

SOME HISTOCHEMICAL STUDIES ON CALICOPHORON CALICOPHORUM AND PARAMPHISTOMUM EPICLITUM (PARAMPHISTOMATIDAE ; DIGENEA : TREMATODA)

PARAMPHISTOMES form a major group of helminths which parasitize many ruminants and other livestock and the immature forms of which are the causative agents of the serious disease, paramphistomiasis. With a view to having a better understanding of the host-parasite relationships, some physiological studies applying histochemical techniques were made on Paramphistomum epiclitum¹ Fiscoeder, 1904 and Cotylophoron cotylophorum² (Fiscoeder, 1901) Stiles and Goldberger, 1910. The present work is a comparative study on Calicophoron calicophorum (Fiscoeder, 1901) Näsmark, 1937 and P. epiclitum, both of which are the common parasites in the rumen of sheep and goats in Simla region.

The adult worms of C. calicophorum and P. epiclitum were collected from the rumen of sheep and goats slaughtered at the local abattoir and were fixed in Carnoy, Rossman, Formol calcium and Bouin³. Paraffin sections, cut at a thickness of 5-7 μ were subjected to histochemical tests as summarised in Table I.

The distribution of carbohydrates, proteins and lipids was found to be almost the same in both the species studied (Table I). In view of the fact that both occupy the same habitat in the same host, this similarity indicates the relationship between the habitat and the type of the metabolism occurring in the parasite.

The tegument, both in C. calicophorum and P. epiclitum, was found positive for carbohydrates of 1:2 glycol group, glycogen, proteins and lipids. The carbohydrates of 1:2 glycol group have also been reported in the tegument of Fasciola hepatica⁴ and in P. epiclitum¹. Being in conformity with these, the present observations are contrary to some earlier reports⁵⁻⁷. The tegument is suggested to be the region of maximum carbohydrate uptake from the host⁸. Proteins have also been revealed in the outermost covering of other digenetic trematodes⁸. Besides having been demonstrated in the tegument of many digenea⁶, the lipids have also been detected in the tegument in Cotylophoron cotylophorum which also parasitizes the same host and habitat as the worms under present studies. It seems likely that the transport of lipophilic substances takes place at this site².

Presence of glycogen, proteins and sudanophilic substances in the subtegument has also been reported earlier⁹. Singh and Gupta¹ found no proteins and lipids in this layer of P. epiclitum and glycogen was found to be missing by Axmann⁵. In view of the variations with regard to the occurrence of glycogen in this layer⁶, the subtegument may be regarded as a temporary site for glycogen storage.

The parenchymal cells have also been found to be containing abundant glucogen^{1,5,7,10} along with the carbohydrates of 1:2 glycol group and also a little amount of lipid and protein material. The parenchymal cells in the suckers, too, appear to be the important storage places^{10,11}.

The gut wall contains some carbohydrates⁷ and lipids¹². Lipids occurring in the lumen of gut may either be from the host diet or may represent the excreted metabolic products².

In both the species studied, the gametogonia and gametocytes were observed to be lacking in glycogen which was present in the mature sperm and uterine eggs only^{5,7}. In the Mehlis' gland and vitellaria carbohydrates, proteins and some sudanophilic substances could be localized. Earlier, lipoproteins and phospholipids have been reported in the secretion of Mehlis' gland and proteins, in the vitellaria of F. hepatica¹³ and P. epiclitum¹.

In accordance with many earlier observations^{1,14-16} lipids were localized in abundance in the excretory system, indicating that such metabolic by-products are expelled from the body.

The presence of some sudanophilic material in the lymph vessels of these flukes may possibly be related with some excretory function performed by this system, as is suggested² in view of its presumed origin from the primary excretory tubules.

The award of JRF from Himachal Pradesh University to one of us (JV) is thankfully acknowledged. Thanks are also due to Professor H. O. Agrawal for providing laboratory facilities.

TABLE I

Summary of histochemical tests applied in *Paramphistomum epiclitum* (A) and *Calicophoron calicophorum* (B)

Metabolite to be demonstrated	Technique	Fixation	Tegument		Subtegument		Parenchyma		Stickers		Oesophagus and Intestine	
			A	B	A	B	A	B	A	B	A	B
1. Carbohydrates	PAS (for 1:2 glycol group)	Carnoy, Rossman	+	++	0	0	+++	+++	+++	+++	+	+
	Best's carmine (for glycogen)		+	+	+	+	+++	+++	+++	+++	+	+
	Mercury-Bromophenol blue	Bouin, Carnoy	+	++	++	++	++	++	+	+	+	+
3. Lipids	Sudan Black-B	Formol calcium (with post-chromation)	+	+	+	+	0	0	+	+	++	+

Metabolite to be demonstrated	Technique	Fixation	Testis/Sperm		Ovary/Ova		Vitellaria		Excretory vesicle		Lymph vessels	
			A	B	A	B	A	B	A	B	A	B
1. Carbohydrates	PAS (for 1:2 glycol group)	Carnoy, Rossman	+	+	+	+	+	++	+	+	+	+
	Best's carmine (for glycogen)		+	+	+	+	++	++	+	+	++	+
	Mercury-Bromophenol blue	Bouin, Carnoy	++	++	++	++	++	+	+	+	+	+
3. Lipids	Sudan Black-B	Formol calcium (with post-chromation)	+	+	+	+	+	+	+++	+++	+	+

+ = slight reaction; ++ = moderate reaction; +++ = intense reaction; 0 = negative reaction.

Department of Biosciences, JAGDISHWARI VAIDYA.
Himachal Pradesh University, VEENA MEHROTRA.
Simla 171 005,
May 19, 1977.

1. Singh, R. P. and Gupta, N. K., *Proc. 60th Ind. Sci. Cong.*, 1973, p. 435.
2. Parshad, V. R. and Guraya, S. S., *J. Helm.*, 1976, **50**, 11.
3. Pearse, A. G. E., *Histochemistry: Theoretical and Applied*, J. & A. Churchill Ltd., London, 1968.
4. Lal, M. B. and Shrivastava, S. C., *Experientia*, 1960, **16**, 185.
5. Axmann, M. C., *J. Morph.*, 1947, **88**, 321.
6. Erasmus, D. R., *The Biology of Trematodes*, Grane, Russac and Co., N.Y., 1972.
7. Halton, D. W., *Comp. Biochem. Physiol.*, 1967, **23**, 113.
8. Monne, L., *Ark. Zool.*, 1959, **12**, 99.
9. Cheng, T., *Biology of Animal Parasites*, Saunders Toppan, 1964.
10. von Brand, T. and Mercado, T. I., *J. Parasit.*, 1961, **47**, 459.
11. Threadgold, L. T. and Gallagher, S. S. E., *Parasitology*, 1966, **56**, 299.
12. Morris, G. P., *Experientia*, 1968, **24**, 347.
13. Guraya, S. S., *Acta Biol. Acad. Sci. Hung.*, 1970, **21**, 3.
14. Gupta, A. N., Guraya, S. S. and Sharma, P. N., *Acta Morph. Nether. Scandinavia*, 1974, **12**, 231.
15. Pantelouris, E. M. and Threadgold, L. T., *La Cellula*, 1963, **54**, 73.
16. von Brand, T., *Biochemistry of Parasites*, Academic Press, N.Y., 1966.