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Lectin-binding Properties of the Surfaces of Three Developmental Stages of *Paragonimus ohirai*

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Abstract

The lectin-binding properties of the surface of live lung flukes, *Paragonimus ohirai,* were studied in newly excysted juveniles, liver stage juveniles, and adult worms. Lectins of the same kind bound to the surface during fluke development. Concanavalin A (Con A), peanut agglutinin (PNA), *Ricinus communis* agglutinin I (RCA_{120}), soybean agglutinin (SBA), and wheat germ agglutinin (WGA) bound to the surface of living flukes, whereas *Dolichos biflorus* agglutinin (DBA), *Limax flavus* agglutinin (LFA), and *Ulex europaeus* agglutinin I (UEA-1) did not. Of the reactive lectins, PNA showed the greatest binding at juvenile stages, and Con A showed the greatest binding at the adult stage. RCA_{120} had the weakest binding throughout all stages. The binding of all reactive lectins except WGA was almost completely inhibited by corresponding competing sugars. Binding of WGA was slightly reduced by 0.2 M N-acetyl-D-glucosamine. On sections frozen and cut after lectin labeling to living adult worms, linear staining was seen at the apical region of the worm surface. All reactive lectins bound to the tegumental syncytium and also bound more weakly to the tegumental cells in the adult frozen sections.

Key words: *Paragonimus ohirai,* Worm surface, Lectins, Developmental stages, Tegument

Introduction

Helminths are known to alter the biochemical and antigenic properties of their body surface during development within the host (Philipp and Rumjaneck, 1984). Lectin is a good probe for analyzing exposed oligosaccharides of glycoconjugates and has been utilized to characterize the complex oligosaccharides exposed on helminth surface. In parasitic trematodes, there have been many reports about lectin binding to the surface of *Schistosoma mansoni* (Stein and Lumsden, 1973; Bennett and Seed, 1977; Murrell *et al.*, 1978; Simpson and Smithers, 1980; Linder and Huldt, 1982; Simpson *et al.*, 1983). However, the genus *Schistosoma* is biologically unique among parasitic trematodes, namely, the fluke is dioecious, develops cercariae which infect by penetrating the host's skin, possesses a tegumental double outer membrane from a schistosomulum stage, and matures in the blood vessels of the host. Therefore, the information on lectin-binding properties in other parasitic trematodes is indispensable for understanding the property of the trematode surface, but there have been very few reports. Lung flukes, *Paragonimus,* are hermaphroditic and initiate infection upon ingestion of the metacercaria, as do the majority of parasitic trematodes. Moreover, the flukes migrate within the host until they reach the lung of their final location. In the present study, we examined the lectin-binding properties of the surface of living *Paragonimus ohirai* and compared the properties among three developmental stages.

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Materials and Methods

Parasite

P.ohirai metacercariae were collected from naturally infected crabs, *Sesarma dehaani,* washed in sterile saline, and then excysted by cultivating overnight in Tyrode's salt solution (pH 8.0) at 37°C. Juveniles migrating in the liver

and adult flukes in the lung were obtained from infected Wistar rats at 2 weeks and 6-8 weeks after infection, respectively. The flukes were washed three times in saline before binding experiments.

Lectin binding

For lectin binding to the surface of intact worms, living flukes were incubated for 30 min at room temperature with fluorescein isothiocyanate (FITC)-conjugated lectins diluted to 1/40 in phosphate-buffered saline (PBS, pH 7.2). After being washed five times in cold PBS, the flukes were fixed with 5% formaldehyde in PBS or some of adult worms were frozen and sectioned in a cryostat as described previously (Ohara *et al.*, 1985). For lectin binding to tegumental structure, frozen sections of unstained adult worms were fixed with 95% ethanol, washed with PBS, and then stained with FITC-lectins as described above. The preparations were examined using a Nikon fluorescence microscope with epi-ultraviolet illumination. Seven kinds of FITC-lectins, Con A, DBA, RCA_{120}, PNA, SBA, UEA-1, and WGA, were obtained as Fluorescein lectin kit I from Vector Laboratories (Burlingame, CA). FITC-LFA was obtained from E. Y. Laboratories (San Mateco, CA).

Results

The binding of FITC-lectins to the exposed surface of living flukes was examined. Fig. 1 shows the lectin reactivity with newly excysted juvenile surface. Con A, PNA, SBA, and WGA showed intense staining and RCA_{120} moderate staining, while little or no staining was detected with DBA, LFA, and UEA-1. Of the positive lectins, PNA showed the intensest staining. Figs. 2 and 3 show the lectin reactivity with liver stage juveniles and adult worms, respectively. The same lectins as those binding to the newly excysted juvenile surface showed positive staining, and with that of RCA_{120} being much weaker than those of the other lectins. On the adult worm surface, Con A gave relatively intenser staining than PNA and SBA and WGA gave moderate, although brighter than RCA_{120}, staining. The surface staining of the above positive lectins exhibited a furrow-like pattern for juveniles and an imbricate one for adult worms (Fig. 2D and Fig. 3A). On sections of the adult worm frozen and cut after lectin labeling, almost uniform staining was observed at the apical region of the worm surface with all reactive lectins (Fig. 3D). The staining with all positive

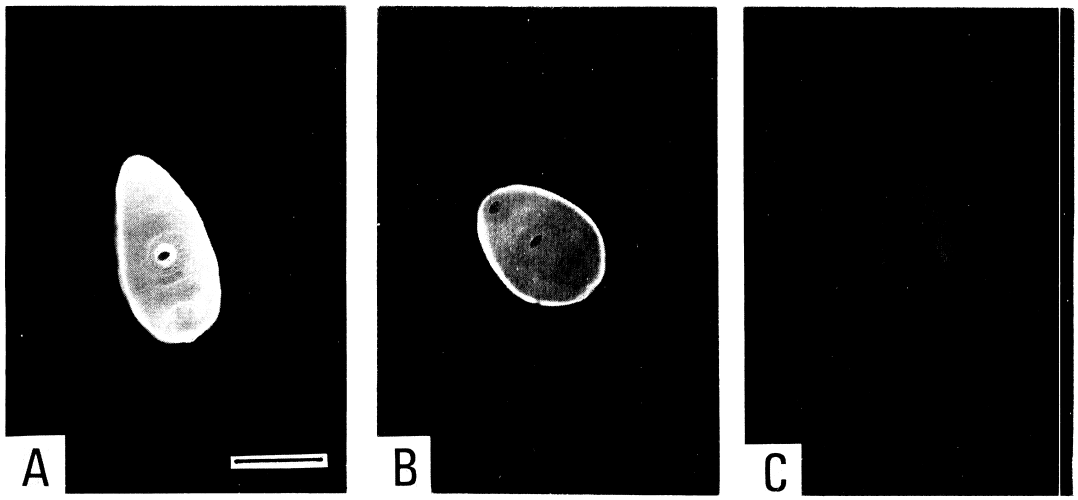

Fig. 1. Lectin binding to the surface of live newly excysted juveniles of *Paragonimus ohirai*. A, intense staining with PNA. Similar intense staining is also seen with Con A, SBA, and WGA. B, moderate staining with RCA_{120}. C, negative staining with UEA-1. DBA and LFA were also negative. Bar = 200 μm

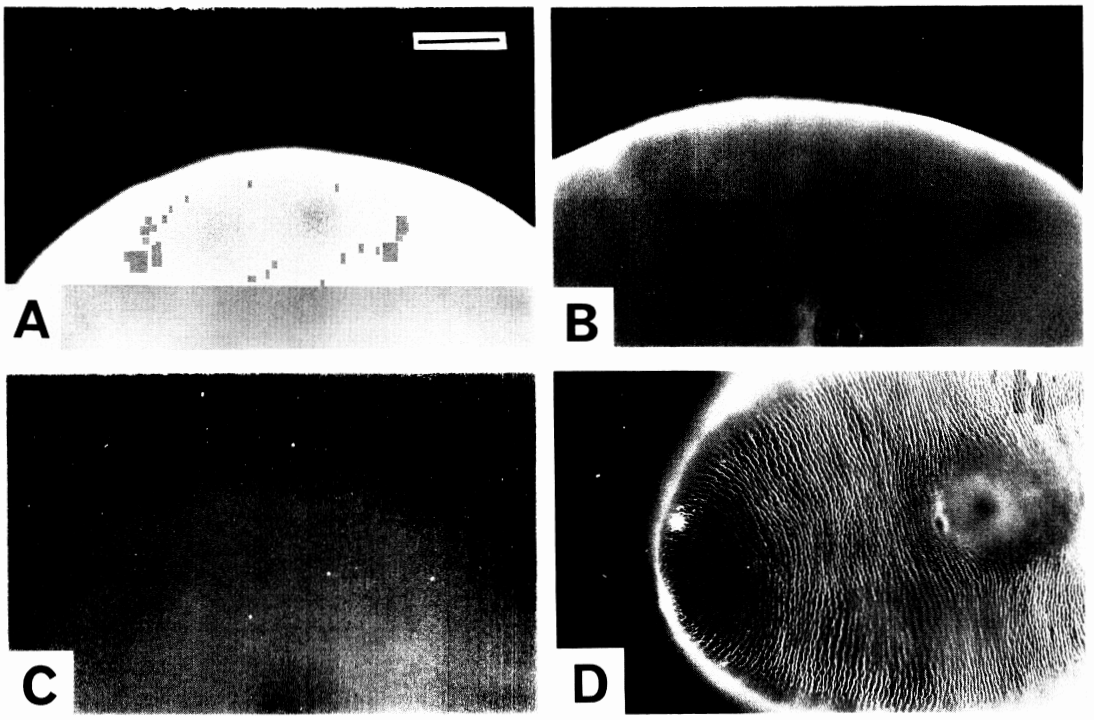

Fig. 2. Lectin binding to the surface of liver stage juveniles of *Paragonimus ohirai*. The lectin-binding properties are similar to those for newly excysted juveniles. A, intense staining. B, moderate staining. C, negative staining. D, characteristic furrow-like pattern of staining. Bar = 200 μm

lectins except WGA was almost completely inhibited by 0.2 M corresponding competing sugars, while the staining with WGA was slightly inhibited by N-acetyl-D-glucosamine (Fig. 4).

Lectin binding to the tegumental structure was observed on adult frozen sections (Fig. 5). All of the lectins reactive with the intact worm surface showed positive staining at the tegumental syncytium and the tegumental cells. The staining at the latter site was significantly weaker than at the former. The staining properties at the apical surface was similar to those on the intact surface. The spines in the syncytium were negative. Con A, SBA, and WGA also bound to the subtegumental muscle layers. Summary on the lectin-binding properties of three developmental stages is shown in Table 1.

Discussion

The present study was demonstrated that a variety of lectins bind to the surface of *P.ohirai* fluke and lectins of the same kind do during development from newly excysted juveniles to adult worms. Con A, PNA, RCA_{120}, SBA, and WGA bound to the fluke surface, but DBA, LFA, and UEA-1 did not bind. The binding of all lectins except WGA was almost completely inhibited by corresponding competing sugars, indicating the specificity of these binding properties. The sugar specificity of lectins (Lis and Sharon, 1986) indicates that the sugars exposed on the fluke surface are α-mannose and/or α-glucose, β-galactose, and N-acetyl-D-galactosamine. However, the presence of α-N-acetyl-D-galactosamine may be excluded since the binding of DBA specific for the sugar was negligible. SBA interacts strongly with α or β -N-acetyl-D-galactosamine and weakly with α-D-galactose (Hammarström *et al.*, 1977). Therefore, SBA binding may be due to β-N-acetyl-D-galactosamine and/or α-D-galactose

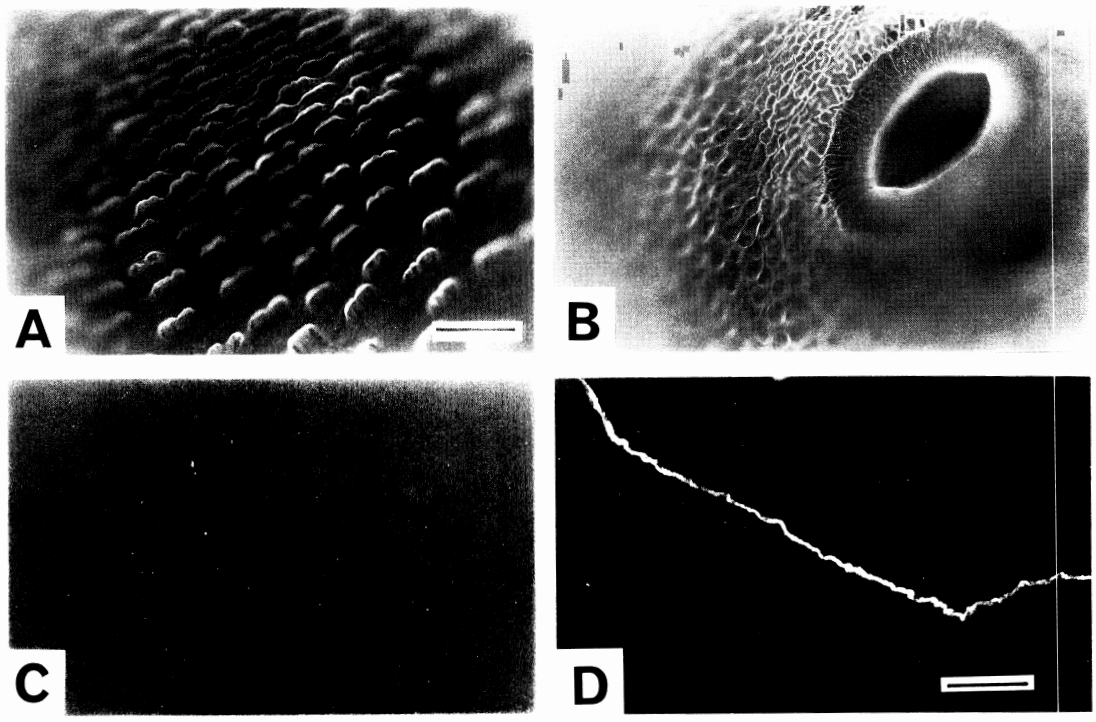

Fig. 3. Lectin binding to the surface of live adult worms of *Paragonimus ohirai*. The lectin-binding properties are similar to those for juvenile stages except that SBA and WGA gave weaker staining. A, characteristic imbricate pattern of the fluorescent staining. B, staining around ventral sucker. C, negative staining. Weak autofluorescence is seen in vitelline cells. D, section frozen and cut after lectin labeling. A, B, C: Bar = 50 μm; D: Bar = 200 μm

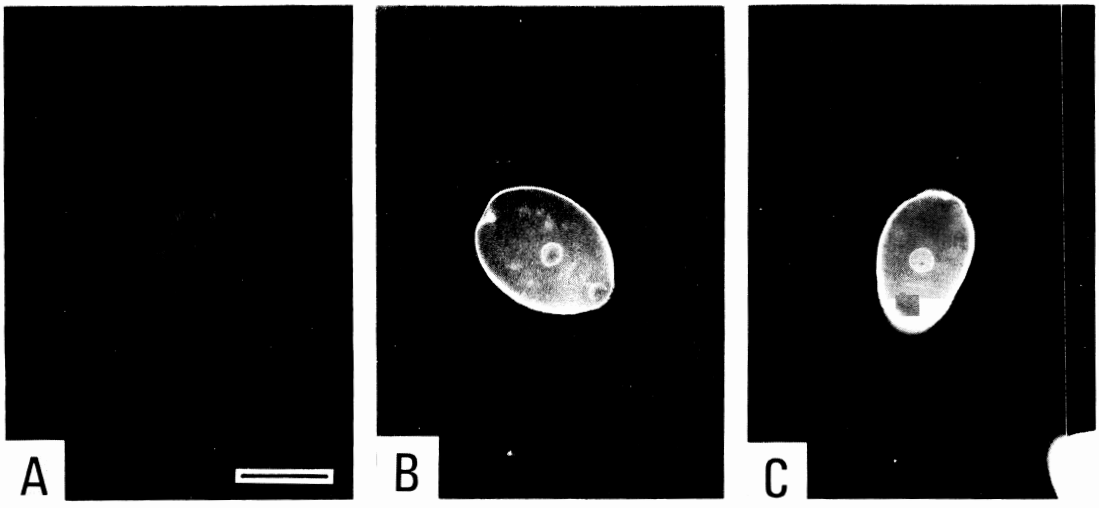

Fig. 4. Lectin binding to the surface of live newly excysted juveniles in the presence of 0.2 M competing sugars. A, almost complete inhibition of PNA staining. Similar inhibition is also seen in Con A, RCA_{120}, and SBA. B, slight inhibition of WGA staining. C, WGA staining without N-acetyl-D-glucosamine. Bar = 200 μm

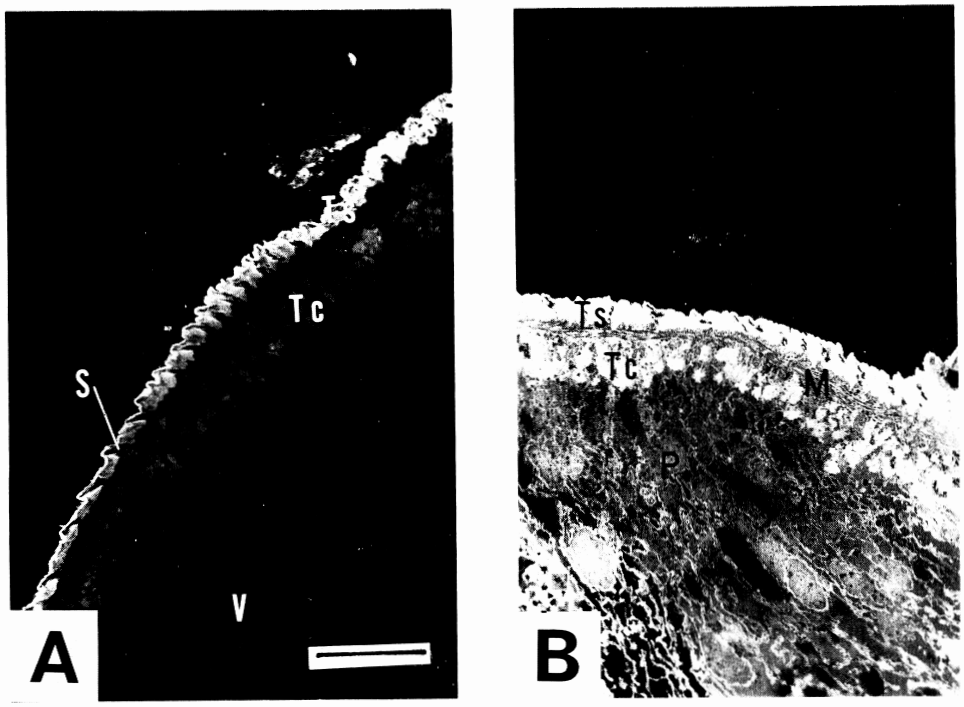

Fig. 5. Lectin binding to the tegumental structure on frozen sections of adult worms. A, PNA staining. The tegumental syncytium (Ts) is strongly stained, the tegumental cells (Tc) weakly stained and the surface spines (S) not stained. Vitelline cells (V) give autofluorescence. B, Con A staining. Intense staining over the tegumental syncytium and positive staining on the tegumental cells, sub-tegumental muscle layer (M), and parenchyma (P) are seen. Bar = 200 μm

Table 1. Comparison of lectin-binding properties of the surface of live *Paragonimus ohirai* flukes among three developmental stages.

Lectin	Major specificity	Newly excysted juvenile	Liver stage juvenile	Adult
Con A	D-mannose	++	++	++
DBA	GalNAc	−	−	−
LFA	Sialic acid	−	−	−
PNA	D-galactose	++	++	++
RCA_{120}	D-galactose	+	+	+
SBA	GalNAc	++	++	+
UEA-1	L-fucose	−	−	−
WGA	GluNAc	++	++	+

The degree of fluorescence on the fluke surface was graded as −, negative; +, moderate; ++ intense.

sugars. Slight inhibition of WGA binding of the competing sugar by N-acetyl-D-glucosamine suggests the presence of small amounts of the sugar. WGA binding may mainly be due to non-specific interaction derived from the positively charged lectin. The absence of any detectable fluorescence with LFA and UEA-1 indicates that little or no sialic acid and α-L-fucose are present on the fluke surface. The two sugars have rarely been identified in helminths (Barrett, 1981).

The staining of the above positive lectins with the intact surface of the liver stage juveniles and adult worms was seen as a furrow-like pattern and an imbricate one, respectively. The peculiar staining patterns seem to be formed by stressed brightness of the outlines along furrows and spine covering tubercles. Because the uniform staining over the intact surface was seen on the adult worm sections frozen and cut after lectin labeling. In staining of worm sections, all lectins reactive with the worm surface bound to the tegumental syncytium and more weakly to the tegumental cells. This implies that the lectin-bound glycoconjugates are of parasitic origin and that, as reported in the tegumental glycocalyx of *F. hepatica* (Hanna, 1980), they appear to be synthesized in the tegumental cells, transported into the syncytium where they accumulate, and finally secreted at the apical plasma membrane.

In *S. mansoni,* during development from schistosomule stage to adult stage, Con A, PNA, RCA, and WGA bound to the fluke surface whereas DBA, SBA, and UEA did not (Simpson and Smithers, 1980; Linder and Huldt, 1982; Simpson *et al.*, 1983). Sialic acid is present at terminal sugars because neuraminidase treatment not only expressed SBA binding but also increased PNA binding to the worms after contact with the host (Simpson *et al.*, 1983). There are two marked differences in the lectin-binding profiles between *P. ohirai* and *S. mansoni,* namely the presence of sialic acid and SBA binding. The sialic acid-containing molecules on *S. mansoni* surface are thought to be derived from the host because of the presence of host glycoconjugate antigens (Goldring *et al.*, 1976; Sher *et al.*, 1978) and a specific sialyl transferase has not been detected from worms. Therefore, one of the two main differences may be reflected by the ability to incorporate the molecules from the host into worm surface. The difference on SBA binding between *P. ohirai* and *S. mansoni* is interesting. It is important to examine whether SBA binding to the worm surface occurs in other parasitic trematodes.

There were no marked alterations in exposed sugars on the fluke surface throughout worm development, but two quantative changes in lectin binding occurred between liver juvenile and adult stages, the shift of the greatest binding lectin from PNA to Con A and the reduction in SBA and WGA binding. We prepared a monoclonal antibody recognizing the *P. ohirai* tegumental antigen which is present from metacercaria to adult stages but decreases with worm maturation (Oikawa and Ikeda, 1989) and observed ultrastructurally that the monoclonal antibody bound to tegumental glycocalyx and tegumental secretory bodies (Fujino *et al.*, 1989). The purified glycocalyx antigen was markedly reactive with PNA, moderately reactive with RCA and unreactive with Con A (Ikeda and Oikawa, unpublished data). Therefore, the relatively reduction in PNA binding at adult stage appears to be due to the decrease of the glycocalyx antigen. Thus, during fluke maturation some modifications of glycoconjugate components occur on the surface. Studies are in progress to determine whether the glycoconjugates bound by Con A, SBA, and WGA have some antigenicities in *P.ohirai* infection.

REFERENCES

1) Barrett, J. (1981): Biochemistry of parasitic helminths. University Park Press, Baltimore.
2) Bennett, J. L. and Seed, J. L. (1977): Characterization and isolation of concanavalin A binding sites from the epidermis of *S. mansoni.* J. Parasitol., 63, 250–258.
3) Fujino, T., Ikeda, T., Oikawa, Y., Fukuda, K. and Ishii, Y. (1989): Expression of a tegumental antigen during the development of *Paragonimus ohirai.* Jpn. J. Parasitol., 38, 263–270.
4) Goldring, O. L., Clegg, J. A., Smithers, S. R. and Terry, R. J. (1976): Acquisition of human blood group antigens by *Schistosoma mansoni.* Clin. Exp. Immunol., 26, 181–187.

5) Hammarström, S., Murphy, L. A., Goldstein, I. J. and Etzler, M. E. (1977): Carbohydrate binding specificity of four N-acetyl-D-galactosamine-"specific" lectins: *Helix pomatia* A hemagglutinin, soy bean agglutinin, lima bean lectin, and *Dolichos biflorus* lectin. Biochemistry, 16, 2750–2755.
6) Hanna, R. E. B. (1980): *Fasciola hepatica*: Autoradiography of protein synthesis, transport, and secretion by the tegument. Exp. Parasitol., 50, 297–304.
7) Linder, E. and Huldt, G. (1982): Distribution of exposed and hidden carbohydrates of *Schistosoma mansoni* adult worms demonstrated by selective binding of fluorochrome-conjugated lectins. Parasitology, 85, 503–509.
8) Lis, H. and Sharon, N. (1986): Lectins as molecules and as tools. Annual Review of Biochemistry, 55, 35–67.
9) Murrell, K. D., Taylor, D. W., Vannier, W. E. and Dean, D. A. (1978): *Schistosoma mansoni*: Analysis of surface membrane carbohydrates using lectins. Exp. Parasitol., 46, 247–255.
10) Ohara, H., Ikeda, T., Oikawa, Y. and Tani, S. (1985): Studies on antibody response to rats infected with *Paragonimus ohirai* by immunofluorescent staining method. Jpn. J. Parasitol., 34, 245–252.
11) Oikawa, Y. and Ikeda, T. (1989): Monoclonal antibodies recognizing the main surface antigens of newly excysted metacercaria or adult flukes of *Paragonimus ohirai*. Jpn. J. Trop. Med. Hyg., 17, 323–329.
12) Philipp, M. and Rumjaneck, F. D. (1984): Antigenic and dynamic properties of helminth surface structures. Mol. Biochem. Parasitol., 10, 245–268.
13) Sher, A., Hall, B. F. and Vadas, M. A. (1978): Acquisition of murine major histocompatibility complex gene products by schistosomula of *Schistosoma mansoni*. J. Exp. Med., 148, 46–57.
14) Simpson, A. J. G. and Smithers, S. R. (1980): Characterization of the exposed carbohydrates on the surface membrane of adult *Schistosoma mansoni* by analysis of lectin binding. Parasitology, 81, 1–15.
15) Simpson, A. J. G., Correa-Oliveira, R., Smithers, S. R. and Sher, A. (1983): The exposed carbohydrates of schistosomula of *Schistosoma mansoni* and their modification during maturation in vivo. Mol. Biochem. Parasitol., 8, 191–205.
16) Stein, P. C. and Lumsden, R. D. (1973): *Schistosoma mansoni*: Topochemical features of cercariae, schistosomula and adults. Exp. Parasitol., 33, 499–514.