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Biochemical Studies of Fascioliasis

(2) Dynamics of Sequential Changes of Serum Protein and Serum Protein Fractions in Rabbits Infected with *Fasciola* sp.

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Key words: experimental fascioliasis in rabbit, serum protein concentration, serum protein fraction, albumin/globulin ratio

We have previously reported that the growth patterns of the Japanese common liver flukes (*Fasciola* sp.) change when they migrate from the liver parenchyma into the bile ducts in the cattle (Akahane *et al.*, 1974). In rabbits, the flukes migrate from the liver parenchyma to the bile ducts 50 to 60 days postinfection (Ono and Isoda, 1972; Kimura, 1961). The presence of flukes in the liver parenchyma induces eosinophilia, and leukocytosis (Akahane, 1975a). Immune responses of the host are stronger in the intrahepatic migratory phase than in the bile duct phase (Akahane, 1975b). Elevations of GOT and GPT activities in infected rabbits are more characteristic in the former phase (Akahane *et al.*, 1980). The present study was carried out to find out possible differences of serum proteins and serum protein fractions between these two phases.

Materials and Methods

Animals used were the same as described previous report (Akahane *et al.*, 1980). Briefly, albino rabbits were orally infected with either 10 or 50 metacercariae of the Japanese common liver fluke. The infected rabbits were divided into two groups. Group 1; 12 heavily infected rabbits which died between 50 to 129 days after the infection. Eight of them were given 50 metacercariae and the rest were 10 metacercariae. Group 2; 8 lightly infected rabbits which were survived up to 200 days. Six of them were infected with 10 metacercariae and the rest were with 50 metacercariae. Seven rabbits served as the control without infection.

Blood samples were collected from the ear-vein at a 10 day interval and sera were stored at −20 C. Total protein was measured by auto analyzer of Japanese Electron Co. Ltd., Model JCA-N3C3R (Biuret method). Serum protein fraction was studied by cellulose acetate electrophoresis with Separax using veronal buffer, PH 8.6 ionic strength 0.06.

An aliquot of 0.06 ml of the serum was placed on each strip with an applicator. The current flow was adjusted to 0.08 mA per centimeter width of Separax and separation was allowed to proceed for 45 min. The strips were stained with Ponceau 3R and scanned with a densitometer. Percentage of each serum protein fraction was calculated

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from areas of the respective part of peak of the curves. Concentrations of each serum protein were calculated.

Results

Total serum protein concentration: Fluctuations of average levels of total serum protein are shown in Fig. 1. No significant difference was observed among animals of heavily and lightly infected groups and of control until 120 days after the infection. Protein concentration became lower in the lightly infected group than in the control 130 days after the infection.

Proportion of albumin to total protein in serum: As shown in Fig. 2, proportion of total albumin to total protein in three groups was not significantly different from one another until 30 days after the infection. After 40 days of infection, however, the percentage of albumin of the infected groups decreased rapidly until 70th day and, thereafter, recovered slightly, although it was consistently lower than that of the control.

Proportion of globulin to total protein in serum: The proportion of total globulin to serum protein in three groups was not significantly different until 30th day of infection. After 40th day, however, that of the infected groups increased markedly until day 70. Moderate hyperglobulinemia was observed in the lightly infected group from 80 to 190 days of infection (Fig. 3).

Albumin/globulin ratio (A/G ratio): A/G ratio in both infected groups was not significantly different from the control group until 30 days after the infection. From 40th day of infection the ratio dropped abruptly but after day 80, it restored gradually, although the values were always smaller than those in the control. From 60 to 70 days after the infection, inversion of A/G ratio was observed (Fig. 4).

Percentage of various globulin fractions to total serum protein: Proportion of α_1-globulin in total serum protein was slightly

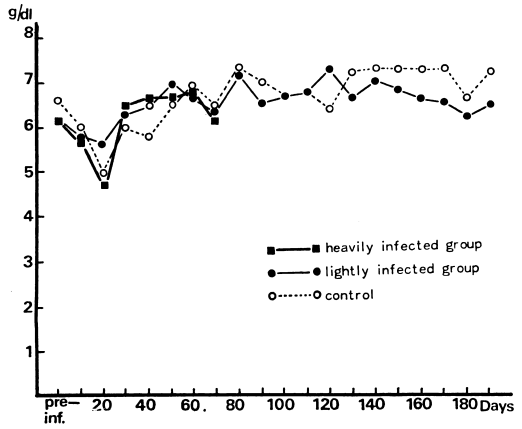


Fig. 1 Total protein concentration of rabbits before and after the infection with *Fasciola* sp.

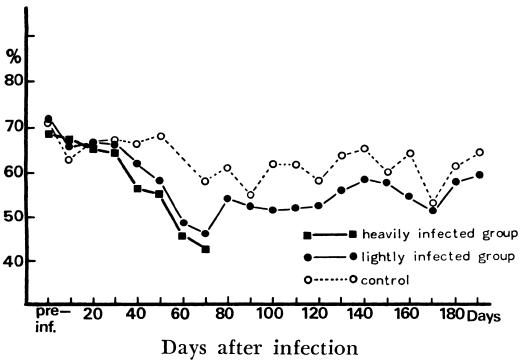


Fig. 2 Proportion of total albumin to total protein of rabbits before and after the infection with *Fasciola* sp.

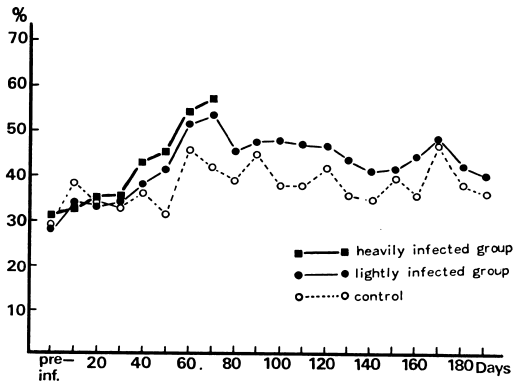
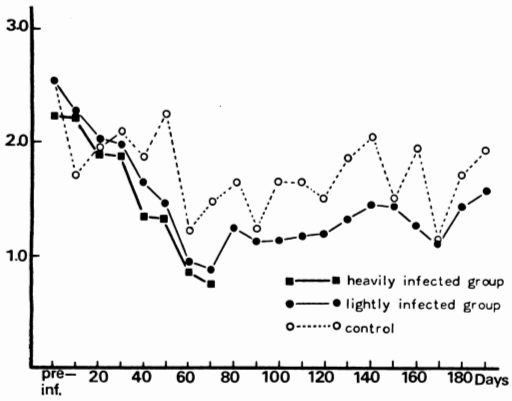
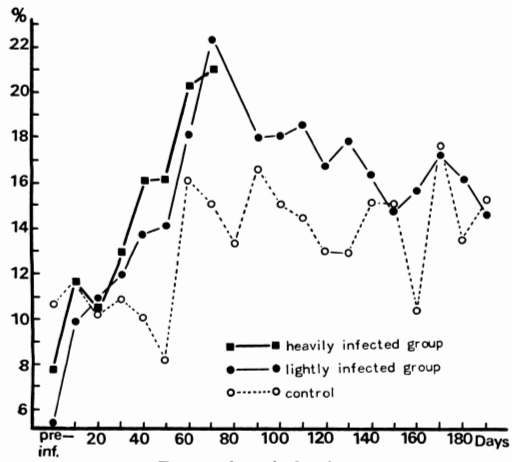


Fig. 3 Proportion of total globulin to total protein of rabbits before and after the infection with *Fasciola* sp.



Days after infection

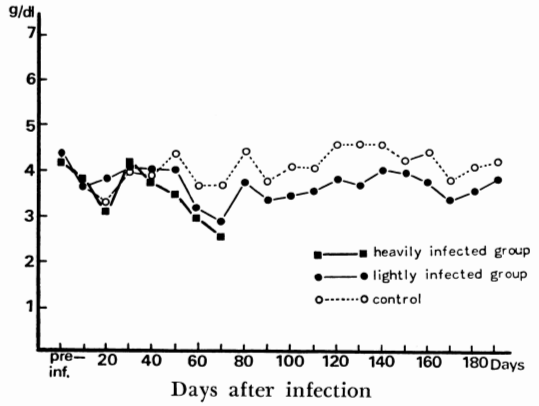
Fig. 4 Albumin/globulin ratio of rabbits before and after the infection with *Fasciola* sp.



Days after infection

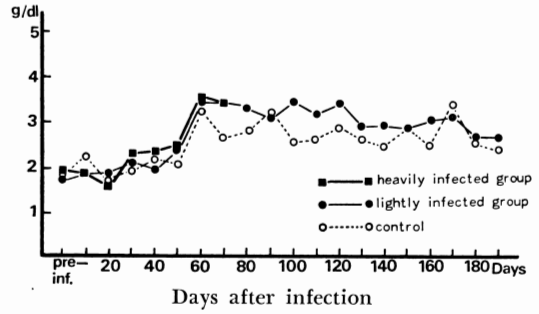
Fig. 5 Proportion of γ-globulin to total protein of rabbits before and after the infection with *Fasciola* sp.

higher in the infected animals than the control between 20th and 120th days of the infection. However, no significant difference was observed thereafter. The values of α_2- and β-globulins were calculated as a whole, and those of the infected rabbits tended to be higher compared with those in the control after 50 days of the infection. Patterns of γ-globulin ratio are shown in Fig. 5. The values in the infected groups began to increase on day 30, and reached the maximum on day 70. The values de-



Days after infection

Fig. 6 Total albumin concentration af rabbits before and after the infection with *Fasciola* sp.



Days after infection

Fig. 7 Total globulin concentration of rabbits before and after the infection with *Fasciola* sp.

creased gradually control level thereafter.

Total albumin concentration: Total albumin concentration in the infected groups began to decrease on day 40 and reached the lowest level in 70th day, keeping lower levels than in the control thereafter (Fig. 6).

Total globulin concentration: Globulin concentration was kept at the same level in all groups until 40 days of the infection. Afterward, the values increased in the infected rabbits but not in the control (Fig. 7).

Concentration of various globulin fractions: The concentration of each of α_1-, and γ-globulin fractions and amount of α_2- and β-globulin were higher in the infected groups than those in the control from 40th to 120th day of the infection. After 130 days of the infection, however, no difference was

observed between the lightly infected group and the control.

Discussion

Changes of serum protein fractions in animals infected with *Fasciola* have been reported by Sinclair (1966), Holmes *et al.* (1968), Roberts (1968) and Kadhim (1975) for sheep, by Ross *et al.* (1966), and Haroun and Hussein (1975) for calves and bovine, by Thorpe (1966) for albino rats, and by Takemoto *et al.* (1977) for monkeys. They have pointed out hypoalbuminemia and hyperglobulinemia as notable changes of serum protein levels in animals infected with *Fasciola*. These changes were observed after 40th day in these papers. In the present paper, we studied on the chronological changes more precisely. The albumin percentage in the infected rabbits decreased from 40th day to 70th day. During the period, the young fluke migrates from the liver parenchyma to the bile ducts (Ono and Isoda, 1952; Kimura, 1961). It was found that coincidently with the period, the albumin proportion started to regain, although the percentage stayed always lower than control levels (Fig. 2). The globulin percentage in the infected rabbits began to increase from day 40, reached the highest value on day 70, and thereafter it was maintained to be higher than that of the control through the experiment. A/G ratio of the infected rabbits decreased lower than 1.0 between 60 and 70 days after the infection. The turnover in this ratio had been observed by many workers on various animals (Sinclair, 1962; Thorpe, 1965; Holmes, 1968; Roberts *et al.*, 1968; Ross *et al.*, 1966; Haround and Hussein, 1975; Takemoto *et al.*, 1975).

Many workers studied on the percentage of albumin and globulin to total serum protein levels, or A/G ratio, but, few studied on albumin concentration. Since the patterns of albumin percentage are affected by the flactuation of globulin levels, characteristic changes of albumin fractions are not obtained from this ratio. The patterns of albumin concentration were more characteristic than those of the albumin ratio in chronic fascioliasis. We noticed that the levels of serum albumin in the infected animals were apparently lower than those of the control animals. Several factors are supposed to be responsible for the marked decrease of serum albumin. As albumin is synthesized in the liver parenchyma, the damage of liver parenchyma due to the migration of young flukes may result in serum hypoalbuminemia. This is supported by our findings that the damage of the liver parenchyma resulted in the increase of GOT and GPT activities in the infected rabbits after 20th day of infection (Akahane *et al.*, 1980). The appearance of hypoalbuminemia delayed in comparison with the elevation of GOT and GPT activities, probably because of long half life span of serum albumin. Holmes *et al.* (1968) also showed that the migration of juvenil flukes in the liver parenchyma caused rapid catabolism of albumin in the infected animals. The half life of albumin in infected animals ranged from 110 to 280 hours, while that in the control animals ranged from 400 to 470 hours.

The inflammatory exudation in the liver may also cause the loss of serum albumin. In the present study, the inflammatory exudation of albumin into abdominal cavity was observed in the infected animals by the necropsy on day 60–70 when hypoalbuminemia of the animals was most apparent. In addition, albumin may be lost from the injured bile ducts. Such a loss of albumin into the gastrointestinal tract has already been demonstrated (Sinclair, 1962).

The proportion of total globulin to the total serum protein began to increase on day 40, reaching the highest ratio on day 70, and 80 days after the infection, the ratio restored slightly although the ratio in the

infected rabbits was higher than that in the control. On the other hand, total globulin concentration began to increase from 50 days and maintained its higher levels until 140 days after the infection. After 150 days of the infection, the concentration recovered to the control levels. It is believed that the hyperglobulinemia is followed after liver damage. From the results, it seems that decrease of A/G ratio is due to both of decrease of albumin and of increase of globulin.

The α_1-, α_2- and β-globulin ratios tended to be higher in both groups of infected animals than in the control, but most animals of these three groups were similar in the absolute levels. It is interesting to note that concentration of α-globulin of infected animals did not decrease during the course of the experiment despite the fact that α-globulin is produced in the liver parenchyma.

On the other hand, there was no significant difference in total serum protein between the control and infected rabbits until 130 days of the infection. The result indicated that the elevation of globulin values matched the fall in albumin concentration. Therefore, it seems apparent the total serum protien values *per se* give no good indication of the hepatic damage in the infected animals as previously reported (Thorpe, 1965; Takemoto *et al.*, 1977). However, total protein values in the infected rabbits showed a moderate decrease after day 140. The deviation from the control level may be due to continuation of hypoalbuminemia, in spite of gradual restoration of globulin up to the control level in this period.

Summary

Dynamics of the fluctuation of the serum levels of the total protein and various protein fractions was studied after rabbits were infected with the Japanese common liver fluke (*Fasciola.*).

After the 40th day of infection when fluke migrated away from the liver parenchyma to the bile ducts, globulin tended to be dominant than albumin. The increase of globulin level was mainly due to the increase of γ-globulin fraction. There was no significant difference in total serum protein concentration between infected animals and the control till day 130. The elevation of globulin concentration matched the decrease of albumin concentration. Thus, the total serum protein values remained normal range.

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肝蛭症の生化学的研究

2. 日本産肝蛭（*Fasciola* sp.）感染ウサギの血清蛋白分画の推移

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生後4カ月のウサギに日本産肝蛭のメタセルカリアを10個または50個または個経口投与し，急性または亜急性肝蛭症により感染後50～129日目に死亡した群（第1群）と慢性に経過して感染後 200 日まで生存した群（第2群）について，経時的に血清蛋白および血清蛋白分画をしらべ，メタセルカリア非投与対照群の検査成績と比較した．結果は次の通りであった．

1. アルブミンの減少，グロブリンの増加は感染後50日以降常にみとめられたが，比較的感染初期の感染後60～70日に顕著であった．

2. A/G 比の逆転がみられたのは比較的短期間で，虫体が肝実質から胆管へ移行する感染後60～70日であった．

3. グロブリンの中で特に増加傾向が著しかったのは γ-グロブリンであった．

4. 血清蛋白は感染後 130 日まで対照群と有意差はみとめられず，宿主の肝障害を示す指標にはなりえなかった．これはアルブミンの減少がグロブリンの増加で償われるためと考えられた．