Experimental Fascioliasis in Monkeys

I. Parasitological, Clinical and Pathological Observations on Monkeys Infected with the "Japanese Species" of Fasciola

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Abstract. Eleven Macaca monkeys were infected experimentally with 20 to 800 metacercariae of the "Japanese species" of the liver fluke, Fasciola sp. They consisted of two monkeys of Macaca irus, one of M. mulatta, three of M. nemestrina, three of M. cyclops and two of M. fuscata. All of them were highly susceptible to infection with Fasciola sp. When infected with 8.8 or more metacercariae per kg of body weight, six monkeys died within 48 to 80 days after infection. The percentage of worm recovery averaged 36.4%, ranging from 16.0 to 62.0%. Adult flukes recovered from the monkeys after infection decreased in number as the duration of infection was prolonged. The recovery rate of adult worms from the monkeys was comparable with that from very susceptible host species, such as mice, rabbits and sheep.

The major clinical signs observed were rough hair coat, pallor of the face, inappetence, emaciation, prostration, abdominal pain, and ascites with scrotal hydrocele. A prominent change in white blood cell picture was eosinophilia which appeared soon after infection, increased in intensity during the migratory phase of flukes, and subsided gradually after flukes had entered the bile passages.

Almost all the monkeys that died during the parenchymal phase of migration of the parasite presented a decrease in the erythrocyte count, the hemoglobin content, and the hematocrit level shortly before death. The main gross pathological findings in these monkeys were numerous hemorrhagic tracts in the liver and a large volume of blood-stained fluid in the peritoneal cavity. Death occurred to these animals as a result of evident internal hemorrhage affecting the erythrocyte count.

The liver flukes of the genus Fasciola are the most wide-spread and important parasites of ruminants in the world. It has been known that patent infection with them can develop also in some other wild and domestic animals and man. Many excellent papers have already been published on experimental fascioliasis [1-8, 10, 11, 13, 14, 16-24] 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 which deal with the course of infection or the degree of liver damage in monkeys [9] infected with metacercariae of the liver fluke.

The incidence of human fascioliasis [12, 14, 15] 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. The purpose of the present experiments was to study the development of fascioliasis by observing parasitological, clinical, and pathological changes in monkeys infected with Fasciola sp. of Japanese origin (hereinafter referred to as the "Japanese species").

Materials and Methods

1. Experimental animals
Eleven monkeys of the genus Macaca were obtained from some zoological gardens in Osaka Prefecture for the present experiments. They consisted of two monkeys of Macaca irus, one of M. mulatta, three of M. nemestrina, three of M. cyclops and two of M. fuscata. Upon arrival, they were isolated individually and their stools examined. When they adjusted themselves to the new environment and became free from eggs of parasites, they were subjected to the experiments (Table 1).

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" of the liver fluke, Fasciola sp. Eggs were collected from the uterus by dissection with a pair of forceps. Metacercariae were produced from the laboratory-bred and artificially infected snails, Bajherlynnnae viridis. A batch of metacercariae, encysted on sheets of cellophane, was stored at 6°C until use for infection of monkeys 2 to 5 weeks later.

3. Infection of monkeys
All the monkeys were infected by feeding a small piece of banana in which pieces of cellophane bearing the required correct number of metacercariae had been embedded. Two monkeys (Nos. 9 and 11) were infected with 20 metacercariae each, another two (Nos. 13 and 14) with 40 metacercariae each, five (Nos. 3, 4, 6, 7 and 8) with 100 metacercariae each, and two (Nos. 1 and 2) with 600 and 800 metacercariae, respectively.

4. Fecal examination
To determine the prepatent period daily fecal examination for Fasciola eggs was initiated in the monkeys at the beginning of the 6th week of infection. After patency, feces were collected daily, weighed, and mixed thoroughly. Two g of sample was placed in a beaker and 60 ml of water added. The mixture was allowed to stand for approximately 2 hours, being stirred occasionally with a glass rod. The fecal suspension was filtered through a 100-mesh sieve. The filtrate was placed in a watch glass 18 cm in diameter and allowed to stand for 10 minutes. The supernatant fluid produced in the peripheral zone of the glass was withdrawn gently by a pipet with a rubber bulb, while giving a slight rotatory motion. This procedure made eggs accumulate at the center of the glass. After the supernatant fluid was withdrawn, the sediment was resuspended in water. These processes were repeated until a transparent suspension of eggs containing a very small amount of fecal debris was obtained. The total number of eggs in the sediment was then counted under the X4 objective of the microscope. The number of eggs per gram of feces (E.P.G.) and the number of eggs per day of feces (E.P.D.) were calculated.

5. Hematological examination
Blood samples were collected from the saphenous vein of these monkeys before infection and at weekly intervals thereafter. Two ml of sample was then placed in a container with disodium ethylenediamine tetra-acetate (EDTA) for routine blood examinations, including hematocrit, hemoglobin determination, and total erythrocyte and leucocyte counts.

Red and white blood cell counts were performed by a Toa microcell counter. Hematocrit level was determined by centrifuging blood-filled capillary tubes in a microcentrifuge at 12,000 rpm for 4 minutes. Hemoglobin evaluation was done by photometric determination of cyanmethemoglobin. Differential white cell count was done by examining 200 leucocytes in peripheral blood smear stained by the Wright-Giemsa method.

6. Necropsy
All the monkeys were necropsied after death or at the end of the experimental period. Their visceral organs were examined carefully for liver fluke infection and gross pathological lesions.

7. Recovery of flukes
After isolated from each animal, the liver was placed in saline at room temperature. The main bile ducts and gallbladder were dissected to collect all the visible flukes by a pair of forceps. The liver was then cut into slices 5 to 7 mm thick. In each slice flukes were carefully squeezed out from the smaller bile ducts and the parenchyma. The location and number of flukes recovered were recorded. Undamaged flukes were placed between two glass slides and fixed in 70% alcohol. These samples were stained later with hematoxylin and mounted in Canada balsam for observation of the size and detailed morphology of the flukes.
Experimental Fascioliasis in Monkeys. I

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

<table>
<thead>
<tr>
<th>Monkey</th>
<th>Body weight (kg)</th>
<th>Interval between infection and necropsy (days)</th>
<th>Dose of metacercariae</th>
<th>No. of flukes recovered</th>
<th>Percent recovery</th>
<th>Prepatent period (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>Species</td>
<td>At infection</td>
<td>At necropsy</td>
<td>between infection</td>
<td>Per monkey</td>
<td>Per kg of body weight</td>
</tr>
<tr>
<td>9</td>
<td>M. irus</td>
<td>M</td>
<td>5.50</td>
<td>4.50</td>
<td>121</td>
<td>20</td>
</tr>
<tr>
<td>1</td>
<td>M. irus</td>
<td>M</td>
<td>1.40</td>
<td>1.20</td>
<td>48*</td>
<td>600</td>
</tr>
<tr>
<td>2</td>
<td>M. mulatta</td>
<td>F</td>
<td>5.00</td>
<td>4.20</td>
<td>66*</td>
<td>800</td>
</tr>
<tr>
<td>8</td>
<td>M. nemestrina</td>
<td>M</td>
<td>6.00</td>
<td>5.50</td>
<td>50*</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>M. nemestrina</td>
<td>M</td>
<td>8.00</td>
<td>7.00</td>
<td>75*</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td>M. nemestrina</td>
<td>M</td>
<td>15.30</td>
<td>13.00</td>
<td>160</td>
<td>100</td>
</tr>
<tr>
<td>11</td>
<td>M. cyclops</td>
<td>F</td>
<td>3.60</td>
<td>4.40</td>
<td>102</td>
<td>20</td>
</tr>
<tr>
<td>7</td>
<td>M. cyclops</td>
<td>M</td>
<td>9.50</td>
<td>9.50</td>
<td>56*</td>
<td>100</td>
</tr>
<tr>
<td>6</td>
<td>M. cyclops</td>
<td>M</td>
<td>11.30</td>
<td>9.50</td>
<td>80*</td>
<td>100</td>
</tr>
<tr>
<td>13</td>
<td>M. fuscata</td>
<td>M</td>
<td>11.30</td>
<td>10.40</td>
<td>105</td>
<td>40</td>
</tr>
<tr>
<td>14</td>
<td>M. fuscata</td>
<td>F</td>
<td>9.60</td>
<td>8.40</td>
<td>106</td>
<td>40</td>
</tr>
</tbody>
</table>

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

Table 2. Egg production of the "Japanese species" of Fasciola, in the early stage of patency

<table>
<thead>
<tr>
<th>Monkey</th>
<th>Interval between onset of patency and necropsy (days)</th>
<th>No. of adult flukes recovered from main bile passages</th>
<th>E. P. G.</th>
<th>E. P. D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td></td>
<td>E. P. G. (per flute)</td>
<td></td>
<td>E. P. D. (days)</td>
</tr>
<tr>
<td>9</td>
<td>32</td>
<td>845 (121)</td>
<td>344</td>
<td>27</td>
</tr>
<tr>
<td>4</td>
<td>64</td>
<td>968 (61)</td>
<td>353</td>
<td>49</td>
</tr>
<tr>
<td>11</td>
<td>37</td>
<td>382 (127)</td>
<td>148</td>
<td>31</td>
</tr>
<tr>
<td>6</td>
<td>27</td>
<td>1,063 (82)</td>
<td>391</td>
<td>17</td>
</tr>
<tr>
<td>13</td>
<td>33</td>
<td>850 (57)</td>
<td>344</td>
<td>16</td>
</tr>
<tr>
<td>14</td>
<td>35</td>
<td>1,118 (93)</td>
<td>493</td>
<td>34</td>
</tr>
</tbody>
</table>

Remarks.
E. P. G. and E. P. D.: Number of eggs per gram of feces and the number of eggs per day of feces, respectively.

Results

1. Susceptibility of monkeys to the "Japanese species" of the liver fluke and its recovery rate

The results of experimental infection with the different numbers of metacercariae are summarized in Table 1. Monkeys exhibited high susceptibility to the infection. Six animals given 8.8 or more metacercariae per kg of body weight died within 48 to 80 days after infection. The percentage of worm recovery ranged from 31.0 to 62.0%, in these animals. In contrast, there were no
deaths throughout the experimental period among the remaining five administered with 6.5 or less metacercariae per kg of body weight. The percentage of worm recovery ranged from 16.0 to 40.0% in these five animals.

2. Prepatent period
There were considerable differences in the prepatent period among the monkeys used. The period was shorter in *M. cyclopis* than in *M. fuscata, M. irus* or *M. nemestrina* (Table 1).

3. Daily egg production in the early stage of patency
The number of fluke eggs in the feces was counted in the six monkeys infected with 20 to 100 metacercariae (Table 2). The E.P.G. increased gradually for the first 10 to 15 days after the first appearance of eggs. Then it fluctuated and reached a peak between 16 and 49 days after patency. As seen in Table 2, the daily egg production per fluke varied from 4,212 to 8,500 eggs and the E.P.G. per fluke from 57 to 127 eggs after a maximum egg production was reached. The number of eggs produced by flukes in a day, as estimated from the E.P.D. and the number of adult worms recovered from the main bile ducts of the liver, was 1,803 on the average, ranging from 1,457 to 2,845, in the early stage of the patent period.

4. Clinical signs
The onset and development of visible clinical symptoms were observed in 11 monkeys infected with 20 to 800 metacercariae. The results obtained from four monkeys are shown in Fig. 1. The common symptoms observed were rough hair coat, pallor of the face, inappetence, emaciation, prostration, and ascites with scrotal hydrocele. They varied in severity considerably depending upon the degree of infection.

Two monkeys, Nos. 9 and 11, which harbored 4 and 7 flukes, respectively, in the liver showed no severe clinical signs, except temporary anorexia, weakness, loss of weight, and pallor of the face, 7 to 9 weeks after infection.

Three monkeys, Nos. 4, 13 and 14, which had 12 to 16 worms in the liver developed moderate or severe clinical signs, such as inappetence, anemia, emaciation, prostration, rough hair coat and ascites, beginning at 6 to 12 weeks after infection. Of these symptoms, inappetence and ascites were improved gradually for the following 2 to 5 weeks, but the others persisted in these monkeys longer than in two monkeys, Nos. 9 and 11.

The remaining six monkeys, Nos. 1 to 3 and 6 to 8, with fluke burdens ranging from 34 to 248 in the liver began to develop such clinical signs as progressing anorexia, anemia and weakness 5 to 8 weeks after infection. They gradually lost body weight over a period from the 6th to 9th week of infection. Thereafter, these symptoms became very severe and were followed by abdominal pain, ascites, and severe emaciation with terminal prostration. The monkeys died 48 to 80 days after infection (Fig. 3).

5. Hematological changes
Nine monkeys infected with 20 to 100 metacercariae were examined for changes in hematological values. The results obtained from four of them are shown in Fig. 1. There was no evidence of anemia in two monkeys, Nos. 9 and 11, which had received 20 metacercariae and harbored 4 and 7 flukes, respectively, in the liver. Seven monkeys, Nos. 3, 4, 6 to 8, 13 and 14, which had received 40 to 100 metacercariae and had fluke burdens of 12 or more developed varying degree of anemia. Of these monkeys, Nos. 4, 13 and 14, which carried 12 to 16 flukes in the liver, two (Nos. 13 and 14) began to show a slight gradual decline
Fig. 1. Hematological changes and clinical features in monkeys infected with the "Japanese species" of Fasciola

- Remarks:
  - ●: Severe clinical signs,
  - ○: Moderate clinical signs,
  - □: Mild clinical signs,
  - ○: No clinical signs.
Table 3. The number and location of flukes recovered at necropsy

<table>
<thead>
<tr>
<th>Monkey</th>
<th>Interval between infection and necropsy (days)</th>
<th>Dose of metacercariae</th>
<th>Percent recovery</th>
<th>No. of flukes recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Abdominal cavity</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Liver parenchyma</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Bile duct Extra-hepatic</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Wall of stomach</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Small intestine</td>
</tr>
<tr>
<td>No.</td>
<td>Species</td>
<td>Sex</td>
<td>121</td>
<td>20</td>
</tr>
<tr>
<td>9 M. irus</td>
<td>M</td>
<td>M</td>
<td>48*</td>
<td>600</td>
</tr>
<tr>
<td>1 M. irus</td>
<td>M</td>
<td>F</td>
<td>66*</td>
<td>800</td>
</tr>
<tr>
<td>2 M. mulatta</td>
<td>M</td>
<td>M</td>
<td>50*</td>
<td>100</td>
</tr>
<tr>
<td>8 M. nemestrina</td>
<td>M</td>
<td>M</td>
<td>75*</td>
<td>100</td>
</tr>
<tr>
<td>3 M. nemestrina</td>
<td>M</td>
<td>M</td>
<td>160</td>
<td>100</td>
</tr>
<tr>
<td>4 M. nemestrina</td>
<td>M</td>
<td>M</td>
<td>102</td>
<td>20</td>
</tr>
<tr>
<td>11 M. cyclops</td>
<td>F</td>
<td>M</td>
<td>56*</td>
<td>100</td>
</tr>
<tr>
<td>7 M. cyclops</td>
<td>M</td>
<td>M</td>
<td>80*</td>
<td>100</td>
</tr>
<tr>
<td>6 M. cyclops</td>
<td>M</td>
<td>M</td>
<td>105</td>
<td>40</td>
</tr>
<tr>
<td>13 M. fuscata</td>
<td>M</td>
<td>F</td>
<td>106</td>
<td>40</td>
</tr>
<tr>
<td>Overall</td>
<td>2020 (36.4)</td>
<td>735</td>
<td>74</td>
<td>532</td>
</tr>
</tbody>
</table>

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

in the erythrocyte count in the 9th and 10th week of infection, respectively (Fig. 1).

On the other hand, monkey No. 4 exhibited a gradual increase in the erythrocyte count starting in the 10th week of infection, the count reaching a peak, or 9.37 millions per mm³, in the 14th week. During this experimental period, hematocrit and hemoglobin levels increased, as a sign of ascites became evident (Fig. 1). Thereafter, a progressive fall occurred to the erythrocyte count, hematocrit and hemoglobin levels.

In four monkeys, Nos. 3, 6, 7 and 8, which carried 34 to 62 flukes in the liver, anemia occurred earlier than in three moderately infected monkeys, Nos. 4, 13 and 14. In those four animals, the erythrocyte count began to decrease in the 3rd or 4th week of infection and continued to decrease until death. Monkey No. 8 developed severe anemia, presenting an erythrocyte count of 3.5 millions per mm³ a week before death (Fig. 1).

Total and differential leucocyte counts were determined in nine monkeys infected with 20 to 100 metacercariae. The leucocyte count ranged from 8.5 to 14.6 thousand per mm³ in the nine animals before infection. It increased gradually to a range of 19.0 to 34.1 thousand per mm³ between the 5th and 13th week of infection. The differential eosinophil count began to increase 3 or 4 weeks after infection and continued to increase progressively to 64% (No. 4; Fig. 1) until the early stage of invasion of flukes into the bile passages. Subsequently, the leucocyte and eosinophil counts decreased gradually to a nearly normal value in all the monkeys, except monkey No. 14 in which they showed high levels even at the end of the experiment.

6. Distribution of flukes in monkeys at necropsy

The number and location of flukes recovered from the infected monkeys at necropsy are shown in Table 3. In five monkeys, Nos. 1, 2, 3, 7 and 8, which died from a heavy infection by 76 days after infection, a large number of flukes were still found in the hepatic parenchyma and ascitic fluid. In six animals, Nos. 4, 6, 9, 11, 13 and 14, which survived for more than
79 days after infection, a large proportion of the flukes was found in the bile passages.

Although the occurrence of ectopic lesion was fairly uncommon in the monkeys studied, a node about 8 cm in diameter was found in the wall of the stomach of monkey No. 13 (Fig. 13). From this node one living immature fluke was detected. Six dead or moribund worms (Fig. 14) were also found in the small intestine of monkey No. 6 autopsied 80 days after infection.

7. Growth of flukes
The measurements of flukes recovered from the monkeys at necropsy are shown in Table 4 and the growth curves of flukes based on the mean worm length and width at various stages of infection in Fig. 2. The mean length and width of the flukes were directly proportional to the duration of infection, with some exceptions. In monkey No. 2 (M. mulatta), for instance, a large burden of flukes resulted in a decrease in mean length and width. In monkey No. 7 (M. cyclopis), in which flukes reach the bile ducts much sooner than in any other species of monkeys, a small burden of flukes resulted in an increase in mean length and width. As shown in Table 4, over a period from 48 to 160 days after infection, the body increased in mean length from 5.0 to 32.0 mm and in mean width from 2.0 to 10.5 mm.

8. Necropsy findings
The most important necropsy findings are shown in Table 5. The characteristic lesion of fascioliasis was found in the liver. The gross pathological involvement was directly related to the survival period of monkeys and the number of flukes recovered at necropsy. Therefore, the necropsy findings during the migratory stage are described separately from those during the

<table>
<thead>
<tr>
<th>Table 4. Measurements of liver flukes recovered from experimentally infected monkeys</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monkey No.</td>
</tr>
<tr>
<td>------------</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>9</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>8</td>
</tr>
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<td>3</td>
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<td>4</td>
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<td>11</td>
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<td>7</td>
</tr>
<tr>
<td>6</td>
</tr>
<tr>
<td>13</td>
</tr>
<tr>
<td>14</td>
</tr>
</tbody>
</table>
stage of maturity of flukes.

a. Migratory stage from 48 to 75 days after infection

To five monkeys, Nos. 1 to 3, 7 and 8, spontaneous death occurred between 48 and 75 days following infection. At necropsy all the animals showed a considerable loss of mesenteric deposition of fat, a large amount of bloody ascitic fluid (Fig. 4), and a variable severity of hydrothorax (3 to 341 ml) and hydropericardium (1 to 5 ml). In connection with multiple perforation of the hepatic capsule caused by some young flukes, fibrinous and fibrous perihepatitis with adhesions to surrounding organs was noted. These changes were more extensive in two monkeys, Nos. 3 and 8, than in any other monkeys.

In the dead monkeys, the liver was slightly enlarged, light in color, and rather flabby in consistency. It had many dark-red or yellowish-brown lesions on the surface, as well as in the deep portion of the parenchyma, resulting from the migration of immature flukes (Figs. 5–7). These fluke tracts formed foci 3 to 5 mm in diameter or tortuous tunnels up to 20 mm in length and 5 mm in width. As the flukes increased in size, the migratory tracts became more numerous and larger. In two monkeys, Nos. 2 and 3, infected with 800 and 100 metacercariae, respectively, to survive until the 66th and 75th day, the liver showed a honeycomb-like appearance with foci (Fig. 6). In monkey No. 7 which died suddenly 56 days after infection, hematomas developed throughout the liver and some of them harbored growing flukes (Fig. 12).

In three monkeys, Nos. 3, 7 and 8, infected with 100 metacercariae, hepatic
lesions were caused by the liver fluke in the late intrahepatic and early bile-duct stages. A moderate enlargement of the extrahepatic bile passages was also noted. Particularly in monkey No. 8, the main bile duct of the hilus underwent a cylindroid dilation and was occluded by clotted blood accompanied by bile stasis, and jaundice was grossly visible (Fig. 7). The hepatic lymph nodes were mostly enlarged to various degrees.

b. Maturity stage from 80 to 160 days after infection

In six monkeys, Nos. 3, 6, 9, 11, 15 and 14, except No. 6 which died 80 days after infection with 100 metacercariae, no spontaneous death occurred during the experimental period. At necropsy, there were more or less extensive fibrous adhesions between the serous surface of the liver and the surrounding omentum, diaphragm and other organs (Fig. 9). The hepatic lobes were distorted and varied in size with frequent adhesions among them. Their surfaces were characterized by the presence of linear or stellate scarred lesions light grayish-brown in color depressed from the general surface. The lesions were severer in the left lobe than in the right one. Lesions of variable severity were seen also in the other hepatic lobes in more heavily affected monkeys (Nos. 4, 6, 13 and 14; Figs. 9–11). In two monkeys, Nos. 9 and 11, infected with 20 metacercariae and killed 102 and 121 days after infection, respectively, such lesions had a slightly irregular surface caused by the streaks of scarred fluke tracts.

In most of the monkeys studied, the liver was lighter in color and firmer in consistency and had a more clearly demarcated lobular pattern than the regular organ. The extrahepatic bile ducts near the hilus showed a cylindroid enlargement with 2 to 16 interleaved adult worms of Fasciola and a large amount of brown viscid bile inside. Their wall was thickened due to fibrosis and their lumen several times larger than normal (Figs. 9–11). They remained pliable, however, without being affected with calcification. The gallbladder was often twice as large as its normal size and filled with brown bile. Most of the hepatic lymph nodes were enlarged with slight hemosiderosis.

The main features of the cut surface of the liver were the progressive maturity of the fibrous tissue in isolated fluke tracts, as well as in confluent areas of severe damage, the formation of cavities by liver flukes which appeared to have failed to enter the main bile passages, and the fibrous thickening of the walls of the intrahepatic bile ducts. These changes varied in degree generally depending on the number of worms harbored. The greater was the number of parasites established, the more extensive was the effect of organization of tunnels produced by pronounced migration accompanied by the formation of worm cysts in the hepatic parenchyma.

In monkey No. 4 infected with 100 metacercariae and killed 160 days after infection, severe fibrosis was found not only in the hepatic parenchyma of the left lobe where superficial lesions were prominent, but also in that of some other lobes which showed a grossly normal surface (Fig. 10). In monkey No. 6 which died 80 days after infection with 100 metacercariae, many cavities were formed in every hepatic lobe. Most of them were poorly defined with a thin connective-tissue capsule and contained brownish necrotic masses and a brown viscid fluid (Fig. 8). From some of these cavities a small number of living flukes were recovered.
Discussion

The primary aim of this work is to determine the sequence of the development of experimental fascioliasis in monkeys, which has not been reported in Japan or in any other country either. The present studies clearly demonstrated that five species of monkeys of the genus Macaca were highly susceptible to infection with the “Japanese species” of the liver fluke, *Fasciola sp.*, and that an infection with 8.8 or more metacercariae per kg of body weight led to death attributable to fascioliasis within 48 to 80 days.

According to Dawes et al. [5], the average recovery rate of adult flukes ranged from 26.6 to 39.1% in large domestic and small laboratory animals experimentally infected with *F. hepatica*. In the present experiments with 11 monkeys which had received 20 to 800 metacercariae, the recovery rate of flukes was 36.4% on the average, ranging from 16.0 to 62.0% (Table 1). These values obtained from the monkeys were comparable with those from very susceptible host species, such as mouse, rabbit and sheep. There was, however, a variation in the recovery rate of adult flukes among the monkeys. This variation may possibly be due to the disintegration of some of the migrating immature flukes which failed to enter the bile ducts, or to the expulsion of some of the adult flukes from the main bile passages into the intestine (Fig. 14), because flukes recovered from the monkeys decreased in number with the advance in the course of infection [2, 21].

The prepatent period of the liver flukes, *Fasciola spp.*, varies considerably from host to host, and also depends on the species of flukes. Watanabe et al. [24] succeeded in producing experimental infection with *F. hepatica*, *F. gigantica*, or the “Japanese species” in many rabbits and sheep. According to them, there was little difference in the prepatent period between the “Japanese species” and *F. hepatica* of English origin; this period was 61 days in a rabbit and 61 or 62 days in sheep in *F. hepatica* infection and 52 to 69 days in rabbits and 62 to 67 days in sheep in “Japanese species” infection. They also reported that *F. gigantica* developed more slowly in rabbits and sheep than *F. hepatica* and the “Japanese species”, and that eggs appeared in the rabbit feces from 73 to 93 days and in the sheep feces from 72 to 79 days after infection. Ono et al. [13, 14], who had infected two cattle with metacercariae of the “Japanese species”, found that eggs appeared in the feces on the 66th day after infection.

In the present observation, the prepatent period was significantly longer in *M. nemestrina* and *M. irus* than in *M. cyclopis* (Table 1). Although no reasons are known for these differences because of the limited number of animals studied, the authors are inclined to attribute such discrepancies only to the difference in the suitable host species; that is, flukes in *M. cyclopis* appeared to grow more quickly than those in *M. irus*, *M. nemestrina* or *M. fuscata*.

Investigating the egg-producing capacity of *F. hepatica* in sheep, Happich et al. [4] found that the daily egg output per fluke depended on the number of flukes in the liver and varied from 4,000 to 50,000 eggs. In the present experiments, the daily egg-producing capacity of flukes was 1,803 eggs on the average, ranging from 1,457 to 2,845 eggs. These values were extremely smaller than those reported by Happich et al. [4].

It was reported by Boray [3] that in sheep infected with 2,000 or more metacercariae, the development of the migrating forms of *F. hepatica* was retarded and the average length of flukes did not increase appreciably.
for 12 weeks after infection. In the present experiments, the same results were obtained in monkey No. 2 as in Boray's sheep; 248 stunted immature flukes were recovered from the hepatic parenchyma of this monkey (Tables 3 and 4; Fig. 2).

Since lesions of fascioliasis are primarily limited to the liver, irrespective of host species, the size of this organ is an important factor in determining the effect of the number of metacercariae given to the host animal. Boray [4] infected sheep with a single or divided dose of 200 to 10,000 metacercariae of *F. hepatica* and found that sheep with not more than 100 flukes in the liver did not produce clinical fascioliasis during an observation period of 25 weeks. Most of the sheep with approximately 100 to 1,000 flukes died of chronic fascioliasis, and most of those with more than 1,000 flukes died of the acute or subacute form of the disease.

In the present experiments, infected monkeys with 16 or less flukes in the liver were not involved in fatal fascioliasis during the experimental period, although all of them showed clinical signs of varying severity. Infected monkeys with 34 or more flukes in the liver were affected with fatal fascioliasis, showing severe inappetence, emaciation, prostration, rough hair coat, abdominal pain, progressing anemia and ascites.

Details in changes in the blood cell picture in experimental infection were provided by Kimura [8] in rabbits, by Tewari [21] in guinea pigs, by Boray [3] and Symons et al. [20] in sheep, and by Ross et al. [16] in calves. As a whole, they suggest that the anemia of acutely infected animals, in which flukes have entered the parenchyma of the liver, may be attributable to the loss of blood in this organ and the abdominal cavity. On the other hand, the anemia of more chronically infected animals, in which flukes have entered bile ducts, may be due to ingestion of blood by these parasites.

In the present experiments, there was no evidence of anemia in two monkeys, Nos. 9 and 11, which had received 20 metacercariae and harbored 4 and 7 flukes, respectively, in the livers. Seven monkeys, Nos. 3, 4, 6, 7, 8, 13 and 14, which had received 40 to 100 metacercariae and had fluke burdens of 12 or more worms, however, developed a varying degree of anemia. Three of them, Nos. 3, 7 and 8, died between 50 and 75 days after infection, showing relatively low erythrocyte counts shortly before death (Fig. 1). At necropsy they had a large amount of bloody exudate in the abdominal cavity (Fig. 4). These findings lend support to Symons et al. [20] who concluded that the anemia of acutely infected sheep, in which flukes had entered the parenchyma of the liver, was primarily due to hemorrhage in the parenchyma of the organ and ascites in the peritoneal cavity.

Eosinophilia was a prominent change in the white blood cell picture of all the monkeys in the present experiments. It became evident shortly after infection, increased in severity during the migratory phase of the fluke, and subsided gradually after flukes had entered the bile passages.

In the infected monkeys, the severity of pathological changes was in proportion to the number of flukes harbored. In five heavily infected animals, Nos. 1, 2, 3, 7 and 8, which died within 75 days after infection, the large volume of peritoneal fluid was invariably blood-stained, and typical hemorrhagic tracts resulting from the migration of immature flukes were pronounced throughout the liver (Figs. 5 and 6). In one (No. 7) of these monkeys, besides the changes described above, various sizes of hematomas developed throughout the liver.
(Fig. 12). In another heavily infected animal, No. 6, which died 80 days after infection, there was no blood-stained fluid in the peritoneal cavity, but different sizes of cavities were formed rather abundantly in each heptic lobe. Thus, it may be imaginable that the direct cause of death of these six monkeys was internal hemorrhage in the liver and the dysfunction of this organ resulting from damage of the hepatic parenchyma.

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References


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Explanation of Figures

Fig. 3. Posture shortly before death of monkey No. 8 which died 50 days after infection with 100 metacercariae, showing rough hair coat, emaciation, prostration, and ascite with scrotal hydrocele.

Fig. 4. Abdominal cavity of monkey No. 8 showing voluminous blood-stained peritoneal fluid and a considerable loss of mesenteric deposition of fat at necropsy.

Fig. 5. Visceral surface of the liver of monkey No. 2 which died 66 days after infection with 800 metacercariae, showing numerous hemorrhagic tracts resulting from the migration of immature flukes and enlargement of the hepatic lymph nodes (arrow).

Fig. 6. Cut surfaces of the right medial lobe of the liver shown in Fig. 5. They had a honeycomb-like appearance with numerous migratory tracts.

Fig. 7. Visceral surface of the liver of monkey No. 8 showing typical hemorrhagic tracts and a cylindrical dilation of the main bile duct with sufficient clotted blood for occlusion of the lumen (arrow).

Fig. 8. Cut surfaces of the right lateral hepatic lobe of monkey No. 6 which died 80 days after infection with 100 metacercariae. Three cystic cavities are seen in the parenchyma of the liver. The wall of the right cavity (arrow) is marked-

Fig. 11. Visceral surface of the liver of monkey No. 13 showing scarring and distortion of the liver lobes and the thickened wall of the main bile duct that contained one adult worm (arrow) 105 days after infection with 40 metacercariae.

Fig. 12. Cut surfaces of the left medial hepatic lobe of monkey No. 7 which died 56 days after infection with 100 metacercariae, showing a subcapsular hematoma containing two immature flukes (arrows).

Fig. 13. Stomach of monkey No. 13 showing cut surfaces of a node (arrows) in the wall from which one living fluke was recovered.

Fig. 14. Dead fluke recovered from the small intestine of monkey No. 6 at necropsy.