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Changes in Activities of Lysosomal Enzymes in Experimental Schistosomiasis Mansoni

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Introduction

Characterization of biochemical aspects of host responses in human and murine schistosomiasis mansoni has demonstrated changes in some enzyme activities. In particular, detailed studies were conducted with β-glucuronidase activity. Saad *et al.* (1977) reported an increment of β-glucuronidase activity in extract of liver and spleen of mice infected with *Schistosoma mansoni*. Fripp (1960) also reported that β-glucuronidase activity increased in the urine of patients of schistosomiasis mansoni. Saad *et al.* (1977) attributed the increased spleen β-glucuronidase activity in mice to hyperplasia of macrophages caused by elevation of estrogenic activities, which resulted from disturbed hepatic metabollism. On the other hand, since β-glucuronidase is primarily localized in lysosomes (Fishman *et al.*, 1967), the increased β-glucuronidase activity in liver was considered to be due to an intensive infiltration of cells, which were rich in lysosomes, associated with granuloma formation in liver (Saad *et al.*, 1977). Moreover, in experimental hepatosplenic bilharziasis, an increase in the number of lysosomes was demonstrated in liver parenchymal cells by electron microscopy (Ramadan, 1971). In addition to β-glucuronidase, some other lysosomal enzymes such as ribonuclease (Zeitoun *et al.*, 1978) and alkaline phosphatase (Awadalla *et al.*, 1975) were also reported to increase in their activities in human and murine schistosomiasis, respectively.

During the course of investigation of biochemical changes in metabolism of mice infected with *S. mansoni*, we found that β-glucuronidase activity significantly increased in serum as well as in extracts of liver and spleen. This observation led us to study changes in acid phosphatase activity, the marker enzyme for lysosomes, in order to verify lysosomal alterations in schistosomiasis mansoni. We further examined lysosomal enzyme activities in mice injected with isolated eggs of *S. mansoni* to eliminate the possibility that adult schistosomes affect lysosomal enzymes in mice.

Materials and Methods

Experimental animals: Male ICR mice, 7–8 weeks of age, were supplied by Charles River Japan (Tokyo). These mice were infected with 150 unisexual or bisexual cercariae of *S. mansoni* (Puerto Rican strain) by the tail-dipping procedure. ICR mice were also injected with eggs of this parasite to produce granulomatous lesions in lung. Eggs were isolated from liver of

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mice 8 weeks after infection with cercariae of *S. mansoni* according to Coker and Lichtenberg (1956). Five thousands eggs were suspended in 0.3 ml of physiological saline containing 200 IU penicillin and injected into tail veins of mice by the method of von Lichtenberg (1962).

Assays of lysosomal enzymes: Three groups of mice, i.e., (1) infected with bisexual *S. mansoni*; (2) infected only with male schistosomes; (3) age-matched uninfected controls, were lightly anesthetized and sacrificed by exsanguination. Liver and spleen were immediately removed and weighed. Subsequently, these specimens were homogenized using a glass homogenizer for 2 min at 4 C in 20-volumes of distilled water to disrupt latency of lysosomal enzymes. Crude extracts were also prepared in the same manner as above from spleen and lung of mice which received the intravenous injection of *S. mansoni* eggs. Serum was isolated from blood obtained by cardiac puncture. All of these specimens were used for assays of β-glucronidase and acid phosphatase.

β-Glucuronidase activity was determined at 37 C as described by Fishman *et al.* (1948) with a reaction mixture containing 1 mM phenolphthalein-mono-β-glucuronic acid, 0.1 ml of the enzyme preparation (1.5–2.5 mg of protein) and 100 mM acetate buffer, pH 4.5, in a final volume of 1.0 ml. Acid phosphatase was also assayed at 37 C. The reaction mixture contained 20 mM β-glycerophosphate, 0.1 ml of the enzyme preparation of the same quantity of protein as above and 100 mM cacodylate-HCl buffer, pH 5.1, in a final volume of 1.0 ml. The content of inorganic phosphate was determined by the method of Fiske and Subbarow (1925) as modified by Furukawa *et al.* (1952). One unit of these enzyme activities was defined as the amount needed to catalyze formation of the products at one μmole per minute under the present assay conditions.

Protein determination: The content of protein was estimated by the method of Lowry *et al.* (1951) with bovine serum albumin as a standard.

Chemicals: β-Glycerophosphate and phenolphthalein-mono-β-glucuronic acid were supplied by Sigma Chemical Co. (St. Louis, Mo). Other chemicals were of the highest purity commercially available.

Statistical analysis: When needed, data were statistically analyzed with student's *t*-test. Differences with a probability value (P) less than 0.05 were considered significant.

Results

Liver: Biochemical characterization of liver extracts of mice 9 weeks after infec-

Table 1 Changes in wet weight, protein content and lysosomal enzyme activities in the liver of mice 9 weeks after bisexual and unisexual infection with *Schistosoma mansoni*

	Bisexual infection (n=12)	Unisexual infection (n=15)	Control (n=13)
Liver wet weight (g)	4.31±0.51*	1.49±0.18	1.45±0.22
Protein (mg/liver)	673±103*	316±56	288±31
β-Glucuronidase			
(munits/g wet weight)	415±35*	275±31	255±32
(munits/mg protein)	2.65±0.21*	1.30±0.18	1.28±0.15
Acid phosphatase			
(units/g wet weight)	1.82±0.33	2.24±0.25	2.05±0.35
(munits/mg protein)	11.8±1.43	10.3±1.50	10.4±1.41

Each datum represents the mean value (±S.D.) of two to three separate experiments. Details of preparation and assay of enzymes were described in the text.
* $P<0.001$

tion with bisexual *S. mansoni* exhibited a significant increase in β-glucuronidase activity, whereas increment of this enzyme was not observed with the specimens prepared from mice infected with only male schistosomes (Table 1). The activity of β-glucuronidase was also found to increase at 7 and 11 weeks after infection with bisexual *S. mansoni* (data not shown). Moreover, wet weight and protein content of liver also significantly increased in mice infected with bisexual *S. mansoni* but not in those infected with only male schistosomes (Table 1). In contrast to β-glucuronidase activity, however, acid phosphatase was within normal limit in the activity in both specimens (Table 1).

Lung: Mice which received the intravenous injection with *S. mansoni* eggs exhibited a significant increase in wet weight and protein content of lung (Fig. 1). The activity of β-glucuronidase also significantly increased in lung extracts of these egg-injected mice (Fig. 2). This increment became first apparent on 8th day after injection, and the activity remained significantly higher than in age-matched controls for at least 30 days (Fig. 5). However, there was no difference in acid phosphatase ac-

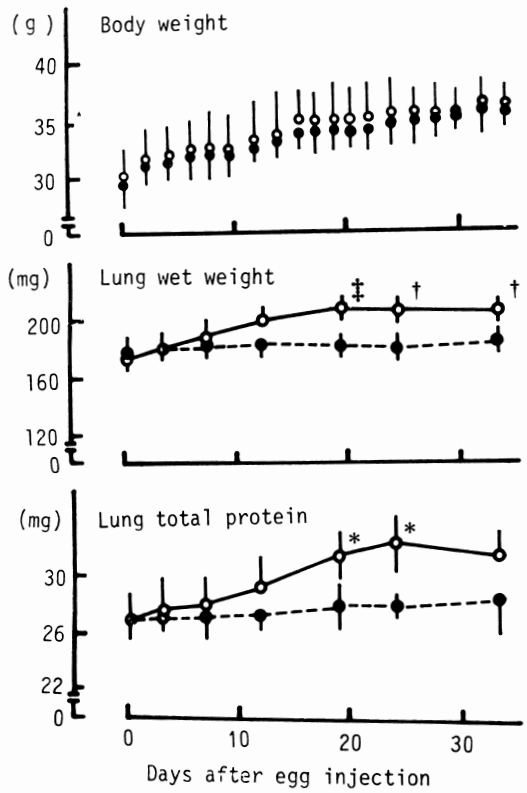

Fig. 1 Changes in body weight, lung wet weight and protein content of mice at various time intervals after intravenous injection of *Schistosoma mansoni* eggs.

Each point represents the mean value of three to five animals; egg-injected mice (○) and saline-treated controls (●). Vertical lines represent standard deviation. An essentially same result was also obtained in the other comparative experiments.

* $P<0.05$, † $P<0.025$, ‡ $P<0.01$

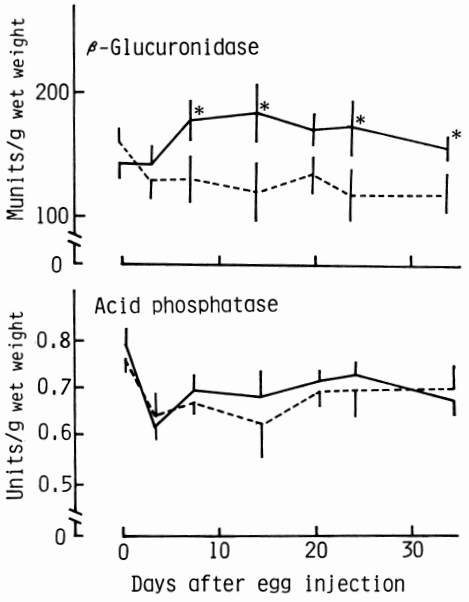

Fig. 2 Change in lysosomal enzyme activities in the lungs of mice at various time intervals after intravenous injection of *Schistosoma mansoni* eggs.

Each point represents the mean value of three to five animals; egg-injected mice (——) and saline-treated controls (----). Vertical lines represent standard deviation. An essentially same result was also obtained in the other comparative experiments.

* $P<0.05$

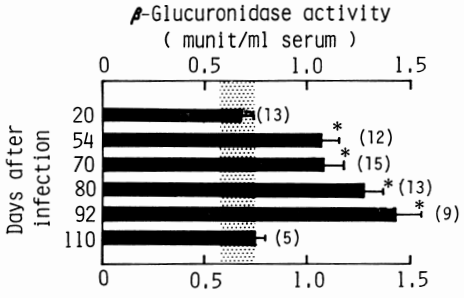

Fig. 3 Change in serum β-glucuronidase activity of mice at various time intervals after bisexual infection with *Schistosoma mansoni*.

Each bar represents the mean value of two to four separate experiments. In each experiment the sera taken from two to five mice were pooled, and then β-glucuronidase activity was assayed. Dotted area shows the activity of β-glucuronidase in the control sera. Vertical lines represent standard deviation with number of mice in parentheses.

* $P<0.01$

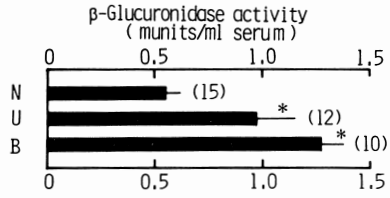

Fig. 4 Change in serum β-glucuronidase activity of mice 11 weeks after bisexual and unisexual infection with *Schistosoma mansoni*.

Each bar represents the mean value of two to three separate experiments, and vertical lines represent standard deviation with number of mice in parentheses. The abbreviations were as follows: B, bisexually infected mice; U, unisexually infected mice; N, uninfected control mice.

* $P<0.01$

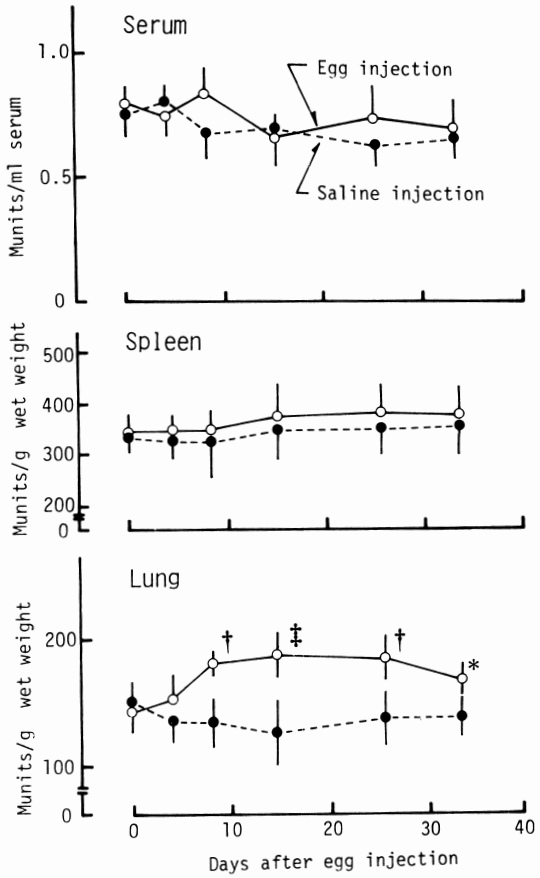

Fig. 5 Change in β-glucuronidase activities in the serum, spleen and lung of mice at various time intervals after intravenous injection of *Schistosoma mansoni* eggs.

Each point represents the mean value of three to five mice; egg-injected mice (○) and saline-treated control (●). Vertical lines represent standard deviation. An essentially same result was also obtained in another comparative experiment.

* $P<0.05$, † $P<0.025$, ‡ $P>0.01$

tivity between these two groups (Fig. 2).

Serum: β-Glucuronidase activity was found to increase significantly in serum of mice infected with bisexual *S. mansoni* (Fig. 3). The activity was already significantly higher than in controls before the 54th day, and was at its maximum around the 90th day after infection. Moreover, mice infected only with male schistosomes also showed a significantly higher activity of this enzyme than in controls (Fig. 4), although the rate of increase in this group was lower than in mice infected with bisexual *S. mansoni* (Fig. 4). However, there was no difference in serum β-glucuronidase activity between egg-injected and saline-injected mice (Fig. 5). In contrast to

Table 2 Changes in wet weight, protein content and lysosomal enzyme activities in the spleen of mice 9 weeks after bisexual and unisexual infection with *Schistosoma mansoni*

	Bisexual infection (n=10)	Unisexual infection (n=9)	Control (n=15)
Spleen wet weight (g)	560±101*	181±40*	96±24
Protein (mg/spleen)	108.1±9.7*	33.3±3.8*	18.7±1.5
β-Glucuronidase (munits/g wet weight)	598±121*	471±59*	342±64
Acid phosphatase (units/g wet weight)	1.80±0.09	1.75±0.11	1.71±0.13

Each datum represents the mean value (±S.D.) of two separate experiments.
* $P<0.001$

β-glucuronidase, acid phosphatase activity was within normal limit in these specimens throughout the course of observation (data not shown).

Spleen: Nine weeks after infection with male schistosomes as well as with bisexual ones, mice exhibited significant increases in wet weight and protein content of the spleen (Table 2). Moreover, the activity of β-glucuronidase increased in the specimen prepared from both groups of mice (Table 2). However, egg-injected mice did not exhibit an increment of spleen β-glucuronidase activity (Fig. 5), although the enzyme activity in lungs increased in these mice (Fig. 2). Acid phosphatase activity was within normal limit in spleen of both groups of infected mice (Table 2).

Histopathological examination of spleen of mice infected with male *S. mansoni* as well as bisexual parasites demonstrated a moderate *reticulo*endothelial hyperplasia and an enlarged white pulp associated with active lymphoid follicles. Granulomatous lesions, however, were barely found in spleen.

Discussion

Our present study demonstrated that the activity of β-glucuronidase increased in liver and spleen extracts of mice infected with bisexual *Schistosoma mansoni*, and it appears to be compatible with that of Saad *et al.* (1977). In addition to this observation, the increased activity of β-glucuronidase in lungs with granulomas induced by injection with *S. mansoni* eggs suggests that granulomatous lesions primarily contribute to increment of this enzyme at least in liver and lung. However, when mice were infected only with male schistosomes, the activity of β-glucuronidase in liver did not increase, whereas that in spleen significantly increased. These findings suggest that the increased β-glucuronidase activity in spleen is not attributable to granuloma formation in liver. This seems incompatible with Saad *et al.* (1977) who reported that the increment of spleen β-glucuronidase activity was due to elevation of estrogenic activities resulting from disturbed hepatic metabolism. Moreover, it was also found that granulomas in lungs did not cause elevation of spleen β-glucuronidase activity. These findings suggest that only the presence of schistosomes is needed for elevation of spleen β-glucuronidase activity, and that elevation of this enzyme may be deeply related with splenomegaly which was observed in mice infected with only male schistosomes as well as with bisexual parasites, but not in those injected with eggs of this parasite.

In contrast to β-glucuronidase, acid phosphatase activity was within normal limit in all specimens examined. Our preliminary experiments also indicated that there was no difference in activity of phosphatase between extracts of isolated granulomas and whole liver of mice infected with bisexual *S. mansoni* (Tanabe, unpublished data). Although Ramadan (1971) found an increase in the number of lysosomes in liver parenchymal cells in hepatosplenic bilharziasis, our present data suggest that biochemical responses of lysosomal enzymes to *S. mansoni* infection are not simple and uniform in mice. Similar observations on different responses of lysosomal enzymes to infectious agents have been made with mice infected with *Mycoplasma fermentans* (Gabridge, 1975).

Although the increased serum β-glucuronidase activity may be closely related to elevation of this enzyme activity in urine of patients with schistosomiasis mansoni (Fripp, 1961), the origin of the enzyme in serum is not fully known. At first, the high activity of this enzyme in fibrotic liver (Table 1) and in extract of isolated granulomas (Tanabe, unpublished data) led us to hypothesize that the enzyme in serum was derived from granuloma-constituting cells in the liver. However, the later findings that the activity was also elevated in serum of mice infected only with male schistosomes (Fig. 4), and that there was no difference in activity of serum enzyme between egg-injected and saline-injected mice (Fig. 5) suggest that this hypothesis was improbable. The other possibility that secretion of this enzyme from adult schistosomes is responsible for the increased serum β-glucuronidase activity also can be eliminated by the following evidence, i.e., (1) the enzyme activity in the extract of adult schistosomes was approximately one-tenth of that of normal liver extract (Tanabe and Asami, 1980); (2) schistosomes did not excrete this enzyme during maintenance *in vitro* for at least 24 hours (Tanabe, unpublished data). As Gabridge *et al.* (1975) attributed the increment of serum β-glucuronidase activity to the reticuloendothelial system in mice infected with *M. fermentans*, our observations that both unisexually- and bisexually-infected mice showed spleen enlargement (Table 2) as well as increment of serum β-glucuronidase activity may indicate that the spleen is at least partially responsible for elevation of this enzyme in serum. This seems to be supported further by our findings that no increase in serum β-glucuronidase activity was demonstrated in egg-injected mice which did not reveal splenomegaly and histological changes in spleen.

Although our present investigation did not give any firm evidences to explain why there is elevation of β-glucuronidase, but not of acid phosphatase, in experimental schistosomiasis mansoni, these data may suggest that lysosomes affect granuloma formation in schistosomiasis mansoni, as previous investigators (Heppleston and Styles, 1967; Allison *et al.*, 1966; Richard and Wustman, 1974) reported that abnormal lysosomes resulting from ingestion of silica particles or asbestos fibers might be responsible for an excessive deposition of collagen in lung with pulmonary silicosis or asbestosis.

Summary

To characterize biochemical aspects of host responses to *Schistosoma mansoni* infection, activities of β-glucuronidase and acid phosphatase, which are lysosomal enzymes, were determined using serum as well as extracts of liver, lung and spleen of mice either infected with cercariae or injected with eggs of this parasite via tail vein. The activity of acid phosphatase was within normal limit with all specimens examined throughout the course of obser-

vation. In contrast, the activity of β-glucuronidase of liver significantly increased in mice infected with bisexual *S. mansoni*, but not in those infected only with male schistosomes. β-Glucuronidase activity also increased in lung with granulomas induced by injection with eggs of this parasite. The activity of this enzyme in spleen and serum also significantly increased in mice infected only with male schistosomes as well as with bisexual parasites but not in egg-injected mice.

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マウスの実験的マンソン住血吸虫症におけるライソゾーム酵素活性の変化

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セルカリアの経皮感染によって作成した実験的マンソン住血吸虫症のマウスおよびマンソン住血吸虫卵を尾静脈から注入して肺に虫卵性肉芽腫を形成させたマウスを用いて，ライソゾーム酵素である β-グルロニダーゼと酸フォスファターゼの活性の変化を経日的に種々の臓器について検べた．

酸フォスファターゼ活性は，感染マウスの肝と脾ホモジネートや血清，虫卵注入マウスの肺ホモジネートのいずれにおいても，全観察期間を通じて正常範囲内にあった．

β-グルクロニダーゼ活性は実験群によって著明な変化を示した．すなわち，肝ホモジネートでは両性感染マウスで有意に活性が上昇したが，単性感染マウスでは正常対照マウスと同様のレベルであった．虫卵注入マウスの肺ホモジネートでは，注入8日後から活性が上昇しはじめ，対照とは有意差を示した．血清では，両性および単性感染のマウスにおいて，対照に比べて有意な活性上昇が認められたが，虫卵注入マウスにおいては上昇は見られなかった．また両性および単性感染マウスの腫大した脾のホモジネートでは有意差をもって活性は上昇したが，虫卵注入マウスの脾においては対照と差がなかった．

このことは，実験的住血吸虫症においてある種の臓器では虫卵性肉芽腫に由来すると思われる β-グルクロニダーゼ活性の上昇が見られるが，肉芽腫とは因果関係のない場合もあり，またその上昇は脾の病態と関係する可能性のあることを示している．