Experimental Fascioliasis in Monkeys : II. Studies on Liver Function Tests and Electrophoretic Pattern of Serum Protein in Monkeys Infected with the "Japanese Species" of Fasciola

Bull. Univ. Osaka Pref., Ser. B, Vol. 29,
大阪府立大学紀要, 農学・生物学, Mar., 1977

Experimental Fascioliasis in Monkeys
II. Studies on Liver Function Tests and Electrophoretic Pattern of Serum Protein in Monkeys Infected with the "Japanese Species" of *Fasciola*

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Fascioliasis is still an important endemic parasitic disease distributed in the world. It is well known that several mammalian species including wild and domestic ruminants and man may serve as potential hosts for the liver flukes of the genus *Fasciola*. Many excellent papers have been published on experimental fascioliasis in large domestic and small laboratory animals, including cattle, sheep, rabbits, guinea pigs, rats and mice, which are distantly related to man. There have been, however, few articles dealing with fascioliasis in monkeys.[2), 5), 7)]

The incidence of human fascioliasis [9)-11)] has been increasing in many endemic areas in recent years. Experimental studies on fascioliasis in the monkey, which is closely related to man, would be not only of value in understanding the host-parasite relationship established in that animal but also helpful for the interpretation of the results of studies on human fascioliasis.

This is the second of a three-part of an investigation to determine the time course of the development of experimental fascioliasis in monkeys. The first report [19)] dealt with the parasitological, clinical and gross pathological observations on infected monkeys. The present paper describes alterations occurring in total and individual serum proteins, total serum bilirubin, and various serum enzyme activities in monkeys infected with *Fasciola* sp. of Japanese origin (hereinafter referred to as the "Japanese species").

Materials and Methods

1. Experimental animals

Six monkeys of the genus *Macaca* were obtained from some zoological gardens in Osaka Prefecture for the present experiment. Of them, one belonged to *Macaca fascicularis* (No. 9), two to *M. nemestrina* (Nos. 4 and 8), one to *M. cyclopis* (No. 7) and two to *M. fuscata* (Nos. 13 and 14). Upon their arrival, they were isolated individually and their stools were examined. When they adjusted themselves to the new environment and became free from eggs of parasites, they were subjected to the experiment. To maintain regular conditions for feeding, each animal was given a limited volume of the pelleted monkey diet (Oriental Co.), fruits and vegetables for two weeks before infection and during the experimental period of 50 to 160 days after infection.

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2. Preparation of metacercariae

Adult worms were obtained at a local slaughterhouse from the bile ducts of the liver of cattle naturally infected with the "Japanese species". Eggs were collected from the uterus by dissection with a pair of forceps. Metacercariae were harvested from the laboratory-bred and artificially infected snail, *Bakerlymnaea viridis*. A batch of metacercariae, encysted on sheets of cellophane, was stored for 2 to 5 weeks at 6°C until infecting monkeys.

3. Infection of monkeys

All the monkeys were infected by feeding a small piece of banana in which pieces of cellophane bearing the required number of metacercariae had been embedded. Monkey No. 9 was given 20 cysts, monkey Nos. 13 and 14 40 cysts each, and monkey Nos. 4, 7 and 8 100 cysts each (Table 1).

Table 1. Results of experimental infection of monkeys with various numbers of metacercariae of the "Japanese species" of *Fasciola*

Monkey			Body weight (kg)		Interval between infection and necropsy (days)	Dose of metacercariae		No. of flukes recovered	Percent recovery	Prepatent period (days)
No.	Species	Sex	At infection	At necropsy		Per monkey	Per kg of body weight			
9	*M. fascicularis*	M	5.50	4.50	121	20	3.6	7	35.0	89
8	*M. nemestrina*	M	6.00	5.50	50*	100	16.6	47	47.0	–
4	*M. nemestrina*	M	15.30	13.00	160	100	6.5	16	16.0	96
7	*M. cyclopis*	M	9.50	9.50	56*	100	10.5	62	62.0	51
13	*M. fuscata*	M	11.30	10.40	105	40	3.5	16	40.0	72
14	*M. fuscata*	F	9.60	8.40	106	40	4.2	12	30.0	71

Remarks.
* : Death occurred on this day of experiment.

4. Serum analyses

Four *ml* of blood sample was withdrawn from the saphenous vein of each monkey before infection and at weekly intervals after infection for serum biochemical analyses. The sample was allowed to clot and centrifuged at 3,000 rpm for 10 minutes. Serum obtained was analysed immediately.

Total protein in serum was measured by the biuret method using a photometer. The protein fractions were ascertained by electrophoresis on cellulose acetate strips and subsequent scanning of Ponceau 3R stained strips on a "Densitron" recording densitometer (Joko Sangyo Co. Ltd.). Total serum bilirubin, serum alkaline phosphatase (SAP), serum glutamate oxaloacetate and pyruvate transaminases (SGOT and SGPT), serum lactate dehydrogenase (SLDH), and serum leucine aminopeptidase (SLAP) were all estimated with a rapid blood analyzer (RaBa) system (Chugai Pharmaceutical Co. Ltd.). No sera were used if hemolysis was present.

These serum enzyme activities and total serum bilirubin levels were expressed in the following units; SGOT and SGPT in Karmen units (K-U), SAP in King-Armstrong units (K-A-U), SLAP in Goldbarg-Rutenburg units (G-R-U), SLDH in Wróblewski units (W-U), and total serum bilirubin in mg per d*l*.

Results

1. Changes in total serum protein and serum protein fractions in the serum

The changes in serum protein constituents occurring after infection are illustrated in Fig. 1. At an early stage, there were no significant changes in the concentration of total

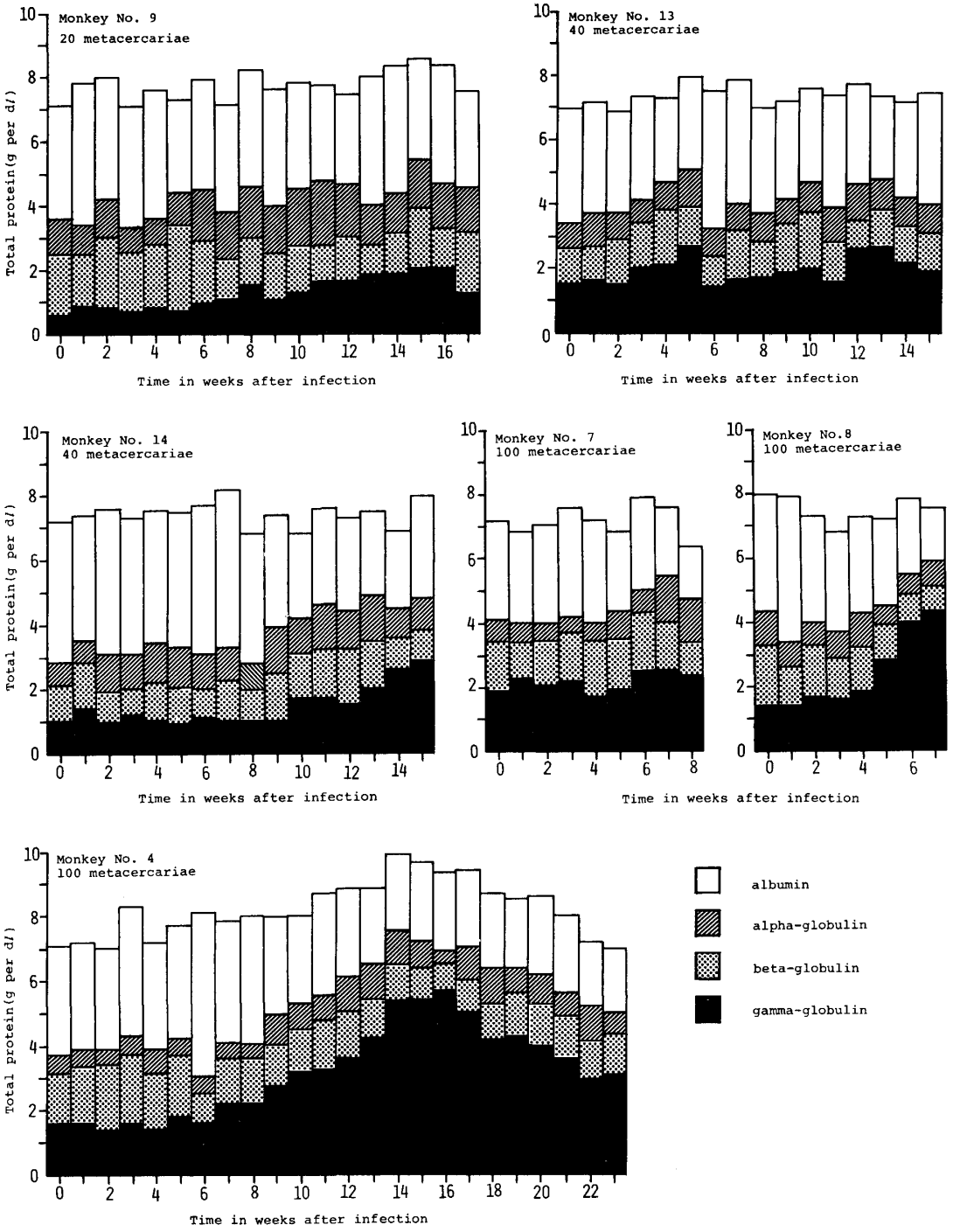

Fig. 1 Changes in total serum protein and various serum protein fractions in monkeys infected with 20 to 100 metacercariae of the "Japanese species" of *Fasciola*

protein or albumin in all six monkeys. Only in monkey No. 4, the concentration of total serum protein reached the highest level, 10.0 g per d*l*, 14 weeks after infection when clinical signs of fascioliasis were prominent. The protein concentration decreased gradually thereafter. In all the other animals, the blood serum showed only a few fluctuations in total serum protein levels until the end of experiment.

Serum electrophoresis showed that the globulin concentration steadily increased to 7.6g per d*l* in monkey No. 4 by 14 weeks, and to 5.9 g per d*l* in monkey No. 8 by 7 weeks immediately preceding death. Such a striking elevation in globulins is to be associated with elevation of the gamma-globulin fraction. The globulin concentrations showed relatively low levels in the other four monkeys, Nos. 7, 9, 13 and 14.

Albumin decreased progressively in concentration in all monkeys; hence the albumin/globulin (A/G) ratio decreased markedly (Table 2). The ratio in three monkeys, Nos. 4, 8 and 13, changed from 0.92, 0.85 and 1.00 prior to infection, to 0.33, 0.25, and 0.54 at 13, 7 and 13 weeks, respectively, after infection. During this period, all monkeys showed typical clinical signs of fascioliasis. The alpha- or beta-globulin concentration showed little change in any monkeys. Since the increased level of gamma-globulin compensated the decreased level of albumin, total serum protein remained unchanged.

Table 2. Changes in the albumin/globulin ratio in the blood serum of monkeys infected with metacercariae of the "Japanese species" of *Fasciola*

Monkey			A/G ratio prior to infection	Minimum A/G ratio after infection	Time of minimum A/G ratio (weeks)
No.	Species	Sex			
9	*M. fascicularis*	M	0.96	0.56	15
8	*M. nemestrina*	M	0.85	0.25	7
4	*M. nemestrina*	M	0.92	0.33	13
7	*M. cyclopis*	M	0.67	0.32	8
13	*M. fuscata*	M	1.00	0.54	13
14	*M. fuscata*	F	1.38	0.54	14

2. Changes in enzymes and total bilirubin in the serum

The changes in various serum enzyme activities and total serum bilirubin levels afer infection are illustrated in Figs. 2 and 3.

a. Serum transaminases (SGOT and SGPT)

Significantly increased SGOT activities and different patterns were detected in the serum of all six monkeys in 6 to 8 weeks after infection. In three monkeys, Nos. 4, 7 and 8, SGOT activity showed significantly high levels, reaching 175 to 357 K-U at maximum, 7 to 15 weeks after infection. In the other three monkeys, Nos. 9, 13 and 14, the blood serum showed relatively low levels of SGOT activity, or 66 to 101 K-U. SGOT activity fell gradually to a normal level in two monkeys, Nos. 4 and 13, 18 and 13 weeks, respectively, after infection. No such a trend was observed in the other animals; it remained at considerably high levels in two monkeys, Nos. 9 and 14, until the end of experiment. SGPT activity fluctuated in essentially the same manner as SGOT activity, and reached a maximum value of 293 to 377 K-U in three monkeys, Nos. 4, 7 and 8, and of 151 to 296 K-U in the other three animals, Nos. 9, 13 and 14. When both transaminase activities were examined, SGPT tended to be higher in

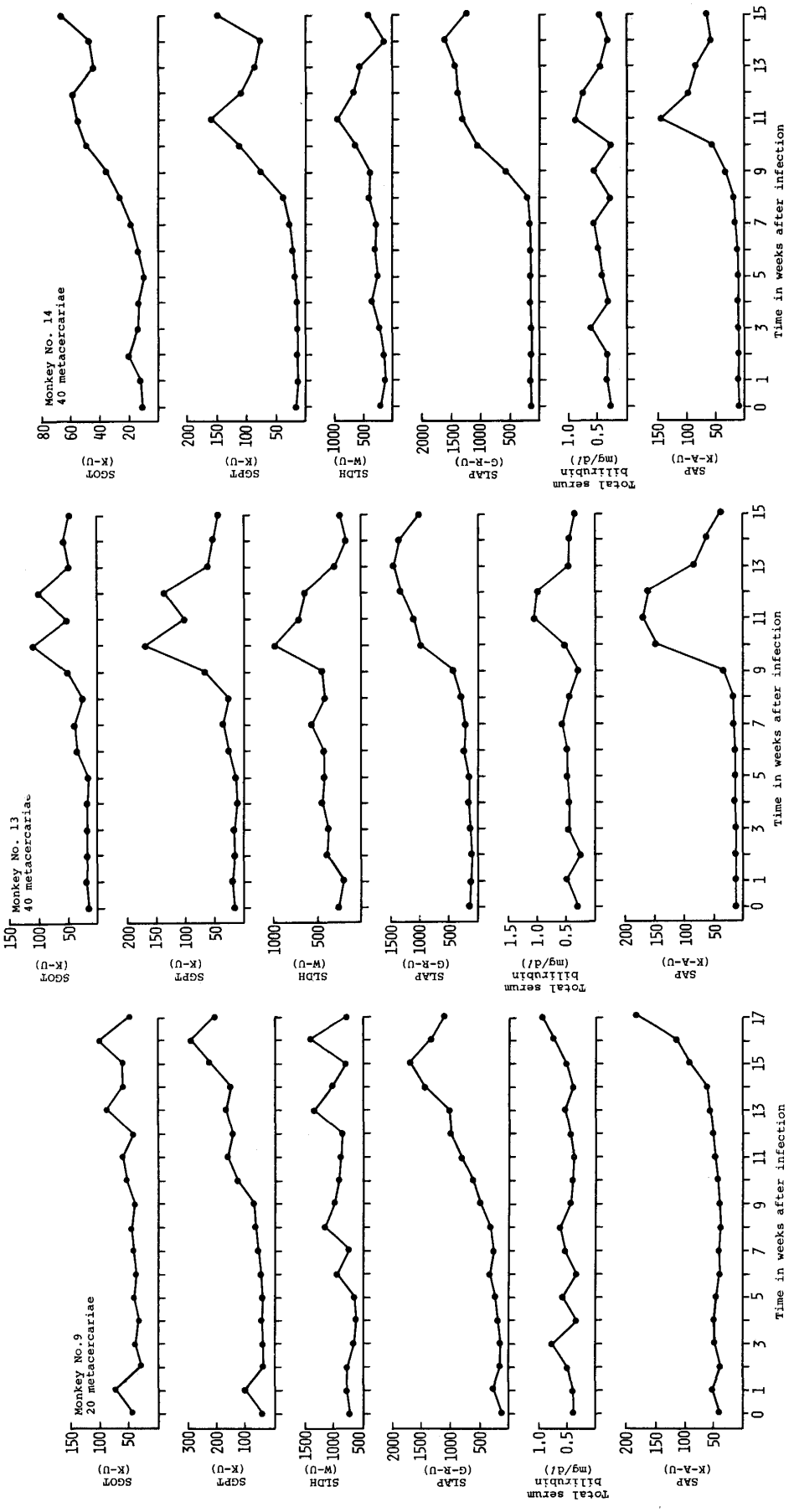

Fig. 2 Changes in various serum enzyme activities and total serum bilirubin levels in monkeys infected with 20 or 40 metacercariae of the "Japanese species" of *Fasciola*

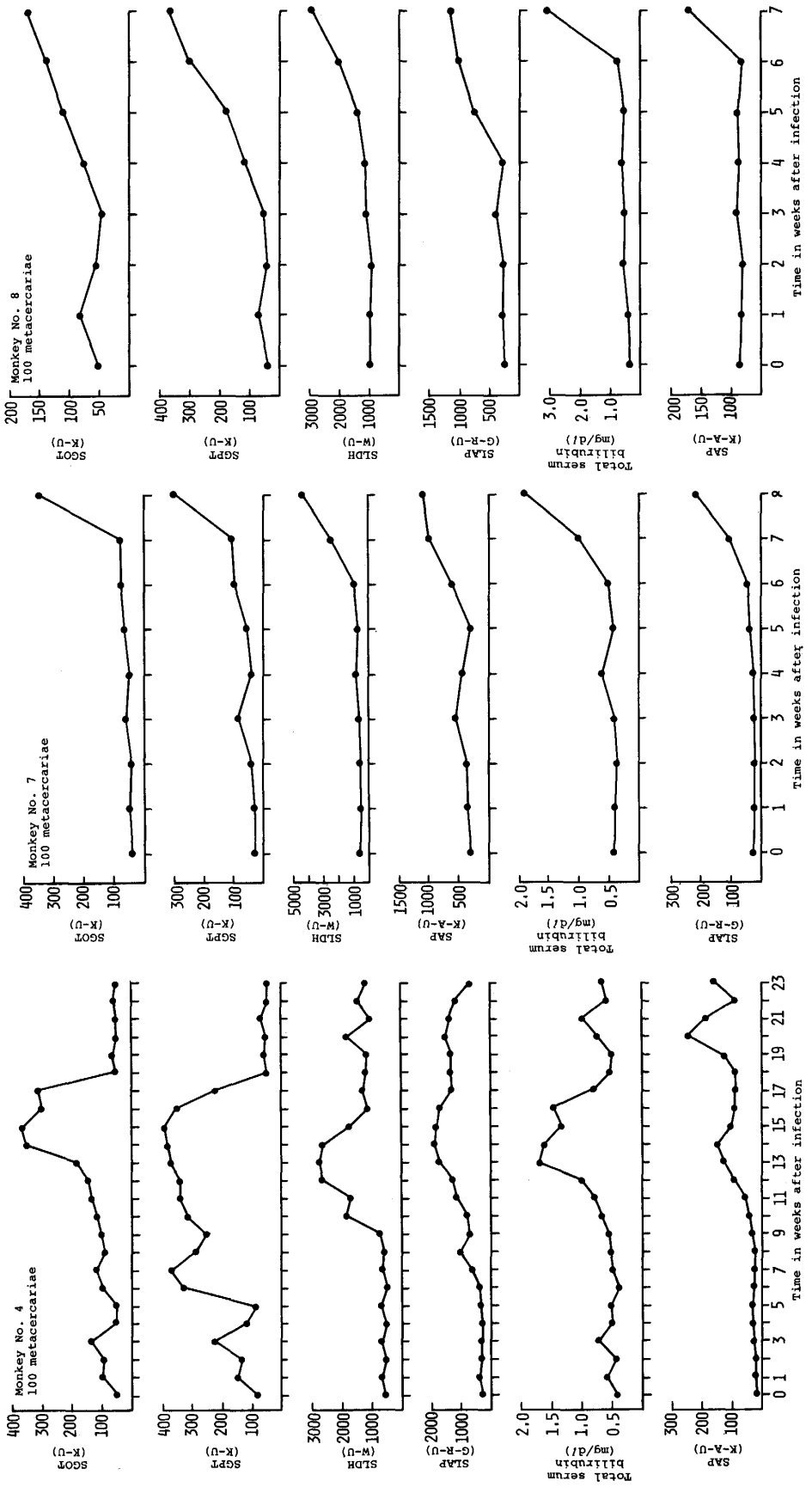

Fig. 3 Changes in various serum enzyme activities and total serum bilirubin levels in monkeys infected with 100 metacercariae of the "Japanese species" of *Fasciola*

level than SGOT in most monkeys.

b. Serum lactate dehydrogenase (SLDH)

An increase in SLDH activities was observed in almost all monkeys in 6 to 9 weeks after infection. In three animals, Nos. 4, 7 and 8, SLDH activity showed significantly high levels, reaching 2,700 to 4,180 W-U at maximum, 7 to 13 weeks after infection. In the other three monkeys, Nos. 9, 13 and 14, the blood serum showed relatively low peak levels of SLDH activity, or 960 to 1,410 W-U, 10 to 16 weeks after infection. SLDH activity in all monkeys fluctuated in essentially the same pattern as SGOT or SGPT activity.

c. Serum leucine aminopeptidase (SLAP) and serum alkaline phosphatase (SAP)

An increase in SLAP and SAP activities was detected in the serum of all six monkeys at later stages of infection. A maximum level ranged from 1,050 to 1,860 G-R-U in SLAP activities, and 146 to 230 K-A-U in SAP activities. Such a strikingly high level in SLAP activities tended to be maintained until the end of the experimental period. On the other hand, SAP activity showed an appreciable depression immediately after the peak values in three monkeys, Nos. 4, 13 and 14, which had survived for longer periods. No such a trend was observed in SAP activities in the other three animals, Nos. 7, 8 and 9.

d. Total serum bilirubin

During the first 6 to 10 weeks after infection, no remarkable deviations were found in total serum bilirubin concentrations in all six monkeys. Thereafter, in three animals, Nos. 4, 7 and 8, the values tended to increase rapidly, reaching 1.73 to 3.13 mg per d*l* at maximum, 7 to 13 weeks after infection. Monkey No. 8 showed an extremely high bilirubin level of 3.13 mg per d*l* just before death. At necropsy, it was found that hemorrhage and blood clot formation in the bile passages caused by migrating liver flukes had resulted in almost complete obstruction of the common bile duct accompanied with bile stasis. In the other three monkeys, Nos. 9, 13 and 14, the blood serum showed relatively low levels of bilirubin, or less than 1.0 mg per d*l* throughout the experimental period.

Discussion

Analysis of the blood serum in six monkeys infected with different numbers of metacercariae of the "Japanese species", showed significantly increased serum enzyme activities, hypoalbuminemia, and hyperglobulinemia. Such changes were more prominent in three monkeys, Nos. 4, 7 and 8, infected with 100 cysts each, than in the other three animals, Nos. 9, 13 and 14, given 20 or 40 cysts.

The results obtained during the first 4 weeks of infection showed that immature liver flukes had little, if any, effect upon the total serum protein values. During this period, there were no significant deviations in the level of serum protein fractions in all infected monkeys. Thereafter, in high-dosed two monkeys, Nos. 4 and 8, globulin concentration showed a remarkable increase, due primarily to a dramatic increase in the gamma-globulin fraction, as the infection progressed. In low-dosed three animals, Nos. 9, 13 and 14, the

blood serum showed relatively low levels of globulin concentration. The increased globulin concentrations may probably have reflected the degree of hepatic involvement and antigenic stimulation by the parasites. No significant increases in total serum protein values occurred except in monkey No. 4, as the infection progressed. It seems, therefore, that total serum protein values *per se* does not indicate the hepatic damage cuased by migrating flukes in infected monkeys. This finding was similar to that previously oberved in fascioliasis of rats,[18] sheep,[12] or cattle,[3),4),21] by other investigators.

Hypoalbuminemia having developed in all infected monkeys was in agreement with observations reported by THORPE [18] for rats, SINCLAIR[17], ROSS *et al.* [14] and ROBERTS[12] for sheep, and WEINBREN *et al.*,[21] ROSS *et al.*[13] and HAROUN *et al.* [3),4)] for cattle. Liver cell damage occurring during the migratory phase of fascioliasis appeared to be the most likely cause of the reduced albumin synthesis and the lowered albumin level in the serum. An additional contributory factor is seemed to be loss of some albumin in the inflammatory exudates in the liver and peritoneal cavity in the present study. Hypoalbuminemia was still detected on the day of necropsy in three monkeys, Nos. 4, 9 and 14, having survived for 160, 121, and 106 days, respectively. This was due perhaps to the persisting inflammatory exudates to some extent. It is possible, therefore, that chronic catarrhal inflammation cuased by the presence of sexually mature flukes in the bile passages, contributed to the low serum albumin level, as was suggested by HOLMES *et al.*[6]

It has been reported that SGOT and SGPT activities may be enhanced by the damage of the hepatic cells entailing the enzyme release in fascioliasis of different host species.[8),12),13),18] From this finding, it is reasonable to assume that these transaminase activities may reflect the intensity of *Fasciola* infection, because the liver tissue is extensively destroyed by the immature liver flukes during their migration to the biliary passages.

In the present investigation, significantly increased SGOT and SGPT activities were detected in all monkeys, at various degrees in different courses. In three monkeys, Nos. 4, 7 and 8, infected with 100 cysts each, the extent of increase in SGOT and SGPT activities was apparently greater than that in the other three animals, Nos. 9, 13 and 14, given 20 or 40 cysts. From this result, it is suggested that SGOT and SGPT levels would be convenient and accurate criteria for evaluating the extent of *Fasciola* infection without sacrificing the host animal.

Since MEYERHOF first discovered in 1919 lactate dehydrogenase (LDH) in the muscular tissue, it has been reported that LDH is widely distributed in the various body tissues, *i.e.*, the skeletal muscles, kidneys, liver, and others. There have been no report on measurement of SLDH activity in fascioliasis of any animal except albino rats.[8] Therefore, in this study the SLDH activities were measured in monkeys; significantly increased SLDH activities were detected in all animals. The increase in SLDH activities in high-dosed three monkeys, Nos. 4, 7 and 8, tended to be greater than in low-dosed three animals, Nos. 9, 13 and 14. The patterns of the increase in SLDH activities showed the same fluctuation as those of SGOT and SGPT activities.

Increased activities of SLDH in three monkeys, Nos. 4, 13 and 14, having survived for 160, 105, and 106 days, respectively, after infection, decreased following the onset of oviposition in the feces, and then returned to the values before infection. Accordingly, this enzyme activity may probably increase at the stage when the liver parenchyma was damaged by migrating immature flukes, rather than eruption of the biliary epithelium caused by the presence of mature flukes following the establishment of the flukes in the

bile ducts.

It has widely been accepted that significant increase in SAP and SLAP activities is observed in liver diseases such as acute hepatitis and obstruction of the biliary tract. On account of the paucity of information regarding SAP and SLAP activities in fascioliasis of any animal species except albino rats,[8), 18)] rabbits,[20)] and cattle,[3)] the authours tried to measure SAP and SLAP activities in the serum of infected monkeys. Significantly increased SAP and SLAP activities were found in all monkeys in 6 to 10 weeks after infection. Levels of SAP and SLAP rapidly reached the peak in accordance with the onset of oviposition in the feces. After that, SAP remained at high levels until the day of necropsy in monkey Nos. 7 and 8, which died in relatively short period, and No. 9. The other three animals, Nos. 4, 13 and 14, survived for long period and exhibited depressed SAP activities in 2 to 4 weeks after the appearance of the first eggs in the feces. In the latter three monkeys, even when SAP values returned to normal, many flukes still packed into the common bile duct when necropsied. It seemed, therefore, that significantly increased SAP levels were not related to obstruction of the larger bile ducts by mature flukes, but were attributed to immature flukes packed in the smaller bile ducts.

Meanwhile, SLAP activities tended to decrease to some extent at later stages in long-survived four animals, Nos. 4, 9, 13 and 14, although the activities were maintained at relatively high levels until the time of necropsy. These results suggest that obstruction to a certain extent of the bile passage by liver flukes after the onset of oviposition is reflected more sensitively in SLAP value than in SAP value.

Bilirubinemia, having developed to a moderate extent at later stages of infection only in heavily infected monkeys, seemed not to be a characteristic feature of fascioliasis in monkeys, because in lightly infected monkeys the bilirubin concentration fluctuated below 1.0 mg per d*l* throughout the experiment period. Serum bilirubin concentration in ruminants [12)] or rats [18)] was not regarded as a sensitive indicator of liver dysfunction resulting from migration of immature flukes, or from obstruction of the bile ducts by mature ones, but bilirubin elevation have been found chiefly in hemolytic crisis.[1)] It seems that the affected liver was still able to remove the excess bilirubin arising from tissue hemorrhage caused by the flukes even in severe infection. Therefore, unless the host is infected with a large number of metacercariae, the serum bilirubin estimation would give no indication for hepatic cell damage or biliary obstruction at any stage and, if these findings could be applied to the natural hosts, no diagnostic or prognostic aid could be expected from this test.

Summary

The alterations in total and individual serum proteins, total serum bilirubin, and various serum enzyme activities were studied in six *Macaca* monkeys infected with 20 to 100 metacercariae of the "Japanese species" of the liver flukes, *Fasciola* sp.

Infections with metacercariae showed no effect on the serum protein during the first few weeks. Thereafter, in heavily infected monkeys, globulin concentration showed a significant increase, due primarily to a dramatic increase in the gamma-globulin fraction, as the infection progressed. In lightly infected animals, the blood serum showed relatively low levels of globulin concentration. Hypoalbuminemia developed in all the infected monkeys to various degrees.

Increased activities of SGOT, SGPT, SLDH, SLAP and SAP were detected in the blood serum of all the infected monkeys, as the infection progressed. Of these enzymes, each pattern of increase in SGOT, SGPT and SLDH was similar. SLAP and SAP activities

reached the maximum levels in accordance with the onset of oviposition in the feces.

Bilirubinemia, having developed to a moderate degree at later stages of infection in only heavily infected monkeys, appeared not to be a characteristic feature of fascioliasis in monkeys, because in lightly infected animals the bilirubin concentration fluctuated below 1.0 mg per d*l* throughout the experiment period.

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