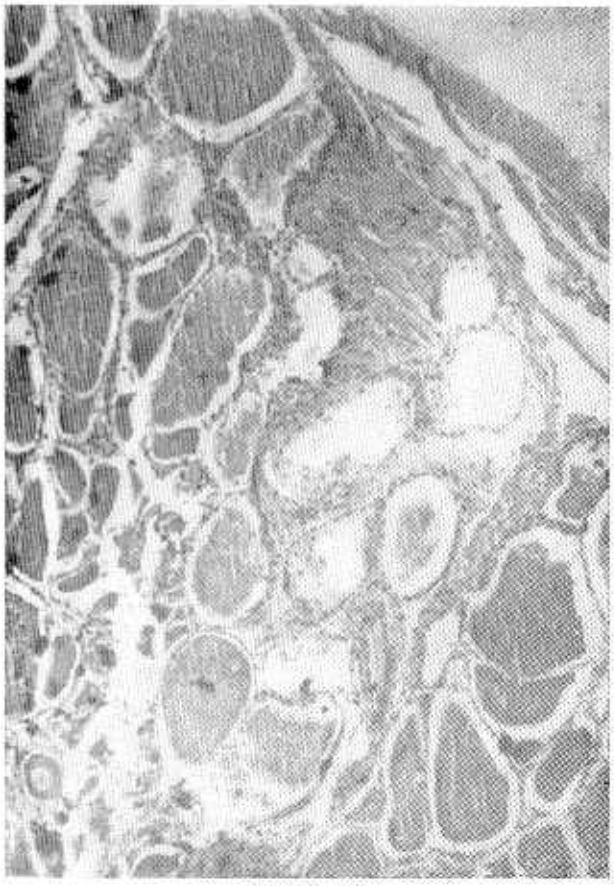

Figure 23