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MORPHOLOGICAL AND HISTOCHEMICAL STUDIES ON THE DEVELOPMENT OF TESTIS AND SPERMATOGENESIS IN THE RUMINAL TREMATODE OF SHEEP, PARAMPHISTOMUM CERVI (DIGENEA: PARAMPHISTOMATIDAE)

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Abstract. Morphological and histochemical changes accompanying testicular development and spermatogenesis have been described in the ruminal trematode *Paramphistomum cervi* during the course of its infection in sheep. Small testes of 4-week-old worms contain a few primordial germ cells. Spermatogonial cells appear first in 6-week-old worms and increase in number by the 10th week when the testes become large and follicular. Mature spermatozoa appear in the testes of 16-week-old worms. General pattern of spermatogenesis is the same as described for other trematodes, i.e. a single spermatogonium gives rise to 32 spermatozoa. Cytophore is formed at secondary spermatogonial stage. Various spermatogenic stages contain proteins, glycogen and phospholipids, however, the amount of phospholipids decreases as the maturation proceeds. The mature spermatozoa stain strongly with PAS. The enucleated residual mass contains HgBB-positive proteins and lipid granules. The morphological and cytochemical changes occurring during various spermatogenic stages have been correlated with corresponding histoenzymological changes in the cytoplasm. Frequency of spermatogenesis has been worked out which indicates that the transformation of secondary spermatogonia into spermatids is relatively fast as compared to transformation of primary spermatogonia into secondary spermatogonia and stages of spermateleosis.

The trematode spermatogenesis shows interesting features in a sense that the cytoplasmic bridges link the spermatogenic cells and the spermatozoa develop in a rosette of 32 spermatids (Gresson 1958, Gresson and Perry 1961, Sato et al. 1967, Burton 1972, 1973, Halton and Hardcastle 1976, Nollen and Pyne 1979, Rees 1979). Previous studies on spermatogenesis in trematodes deal mainly with cytological changes. Information is scarce regarding the chemical and metabolic features of different stages (Guraya and Gupta 1970) for the understanding of which detailed histological, histochemical and histoenzymological studies are necessary. Such studies have now been carried out in *Paramphistomum cervi*, as little information is available on the spermatogenesis in paramphistomes. The frequency of spermatogenesis in the testes of mature *P. cervi* has been described in detail. The worms attain maturity at 16 weeks of their in vivo development in the definitive host, but the testicular rudiments appear in one-week-old worms (Gupta 1982). Therefore, it is of interest to describe the cellular changes during the testicular development along with the spermatogenesis in *P. cervi*.

MATERIAL AND METHODS

Paramphistomum cervi of 4, 6, 8, 10 and 16 weeks were raised according to Gupta et al. (1983). Adult specimens of *P. cervi* were collected in cold saline from the rumen of sheep slaughtered at local slaughter house. They were thoroughly washed in saline and fixed immediately in alcoholic Bouin's, Carnoy's fluid and calcium formol. 5—7 μm thick paraffin sections were used for histo-

logical as well as histochemical preparation of proteins and carbohydrates. Frozen 10—14 μm thick sections were used for various tests for lipids and enzymes with appropriate controls. Paraffin sections were stained with haematoxylin-eosin (Humason 1979), iron-haematoxylin (Bird 1971) and with polychrome (Veterling and Thompson 1972) for general histology. Various histochemical and histoenzymological tests were used (Pearse 1972, Chayan et al. 1973, Lojda et al. 1979). The % frequency of various spermatogenic cells was worked out according to Guraya and Bilaspuri (1976).

RESULTS

DEVELOPMENT OF TESTES DURING THE COURSE OF INFECTION

The germinal mass appeared in the posterior region of the worms before 4 weeks. In 4-week-old worms a pair of small testes, lying close to the posterior sucker, contained few morphologically similar primordial germ cells (Fig. 1A). Their large nuclei contained condensed chromatin material. Thin testicular coat of 4-week-old worms was syncytial at 6 weeks and showed intense staining for proteins and lipids. The number and size of primordial germ cells also increased. At this stage, few large cells corresponding to the spermatogonial cells of mature testes appeared in the centre of the testes (Fig. 1B). Their cytoplasm contained HgBB-positive material. The number of spermatogonial cells increased in the testes of 8-week-old worms (Fig. 1C). The testes in (Fig. 1D) 10-week-old worms increased in size and became follicular in shape. Grouping of spermatogonial cells occurred at this stage. Their cytoplasm stained for HgBB and

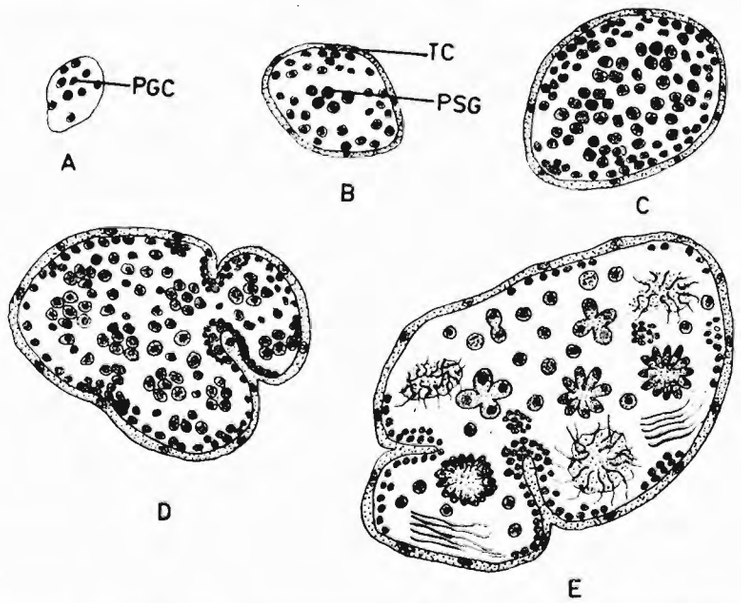


Fig. 1. Schematic representation of development of testis. A — Testis of 4-week-old worms containing for primordial germ cells (PGC). B — Testis of 6-week-old worms covered by testicular coat (TC). Few primery spermatogonia (PSG) also appear. C — Number of primary spermatogonia increases in 8-week-old worms. D — In 10-week-old worms grouping of primary spermatogonial cells begins. E — Mature testis of 16-week-old worms containing spermatogenic cells at various stages of spermatogenesis as well as mature spermatozoa.

PAS-positive material. The large follicular testes at 16th week showed deep invaginations of the testicular coat (Fig. 1E) along with various stages of spermatogenesis and mature spermatozoa, comparable to the testes of adult worms.

MORPHOLOGY AND HISTOCHEMISTRY OF TESTES OF MATURE WORMS

Testicular coat

The testis of mature worm was surrounded by a thick syncytial coat which contained several round to oval nuclei with granular chromatin material and was guarded on either sides by fibrous layers. The testicular coat stained strongly for proteins with free and bound NH_2 groups, carbohydrates containing glycogen and mucopolysaccharides and sudanophilic neutral lipids. It showed weak activities of acid phosphatases, alkaline phosphatase, 5'-nucleotidase and glucose-6-phosphatase, moderate activities of lactate dehydrogenase, glycerophosphate dehydrogenase, glucose-6-phosphate dehydrogenase and strong activities of adenosine triphosphatase, esterase, tetrazolium reductase, isocitrate dehydrogenase, glyceraldehyde dehydrogenase and malate dehydrogenase.

Primordial germ cell (PGC)

They were smallest of all cell types located mostly along the testicular coat and its invaginations (Plate I, A) and in the small groups among the rosettes of spermatogenic cells (Plate II, C). Their round to oval nuclei contained compactly arranged chromatin material staining with Feulgen and methyl green. Some of the PGC showed increased dimensions with relatively large nuclei containing dispersed chromatin material (Fig. 2) and a distinct nucleolus and were considered as differentiating spermatogonial cells.

Primary spermatogonia (PSG)

Round primary spermatogonia occurred mainly in the peripheral region of the testis. On the basis of chromatin morphology, five different stages of primary spermatogonia have been identified (Fig. 2):

PSG_1: Each PSG_1 possessed a nucleus with a distinct nuclear envelope and thread-like chromatin material (Fig. 2, Plate I, B). The chromatin material stained lightly with Feulgen and green with methyl green.

PSG_2: The nuclear envelope disappeared (Fig. 2, Plate I, C). Condensed chromatin material in the centre stained violet with methyl green-pyronin.

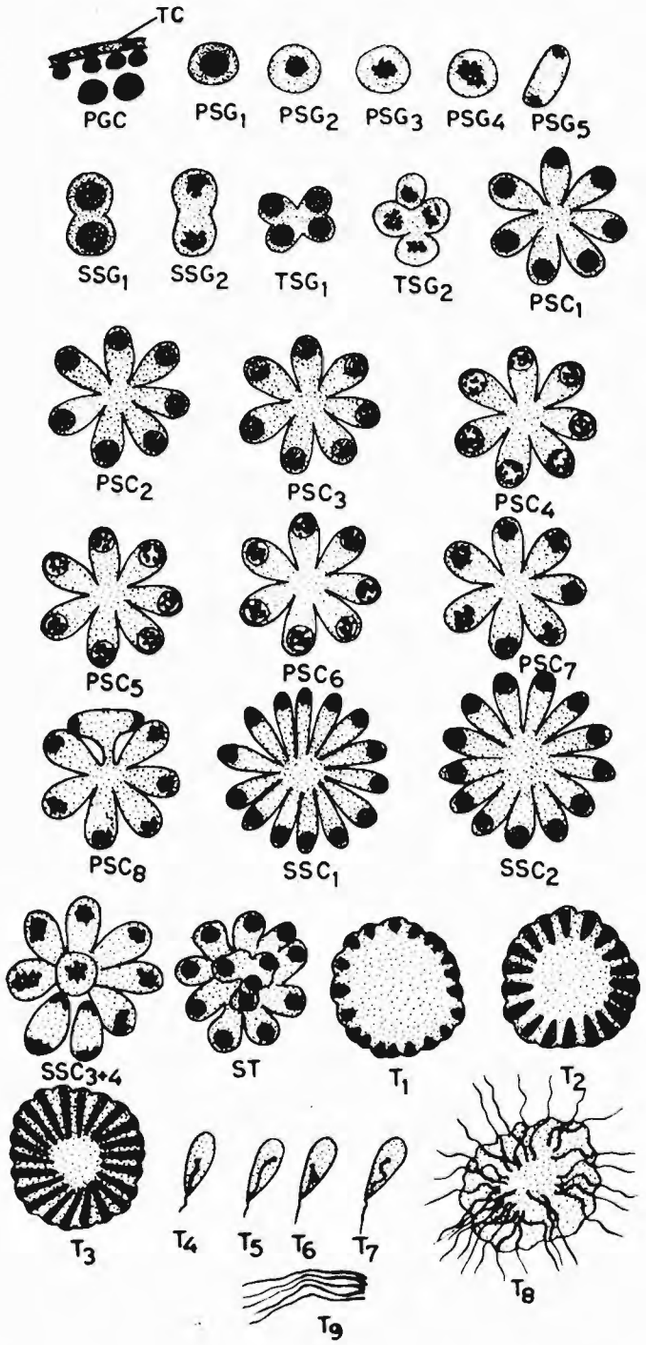
PSG_3: The chromatin material was further condensed (Fig. 2, Plate I, D). It stained strongly with Feulgen and methyl green-pyronin.

PSG_4: Distinct-rod shaped chromosomes, corresponding to metaphase of mitosis were present in the centre (Fig. 2, Plate I, E). Their staining behaviour remained similar to PSG_3 chromosomes.

PSG_5: They were characterized by anaphase of mitosis (Plate I, F). As compared to PSG_3 and PSG_4 their chromosomes showed decreased affinity for Feulgen and methyl green-pyronin.

Histochemical features of primary spermatogonia

Various histochemical features of primary spermatogonia were summarized in Table 1. Their basophilic cytoplasm contained proteins with free and bound NH_2 groups, few PAS-positive glycogen granules and sudanophilic substance showing histochemical reactions for phospholipids.



Secondary spermatogonia (SSG)

The completion of first mitosis resulted in the formation of two cells with incomplete cytokinesis marking the end of primary spermatogonial stage. The initiation of second mitotic division marked the beginning of the secondary spermatogonial stage. Two round SSG were joined in the centre by a broad cytoplasmic bridge (Fig. 2). They were divided into two stages:

SSG_1: Round nucleus of SSG_1 contained thread-like chromatin material (Plate I, G) staining weakly with Feulgen and methyl green.

SSG_2: Condensed rod-like chromosomes (Plate I, H) stained darkly with Feulgen and violet with methyl green-pyronin.

Except for the above stages, other stages of SSG could not be seen, which appeared to be of short duration. The cytoplasm of secondary spermatogonia stained for proteins and weakly for glycogen and lipids (Table 1).

Tertiary spermatogonia (TSG)

The completion of second mitotic division resulted in the formation of four tertiary spermatogonia joined with each other through the cytoplasmic bridges (Fig. 2). Two stages of TSG were distinguished:

TSG_1: They had a round prominent nucleus with intact nuclear envelope (Plate II, A). Thread-like chromatin material stained weakly with Feulgen and methyl green.

TSG_2: The nuclear envelope disappeared. The chromosomes showed metaphase configuration (Plate II, B). They stained strongly with Feulgen and violet with methyl green-pyronin.

Other stages of third mitotic division could not be observed. The histochemical features of the cytoplasm of tertiary spermatogonia remained similar to secondary spermatogonia (Table 1).

Primary spermatocytes (PSC)

The completion of the third mitotic division in four TSG resulted in a cluster of eight primary spermatocytes which were joint with each other through cytoplasmic bridges. Each PSC was conical in shape with outer spherical body tapering into a cytoplasmic bridge, which was joined with similar extensions of other seven PSC cells (Fig. 2). PSC could be divided into eight stages:

PSC_1: The large-sized nucleus present at the broader end of the primary spermatocyte contained a distinct nucleolus and evenly distributed granular chromatin (Plate II, C) staining weakly with Feulgen and green with methyl green. All cells of the cluster were at interphase.

Fig. 2. Schematic representation of process of spermatogenesis. Primordial germ cells (PCC) are present near the testicular coat (TC). Note few large PGC. They appear to represent a stage between PGC and primary spermatogonia (PSG). Five stages of primary spermatogonia (PSG_1, PSG_2, PSG_3, PSG_4 and PSG_5) have been recognized on the basis of their cytomorphology. Each PSG divides to give rise to two secondary spermatogonia (SSG_1 and SSG_2) which in turn divide to form four tertiary spermatogonia (TSG_1 and TSG_2). Four TSG divide to form eight primary spermatocytes (PSC) which can be seen in interphase (PSG_1), leptotene (PSC_2), zygotene (PSC_3), pachytene (PSC_4), diplotene (PSC_5), diakinesis (PSC_6), metaphase (PSC_7) and anephase (PSC_8). Eight PSC divide to form sixteen secondary spermatocytes (SSC) which can be seen at interphase (SSC_1), prophase (SSC_2), metaphase (SSC_3) and anaphase (SSC_4). Sixteen SSC divide to form 32 spermatids (ST). The process of spermateleosis involves the condensation and elongation of nucleus (T_1 to T_8) which leads to the formation of mature spermatozoa (T_9).

PSC_2: The chromatin material of each PSC_2 started condensing and appeared in the form of small dots. In addition to Feulgen positive chromatin dots, few pyronin-positive patches appeared in the nucleus.

PSC_3: The chromatin material moved toward one side of the nucleus. The staining affinity of chromatin material with Feulgen increased and the patches stained violet with methyl green-pyronin. All the cells of the cluster showed similar cytological features.

PSC_4: Distinct chromosomes, staining strongly with Feulgen and violet with methyl green-pyronin, were formed.

PSC_5: Some PSC_5 of the rosette showed diplotene configuration, while other remained in pachytene stage indicating that synchrony among different cells of the group breaks at this stage. The chromosomes stained strongly with Feulgen and violet with methyl green-pyronin.

PSC_6: Thick chromosomes appeared in the form of tetrads. Though most of the cells in the rosette were at diakinesis, a few cells still showed pachytene stage. Staining behaviour of the chromosomes remained similar to PSC_6 chromosomes.

PSC_7: Thick rod-shaped chromosomes were present in the centre of the broader end of the PSC_7 (Plate II, D).

PSC_8: The chromosomes moved towards the poles and stained darkly with Feulgen and violet with methyl green-pyronin. Though spindle fibres were not visible but from the location of the chromosomes, it was clear that the plane of division was parallel to the long axis of primary spermatocytes. The amounts of glycogen increased in primary spermatocytes (Table 1). First meiotic division in primary spermatocytes gave rise to a rosette of sixteen secondary spermatocytes.

Secondary spermatocytes (SSC)

Sixteen cone-shaped secondary spermatocytes were joined to each other by their narrower ends (Fig. 2) in a rosette. They could be divided into four stages:

SSC_1: The round and small sized nucleus was displaced towards the broader end of the SSC (Plate II, E). It contained a single pyroninophilic nucleolus and condensed chromatin material staining weakly with Feulgen and green with methyl green.

SSC_2: The chromatin material becomes thread-like and violet patches staining with methyl green-pyronin appeared in the nucleus.

SSC_3: The nuclear envelope and the nucleolus disappeared. Rod-shaped chromosomes were present in the centre of the cells. They stained darkly with Feulgen and violet with methyl green-pyronin.

SSC_4: The chromosomes moved toward the opposite poles and stained darkly with Feulgen and violet with methyl green-pyronin. From the location of the chromosomes, it appeared that the plane of division is at right angle to the long axis of the secondary spermatocytes.

The histochemical features of SSC were similar to PSC (Table 1), however, there was a slight decrease in the amount of proteins. Second meiotic division in SSC resulted in the formation of a rosette of 32 similar spermatids.

Spermatids (ST)

They were club-shaped, with broader distal and narrower proximal ends by which they were joined to other ST (Fig. 2). They contained nuclei at their broader ends. All the thirty-two spermatids simultaneously underwent the process of differentiation.

Table 1. Histochemical observations of the cytoplasm of spermatogenic cells of *P. cervi*

Methods	Primordial germ cells	Primary spermatogonia	Secondary spermatogonia	Tertiary spermatogonia	Primary spermatocytes	Secondary spermatocytes	Spermatids	Spermatozoa	Remarks
Mercuric bromophenol blue (HgBB)	+++	+++	+++	+++	+++	++	++	++	Shows the presence of proteins
Ninhydrin Schiff	+	++	++	++	++	++	++	+	Presence of proteins with free-NH_2 groups
Chloramine T-Schiff	++	++	++	++	++	++	++	+	Presence of proteins with bound-NH_2 groups
Periodic acid-Schiff (PAS)	+	+	+	+	+	+	+	+++	Shows the presence of carbohydrates
Best's carmine	+	+	+	+	++	++	++	++	Small amount of glycogen
Colloidal iron	–	–	–	–	–	–	–	–	Absence of acid mucopolysaccharides
Alcian blue pH 2.5	–	–	–	–	–	–	–	–	Absence of acid mucopolysaccharides
Sudan black B (SBB)	+++	++	++	++	++	++	++	+	Presence of lipids
Acid haematin	+++	++	++	++	++	++	++	+	Presence of phospholipids
Nile blue sulphate	+++	++	++	++	++	++	++	+	Presence of phospholipids
Oil Red 0	+++	++	++	++	++	++	++	+	Presence of neutral lipids
Methyl green-pyronin	+++P	+++P	+++P	++P	++P	++P	++P	++G, H	Presence of basophilia
Feulgen	–	–	–	–	–	–	–	+P, H	Presence of DNA

Key to abbreviations: – no activity, + weak activity, ++ moderate activity, +++ strong activity, G green, H head, P pink

Spermateleosis

During the process of differentiation, the nucleus started elongating. The cytoplasm of each spermatid also elongated peripherally accommodating the lengthening of the nucleus. From each cytoplasmic tip a strand began to form, which became progressively longer and was retained by the mature spermatozoon as flagellum (Fig. 2). The process of spermateleosis could be divided into nine stages:

T_1 — The nucleus containing condensed chromatin material moved toward the broader distal end (Plate II, F).

T_2 — The nucleus started elongating. It was broader towards the broader end of the spermatid and narrow towards the central cytophore (Plate II, G).

T_3 — The nucleus elongated further and reached up to the centre of the spermatid (Plate II, H).

T_4 — The elongating nucleus became elongated and curved (Plate III, A). A small tail appeared.

T_5 — The nucleus elongated further (Plate III, B).

T_6 — The elongating nucleus showed coiling (Plate III, C). The size of the tail increased.

T_7 — The elongated nucleus started straightening but still showed bends in it (Plate III, D).

T_8 — The spermatozoon attained its final shape but was still present in the form of rosette (Plate III, E).

T_9 — As the process of spermateleosis was completed, the nucleus straightened and each spermatozoon emerged from a cytoplasmic mass (Plate III, F).

Till T_5 stage, the heads of the developing spermatogonia were directed toward the centre of the cytophore. During the latter stages, the sperm heads showed bendings and coilings which resulted in the change in their orientation. In T_6 and T_7 stages, some of the sperm heads were lying parallel to the periphery of the rosette. Further straightening of the sperm heads changed their orientation, as a result of which the sperm heads were directed towards the centre and tail outer to the rosette.

The enucleated residual mass contained HgBB-positive and lipid granules. The residual mass disintegrated rapidly. It showed strong activity of esterase and weak to moderate activities of alkaline and acid phosphatases.

Features of spermatozoa

The testicular spermatozoa occurred in bundles as well as separately (Plate III, G). It appeared that after emerging from the rosette, spermatozoa separated, as they were seen scattered among the spermatogenic cells.

The mature spermatozoon was 20—30 μm long. It consisted of an elongated methyl green and Feulgen-positive head and a Feulgen-negative tail. The anterior end of the head was blunt while the posterior end appeared to taper to a point from which the flagellum arised. Mature spermatozoa stained moderately with HgBB and PAS. Spermatozoa present in the vas deferens stained strongly with Best's carmine for glycogen.

HISTOENZYMOLOGY

Histoenzymological features of various stages of spermatogenic cells and the spermatozoa present in the testes, were summarized in Table 2. The spermatozoa present in the vas deferens showed weak activities of alkaline phosphatase, ATPase, 5'-nucleotidase, GPD, GLD, NADH-diaphorase, SDH, MDH and moderate activities of acid phosphatase, alkaline phosphatase (Plate IV, A), and ATPase (Plate IV, C). Strong activity of

Table 2. Histoenzymological observations of the spermatogenic cells of *P. cerri*

Enzymes	Substrates	Reference	Spermatogonia	Primary spermatocytes	Secondary spermatocytes	Spermatids	Spermatozoa
Acid phosphatase	Sodium β-glycerophosphate	Pearse 1972	+	+	+	+	+
Alkaline phosphatase	Sodium β-glycerophosphate	Pearse 1972	+	+	+	+	+
Adenosine triphosphatase (ATPase)	Adenosine triphosphate	Pearse 1972	+	+	+	+	+
Esterase	α-naphthyl acetate	Chayan et al. 1973	−	−	−	−	−
NADH-diaphorase	NAD	Pearse 1972	++	+	+	+	+
Malate dehydrogenase (MDH)	Disodium malate	Lojda et al. 1979	+++	++	++	++	+
Succinate dehydrogenase (SDH)	Disodium succinate	Lojda et al. 1979	++	+	+	+	+
Glycerophosphate dehydrogenase (GPD)	Glyceraldehyde-3-phosphate	Pearse 1972	+++	++	++	++	+
Glyceraldehyde dehydrogenase (GLD)	Sodium-L-glutamate	Pearse 1972	+++	++	++	+	+

Key to abbreviations: − no activity, + weak activity, ++ moderate activity, +++ strong activity

esterase was seen in the testicular wall (Plate IV, B) and strong to moderate activities of NADH diaphorase (Plate IV, D), MDH (Plate IV, E), GLD (Plate IV, F) and SDH were observed in spermatogenic cells.

FREQUENCY OF SPERMATOGENESIS

The frequency of occurrence of different stages of spermatogenesis had been tabulated in Table 3. When the data was analysed statistically, no variability was found as far as the frequency of various stages of spermatogenesis in different worms was concerned.

Table 3. Percentage frequency of various stages of spermatogenesis

Stages of spermatogenesis		1	2	3	4	5	Total	% frequency	Total % frequency
Primary spermatogonia	PSG_1	52*	14	45	57	65	233	26.51	
	PSG_2	15	12	4	10	14	55	6.26	
	PSG_3	3	28	6	3	2	42	4.78	44.49
	PSG_4	3	9	8	4	3	27	3.07	
	PSG_5	9	4	12	3	6	34	3.87	
Secondary spermatogonia	SSG_1	4	3	3	3	6	19	2.16	3.07
	SSG_2	2	2	1	2	1	8	0.91	
Tertiary spermatogonia	TSG_1	9	9	17	9	6	50	5.69	7.28
	TSG_2	3	2	2	4	3	14	1.59	
Primary spermatocytes	PSC_1	2	1	4	4	6	17	1.93	4.09
	PSC_7	4	4	2	5	4	19	2.16	
Secondary spermatocytes	SSC_1	4	7	3	6	3	23	2.62	2.62
Spermatids	ST	5	4	5	7	6	27	3.07	3.07
Spermateleosis	T_1	3	3	3	4	3	16	1.82	
	T_2	4	3	3	4	3	17	1.93	
	T_3	7	2	2	4	7	22	2.50	
	T_4	7	7	5	6	7	32	3.64	35.38
	T_5	6	4	4	5	4	23	2.62	
	T_6	2	4	8	4	3	21	2.30	
	T_7	4	2	4	3	5	18	2.05	
	T_8	15	8	15	15	14	57	6.48	
	T_9	24	19	25	13	24	105	11.95	

* Mean of ten replications (for all readings)

ANNOVA

Source of error	d.f.	S.S.	M.S.S	Fcal.	Ftab. at 5 %
Worms	4	50.9	12.73	0.46	2.48
Stages	21	9366.82	446.04	15.95	
Error	84	2394.5	27.97		
Total	109				

This showed that the frequency of spermatogenesis was similar in all the worms. However, statistically, the variability between the various stages of spermatogenesis in the same worm was found to be highly significant. If we see the total % frequency of various stages, it is quite clear that primary spermatogonia took more time to divide to form secondary spermatogonia. Thereafter, the divisions occurred quite rapidly till spermatids were formed. The % frequency of various stages during the process of spermatogenesis was very high, as compared to other stages of spermatogenesis, this showed that the spermatids took longer time to differentiate into mature spermatozoa.

DISCUSSION

The basic structural organization of the testes of *P. cervi* and the developmental sequences of spermatozoa agree with the general spermatogenic pattern established so far in digenetic trematodes (Dhingra 1954a, b, 1955a, b, Nez and Short 1957, Gresson 1965, Halton and Hardcastle 1976). The primary spermatogonia through three mitotic and two maturation (meiotic) divisions form thirty two spermatids, which differentiate into thirty two spermatozoa.

Cytophore formation, due to incomplete cytokinesis, occurs at secondary spermatogonial stage, as also reported in *Parorchis acanthus* (Rees 1939), *Isoparorchis erythremum* (Dhingra 1954a) and *Haematoloechus medioplexus* (Burton 1960). The significance of cytophore formation in trematodes appears to hasten the process of spermatogenesis as well as the production of male gametes at a lesser consumption of cytoplasm.

Various cytological changes during the spermatogenesis are poorly understood in trematodes. Green staining of the chromatin material with methyl green-pyronin during the initial stages, together with its violet staining afterwards, indicate the degree of polymerization and depolymerization, respectively, of DNA (Pearse 1972). The basophilic cytoplasm of spermatogonia and spermatocytes stains strongly for proteins, the amount of which decreases during the subsequent stages of spermatogenesis. Also in *Diclidophora merlanghi*, the ribosomes are in abundance in the spermatogonia, but their frequency decreases during the latter stages (Halton and Hardcastle 1976). The decreased affinity of the spermatogenic cells for PAS after salivary amylase treatment, together with their affinity for Best's carmine, is suggestive of the presence of glycogen in them. This observations is in agreement with Sharma and Sharma (1980) and also gains support from the observations of Halton and Hardcastle (1976). The glycogen may be required for the general metabolism of the spermatogenic cells. The presence of lipids in the spermatogenic cells, together with their decreased amount in the spermatozoa, gain justification from the fact that actively dividing spermatogenic cells synthesize and need more building material like phospholipids and lipoproteins for the formation of various cellular organelles (Sharma and Sharma 1980, Lehninger 1975) and their apparent decrease during spermateleosis occurs because most of the lipoproteinous organelles, i.e. mitochondria are shifted to the cytoplasmic residue (Guraya and Gupta 1970, Sharma and Sharma 1980).

The elongation of the nucleus during spermateleosis in the longitudinal axis of the spermatid, together with the condensation of the chromatin material into dense coiled lamellae in a spiral, have also been reported by a number of workers (Gresson and Perry 1961, Burton 1972, Halton and Hardcastle 1976, Grant et al. 1976). The mature spermatozoon contains a Feulgen-positive head and a Feulgen-negative tail revealing its nuclear as well as cytoplasmic origin, which supports the observations of other workers (Gresson 1958, Gresson and Perry 1963, Burton 1972, Halton and Hardcastle 1976, Rees 1979).

The reason for the increased affinity of mature spermatozoa present in the vas deferens

for Best's carmine is that the glycol group in the testicular spermatozoa may be masked and as the spermatozoa reach the vas deferens a masking of the glycol group occurs. Threadgold (1975) in *Fasciola hepatica* has reported that the secretion from the cells present in the wall of seminal vesicle may bring about some maturational changes in the spermatozoa. Glycogen in the mature spermatozoa has been reported by various workers (Halton and Hardcastle 1976, Sharma and Sharma 1980), it represents a potential energy source for the use in the absence of external metabolites (Halton and Hardcastle 1976) and is also used during the relatively long distance the sperm travels (Rees 1979).

The activities of various phosphatases in the spermatogenesis cells of *P. cervi* are particularly apparent in the peripheral cell population and their presence may be considered as the site of synthetic activity for extracellular material needed to promote spermatogenic activity (Sharma and Sharma 1980). The moderate activity of acid phosphatase in the spermatozoon, due to its lytic activity, may help it to penetrate the ova during fertilization. The intense activity of esterases, as observed in the testicular coat of *P. cervi*, has also been observed by Halton (1967) and Mandawant and Sharma (1978).

NADH-disphorase, MDH and SDH enzymes are the marker of mitochondrial activity in the cells and the presence of mitochondria has been reported by Halton and Hardcastle (1976) in the spermatogenic cells of *D. merlangi*. Strong to moderate activities of various dehydrogenases observed in spermatogonia and spermatids are suggestive of their high metabolic rate.

An analysis of frequency of spermatogenesis reveals that after primary spermatogonia, the spermatogenic divisions occur rapidly till spermatid stage. Thereafter the process becomes slow. Similar observations have been made by Nollen (1975) in *Hymenolepis diminuta* and Moseley and Nollen (1973) in *Philophthalmus hegenesi*. The study of frequency of spermatogenesis is of significance that it shows the duration of occurrence of various stages of spermatogenesis, which can help to restrict our choice to those chemosterilants.

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МОРФОЛОГИЧЕСКИЕ И ГИСТОЛОГИЧЕСКИЕ ИЗУЧЕНИЯ РАЗВИТИЯ СЕМЕННИКА И СПЕРМАТОГЕНЕЗА У ТРЕМАТОДЫ *PARAMPHISTOMUM CERVI* (DIGENEA: PARAMPHISTOMATIDAE) ПАРАЗИТИРУЮЩЕЙ У ОВЕЦ

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Резюме. Описаны морфологические и гистохимические изменения, сопровождающие развитие семенника и сперматогенез у трематоды *Paramphistomum cervi* в течение заражения овец. Малый семенник трематоды в возрасте 4 недель содержит небольшое количество первичных эмбриональных клеток. Сперматогониальные клетки появляются у трематод в возрасте 6 недель и их количество возрастает до 10-й недели, когда семенник станет большим и фолликулярным. Зрелые сперматозоиды появляются в семеннике трематод в возрасте 16 недель. Общий характер сперматогенеза одинаковый как и у других видов трематод, т. е. из одного сперматогония возникают 32 сперматозоида. Цитофор образуется во второй стадии сперматогенеза. Отдельные сперматогенные стадии содержат белки, гликоген и фосфолипиды, но количество фосфолипидов понижается в течение созревания. Зрелые сперматозоиды сильно окрашиваются методом PAS. Безъядерное остаточное вещество содержит HgBB-положительные и липидные гранулы. Морфологические и цитохимические изменения, встречающиеся в течение разных стадий сперматогенза находятся в корреляции с отвечающими гистоэнзиммологическими изменениями цитоплазмы. Обнаружено, что трансформация вторичных сперматогониев в сперматиды протекает сравнительно быстро, по сравнению с трансформацией первичных сперматогониев и сперматотелеозом.

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L. F. Borgarenko: Gelminty ptits Tajikistana. Kniga 2. Trematody. (Helminths of birds of Tajikistan. Book 2. Trematodes.) *Publ. House Donish, Dushanbe 1984, 210 pp., 94 Figs. Price 3. 10 R.*

After the successful first volume dealing with cestodes, this book is a further part of the series, devoted to trematodes. It is based on the author's 25 years' scientific work during which more than 3,000 specimens of birds have been examined. The author belongs to the pupils of Academician Skryabin and she has written for both the present time and history a scientific work concerning bird trematodes in one of the republics of the USSR.

The trematodes found in birds of Tajikistan belong to 176 species and parasitize 235 species of birds, both wild-living and domestic ones. Hosts, localization within the host body, incidence and intensity of infection, locality and sometimes also distribution in the republics of Central Asia and Kazakhstan are given for each parasite species. Almost all of the trematode species are characterized by a detailed diagnosis with figures. Of importance is the evaluation of morphological-anatomical characters and peculiarities. Even some new hosts of trematodes are reported. The author presents the reader not only the systematics, but also information concerning the life cycles. Due to the large number of intermediate hosts examined (belonging to more than 20 orders of invertebrates), the author managed to elucidate the hitherto unknown life cycle of *Mosesia microstoma* (Sing, 1962) Khotenovsky, 1970. Fourteen trematode species are recorded for the first time in the USSR and 120 species in the territory of Tajikistan.

The material is discussed also from the practical viewpoint, particularly with regard to the fowl and fish breeding. Some species of the bird trematodes (adults or larvae) are evaluated as potential parasites of man. The whole concept and contents of the book suggest that it is another valuable income to the treasury of the world helminthology.

Prof. Dr. F. Tenora, D.Sc.

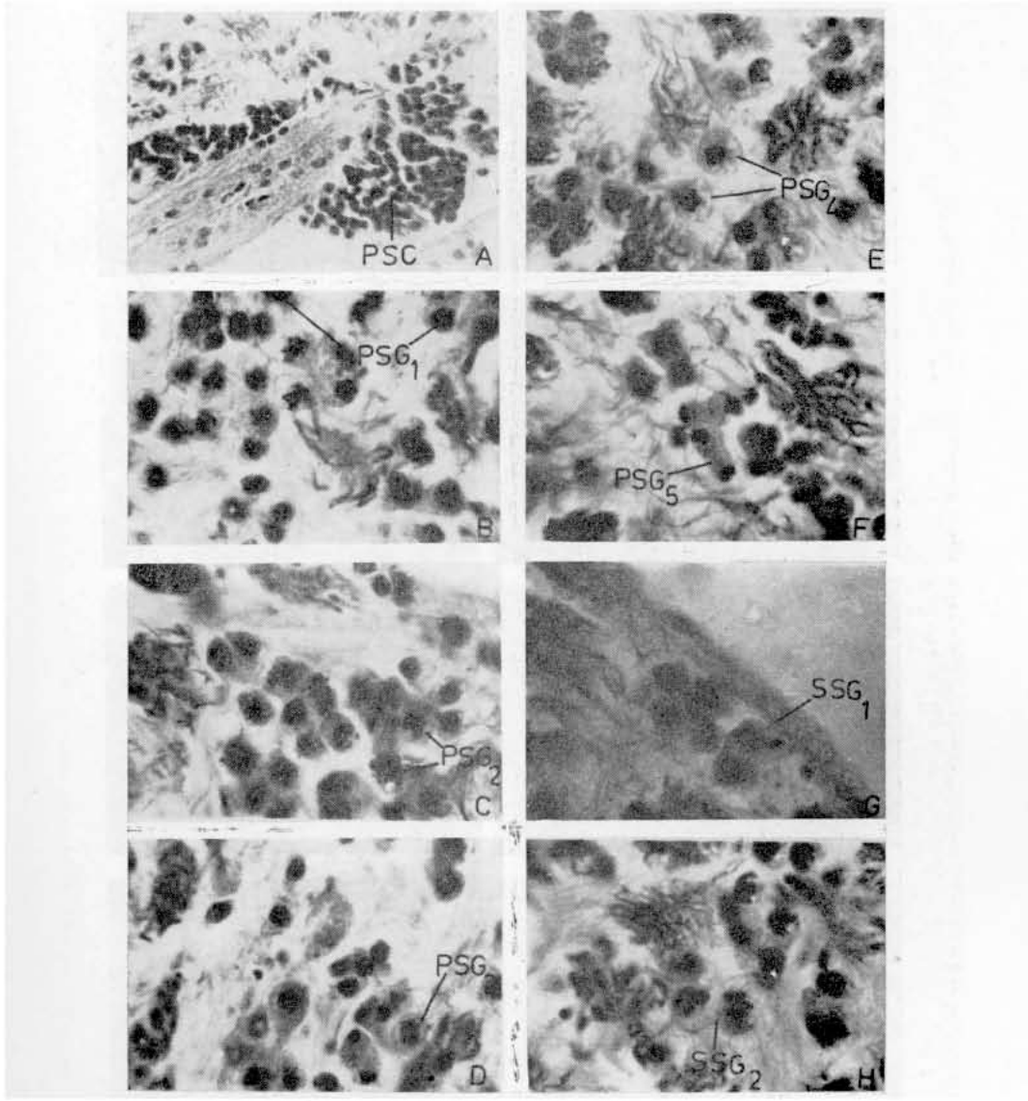

Fig. A. Primordial germ cells (PC) present along the testicular evagination. Note their compact nuclei. Polychrome × 675. **Fig. B.** Stage 1 of the primary spermatogonia (PSG_1). Note the compactly packed chromatin material. Polychrome × 675. **Fig. C.** Stage 2 of the primary spermatogonia (PSG_2). Condensation of chromatin material begins. Polychrome × 675. **Fig. D.** Stage 3 of primary spermatogonia (PSG_3). Note the appearance of distinct chromosomes. Polychrome × 675 **Fig. E.** Stage 4 of primary spermatogonia (PSG_4). Note rod-shaped chromosomes present in the centre of the cells. Polychrome × 675. **Fig. F.** Stage 5 of the primary spermatogonia (PSG_5). Note the chromosomes at opposite poles. Polychrome × 675. **Fig. G.** Stage 1 of the secondary spermatogonia (SSG_1). Note compact chromatin material and the formation of cytophore. Polychrome × 675. **Fig. H.** Stage 2 of the secondary spermatogonia (SSG_2). Note rod-shaped chromosomes. Polychrome × 675.

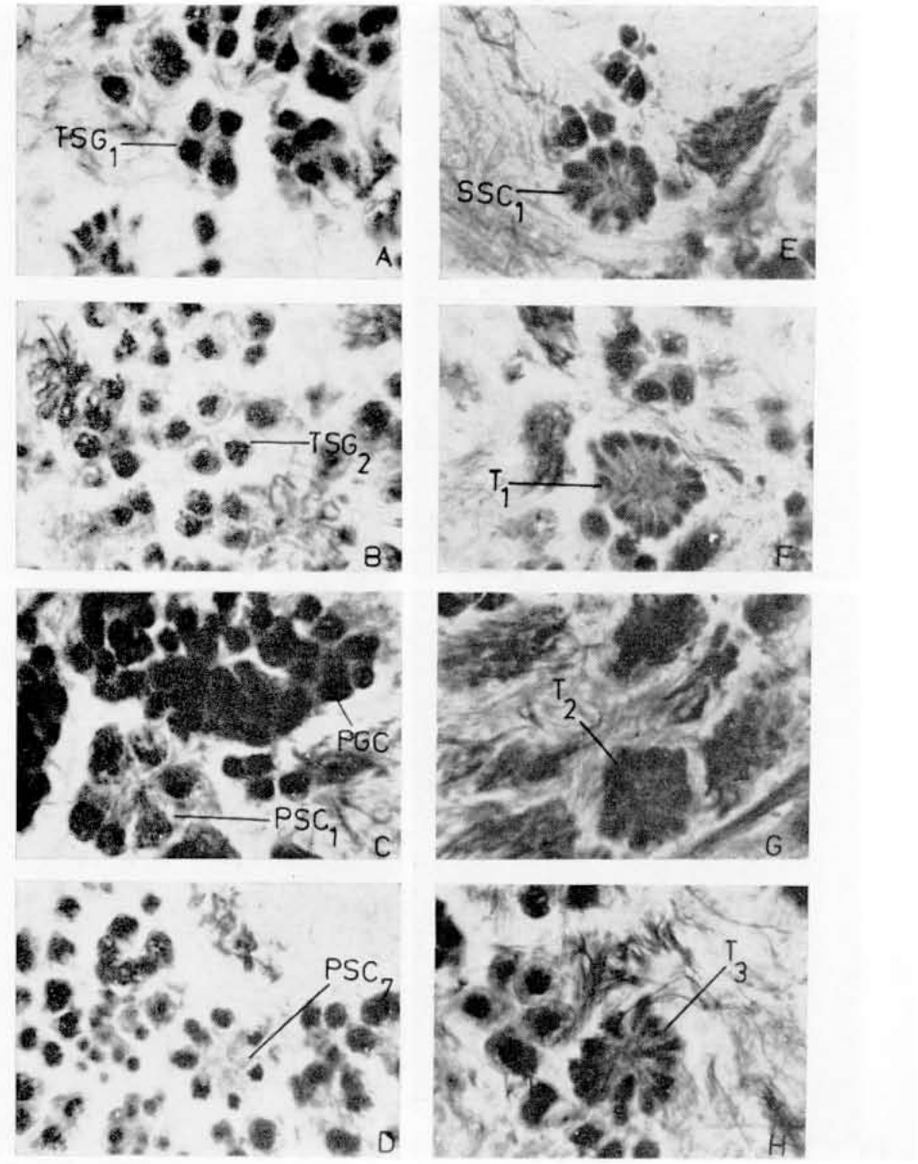

Fig. A. Stage 1 of tertiary spermatogonia (SSG_1). Note their compact chromatin material. Polychrome × 685. **Fig. B.** Stage 2 of tertiary spermatogonia (TSG_2). Note rod-shaped chromosomes. Polychrome × 685. **Fig. C.** Primordial germ cells (PGC) and stage 1 of the primary spermatocytes (PSC_1). Note the interphase nuclei of the PSC. Polychrome × 685. **Fig. D.** Stage 7 of primary spermatocyte (PSC_7). Note the metaphase chromosomes. Polychrome × 685. **Fig. E.** Stage 1 of secondary spermatocyte (SSC_1). Note the compact chromatin material. Polychrome × 685. **Fig. F.** Thirty-two spermatids showing stage 1 (T_1) of spermateleosis. Note their darkly stained nuclei present toward the broader ends. Polychrome × 685. **Fig. G.** Stage 2 of spermateleosis (T_2) showing slight elongation of nuclei. Polychrome × 685. **Fig. H.** Stage 3 of spermateleosis (T_3). Note the elongation nuclei in the centre. Polychrome × 685.

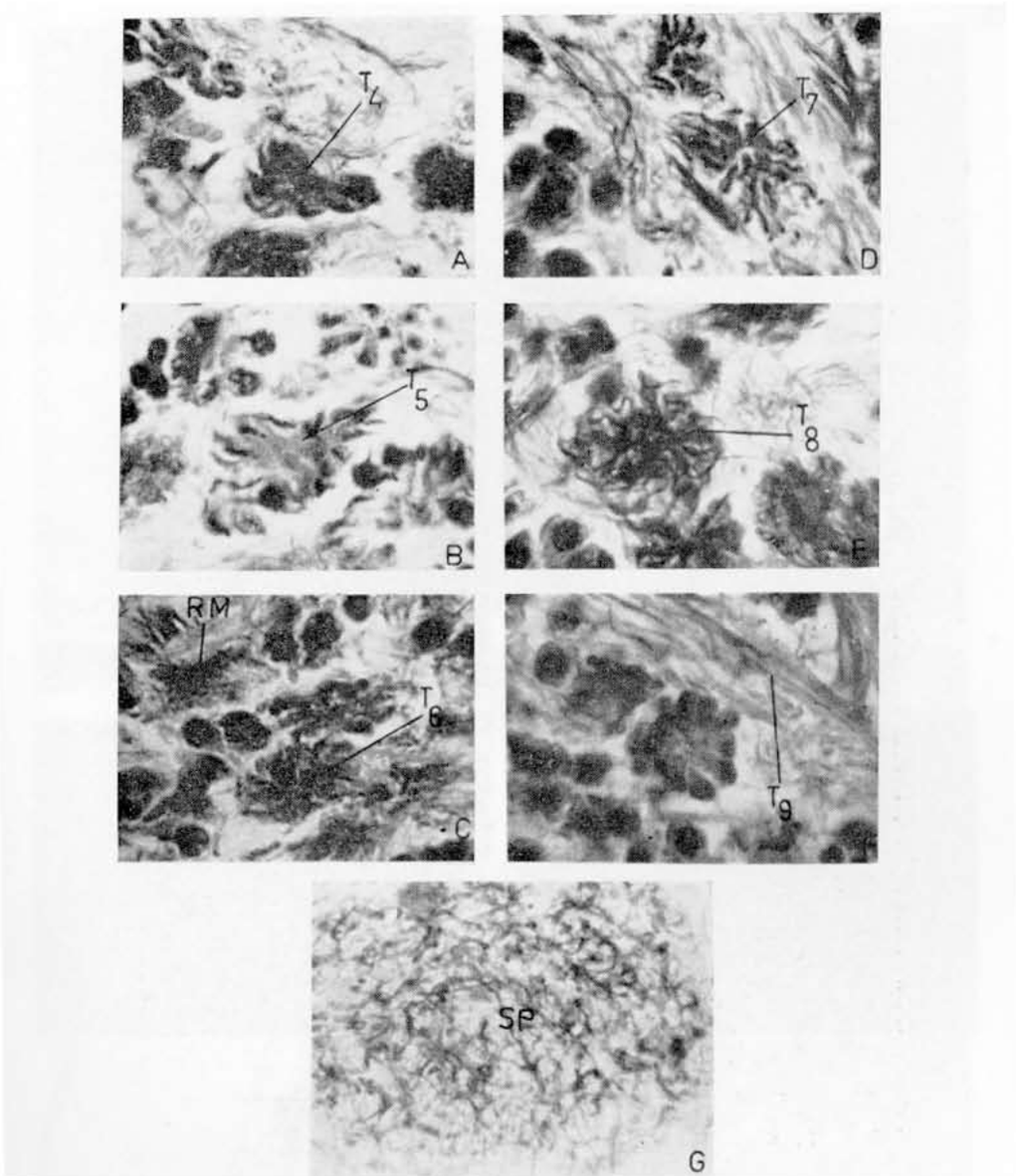

Fig. A. Stage 4 of spermateleosis (T_4). Note the elongating nuclei. Polychrome × 675. **Fig. B.** Stage 5 of spermateleosis (T_5). Polychrome × 675. **Fig. C.** Stage 6 of spermateleosis (T_6). Note the coiling of the nuclei and the presence of darkly stained residual mass (RM). Polychrome × 675. **Fig. D.** Stage 7 of spermateleosis. Note some of the spermatozoa which changed their orientation. Polychrome × 675. **Fig. E.** Stage 8 of spermateleosis. Note the formation of mature spermatozoa but still present in cytophore. Polychrome × 675. **Fig. F.** Stage 9 of spermateleosis (T_9). Note mature spermatozoa present in bundles in the testis. Polychrome × 675. **Fig. G.** Mature spermatozoa are present separately in the vas deferens. Polychrome × 675.

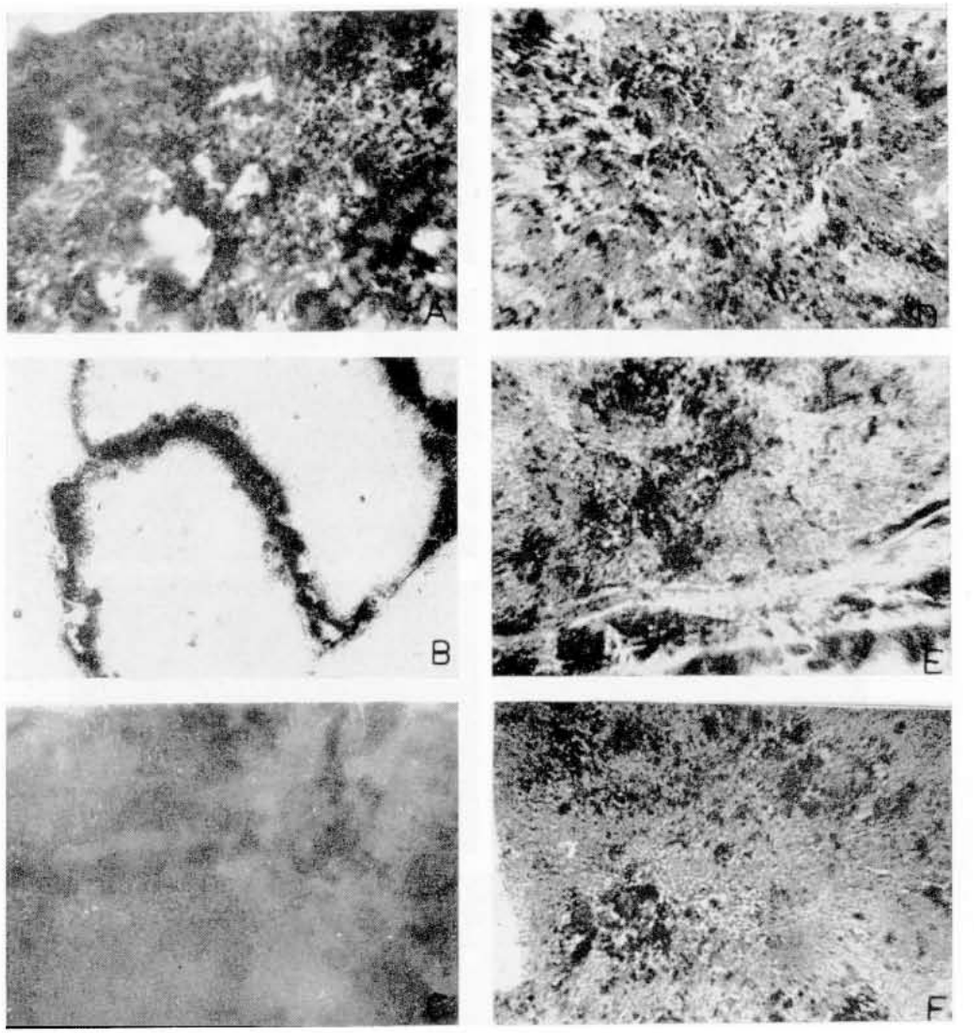

Fig. A. Spermatogenic cells showing the activity of alkaline phosphatase. × 330. **Fig. B.** Intense activity of esterase in the testicular wall. Note the absence of activity in the spermatogenic cells × × 80. **Fig. C.** Activity of ATPase in the testicular wall and the spermatogenic cells. × 330. **Fig. D.** Activity of NADH-diaphore. × 330. **Fig. E.** Strong activity of MDH. × 330. **Fig. F.** Activity of GLD in the spermatogenic cells. × 330.