[Jap. J. Parasit., Vol. 29, No. 2, 61-68, 1980]

Biochemical Studies of Fascioliasis (1) Results of Liver Function Tests in Rabbits Infected with *Fasciola* sp.

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(Received for publication; December 16, 1978)

The taxonomical analysis on the common liver fluke (*Fasciola* sp.) distributing among cattle, sheep and goat in Japan has not yet been established. Although the presence of morphological differences of the fluke has been pointed out (Watanabe, 1965), the migration behaviour of the Japanese common liver fluke in the final hosts are similar to those *Fasciola hepatica* and *F. gigantica*; orally administered metacercariae excyst in the duodenum and the young flukes enter into the body cavity through the intestinal wall, and then, they invade the liver, and migrate into the bile duct. In rabbits, it takes 50 to 60 days to enter the bile ducts after the administration of metacercariae of the Japanese common liver fluke (Ono and Isoda, 1952; Kimura, 1961). It is important to note that the migration of the flukes from the liver to the bile ducts affects their own biological development as well as clinicopathological change in the infected hosts.

Previously, we have shown that patterns of relative growth (Huxley and Teissier, 1936) of the flukes in the liver parenchyma are quite different from those in the bile duct (Akahane *et al.*, 1974). Soon after the flukes migrated from the parenchyma to the bile ducts, their reproductive organs grew more rapidly than the other organs, and the juvenile flukes became mature. In respect to responses of the final host, most of heavily infected rabbits died during the intra-hepatic migratory phase of the flukes, but many of lightly infected rabbits survived during this phase and restored slowly. Marked anemia, eosinophilia, and leukocytosis appeared from 40 to 80 days after the infection but restored slowly after 100 days in the latter (Akahane, 1975a). Immune responses were also enhanced in the migration period of liver parenchyma but gradually decreased in the bile duct phase (Akahane, 1975b).

The present study was carried out to know the possible changes of liver functions of the rabbits occuring in association with different phases of migration of the flukes, especially with the phase of migration from the liver to the bile ducts. The results obtained from various liver function tests suggest that damages of the liver parenchyma and impairment of the bile ducts occur almost at the same time when the flukes begin to enter the bile duct, and that rapid development or increase of activity of the flukes at this period is the major cause of acute fascioliasis.

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Materials and Methods

Twenty-seven albino rabbits of 4 months old were used and reared in the fluke free condition in closed environment at 23 C. They were orally administered either 10 or 50 metacercariae of the Japanese common fluke. As difference of susceptibility to the infection was observed among individual animals, infected rabbits were divided into two groups by the clinical course of the infection. Group 1 (heavily infected group) consists of 4 rabbits administered 10 metacercariae and 8 rabbits given 50 metacercariae ; all of them died of acute or subacute fascioliasis between 50 to 129 days after infection. Group 2 (lightly infected group) consists of 8 animals, all of which survived up to 200 days after infection. Among them, 2 rabbits given 50 metacercariae were included. Seven rabbits were left without infection as control.

Blood samples were collected from the ear-vein of all rabbits on every 10 days. Liver function tests examined are as follows: 1) Activities of serum glutamic oxaloacetic transaminase (GOT), serum glutamic pyruvic transaminase (GPT), and lactate dehydrogenase (LDH) were tested using an auto-analizer of Japanese Electron Co. Ltd., model JCA-N3C3R. 2) Alkaline phosphatase (ALP) activities were measured using an auto-analizer of Hitachi Co, Ltd., model 500. 3) Zinc sulfate (ATT) and thymol turbidity tests (TTT) were also carried out and leucine amino peptidase (LPA), cholesterol, bilirubin levels were determined by RaBA-System of Chugai Co. Ltd. (Hirano *et al.* 1976).

Results

Serum glutamic oxaloacetic transaminase (GOT): Averages of GOT activity are shown in Fig. 1. The activity of the control rabbits remained within normal range during the course of the experiment. On the 20th day of infection, the activity of infected animals became higher than that of the control. Animals of heavily infected group showed the highest activity on day 50 but the value decreased just before death. The activity of lightly infected rabbits elevated from days 20 to 80, but the value became similar to that of the control on day 90.

Serum glutamic pyruvic transaminase (GPT): The GPT activity of the three groups was within normal range until 10 days after the infection. But on day 20, the activity of animals of both infected groups increased rapidly and reached to the maximum 50 days after infection. Thereafter, the activities of lightly infected group decreased slightly, and returned to the control level in 120 days after infection (Fig. 2).

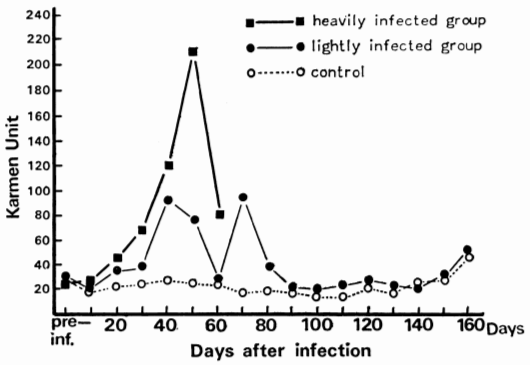

Fig. 1 GOT activities in rabbits before and after the infection with *Fasciola* sp.

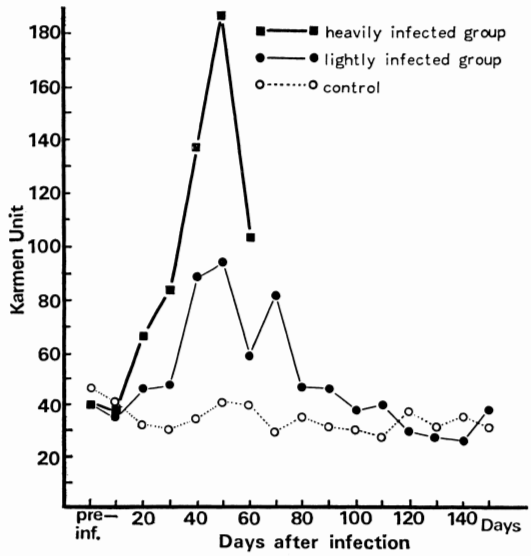

Fig. 2 GPT activities in rabbits before and after the infection with *Fasciola* sp.

Zinc sulfate test (ZTT): Results of ZTT in the three groups are summarized in Fig. 3. No significant difference was recognized among the values of the three groups during the experiment.

Thymol turbidity test (TTT): As shown in Fig. 4, the values of TTT were almost identical among the three groups except the fact that the values of the infected groups elevated slightly 50–70 days after infection.

Lactate dehydrogenase (LDH): The LDH activity of the infected rabbits was slightly higher than that of the control 50–100 days after infection, and became similar to the level of the control on day 120.

Alkaline phosphatase (ALP): The ALP activity of the heavily infected animals increased rapidly on day 50 and remained higher until day 60. The activity of Group 2 was not significantly different from that of the control during the course of the experiment (Fig. 6).

Lecine amino peptidase (LAP): Forty

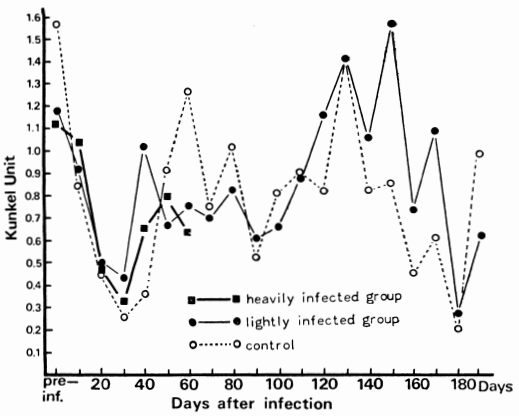

Fig. 3 ZTT values in rabbits before and after the infection with *Fasciola* sp.

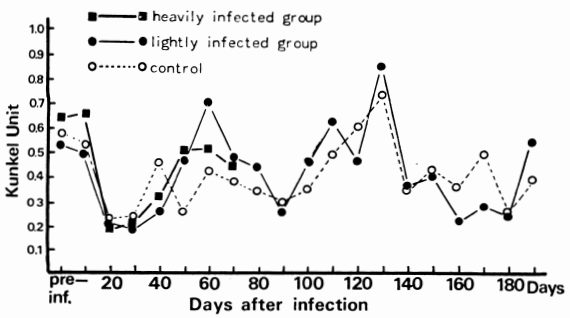

Fig. 4 TTT values in rabbits before and after the infection with *Fasciola* sp.

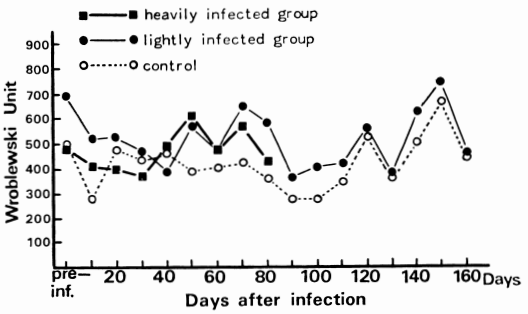

Fig. 5 LDH activities in rabbits before and after the infection with *Fasciola* sp.

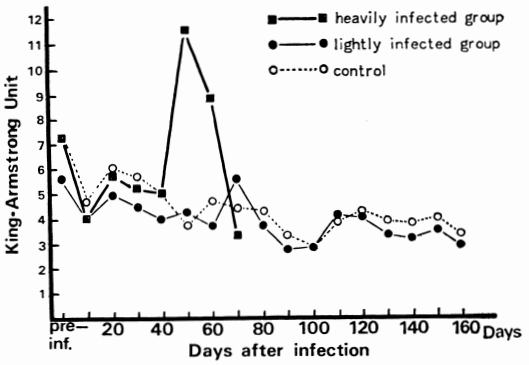

Fig. 6 ALP activities in rabbits before and after the infection with *Fasciola* sp.

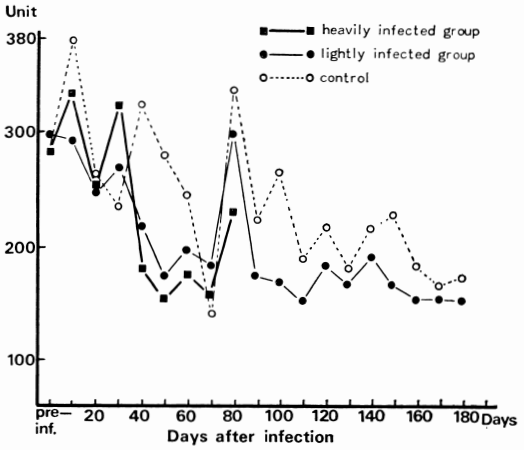

Fig. 7 LAP activities in rabbits before and after the infection with *Fasciola* sp.

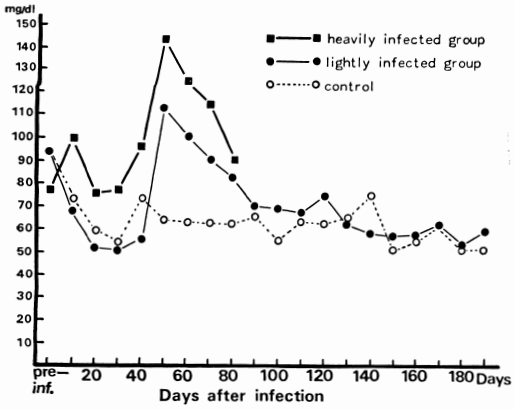

Fig. 8 Cholesterol levels in rabbits before and after the infection with *Fasciola* sp.

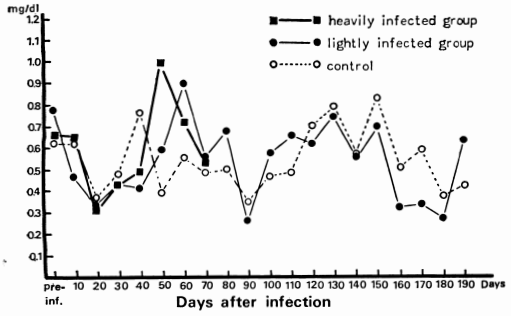

Fig. 9 Bilirubin levels in rabbits before and after the infection with *Fasciola* sp.

days after infection, the LAP activity became slightly lower in both infected groups than in the control, though the difference was not significant (Fig. 7). There were considerable variations of the activity among individual animals.

Cholesterol : Serum levels of cholesterol in the heavily infected rabbits began to increase 40 days after infection, and reached to the maximum value on day 50. On the other hand, the values of the lightly infected rabbits rose suddenly on day 50, and remained higher levels until day 80 (Fig. 8).

Bilirubin : As shown in Fig. 9, bilirubin levels of both infected groups had a tendency of increasing between 50 and 60 days after infection, but no significant difference was observed among the three groups.

Discussion

Changes of liver function tests have been reported using laboratory and domestic animals, which were infected with *Fasciola* (Thorpe, 1965 ; Ross *et al.*, 1966 ; Campbell and Barry, 1970 ; Bundesen and Janssens, 1971 ; Takemoto *et al.*, 1977). In these studies, practical numbers of the liver function test were not enough to understand the characteristic symptoms of fascioliasis. In the present study, nine liver function tests were chosen to estimate the degree of liver damage and impairment of liver functions caused by *Fasciola* infection.

The tests used are separable into two indicator groups ; the first is the indicator for liver parenchymatous damage and includes GOT, GPT, LDH, ZTT and TTT. The second is for impairment of the bile duct ; ALP, LAP, cholesterol and bilirubin levels belong to the latter.

In the examination of liver parenchymatous damage in fascioliasis, Thorpe (1965) reported that serum GOT activity was the most sensitive indicator of hepatic damage in fluke-infested rats. In mice infected with *F. hepatica*, Bundesen and Janssens (1971) reported that serum GPT activity reached the maximum value on the 28th day after the infection, which is in agreement with a finding that the maximum liver damage occurred approximately 28 days after infection and thereafter the damage decreased (Dawes, 1963).

In the present experiment, serum GOT, and GPT activities showed the most characteristic change in both of the heavily and lightly infected groups. Serum GOT and GPT activities of the infected rabbits increased from day 20. GOT activity of the heavily infected group and GPT activity of the both infected groups reached to the maximum values on day 50. GOT and GPT activities in the lightly infected animals showed another peak on day 70 and thereafter, those activities returned to approximately normal values. Ono and Isoda (1952) found that the Japanese common liver flukes migrate

from liver parenchyma to bile duct in 50–60 days after infection. Accordingly, the time of the increase of GOT and GPT may well correspond to the time when the flukes begin to enter the bile duct.

In the present study, the elevation of GOT, GPT activities delayed as compared with the results obtained in the mice infected with *F. hepatica* (Bundesen and Janssens, 1971). It is known that the time required for the fluke to arrive and develop to adults in the bile duct varies from host species to species (Sinclair, 1967; Dawes and Hughes, 1964). In mice, the flukes mature much more quickly than in other hosts and settlement in the bile ducts may take place 24 days after infection or in some instances 29–32 days after infection (Dawes, 1961; 1962). Urquhart (1956) showed that the flukes of *F. hepatica* may settle in the bile ducts of rabbits as early as 5 weeks after infection. It is also known that the migration and development of Japanese common liver fluke in the final host differ from those of *F. hepatica*, although the prepatent period of the former was shown to be 61 days in rabbits and similar to that of the latter (Watanabe, 1965). However, Ono and Isoda (1952) reported that the prepatent period of the Japanese common liver fluke was 65 to 80 days after infection. Our previous data indicate the prepatent period of the fluke was 60–70 days postinfection in rabbits in the case of the small egg strain, and 78–81 days in the large egg strain (Akahane *et al.*, 1976). We confirmed the prepatent period to be 77–86 days in further experiments in which rabbits were infected with the large egg strain (unpublished data).

Results of TTT are considered to be an indicator of the degree of liver parenchymatous damages (Watson, 1944: Recant *et al.*, 1945), and it is well known that serum LDH activities increase in the early stage of acute infectious hepatitis. Oda *et al.* (1967) reported that values of TTT in the human fascioliasis reached to higher levels than that in normal control. In the present study, levels of TTT and LDH activities in both infected groups were slightly higher than those of the control during the intrahepatic migratory phase, though the values remained within normal range. Difference of values of ZTT was not significant between the infected and the control rabbits in the present experiment.

There are considerable literatures on the liver function tests in fascioliasis but little work has been done about the impairment of the bile duct in fascioliasis. Thorpe (1965) reported that in rats infected with *F. hepatica*, the serum bilirubin level was not significantly elevated, but ALP activity was elevated at 4 weeks and fell down in 6 weeks after infection. In our hands, ALP activities of heavily infected animals increased 50–60 days after infection, but the activities in lightly infected animals returned to normal range when the flukes moved into the bile ducts. Cholesterol levels showed the maximum values on 50 days postinfection but were restored slowly thereafter. There was a tendency of elevation of bilirubin levels which elevated during the intrahepatic migratory phase. LAP activities of the infected groups showed slightly lower levels from 40 to 180 days postinfection as compared with those of the control. This was unexpected finding and the reason has not yet been clarified.

As mentioned above, ALP activities, cholesterol and bilirubin levels were elevated approximately 50–60 days after infection when the fluke migrates from the liver parenchyma to the bile duct. After settlement of the fluke in the bile ducts, those values of the lightly infected rabbits returned to normal range. However, it seems likely from our observations on liver function tests that in the early period of the infection, the parenchymatous damage may be associated with the impairment of bile ducts in the infected rabbits. The results suggest that the flukes destroy the liver parenchyma and intrahepatic ducts especially in the period of their migration into the bile ducts.

Summary

Rabbits orally administered either of 10 or 50 metacercariae of the Japanese common liver fluke were divided into two groups, heavily infected (Group 1) and lightly infected rabbits (Group 2), according to their susceptibility to the infection. Control animals were left without infection. Blood samples were collected from the ear-vein in every ten days, and were examined for GOT, GPT, ZTT, TTT, LDH, ALP, LAP, cholesterol and bilirubin levels in the serum. Results are summarized as follows;

1) GOT, GPT activities reached to abnormal values on 20–90 days after infection which were considered to indicate liver parenchymatous damage due to the infection. LDH, TTT, ZTT levels were remained within normal range.

2) ALP activities, cholesterol and bilirubin levels, well-known indicators of impairment of the bile duct were elevated approximately 50–60 days after infection corresponding to the time of the migration of the fluke from the liver parenchyma to the bile ducts, but, thereafter, these levels returned to normal range.

These results suggest that in the early period of infection of the Japanese common liver fluke, liver parenchymatous damage is associated with impairment of the bile ducts, especially when the fluke migrates into the bile ducts.

Acknowledgement

We wish to express our thanks to Professor Tomoo Oshima, Department of Parasitology, School of Medicine, Yokohama City University, Prof. Somei Kojima, Department of Parasitology, Faculty of Medicine, Shinshu University, and Prof. Teiji Kifune, Department of Parasitology, School of Medicine, Fukuoka University, for their helpful discussions and suggestions during this work. Thanks are also due to Mr. Shigeru Hayashi, Mrs. Mitsue Okura and the staff of the Clinical Examination Center, Matsumoto Medical Association, and Miss Atsuko Ushimaru, Shinshu University, for their assistance in the liver function tests, and to Miss Rieko Okae for secretarial assistance.

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肝蛭症の生化学的研究
1. 日本産肝蛭（*Fasciola* sp.）感染ウサギの肝機能検査結果

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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日目迄生存した群（第2群）について，経時的に肝機能検査を実施し，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る．

1. 肝実質障害の指標

肝機能検査の結果，肝実質障害とみられる変化は感染後20～90日の間にみられ，GOT，GPT，活性の上昇がみられた．しかし，LDH，TTT，ZTT，値に著しい変化はみられなかつた．

2. 胆管障害の指標

肝機能検査の結果，胆管障害とみられる変化は感染後50～60日の間に一過性にみられ，虫体が胆管に定着後は検査成績は正常値に復した．すなわち，感染後50～60日の間，ALP，Cholesterol，Bilirubin 値は高値を示した．しかし，全期間を通じ LAP 値は逆に低値を示した．

以上の肝機能検査の成績から，虫体が肝実質から胆管へ移行する時期に肝実質障害と胆管障害がともにかつ最も強くみとめられるものと考えられた．