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MORPHOLOGICAL AND HISTOCHEMICAL STUDIES ON THE OVARIAN DEVELOPMENT AND OOGENESIS IN PARAMPHISTOMUM CERVI (DIGENEA: PARAMPHISTOMATIDAE)

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Abstract. The ovary in 4-week-old worms contains undifferentiated germ cells. The oogonial differentiation, together with the appearance of some PAS-positive material in the ooplasm occurs in the ovary of 8-week-old worms. The ovary of 16-week-old worms contains only oogonia and oocytes and no germ cells. In adult worms, the oocytes reach the pachytene stage and further development is arrested at this stage. Nucleolar fragmentation and subsequent transport of nuclei to the ooplasm have been observed. The ooplasm contains nutritive material rich in proteins, carbohydrates and lipids. Its amount varies with different stages of oogenesis. The localization of various phosphatases and dehydrogenases was studied during oogenesis and their functional significance was discussed.

The oogenesis of trematodes has been the subject of several studies by light (Yosufzai 1953, Koulish and Kleinfield 1954, Nez and Short 1957, Hanumantha Rao and Madhvi 1966, Saxena 1979, Kanwar et al. 1980) and electron microscope (Bjorkman and Thorsell 1964, Gresson 1964, Halton et al. 1976). In general, the developmental changes in the oocytes of adult trematodes involve marked alterations in the nucleus and cytoplasmic organelles accompanied by the accumulation of nutrient material in their cytoplasm. Though some information is available about the cytoplasmic changes in the nutrient components during oogenesis in a few species (Yosufzai 1953, Koulish and Kleinfield 1954, Kanwar et al. 1980), nothing is known about the enzymatic changes during the trematode oogenesis. The morphological and histochemical features of the developing ovary of the trematode are also unknown. Therefore, the present paper describes the morphological, histochemical and histoenzymological changes in the ovary during in vivo development of *Paramphistomum cervi* in sheep and during the oogenesis in the adult worms.

MATERIAL AND METHODS

Paramphistomum cervi Zeder, 1890 at the age of 4, 6, 8, 10 and 16 weeks were raised according to Gupta et al. (1983). Adult specimens were collected from the rumen of sheep from the local slaughterhouse. After washing with saline, they were fixed in alcoholic Bouin's fluid, Carnoy's fluid and calcium formol. Paraffin sections (5—6 μm thick) were used for histological and histochemical preparations. Frozen sections (10—14 μm thick) were subjected to various histochemical tests for lipids and enzymes with appropriate controls. For general histology, sections were studied with a heamatoxylin-eosin (Humason 1979), iron haematoxylin (Bird 1971) and whipt polychrome (Veterling and Thompson 1972) techniques. Various histochemical and histoenzymological tests were used (Pearse 1972, Chayan et al. 1973, Lojda et al. 1979) and their results are summarized in Tables 1 and 2, respectively.

RESULTS

The ovary of 4-week-old worms consists of a small oval to round mass surrounded by a thin covering. It contains some undifferentiated germ cells (14 μm in diameter) with nucleus (10 μm in diameter) containing condensed chromatin material (Fig. 1). At six weeks, the germ cells divide mitotically and increase in number. Their cytoplasm is devoid of PAS and SBB-positive material. All the cells in the ovary of 6-week-old worms show similar cytological features. The oogonial differentiation occurs at 8 weeks, as differentiating oogonia and polygonal fully formed oogonia similar to those of adult ovary can be seen at this stage along with primordial germ cells (Fig. 1). In contrast to the cytoplasm of germ cells at 4- and 6-week-old stages, PAS and SBB-positive material appears in oogonial cytoplasm in 8-week-old worms. The intensity of staining with PAS and HgBB increases in 10-week-old worms. Simultaneously, oogonial multiplication by mitosis can be se enat this stage, but the oocyte development seems to begin after 10 weeks, as the ovary of 16-week-old worm contains all stages of oogenesis as in the adult worm from the natural infection.

The ovary of adult worms is a flask-shaped structure with a narrow proximal and broader distal ends. It is covered by a syncytial ovarian coat (Plate I, Fig. A), which is supported on the inner side by a thick basal lamina. The ovarian coat stains moderately for proteins containing free and bound NH_2 groups, tyrosine and keratin, for carbohydrates containing glycogen and small amount of mucopolysaccharide, and for sudanophilic lipids. Weak activities of alkaline phosphatase, ATPase, esterase, GPD,

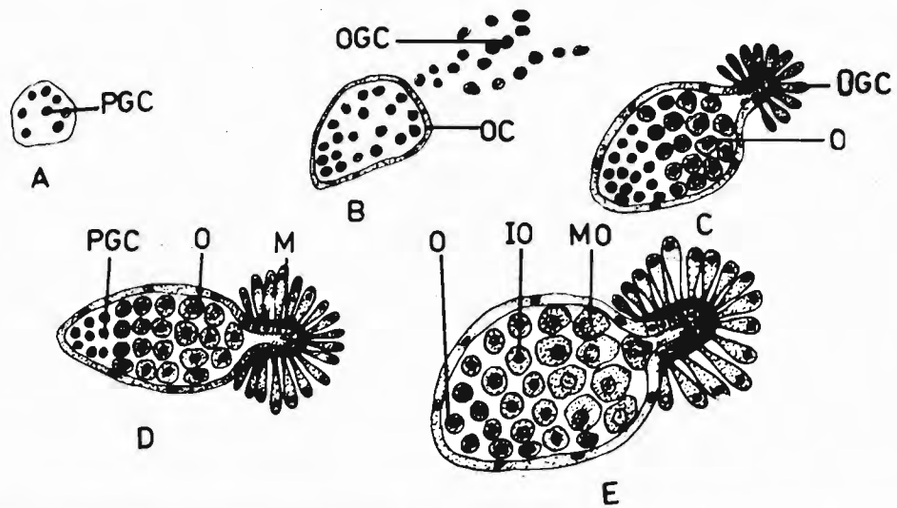

Fig. 1. Diagrammatic representation of development of the ovary and oocapt gland. A — In 4-week-old-worms, the ovary contains a few primordial germ cells (PGC). B — Number of primordial germ cells increases in 6-week-old worms. Ovarian coat (OC) is formed. Note the appearance of a few nuclei in the vicinity of the ovary marking the presence of oocapt gland cells (OGC). C — In 8-week-old worms, the ovary contains primordial germ cells towards the narrower end and the oogonia (O) towards the broader end. Oocapt gland cells are formed (OGC). D — In 10-week-old worms, the ovary contains mainly oogonia (O) and a few primordial germ cells (PGC). Musculature (M) develops around the oocapt. E — in 16-week-old worms the ovary contains oogonia (O), immature primary oocytes (IO) and mature primary oocytes (MO).

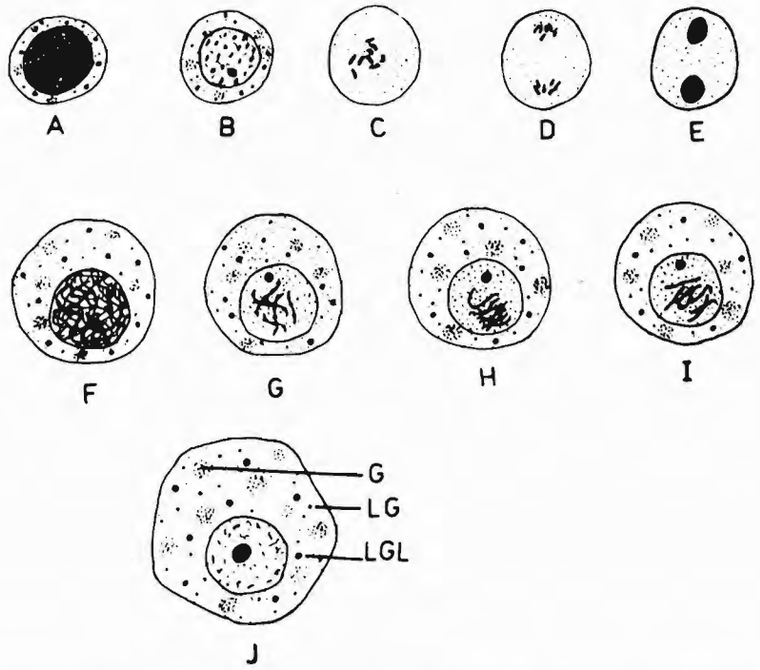

Fig. 2. Diagrammatic representation of various stages of oogenesis. A—E — various stages of mitosis in the oogonia, F — primary oocyte at interphase, G — leptotene stage, H — zygotene stage, I — pachytene stage, J — mature primary oocyte showing diffused chromatin material. G, glycogen, LG, lipid granules. LGL, lipid globules.

ICDH, MDH, NADH-diaphorase, GLD and moderate activity of TR have been observed in the ovarian coat.

The proximal portion of the ovary contains oogonia and few immature primary oocytes, while the central portion contains immature and few maturing primary oocytes, and the distal portion contains mainly the mature primary oocytes (Plate I, Fig. A). A continuous morphological series can be traced from the oogonium to the mature primary oocytes, but for descriptive purpose the process is confined to four different developmental stages.

1. Oogonia. The oogonia are present towards the narrower end of the ovary, interspread among the immature primary oocytes (Plate I, Fig. B). They are round in shape, with a diameter of 10—14 μm. Their nucleus: cytoplasm ratio is high. Most of the oogonia are at interphase but some show mitotic divisions. The basophilic cytoplasm of oogonia contains RNA, as revealed by methyl green-pyronine (Table 1). The cytoplasm stains moderately after HgBB for proteins which is rich in both free and bound amino groups as well as tyrosine-containing protein. The cytoplasm also contains PAS-positive material, which becomes negative after acetylation and again gives positive staining after KOH treatment, revealing 1 : 2 glycol group. A small amount of glycogen is also present in the cytoplasm as it is evident after Best's carmine staining. The ooplasm does not stain after colloidal iron but stains weakly after Alcian blue-PAS, Alcian blue, pH 2.5, and Azure A, pH 1.5, revealing the presence of small

amount of mucopolysaccharides. Two types of granules staining intensely after SBB have been observed. The smaller granules stain moderately for neutral lipids, but the larger ones contain both neutral lipids and phospholipids as revealed by their reactions with acid haematin, Nile blue sulfate, Oil Red 0 and Sudan III and IV tests (Table 1).

2. Immature primary oocytes. The immature primary oocytes are round, 14—17 μm in diameter and intermixed with the oogonia (Plate I, Figs. B, C). Their number is relatively high. The nucleus: cytoplasm ratio decreases. The nucleus is round in outline and is placed slightly eccentrically. The chromatin material is granular and is dispersed in the nucleoplasm. One or two nucleoli are present either centrally or towards one side. The size of the nucleus, as well as of nucleolus, increases during the oocyte growth. In addition to the nucleolus, a small darkly stained body is present in some oocytes near the leptotene and zygotene chromosomes. The immature primary oocytes show meiotic prophase and their division is arrested at pachytene stage (Fig. 2).

The immature oocytes show accumulation of some HgBB-positive material in their juxtanuclear cytoplasm. The staining intensity of tyrosine containing proteins increases but that of RNA decreases (Table 1). The amount of protein containing free and bound NH_2 groups remains the same. The amount of PAS-positive material and glycogen also slightly increases. The staining with Alcian blue-PAS, Alcian blue, pH 2.5, and Azure A, pH 1.5, also slightly increases. The ooplasm contains mainly large granules consisting of both phospholipids and neutral lipids. Few small granules containing mainly neutral lipids are also present.

3. Maturing primary oocytes. The size of maturing primary oocytes increases to about 18—22 μm. They are round in out line (Plate I, Fig. D). The nucleus: cytoplasm ratio further decreases. The nucleus contains diffused chromatin material. The amount of tyrosine-containing proteins further increases in the cytoplasm while that of RNA decreases. More PAS-positive material and glycogen appear in the cytoplasm (Table 1). The amount of mucopolysaccharides also increases as revealed by their increased affinity for Alcian blue-PAS, Alcian blue, pH 2.5 and Azure A, pH 1.5 and colloidal iron. The number of small granules containing neutral lipid increases.

4. Mature primary oocytes. Mature primary oocytes are present towards the broader distal end of the ovary having a diameter of 26—28 μm. Their number is relatively high and they are compactly arranged. They form the largest cell type of the ovary. Due to their large size and compact arrangement, the primary oocytes appear polygonal in shape (Plate I, Fig. E). Their nucleus: cytoplasm ratio is quite low. The nucleus is round and is generally placed eccentrically. Diffuse chromatin material is present. The nucleus usually contains a single prominent nucleolus, but occasionally two nucleoli are also seen. The amount of tyrosine containing proteins further increases in the cytoplasm while that of RNA decreases. The juxtanuclear cytoplasm of mature primary oocytes contains accumulation of more HgBB-positive material (Plate I, Fig. F). The amount of protein containing free NH_2 groups slightly increases, while that containing bound NH_2 groups decreases. The amount of PAS-positive material and glycogen further increases (Table 1). The staining intensity of Alcian blue-PAS, Alcian blue, pH 2.5, and Azure A, pH 1.5, also slightly increases. The cytoplasm of primary oocytes contains mainly the small granules with neutral lipids, while the larger granules containing both phospholipids and neutral lipids are very few in number.

During the course of oogenesis, no evidence of the transport of intact nucleoli into the cytoplasm was obtained. However, the disintegrating nucleoli can be seen in the nucleus and their material is possibly transported into the ooplasm. This is supported

Table. 1. Histochemical observations on the oogenesis of *P. cervi*

Methods	Fixative	Oogonia		Immature oocyte		Maturing oocyte		Mature oocyte		Remarks
		Nu.	Cyto.	Nu.	Cyto.	Nu.	Cyto.	Nu.	Cyto.	
Mercuric bromophenol blue (Hg BB)	AB	++	++	++	++	++	++	+++	+++	Shows the presence of proteins
Ninhydrin-Schiff	AB	++	+	++	+	+	+	+	++	Reveals the presence of free NH_2 groups
Chloramine T-Schiff	AB	+++	++	++	++	++	++	+	++	Reveals the presence of bound NH_2 groups
Millon's reaction	AB	+	+	+	++	+	++	+	++	Tyrosine present
Performic acid-Schiff	AB	−	−	−	−	−	−	−	−	Keratin absent
Periodic acid-Schiff (PAS)	C	−	+	+	++	−	++	−	++	Presence of carbohydrates
Best's carmine	C	−	+	+	++	−	++	−	++	Glycogen present
Colloidal iron	C	−	−	−	−	−	−	−	−	Absence of acid mucopolysaccharide
Alcian blue, pH 2.5	C	−	++	−	++	−	++	−	++	Presence of mucopolysaccharide
Alcian blue — PAS	C	−	+	+	++	−	++	−	++	Reveals sulphated mucosubstances
Azure A, pH 1.5	C	−	+	+	++	−	++	−	++	,,
Sudan black B (SBB) in 70 % ethanol	CF+Pc	−	++	−	+++	−	+++	−	+++	Reveals lipids
Aceton SBB	CF+Pc	−	+++	−	+++	−	+++	−	+++	Shows masked lipids
Oil Red O	CF+Pc	−	++	−	+++	−	+++	−	+++	Shows neutral lipids
Sudan III & IV	CF+Pc	−	++	−	++	−	++	−	+++	Shows neutral lipids
Acid haematin	CF+Pc	−	+++	−	+++	−	++	−	++	Reveals phospholipids
Methyl green-pyronin	C	++G	+++P	+G	++P	++G	++P	+G	+P	Reveals basophilia
Feulgen	C	++	−	++	−	+	−	+	−	Reveals DNA

Key to abbreviations: − no activity, + weak activity, ++ moderate activity, +++ strong activity, ++++ intense activity; AB − Alcoholic Bouin's fluid; C − Carnoy's fluid; CF+Pc − Calcium formol postchromatin; G − green; P − pink.

Table 2. Histoenzymological observations on the oogenesis of *P. cervi*

Enzymes	Substrates	References	Oogonia	Maturing oocytes	Mature oocytes
Acid phosphatase	Sodium β-glycerophosphate	Pearse 1972	+	+	+
Alkaline phosphatase	Sodium β-glycerophosphate	Pearse 1972	+	+	+
ATPase	Adenosine triphosphate	Pearse 1972	+	+	+
5'-nucleotidase	Adenosine-1-phosphate	Pearse 1972	+	+	+
Glucose-6-phosphatase	Glucose-6-phosphate	Pearse 1972	+	+	+
Esterase	α-naphthylacetate	Chyan et al. 1973	++	++	+
Lipase	Tween 80	Chyan et al. 1973	+	+	+
Malate dehydrogenase (MDH)	Disodium malate	Lojda et al. 1979	++	+++	++++
Isocitrate dehydrogenase (ICDH)	Sodium citrate	Lojda et al. 1979	+++	+++	++++
NADH-diaphorase	NAD	Pearse 1972	+++	+++	++++
Glyceraldehyde dehydrogenase (GLD)	Sodium-L-glutamate	Pearse 1972	+++	+++	++++
Glycerophosphate dehydrogenase (α-GPD)	Glyceraldehyde-β-phosphate	Pearse 1972	++	+++	++++
Tetrazolium reductase (TR)	NADPH	Lojda et al. 1979	++	+++	++++
Glucose-6-phosphate dehydrogenase (G-6-PDH)	Glucose-6-phosphate	Lojda et al. 1979	++	++	+++
Glycerol-3-phosphate dehydrogenase	Sod. β-glycerophosphate	Lojda et al. 1979	+	+	+

Key to abbreviations: + weak activity, ++ moderate activity, +++ strong activity, ++++ intense activity.

by the presence of the granular material in the ooplasm showing cytochemical properties similar to those of nucleoli.

Weak activities of acid phosphatase, alkaline phosphatase, lipase, ATPase, 5'-nucleotidase and glucose-6-phosphatase are present in the oogonia (Table 2). However, no change in the enzyme activity is observed with their further maturation. There is a strong activity of esterase in the oogonia, while the primary oocytes show a weak esterase activity. Of the various dehydrogenases studied, MDH, ICDH-diaphorase, GLD and α-GPD show strong activities in the oogonia (Plate II, Table 2). However, activities of these enzymes further increase with the maturation process, as the mature primary oocytes show intense enzyme activities. The oogonia show moderate activities of TR and G-6PDH. However, their activities further increase in the primary oocytes. Both oogonia and primary oocytes show weak activities of glycerol-3phosphate dehydrogenase.

DISCUSSION

Though a few workers have attempted to study the gross development of the reproductive system in vivo (Dawes 1962, Ghandour 1978), the literature regarding the development of ovary in vivo is scarse. The primordial germ cells present in 4-week-old worms correspond to the immature cells present in the immature ovary of *Raillietina cesticillus* (Sushma Rani 1982). In *R. cesticillus* these cells multiply by mitosis resulting in an increase in their number as well as the size of the ovary. Similar mitotic divisions have also been observed in *P. cervi* in 6-week-old worms. In 8-week-old worms, oogonial cells appear and their number increases considerably in 30-week-old worms. Sixteen-week-old worms contain only oogonia and primary oocytes. No primordial cell has been seen at this stage. Even Halton et al. (1976) in their electron microscopic studies could not observe primordial germ cells.

In adult worms, the sequence of events, which take place during the development from the early oogonia to a mature primary oocyte, as well as the distribution of various stages of oogenesis (oogonia, immature, maturing and mature oocytes), followed a similar pattern as described for other trematodes (Nez and Short 1957, Gresson 1964, Halton et al. 1976, Saxena 1979, Kanwar et al. 1980). The immature stages of oogenesis are present towards the narrower end of the ovary, while more mature stages are towards the broader end. The oogonia have a high nucleus: cytoplasm ratio which decreases during the later stages of oogonia. Halton et al. (1976) in their electron microscopic studies have also reported similar observations.

The number of oogonia increases by mitotic divisions. Similarly Halton et al. (1976) in monogeneans and Sushma Rani (1982) in cestodes have reported mitotic division of oogonia. First maturation division begins in primary oocytes. Leptotene, zygotene and pachytene stages of meiotic prophase appear mainly in the immature primary oocytes which undergo a resting phase at pachytene stage. Later stages of prophase of first meiosis and all stages of second meiosis are completed after the oocyte has been penetrated by the sperm (unpublished observations). Halton et al. (1976) have reported that the prophase of first meiotic division in early primary oocytes is marked by the appearance of synaptonemal complexes in the nucleus.

The cytoplasm of oogonia and immature oocytes is basophilic. Bjorkman and Thorsell (1964) in *Fasciola hepatica*, Erasmus (1973) in *Schistosoma mansoni* and Halton et al. (1976) in *Diclidophora merlangi*, *Diplozoon paradoxum* and *Calicotyle kroyeri* have also reported the abundance of ribosomes in these cells. Recent studies by Kanwar et al. (1980) also support the above observation. Oogonial cytoplasm shows a moderate amount of proteins, small amount of glycogen and lipid granules

composed of neutral lipids and phospholipids, as also reported by Kanwar et al. (1980). Basic proteins and mucopolysaccharides present in the cytoplasm may be used for the formation of cortical granules, as suggested by Boyer (1972) in his electron microscopic studies on *Prostheceraeus floridanus*.

As the oocyte grows the amount of glycogen and proteins increases as also reported by Kanwar et al. (1980) in the oocytes of *Gastrothylax crumenifer* and *Ceylonocotyle dawesi*. Halton et al. (1976) reported that with the oocyte growth, the number of ribosomes, mitochondria, GER and Golgi complex increases. Most of the phospholipids present in the oocyte appear to be used in the synthesis of membranes for the formation of various cellular organelles (Lehninger 1975).

The presence of alkaline phosphatase, acid phosphatase, ATPase, 5′-nucleotides and esterase has also been reported by a number of workers in the ovary of the trematodes (Halton 1967a, b, Patil and Rodgi 1976, Rodgi et al. 1976, Sharma 1976, Mandawant and Sharma 1978). According to Lobel and Levy (1968), alkaline phosphatase may be involved in growth processes and differentiation of cells. ATPase is concerned with the energy liberation processes and is responsible for the breakdown of ATP to ADP. Acid phosphatase may represent autolytic processes associated with the rapid turnover of membranes and macromolecules in the growing oocytes. It has also been suggested that acid phosphatase is concerned with the nucleic acid synthesis (Hashimoto and Ogawa 1963) and may also be involved in the active protein synthesis Eranko 1951, Novikoff 1961, Pearse 1972). The high activity of esterase in the oogonia shows the rapid hydrolysis of lipids, which may be used for the energy production.

MDH, ICDH, NADH-diaphorases and TR have been established as mitochondrial markers (Nachlas et al. 1967, Pearse and Scarpelli 1958). Mitochondria have been reported by Halton et al. (1976) in the oocytes of *D. merlangi*. Strong activities of various dehydrogenases in the oogonia and intense activity in the oocytes indicate that they require a continuous supply of energy for the synthesis of various cellular materials during their division and growth. However, the presence of glucose-6-phosphate dehydrogenase indicates that some glucose may be channeled via pentose phosphate pathway for energy production.

The histochemical studies of Koulish and Kleinfield (1964) on the oocytes of *Gorgoderina attenuata* have shown a nucleolus-like body (rich in ribonucleoprotein) in the ooplasm. Yosufzai (1953) in *F. hepatica*, Burton (1960) in *H. medioplexus* and Kanwar et al. (1980) in *G. crumenifer* and *C. dawesi* have shown that the nuclear material in considerable quantities is extruded in the cytoplasm of the oogonia and the extensive extrusion of such material forms marked feature of the growing oocytes. During the present studies, no evidence of extrusion of intact nucleolus into the cytoplasm could be obtained. However, nucleolar fragmentation in nucleus and accumulation of similar material in the cytoplasm of immature and maturing oocytes indicate that the nucleolar material in granular form is extruded.

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МОРФОЛОГИЧЕСКОЕ И ГИСТОХИМИЧЕСКОЕ ИЗУЧЕНИЕ РАЗВИТИЯ ЯИЧНИКА И ООГЕНЕЗА У *PARAMPHISTOMUM CERVI* (DIGENEA: PARAMPHISTOMATIDAE)

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Резюме. Яичник трематод в возрасте 4 недель содержит недифференцированные зародышевые клетки. Дифференцировка и образование ШИК-положительного материала в ооплазме встречаются в яичнике трематоды в возрасте 8 недель. Яичник 16-недельной трематоды содержит только оогонии и ооциты, тогда как зародышевые клетки отсутствуют. У половозрелой трематоды ооциты достигают стадии пахитены и дальнейшее развитие прекращается. Наблюдалась фрагментация ядер и их последующий транспорт в ооплазму. Ооплазма содержит питательный материал, богатый белками, углеводами и липидами. Количество этого материала различно в различных стадиях оогенеза. Изучали локализацию фосфатаз и дегидрогеназ в течение оогенеза. Их значение и функция обсуждаются.

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Ž. Černá: Kokcidie některých domácích a užitkových zvířat a kokcidie člověka. (Coccidia of some domestic and productive animals and of man). *Studie ČSAV 3.83, Academia, Praha 1983, 160 pp., 132 Figs., 18 Tables. Price 24,— Kčs.*

Coccidia are widely distributed and numerous protozoans parasitizing the tissues of various hosts and causing economic losses. The author tried to conform the present ideas and knowledge of monoxenous coccidia with the new data on heteroxenous members of this group. Of current interest are these problems in coccidia of domestic and productive animals, but also in man. Cat, dog and man are included in the life cycles of heteroxenous coccidia as final hosts, but at the same time they are infected also by their own species of monoxenous coccidia. Many of the productive animals (e.g., rabbits, hares, sheep, pigs, cattle) are intermediate hosts of heteroxenous species of coccidia, but they are often infected by many other species of monoxenous coccidia, harmless, but often very pathogenic.

The book by Ž. Černá is divided into two main parts: general and systematic one. The general part includes information on the morphology and ultrastructure of coccidial cells, life cycles and detection of monoxenous and heteroxenous species. The systematic part deals in detail with monoxenous and heteroxenous coccidia of rabbits, hares, pigs, cattle, sheep, cats, dogs and man. The endogenous development, oocysts, prepatent period and pathogenicity of each coccidian species are described. For individual host species are given tables with a survey of their coccidia. The tables, as well as excellent illustrations and photomicrographs of individual stages of the life cycles of diagnostic importance, make the book an important basic tool for classical microscopical diagnostics. Moreover, it offers new stimuli for the studies on the serological detection of antibodies in coccidial infections.

The author made use of her many-years' studies and experience in this complicated topic. The readers thus get a theoretically perfectly founded manual providing information on the most recent results achieved in the studies on coccidia in the last years. It will be very valuable for those dealing with the basic research, students of parasitology, as well as for the diagnostic practice, all the more that it includes the problems of coccidia infecting economically important groups of hosts. These problems are now in the limelight of parasitologists, particularly in large-scale breeding technologies with a great concentration of animals.

Assoc. Prof. Dr. J. Bejšovec, C.Sc.

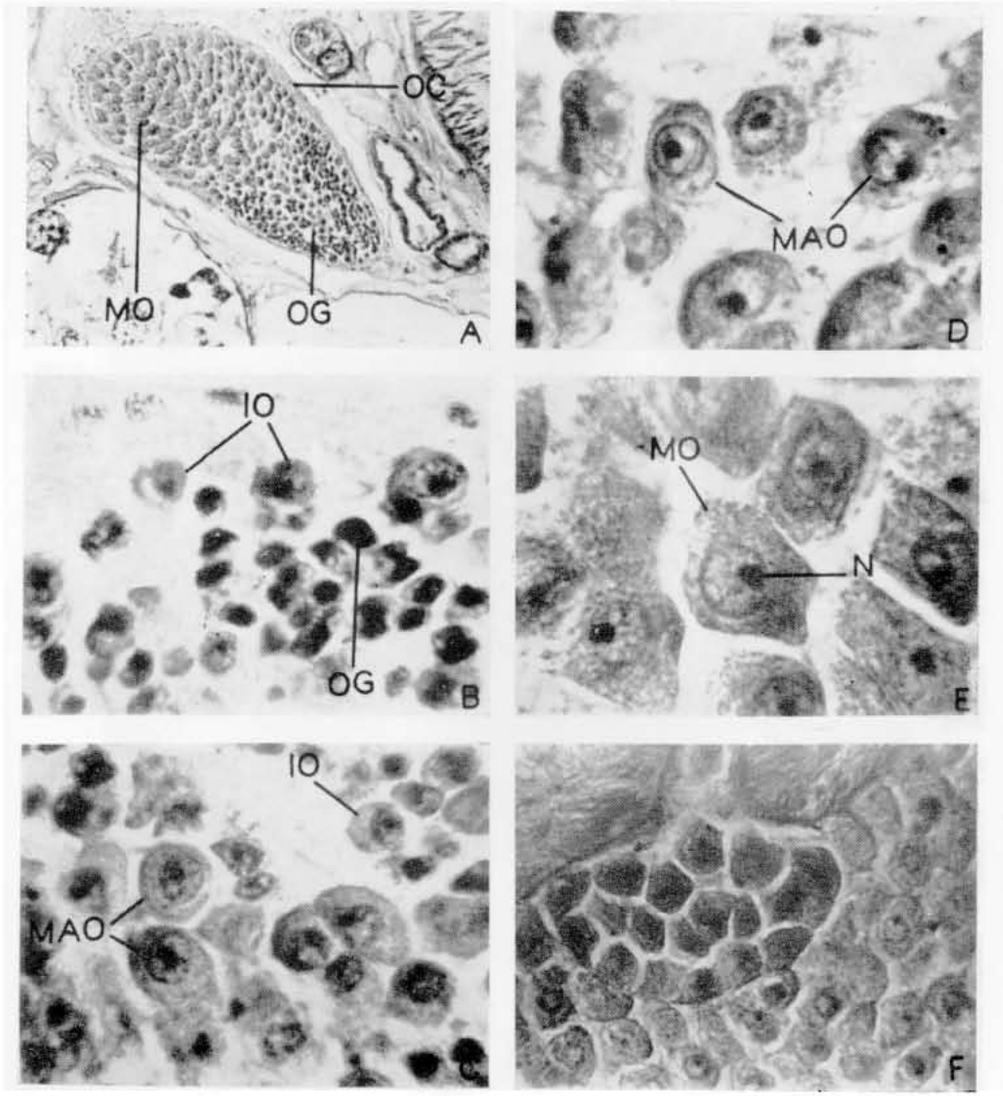

Fig. A. The ovary of adult worm covered by an ovarian coat (OC). Note the presence of oogonia (OG) towards the narrower end and mature primary oocytes (MO) towards the broader end. Polychrome (×80). **Fig. B.** The ovary contains oogonia (OG) and immature primary oocytes (IO). Note the compact nuclei of oogonia. Polychrome (×810). **Fig. C.** Immature (IO) and maturing primary oocytes (MAO) present in the ovary. Note the increased size of maturing primary oocytes. **Fig. D.** Maturing primary oocytes (MAO) in the ovary. Polychrome. (×810). **Fig. E.** Mature primary oocytes (MO) in the ovary. Note their polygonal shapes and prominent nucleoli (N)-Polychrome (×810). Fig. **F.** Mature primary oocytes stained strongly with HgBB. (×325).

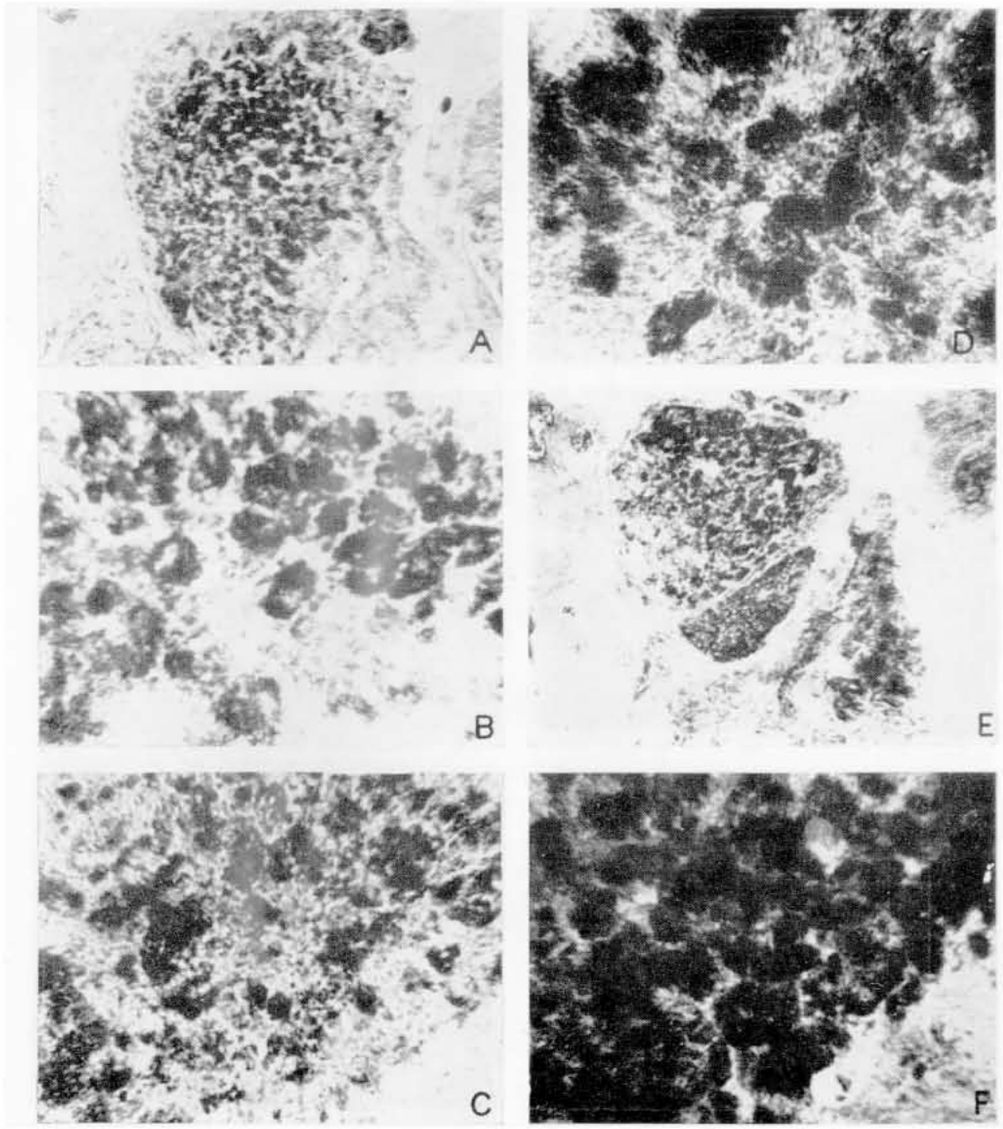

Fig. A. Complete ovary showing activity of MDH. Note strong activity in mature primary oocytes (×80). **Fig. B.** Strong activity of TR in the mature primary oocytes. (×325). **Fig. C.** Strong activity of GLD in mature primary oocytes. (×325). **Fig. D.** Strong activity of GDP in mature primary oocytes. (×325). **Fig. E.** Complete ovary showing stronger activity of NADH-diaphorase in the mature primary oocytes than the oogonia (×80). **Fig. F.** Strong activity of ICDH in the mature primary oocytes. (×325).