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Clinico-Pathological Changes of Mice Following Primary and Secondary Infections with *Hymenolepis nana* Eggs
2. Changes in Clinico-Chemical Values

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Key words: Clinico-chemical values, infection, mice, *Hymenolepis nana*, hypoalbuminemia

Introduction

We have previously reported that laboratory mice showed several changes in hematological values following primary and secondary infections with *Hymenolepis nana* eggs (Shimoda *et al.*, 1982). These changes included a slight anemia as evidenced by a slight decrease in hemoglobin and mean corpuscular hemoglobin values in the infected mice, and an apparent leukocytosis after secondary infection. These observations appear to provide an experimental basis for clinico-pathological changes in human hymenolepiasis. In the present study we have further investigated the changes in clinico-chemical values of sera of mice at various times after primary and secondary infections with *H. nana* eggs.

Materials and Methods

Age, sex and experimental conditions of animals (ddY mice), preparation and administration of *H. nana* eggs, experimental design for primary and secondary infections, and collection of blood have been described previously (Shimoda *et al.*, 1982).

Preparation of mouse sera: Blood sample were stood at 10 C for several hours to allow clot formation. The sera were collected by centrifugation and were analysed immediately or were stored at −35 C for later analysis.

Clinico-chemical examination: All the clinico-chemical examinations were done using commercially available kits (Wako Pure Chemical Co. Ltd., Osaka, Japan). Analytical methods used in the present study were; the biuret method for determination of serum protein content, the bromcresol green method for serum albumin content, the urease indophenol method for blood urea nitrogen content, the POP-p-chlorophenol method for activities of serum glutamic pyruvic transaminase and serum glutamic oxalacetic transaminase, and the specific enzyme methods for activities of cholinesterase and creatine phosphokinase. Results obtained were analysed by a procedure similar to that described in the preceding report (Shimoda *et al.*, 1982). The data were expressed by mean ± standard deviation of the mean.

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Results

Results obtained are shown in Figures 1–7. The following descriptions on the changes of clinico-chemical values are mainly based on the results of male mice since, unless otherwise stated, both male and female mice in experimental groups showed a nearly similar pattern of clinico-chemical changes during progressive infection.

Total serum protein (S.Pr), *serum albumin* (Alb) and *the ratio of albumin to globulin* (A/G ratio); (Fig. 1): The contents of both S.Pr and Alb in sera of primary and secondary infection groups were significantly lower than that of uninfected control groups throughout the course of the experiment. The decrease in these values became more prominent after secondary infection. A/G ratio of infected groups showed no significant difference as compared with that of control group.

Serum glutamic pyruvic transaminase (GPT); (Fig. 2): GPT activity in primary infection group increased gradually and showed a zenith at 14 days after infection. In secondary infection group the activity remained within in the normal range.

Serum glutamic oxalacetic transaminase (GOT); (Fig. 3): A transient decrease with a quick restoration in the activity of GOT was observed 1 day after primary infection. The GOT activity increased immediately

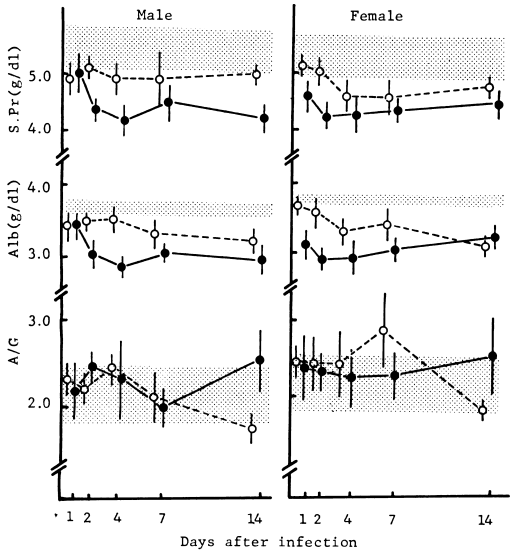

Fig. 1 Contents of total serum protein (S. Pr) and serum albumin (Alb), and value of the ratio of albumin to globulin (A/G) following infection with *H. nana* eggs. ○·····○ ; primary infection ●——● ; secondary infection. Dotted areas indicate the range (mean ± SD) of uninfected control mice.

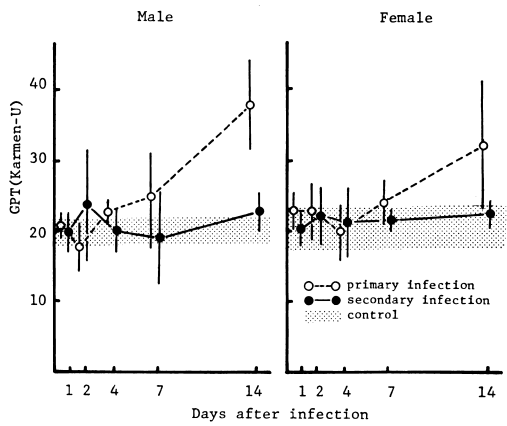

Fig. 2 Serum glutamic pyruvic transaminase (GPT) activities in mice following primary and secondary infections with *H. nana* eggs.

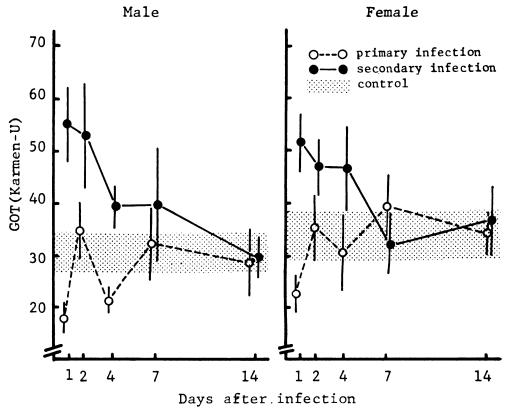

Fig. 3 Serum glutamic oxalacetic transaminase (GOT) activities in mice following primary and secondary infections with *H. nana* eggs.

after secondary infection, rapidly declined thereafter, and returned to the normal range at about 7 days after secondary infection.

Cholinesterase (Ch.E) and *lactate dehydrogenase* (LDH); (Figs. 4 and 5): No significant difference was observed among the activities of experimental and control groups throughout the course of the experiment.

Creatine phosphokinase (CPK); (Fig. 6): The CPK activity of primary infection group remained within normal range, while that of secondary infection group increased 1 day after infection and returned abruptly to the normal range at 2 days after infection.

Blood urea nitrogen (BUN); (Fig. 7): The change of BUN value in both primary and secondary infection groups was biphasic. There was a first peak at 2 days after primary and secondary infections, followed by a second peak at 7 days of infection. Increase in BUN value of female mice at 2 days after primary infection was particularly prominent.

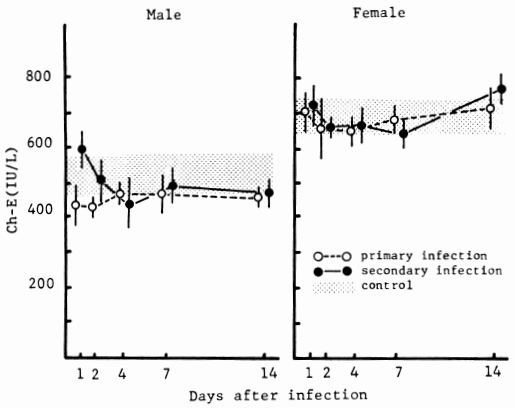

Fig. 4 Cholinesterase (Ch. E) activities in mice following primary and secondary infections with *H. nana* eggs.

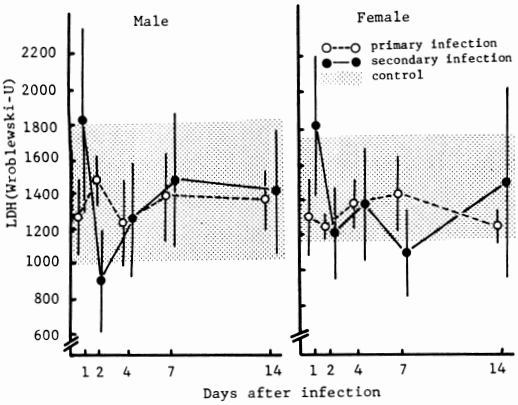

Fig. 5 Lactate dehydrogenase (LDH) activities in mice following primary and secondary infections with *H. nana* eggs.

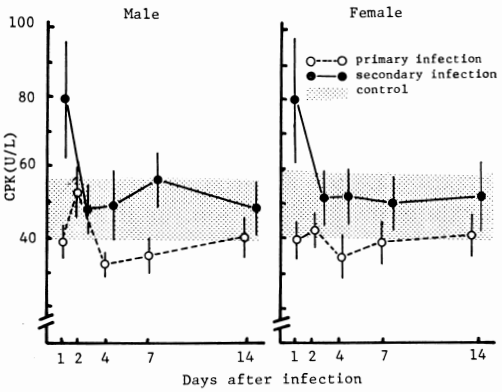

Fig. 6 Creatine phosphokinase (CPK) activities in mice following primary and secondary infections with *H. nana* eggs.

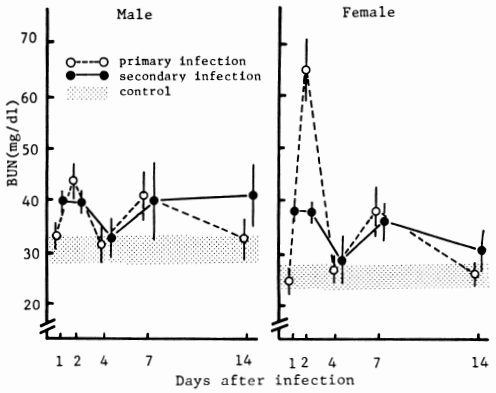

Fig. 7 Blood urea nitrogen (BUN) values in mice following primary and secondary infections with *H. nana* eggs.

Discussion

The laboratory mice infected with *H. nana* eggs showed a slight anemia and apparent leukocytosis with peripheral eosinophilia, and these changes became more apparent after secondary infection (Shimoda *et al.*, 1982). The present study with the same experimental host-parasite interaction also showed that there were several changes in the clinico-chemical values, such as S.Pr, Alb, GPT, GOT, CPK and BUN.

The decrease of S.Pr and Alb contents was apparent after primary and secondary infections with *H. nana* eggs, especially after secondary infection, suggesting that the infected mice had a hypoalbuminemia. The cause of hypoalbuminemia has been suggested to come from excessive protein breakdown, proteinuria, disturbances of metabolism or absorption, or deficient intake of protein (Seward *et al.*, 1971). It was possible that a hypoalbuminemia in mice infected with *H. nana* may be due to malnutrition of the host which was caused by the histological damage of the intestine. It was shown that the upper intestine of mice was damaged by the invasion and rapid growth of *H. nana* larvae, and that the histological damage of intestine was more apparent in immunized and challenged mice (Friedberg *et al.*, 1979; Miyazato *et al.*, 1979; Furukawa *et al.*, 1981). In addition, the chronic infection of adult worms in the lower intestinal tract, occuring about 10 days after administration of eggs and continuing throughout the experimental period, may be related to the continuous hypoalbuminemia. Thomas (1964) described that patients with heavy chronic infection of *H. nana* sometimes complain of vomiting, weight loss, and diarrhea, suggesting that infection of adult worms in the lower intestine may induce a state of malnutrition probably due to the histological damage of intestine. We have already reported that malnutrition or malabsorption after *H. nana* infection caused a slight anemia in infected mice (Shimoda *et al.*, 1982). An additional cause of hypoalbuminemia in infected mice may be the increased and competitive requirement of nutrients by the larvae and adult worms. This may cause an increased catabolism of serum protein and may result in lower S.Pr and Alb contents with simultaneous increase in BUN value as observed in the present study. Frances (1979) described that increased protein catabolism caused an increased urea formation. For further assurance, the changes of body weight of mice, rate of food intake, and examination of occult blood in feces are under investigation.

The gradual increase of GPT activity in primary infection group and rapid increase with gradual decline of GOT activity in secondary infection group are indicative of liver dysfunction due to degeneration and necrosis of liver cells. There is a circumstantial evidence that *H. nana* infection may influence the liver function. Inoue *et al.* (1979) have reported that a part of oncospheres of *H. nana* infected in normal mice can migrate to the liver, where they develop into mature cysticercoids and remain viable in the tissue for up to 10 days or so. However, they also showed that only a very small number of oncospheres can migrate to the liver of normal mice and that the growth of the challenge larvae in the liver of immunized mice is not observed after secondary infection. There is a little doubt, therefore, that whether the infection of *H. nana* eggs at a dose level of 2,000 should exert some influences against the liver function. In addition, LDH and Ch.E activities after infection were always within their normal ranges, suggesting that there was no parenchymatous damage of the liver in infected mice. It is possible that change in the liver function observed in the present

study was nonspecific and transient phenomenon.

The increase of CPK activity at 1 day after secondary infection was prominent. Circumstantially, the change in CPK activity may be related to the immunological reaction of host against challenge larvae since transient increase of CPK activity was coincident with the period of death and expulsion of oncospheres in immunized mice (Friedberg *et al.*, 1979; Miyazato *et al.*, 1979; Furukawa *et al.*, 1981). However, the phenomenon observed requires further study since CPK activity is accepted as a sensitive and specific indicator of the damage of heart and voluntary muscle cells, while *H. nana* infection appears to exert no influence against these organ and tissues.

In conclusion, a series of our experiments revealed that the laboratory mice showed a number of alterations in hematological and clinico-chemical values after primary and secondary infections with *H. nana* eggs. It appears that malnutrition and malabsorption probably due to the histological damage of intestine and nutritional requirement of the infected worms are the most important factors in inducing several alterations and state of illness such as anemia and hypoalbuminemia with slight azotemia. In addition, a marked immunogenicity of *H. nana* caused a leukocytosis with a peripheral eosinophilia in relation to the acquired immunity of mice to this parasite. Finally, the influence of *H. nana* infection against the liver function and other organs or tissues requires further investigation.

Summary

The changes of clinico-chemical values of ddY mice following primary and secondary infections with *Hymenolepis nana* eggs were studied. Blood samples were collected from postcaval vein at various times after infection and the contents of S.Pr, Alb and BUN, and the activities of GPT, GOT, Ch.E, LDH and CPK in the sera were examined.

1. The contents of S.Pr and Alb after primary and secondary infections were lower than that of uninfected control mice. A/G ratio of the infected groups showed no difference as compared with that of control group.
2. GPT activity increased gradually after primary infection.
3. GOT activity increased immediately after secondary infection, rapidly declined, and returned to the normal range at about 7 days after secondary infection.
4. Ch.E and LDH activities in infected groups remained within normal ranges.
5. CPK activity increased 1 day after secondary infection and returned abruptly to the normal range at 2 days of infection.
6. BUN value showed a biphasic increase in both primary and secondary infection groups.

It appears that malnutrition and malabsorption probably due to the histological damage of intestine and nutritional requirement of the infected worms are responsible for the changes of S.Pr, Alb and BUN. The influences of *H. nana* infection against the liver function and other organs or tissues are still questionable.

Acknowledgements

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小形条虫の初感染および再感染時におけるマウスの臨床病理学的検討

2. 臨床化学的所見

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マウスに実験的に小形条虫卵を経口感染させ，初感染および再感染の初期における宿主の臨床化学的変化について検討した．

下大静脈から血液を採取し，血清蛋白，血清アルブミン，尿素窒素，トランスアミナーゼ，コリンエステラーゼ，乳酸脱水素酵素，およびクレアチンホスフォキナーゼなどの測定を行なった．

結果は次の通りである．

1. 血清蛋白および血清アルブミン量の減少が，初感染，再感染のいずれにも認められた．A/G 比は，差は認められなかった．

2. GPT 値のゆるやかな上昇が，初感染後にみられた．

3. GOT 値は再感染の直後に上昇し，直ちに低下した．そして，約7日後には正常値範囲内に戻った．

4. コリンエステラーゼおよび乳酸脱水素酵素の活性値は，実験期間中を通して正常値範囲内であった．

5. クレアチンホスフォキナーゼ活性は，再感染1日後に上昇したが，2日後には正常値範囲内に戻った．

6. 尿素窒素値は，初感染，再感染のいずれにおいても感染の2日後と7日後に増加するという二峰性増加が認められた．

血清蛋白，血清アルブミン，および尿素窒素値の変化は，腸管の組織学的傷害による吸収不良，さらに感染した寄生虫の栄養物摂取による栄養障害が原因となっているものと考えられる．肝機能あるいはその他の臓器，組織に小形条虫の感染がどのような影響を及ぼすかはまだ明らかではない．