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Histochemical studies on *Lytocestus indicus* and *Djombangia penetrans*, caryophyllidean cestode parasites of *Clarias batrachus* (L.)*

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Summary

Histochemical studies have been carried out on two caryophyllidean cestodes, *Lytocestus indicus* Moghe, 1931 and *Djombangia penetrans* Bovien, 1926, both common parasites of the catfish, *Clarias batrachus* (L.). While in both the carbohydrates, proteins and lipids showed a generalized pattern of distribution in the tegument, parenchyma, reproductive organs as well as the scolex gland cells, differences occur with regard to glycogen and lipid in the eggs and vitelline lobes of the two species. DNA concentration appeared to be higher in the eggs of *D. penetrans* as compared with *L. indicus*. The possible functional significance of the various metabolites in relation to the site of their occurrence has been discussed.

Key words: histochemistry; caryophyllids; cestodes; Lytocestus indicus; Djombangia penetrans; parasites; catfish

Introduction

The studies carried out so far on cestode physiology mainly pertain to cyclophyllidean and pseudophyllidean groups and are based on biochemical observations (Smyth, 1969; Barrett, 1981). An examination of the literature shows that relatively few histochemical studies have been made, still lesser concerning the adult worms.

With regard to caryophyllids, the histochemical studies done in the past related mainly to glycogen and to some exent to lipid distribution in the worm (Mackiewicz, 1968, 1972; Ginetsinskaya and Uspenskaya, 1965; Swiderski and Mackiewicz, 1976). Enzyme histochemistry has received relatively better attention in respect of this group (Gaur and Agarwal, 1981; Rasheedunnisa and Simha, 1982). Thus, further investigation into the car-

* Running title: Histochemical studies on caryophyllidean cestodes

Table 1

Histochemical localization of various substances in *Lytocestus indicus and *Djombangia penetrans*****

Metabolites	Reaction	Tegument	Scolex	Sucker	Frontal glands	Neck
Carbohydrates	PAS	+++ (+++)	++ (++)	(++)	− (−)	++ (++)
Glycogen	Bests' carmine method	− (−)	++ (++)	(++)	− (−)	++ (++)
Mucosubstances	Mucicarmine	++ (+++)	− (−)	(−)	− (−)	− (−)
Acid mucosubstances	Alcian Blue method	+++ (+++)	+ (+)	(+)	+ (+)	+ (+)
Acid mucosubstances	Azur 'A'	+++ (++)	+ (+)	(+)	+ (+)	+ (+)
Acid mucosubstances	Toluidine Blue	− (−)	− (−)	(−)	− (−)	− (−)
Proteins	Mercuric Bromophenol Blue method	+++ (+++)	++ (++)	(++)	+++ (+++)	+ (++)
Disulphides	Performic acid Alcian Blue method	++ (+++)	+ (+)	(+)	+ (+)	+ (+)
Arginine	Sakaguchi method	− (−)	− (−)	(−)	− (−)	− (−)
Tyrosine	Millon's (1849)	+ (+)	+ (+)	(+)	+ (+)	+ (+)
Histones	Alkaline Fast Green	+++ (+++)	+ (++)	(+++)	+++ (+++)	+ (++)
Lipids (general)	Sudan Black B	+++ (+++)	++ (+)	(++)	+++ (+)	+ (+)
Unsaturated lipids	Perfomic Acid-Schiff's	− (−)	− (−)	(−)	− (−)	− (−)
Glycolipids	PAS	+++ (+++)	+ (+)	(+)	− (−)	+ (+)
DNA	Feulgen nucleal	+ (+)	+ (+)	(+)	+ (+)	+ (+)
RNA	Methyl Green Pyronin Y	+ (+)	+ (+)	(+)	+ (+)	+ (+)
Haemoglobin	Benzidine method	—	—	—	—	—

+ = weakly positive reaction; ++ = strongly positive; +++ = very strongly positive;
− = negative
* = data without parentheses
** = data given in parentheses

Parenchyma	Subt. layer	Longitudinal muscules	Ovary	Mehlis' gland	Uterus	Uterine glands	Testes	Vitellaria	Eggs	Vitelline cells
+++	+++	+	−	−	++	−	−	−	+++	+++
(+++)	(++)	(+)	(−)	(+)	(++)		(−)	(−)	(+++)	(+++)
+++	+	−	−	−	++	−	−	++	+++	+++
(+++)	(+)	(−)	(−)	(−)	(+)		(−)	(+)	(+)	(+)
+	+	+	−	−	+	−	−	−	−	−
(+)	(+)	(+)	(−)	(−)	(+)		(−)	(−)	(+)	(−)
+	−	−	+	+	+	+	+	+	++	+
(++)	(−)	(−)	(+)	(+)	(+)		(+)	(+)	(+++)	(+)
+	−	−	+	+	+	+	+	+	++	+
(++)	(−)	(−)	(+)	(+)	(+)		(+)	(+)	(+)	(+)
−	−	−	−	−	−	−	−	−	−	−
(−)	(−)	(−)	(−)	(−)	(−)		(−)	(−)	(−)	(−)
+	+++	+++	++	+++	+++	+++	+++	+++	++	+
(++)	(++)	(+++)	(+++)	(+++)	(++)		(++)	(++)	(+)	(++)
+	−	−	+	+	+	+	+	+	++	+
(+)	(−)	(−)	(+)	(+)	(+)		(+)	(+)	(+)	(+)
−	−	−	−	−	−	−	−	−	−	−
(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)
+	+	+	+	+	+	+	+	+	−	−
(+)	(+)	(++)	(+)	(+)	(+)		(++)	(++)	(−)	(−)
+	+++	+++	+++	+++	+++	+++	+++	+++	++	+++
(+)	(+++)	(+++)	(+++)	(+++)	(++)		(+++)	(+++)	(+++)	(+++)
+	++	++	+++	+++	+++	+++	+++	+++	+	+
(+)	(++)	(+)	(+++)	(+++)	(+++)		(+++)	(+++)	(+++)	(++)
−	−	−	+++	−	−	−	+++	+++	−	−
(−)	(−)	(−)	(++)	(−)	(−)		(++)	(++)	−	−
+	−	−	−	−	−	−	−	+	++	++
(+)	(−)	(−)	(−)	(−)	(−)		(−)	−	(++)	(+)
+	+	+	+++	+	+	+	+++	+++	+	+
(+)	(+)	(+)	(++)	(++)	(+)		(+++)	(+++)	(+++)	(++)
+	+	+	+	+	+	+	+	+	+	+
(+)	(+)	(+)	(+)	(+)	(+)		(+)	(+)	(+)	(+)
—	—	—	—	—	—	—	—	—	—	—

yophyllidean group seemed desirable, as the information obtained would be an asset in obtaining and integrated picture of cestode physiology.

Material and methods

Live specimens of *Litocestus indicus* and *Djombangia penetrans* were recovered from the freshly-killed host fish, *Clarias batrachus* (L.). For localization of carbohydrates, proteins, lipids and nucleic acids in the various tissues and organs of the parasite, specific fixatives were used and specific histochemical tests performed (after Pearse, 1968) as mentioned in Table 1. All the paraffin sections were cut at 6—7 μm thickness.

Results and discussion

Based on the specific histochemical tests various metabolites could be demonstrated. Their distribution in respect of the various parts of the body is presented in Table 1.

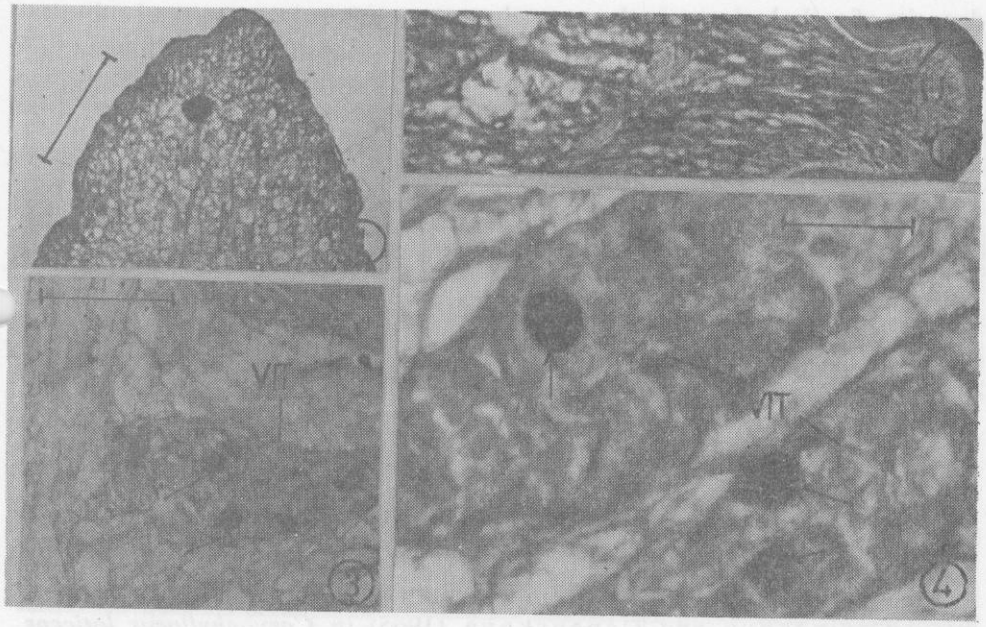
These results indicate towards a similarity in distribution of the different metabolites in the two species.

The tegument, scolex, neck, parenchyma, subtegumental muscle layer, vitelline cells of the egg, and uterus in both the species stained positively with the PAS reaction, though the reaction was mild in the parenchymatous longitudinal muscles (Figs 1, 2, 17—19). This observation of the occurrence of rich

Figs 1—10. *Lytocestus indicus*: Localization of carbohydrates and lipids

1. Scolex showing positive reaction for PAS (scale bar = 0.15 mm)
2. Tegument showing strong intensity for PAS (scale bar = 0.15 mm)
3. Vitelline lobes showing glycogen masses (arrows). Bests' carmine (scale bar = 0.15 mm)
4. Glycogen masses (arrows) in vitelline lobes under higher resolution (scale bar = 0.02 mm)
5. Strong reaction for glycogen in the vitelline cells within the egg. The area appearing dark in the photomicrograph stained deep red and the cell nuclei did not pick up haematoxylin stain (scale bar = 0.02 mm)
6. Tegument showing positive reaction for acid mucopolysaccharides. Alcian Blue (scale bar = 0.15 mm)
7. General parenchyma of the scolex region showing sudanophilic lipids (scale bar = 0.15 mm)
8. Vitelline cells within the eggs showing positive reaction for lipids. Sudan Black B (scale bar = 0.02 mm)
9. Subtegument, parenchymal longitudinal muscles and testes showing intense reaction for lipids. Sudan Black B (scale bar = 0.15 mm)
10. Testes and vitellaria showing strongly positive reaction for unsaturated lipids. Performic Acid — Schiff's reaction (scale bar = 0.15 mm)

Abbreviations: FGL — frontal glands; MGL — Mehlis' gland;
PLM — parenchymal longitudinal muscles;
SC — scolex; STU — subtegument; SUC — sucker;
T — testes; TU — tegument; UGL — uterine glands;
VIT — vitellaria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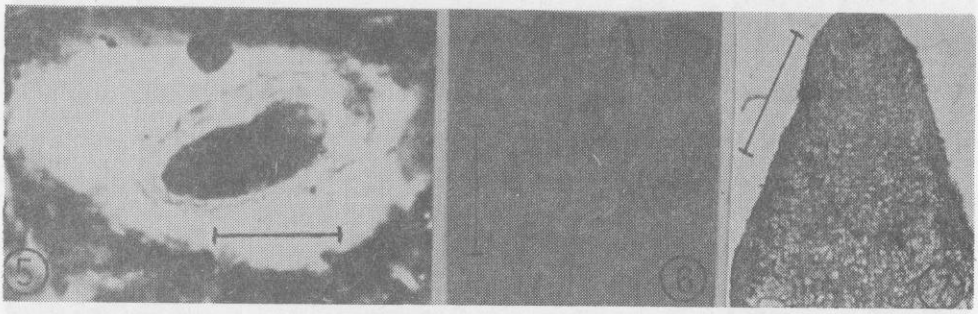
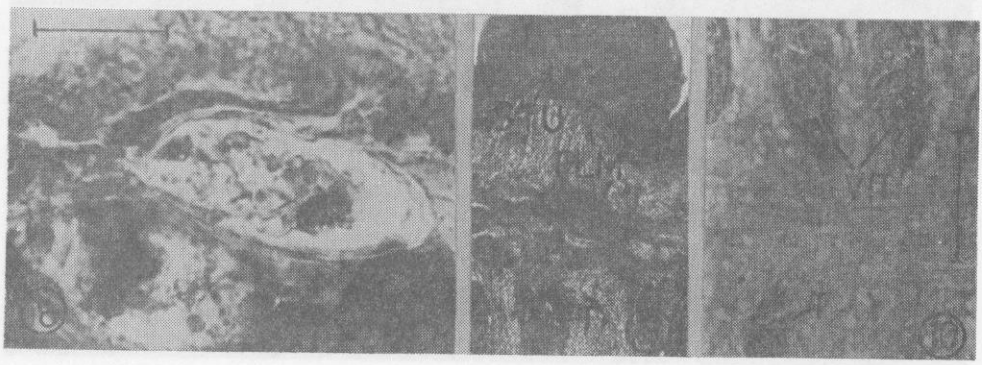




deposits of carbohydrates in the parenchymatous tissue, tegument and subtegumental layer of *L. indicus* and *D. penetrans* is in conformity with those of Gupta and Kapoor (1979) who also found the same tissues of *Cotugnia digonopora* to be rich in carbohydrates.

It is known that in the adult parasitic helminths carbohydrate is the major energy reserve and has manifold functions in the tissues, but carbohydrate levels in the worm may show alterations in accordance with the nutritional status or phase of the life cycle and among different cestode species. This metabolite is known to exhibit diurnal and annual cyclic changes as well. Yet carbohydrates are a major energy reserve, form important structural components and as phosphorylated intermediates are most important for energy metabolism. Further, they are also important as constituents of nucleotides, glycolipids and glycoproteins. However, of the carbohydrates, the main reserve polysaccharide in helminths is glycogen (Barrett, 1981).

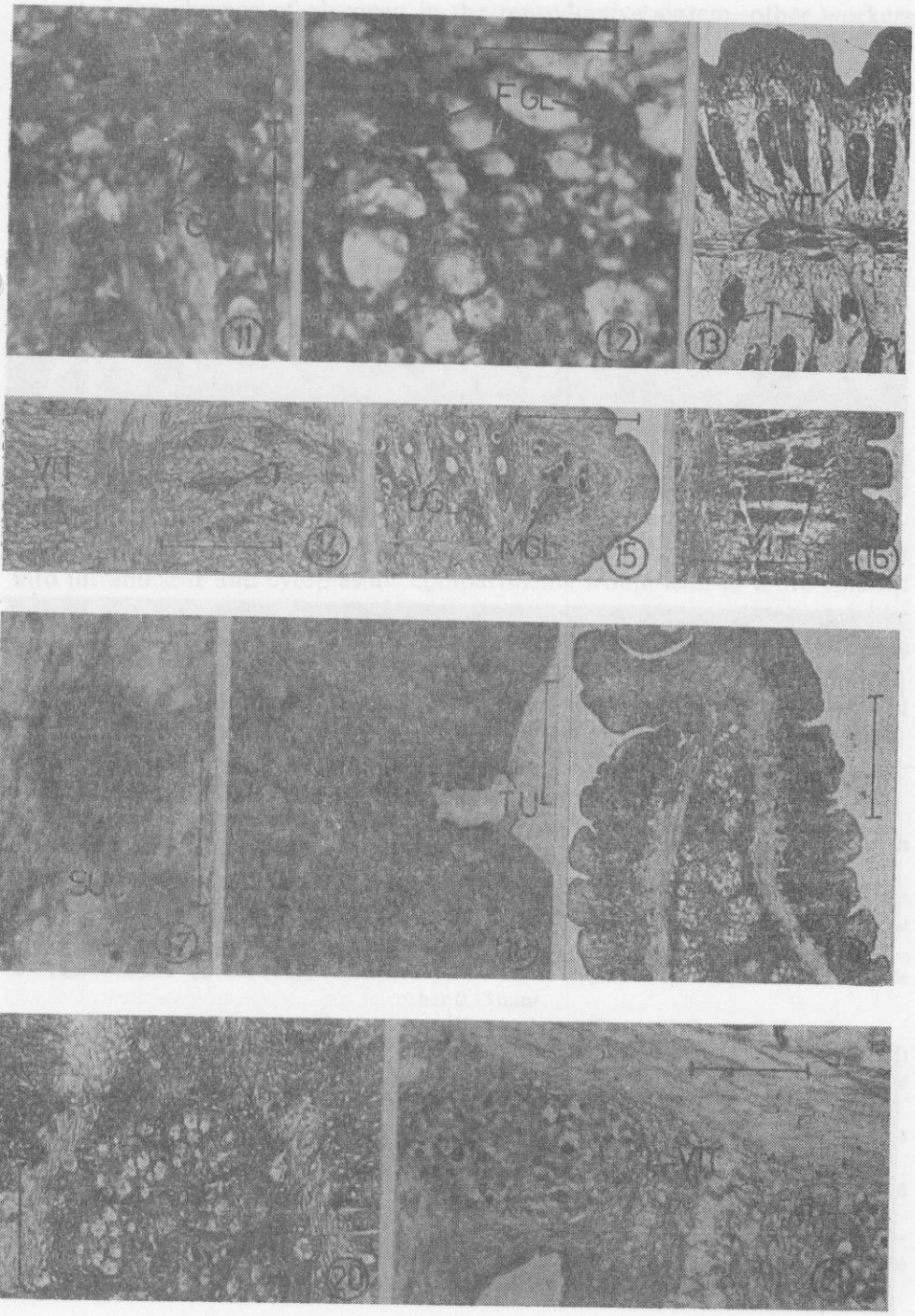
In *L. indicus* and *D. penetrans*, maximum concentration of glycogen was observed in the parenchyma. Presence of glycogen was also seen in the scolex, neck region and vitellaria but it was absent in the tegument, subtegument, muscles and gonads (Figs 3, 20, 21). This observation is in accordance with that of Ginetsinskaya and Uspenskaya (1965) in *Caryophyllaeus laticeps*. However, among cyclophyllidean members the highest content of glycogen is reported in the parenchymatous tissue (Smyth, 1947, 1949; Yamao, 1952a, b); there are conflicting reports of its occurrence in the reproductive systems. Thus, while Hedrick and Daugherty (1957) and Kilejian *et al.* (1961)

Figs 11—16. *Lytocestus indicus*: Localization of proteins

11. Frontal gland cells (arrows) in the scolex region showing proteinaceous contents. Bromophenol Blue (scale bar = 0.05 mm)
12. The same at higher magnification (scale bar = 0.02 mm)
13. Tegument, muscles, testes and vitellaria showing positive reaction. Bromophenol Blue (scale bar = 0.02 mm)
14. A portion of the midbody showing positive reaction for tyrosine in testes, vitellaria and parenchymal longitudinal muscles. Millon's reaction (scale bar = 0.15 mm)
15. The posterior third of the body showing a positive reaction for disulphides in the reproductive components. The Mehlis' gland, uterine glands and eggs in the uterus exhibit deep staining. Performic acid-Alcian Blue (scale bar = 0.15 mm)
16. Tegument vitellaria and parenchymal longitudinal musculature showing strong reaction for basic proteins. Alkaline fast green (scale bar = 0.15 mm)

Figs 17—21. *Djombangia penetrans*: Localization of carbohydrates

17. The sucker region in the scolex. PAS (scale bar = 0.05 mm)
18. Tegument showing positive reaction for PAS (scale bar = 0.1 mm)
19. General parenchyma showing high concentration of carbohydrates. PAS (scale bar = 0.15 mm)
20. Rich deposites of glycogen in the parenchymatous tissue. Best's carmine (scale bar = 0.15 mm)
21. Vitellaria showing glycogen-rich nuclei. Best's carmine (scale bar = 0.15 mm)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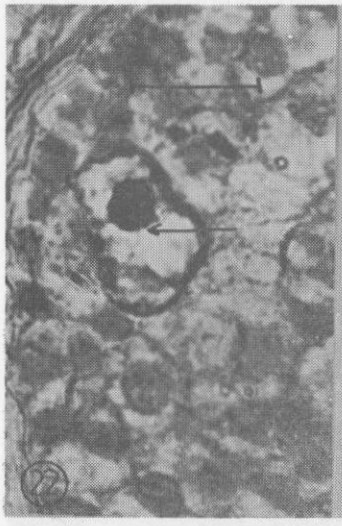

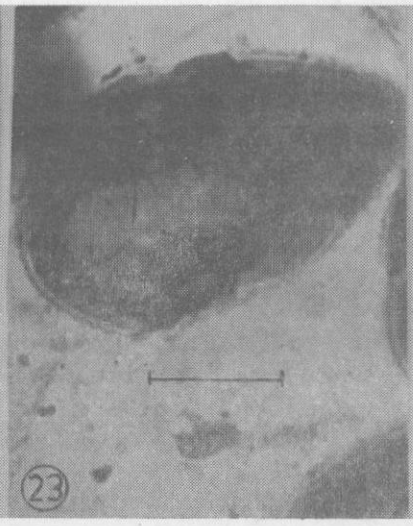

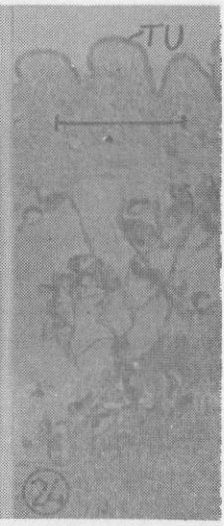
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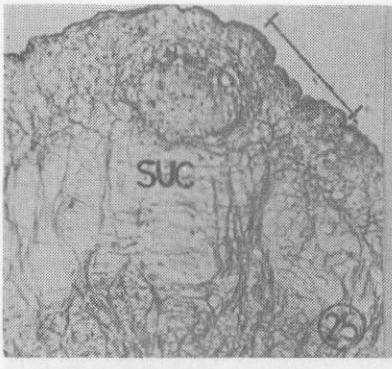
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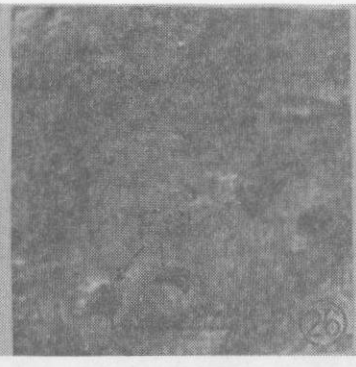

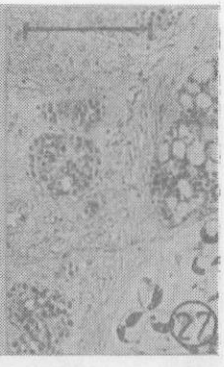


VIT
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reported the absence of glycogen in the reproductive system, other workers observed otherwise (Moczon, 1975; Baugh and Singh, 1979).

As a characteristic feature of caryophyllids (Mackiewicz, 1968) a large glycogen vacuole in the nuclei of mature vitelline cells in the eggs was observed in both the species (Figs 5, 22, 23); while it was more prominent and ubiquitously occurring in *L. indicus* eggs, not all the vitelline cell nuclei of the eggs in *D. penetrans* showed such a character. However, regarding the whole vitelline follicles while in *L. indicus* glycogen was observed as conspicuous intercellular globular masses, 2—5 in number, in *D. penetrans* it appeared to be confined to the nuclei of only a few cells (Figs 4, 21).

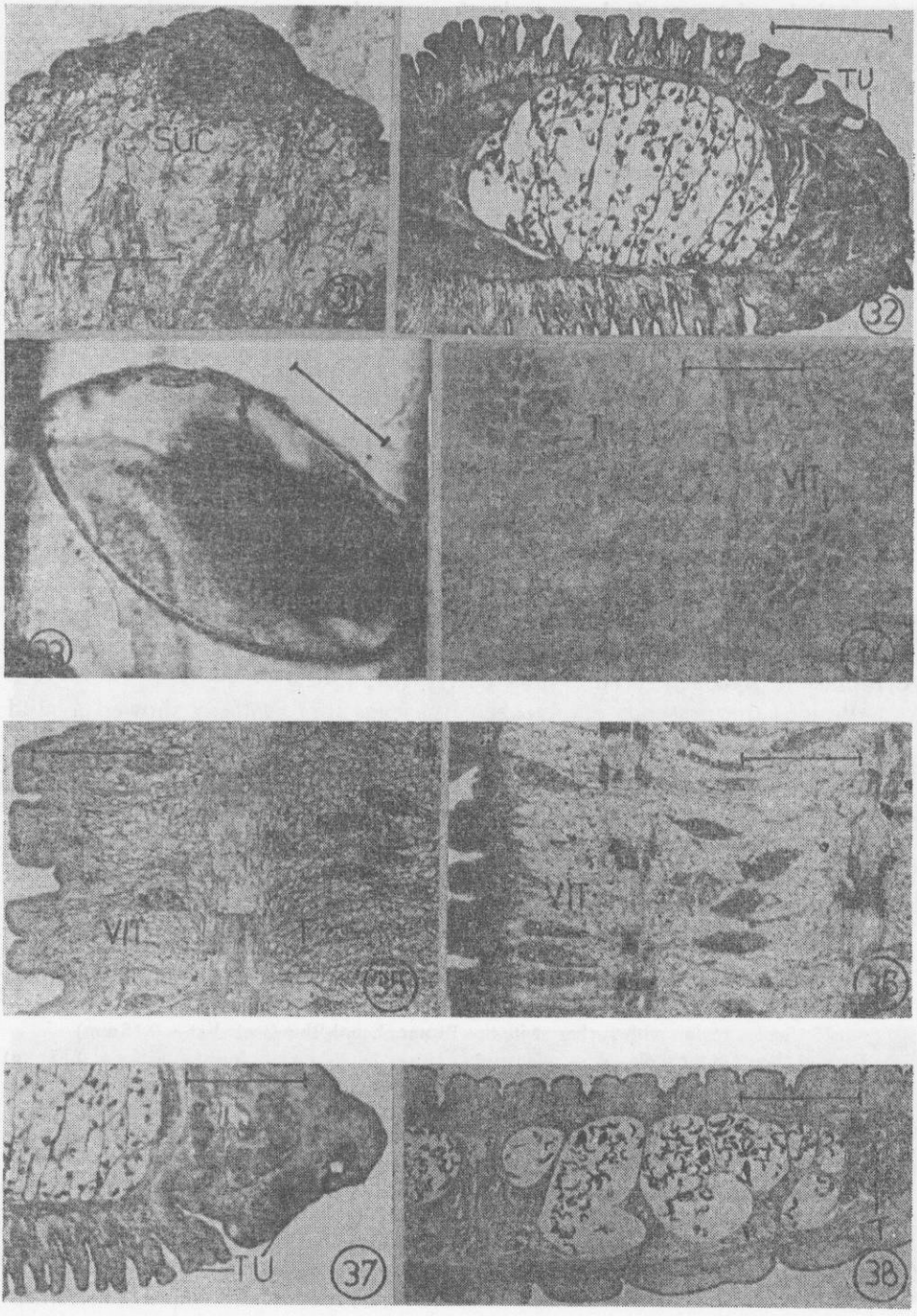
Depending on the properties of glycogen, namely its non-diffusable nature, low osmotic pressure and its highly branched structure which provides large number of chain ends of enzymes to work on, glycogen as a reserve polysaccharide is advantageous (Barrett, 1981).

An enhancement of glycogen content without having any increase in egg size or in the number of vitelline cells per egg seems to have a selective value and adaptational significance (Mackiewicz, 1968). Further, as hypothesized by Swiderski and Mackiewicz (1976), the partitioning of glycogen content into intranuclear and cytoplasmic compartments would lead to more efficient use of this energy reserve. Such a situation occurs among vertebrates like starving tadpoles where the nuclear glycogen is utilized after the depletion of the cytoplasmic glycogen (Himes and Pollister, 1962).

Besides the presence of glycogen, the eggs in *L. indicus* showed a mild

Figs 22—30. *Djombangia penetrans*: Localization of carbohydrates, proteins

22. An enlarged view of a vitelline gland cell. The nucleus shows a positive reaction for glycogen (scale bar = 0.02 mm)
23. Egg under oil immersion. While most of the dark area in the photomicrograph represents intense haematoxylin staining, only a few spots (arrows) stain for glycogen (scale bar = 0.02 mm)
24. Tegument and eggs showing positive reaction for acid mucopolysaccharides. Alcian Blue (scale bar 0.15 mm)
25. Scolex region with sucker at its tip. Bromophenol Blue (scale bar = 0.15 mm)
26. Frontal glands in the scolex showing intense reaction. Bromophenol Blue (scale bar = 0.15 mm)
27. Testes, vitellaria and eggs showing positive reaction for tyrosine. Millon's reaction (scale bar = 0.15 mm)
28. The same structures showing a positive reaction for disulphides. Performic acid — Alcian Blue (scale bar = 0.15 mm)
29. Tegument, testes, vitellaria and parenchymal longitudinal musculature showing intense staining for proteins. Bromophenol Blue (scale bar = 0.15 mm)
30. A position of the posterior half of the body — tegument, parenchymal longitudinal musculature, ovary and eggs show a strong reaction for basic proteins. Alkaline Fast Green (scale bar = 0.15 mm)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occurrence of lipids, whereas in *D. penetrans* eggs this metabolite was more prominently present (Figs 8, 33). Eggs containing both glycogen and lipid have been reported in *Diphyllobothrium latum* and those containing only lipid, in other pseudophyllideans, like *Ligula columbi* and *Triaenophorus nodulosus*, by Ginetsinskaya *et al.* (1971). According to Calow and Jennings (1974) the lipids are a major energy source in free-living platyhelminths, whereas the enteric parasites have an energy source rich in glycogen. While Jennings (1973) looked upon high glycogen as of adaptive value towards low or enhancing oxygen tensions prevailing in their habitat (i.e., gut), Jennings and Calow (1975) regarded this factor to be related to the high fecundity of the worm. However, in respect of caryophyllids, being monozoic cestodes, the requirement of large quantities of glycogen, instead of lipids, as an energy source seems to be related more to the biology of the egg and life cycle of the worm than to its fecundity. All the caryophyllids for which the complete life cycle has been worked out are known to utilize benthic intermediate hosts like tubificid annelids (Mackiewicz, 1981). Glycogen being heavier than lipid and also because it can be metabolized under anaerobic conditions, caryophyllidean eggs with more glycogen content seem to be better adapted to be approached by the intermediate host (Mackiewicz, 1981).

The difference regarding the lipid and glycogen content in the eggs between the two species can be explained on the basis of differences in their fecundity.

Figs 31—34. *Djombangia penetrans*: Localization of lipids

31. Scolex region. The sucker at the tip of the scolex shows a strong reaction of Sudan Black B (scale bar = 0.1 mm)
32. The lipid distribution in the various regions of the body tegument, parenchymal musculature, ovary and eggs exhibit sudanophilic reaction (scale bar = 0.15 mm)
33. Egg, under oil immersion, showing high concentration of lipid in the vitelline cells (scale bar = 0.02 mm)
34. Testes and vitellaria showing a mild reaction for unsaturated lipids. Performic acid — Schiff's reaction (scale bar = 0.15 mm)

Figs 35—36. *Lytocestus indicus*: Localization of nucleic acids

35. Reproductive organs showing a high concentration of DNA. Feulgen nucleal reaction (scale bar = 0.15 mm)
36. A portion of the body, showing intense reaction for RNA. Methyl Green — Pyronin Y (scale bar = 0.15 mm)

Figs 37, 38. *Djombangia penetrans*: Localization of nucleic acids

37. A portion of the body showing strong intensity for RNA. Methyl green — Pyronin Y (scale bar = 0.15 mm)
38. DNA in the various organs. Feulgen nucleal reaction (scale bar = 0.15 mm)

The number of eggs in *D. penetrans* is greater than in *L. indicus*. The uterine coils of *D. penetrans*, which run almost throughout the body, remain full of eggs, whereas in *L. indicus* eggs whenever present are found in a limited number within the uterine coils that occupy only a few milimeters of length in the posterior extent of the body. The fecundity rate seems much higher in *D. penetrans* than *L. indicus* and since the number of eggs is more in *D. penetrans*, glycogen storage does not seem really essential for prolonging the period of infectivity as in other caryophyllid cestodes. However, the occurrence of intranuclear glycogen in a few vitelline cells in *D. penetrans* appears to be related to the low or variable oxygen tension in the gut of the host (Mackiewicz, 1981).

Apart from the vitelline cells of the egg, the sucker at the tip of *D. penetrans*, the frontal, Mehlis' and uterine glands, and the gonads of *L. indicus* and *D. penetrans* all stained positively for lipids. Droplets of lipids were also found scattered throughout the parenchyma and subtegumental muscle layer (Figs 7—9, 31, 32). Unsaturated lipids, however, were found in the gonads and vitelline lobes of the two species (Figs 10, 34) and glycolipids were present in the tegument, parenchyma, scolex and neck. The occurrence of lipids in the tegument and subtegumental layer is in conformity with the observations of Waitz (1963) in *Hydatigera taeniaeformis* and of Gupta and Kapoor (1979) in *C. digonopora*. Scattered droplets of lipid in the parenchyma have been reported in several cyclophyllidean cestodes (von Brand, 1952).

A variety of functions is performed by the lipids in the tissues. Thus, lipid forms a major structural component of the cell membrane and as activators or glycosyl carriers lipids are also found associated with enzyme reactions. In the cytochrome chain and membrane transport mechanisms, lipid forms a component part and acts as important energy reserve (Barrett, 1981). Though, in most caryophyllidean eggs glycogen acts as major energy reserve, in forms like *D. penetrans*, lipid also seems to assume this function.

Almost all the organs except the vitelline cell nuclei showed the presence of proteins, though their maximum concentration was seen in the tegument and the various gland cells of the two species (Figs 11—13, 25, 26, 29). Considerable concentration of protein is found in most of the endoparasites because proteins form a substantial part of the normal diet of a vertebrate, which as a host provides an environment rich in proteins and their related break down products for the cestode parasites (Smyth, 1969). Thus, along with the general proteins, the tegument, scolex, neck, parenchyma and the eggs showed the presence of disulphides (Figs 14, 27). These tissues also show the presence of tyrosine, though in traces but a large concentration of it was found in the gonads (Figs 15, 28). Rich concentration of proteins in the tegument, subtegument and muscle layers is in conformity with the observations of Gupta and Ka-

poor (1979) in *C. digonopora*. Also the occurrence of tyrosine in the species under present investigation is in accordance with the findings of Monne (1959), Chowdhury *et al.* (1962) and Gupta and Kapoor (1979) who made similar observations in *H. taeniaeformis, Taenia saginata* and *C. digonopora*, respectively.

Several biological functions are attributed to proteins. In general, proteins are associated in contractile systems, in transport, as protective agents, toxins, hormones, amino-acid reserves and as important structural components (Barrett, 1981).

The occurence and distribution of the basic proteins like histones has not been studied in parasitic helminths (Barrett, 1981). However, histones being nucleoproteins, in the present investigation also, the nuclei of all the cells showed the presence of this basic protein in the two species (Figs 16, 30). Similarly, DNA appeared to be present in the nuclei of all the cells, but a high concentration of it was found in the reproductive organs (Figs 35, 38). The eggs of *D. penetrans* had considerable amount of DNA in the vitelline cells but a very less concentration of it was found in the eggs of *L. indicus*. Burton and Bogitsh (1963) and Ohman-James (1968) showed that DNA is confined to the nuclei of all the cells in *H. microstoma* and *D. dendriticum*, respectively. However, because of the presence of glycogen in the vitelline cell nuclei in *L. indicus* the concentration of DNA was less. This observation seems in agreement with those of Mackiewicz (1968) on *C. laticeps* and *C. fennica*. Comparatively higher concentration of DNA was found in the vitelline cells of the eggs of *D. penetrans* because it had only a few cells with intranuclear glycogen vacuole, most of them being rich in lipid.

Abundance of RNA was recorded in the tegument, muscles and reproductive organs of the species under investigation (Figs 36, 37). Similar observations were made by Burton and Bogitsh (1962), and Gupta and Kapoor (1979). Besides tegument, muscles and reproductive organs, all the other organs and tissues of the two species also had positive reaction for RNA. This seems quite usual in view of the well established functions, like the storage, transmission and translation of the genetic material, by these nucleic acids.

In gut parasites like cestodes, the tegument is the most important interface since it comes into immediate contact with the host tissue. In both the species under present investigations the tegument was observed to be highly positive for acid-mucopolysaccharides (Figs 6, 24). On the basis of what is known of the biochemistry and function of the mucopolysaccharides, a protective function from the digestive enzymes of the host can be assigned to them (Barrett, 1981). The eggs of *L. indicus* and *D. penetrans* were also found to be positive for acid-mucopolysaccharides. Since mucopolysaccharide molecules can contain upto 500 times their own weight of water, such a property of theirs is used in

egg hatching in trematodes (Barrett, 1981). It is suggested that the mucopolysaccharides perform a similar function in *L. indicus* and *D. penetrans*, as well.

A single specimen with pink colouration of the body of *L. indicus* was obtained during the present study. As the investigation did not reveal it to be due to haemoglobin, this colouration might possibly be on account of vitamin B_{12}. Such pink colour was earlier reported for some specimens of *Biacetabulum infrequens* and *Glaridacris laruei* by Mackiewicz (1972) on which assays for vitamin B_{12} had been done. Mackiewicz (1981) considered that caryophyllidean cestodes have high concentrations of vitamin B_{12} like the pseudophyllidean cestodes. It is known that cestodes with vitamin B_{12} are capable of forming propionate from succinate as an end product of anaerobic energy metabolism, which leads to an increased energy yield from their substrates (Tkachuck *et al.*, 1977). Therefore, since the presence of vitamin B_{12} is recorded from the caryophyllids, it may be assumed that caryophyllids also form propionate which functions to increase the energy available for their egg production which is otherwise low because of their monozoic body plan (Mackiewicz, 1981).

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References

BAUGH, S. C., SINGH, J. P. (1979): Glycogen distribution in *Raillietina echinobothrida* (Magnin, 1880). *Rev. Iber. Parasitol.*, 39: 223—232

BARRETT, J. (1981): *Biochemistry of Parasitic Helminths*. The Scientific and Medical Division McMillan Publisher Ltd. London and Basingstoke (380 pp.)

BRAND, T. von (1952): *Chemical Physiology of Endoparasitic Animals*. Academic Press, Inc. New York

BURTON, J., BOGITSCH (1963): Histochemical studies on *Hymenolepis microstoma* (Cestoda: Hymenolepidae). *J. Parasitol.*, 49: 989—997

CALON, P., JENNINGS, J. B. (1974): Calorific values in the phylum platyhelminthes. The relationship between potential energy, mode of life and the evolution of entoparasitism. *Biological Bulletin*, 147: 81—94

CHOWDHURY, A. B., DASGUPTA, B., ROY, H. N. (1962): On the nature and structure of the calcareous corpuscles in *Taenia saginata*. *Parasitology*, 52: 153—157

GAUR, A. S., AGARWAL, S. M. (1981): Non-specific phosphomonoesterases in three species of caryophyllids from *Clarias batrachus* (Linn.). *Proc. Ind. Acad. Parasitol.*, 2: 71—75

GINETSINSKAYA, T. A., USPENSKAYA, Z. I. (1965): The characteristics of glycogen and fat stores in the tissues of some fish helminths, regarding their location in the body of the host. *Helminthologia*, 6: 319—333