Experimental Visceral Larva Migrans in Monkeys

I. Clinical, Hematological, Biochemical and Gross Pathological Observations on Monkeys Inoculated with Embryonated Eggs of the Dog Ascarid, *Toxocara canis*

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Abstract. Nine Macaca monkeys were inoculated experimentally with 100,000 to 400,000 embryonated eggs of *Toxocara canis*. Of them, four belonged to *Macaca fascicularis*, one to *M. nemestrina*, two to *M. cyclopis*, and two to *M. fuscata*.

Six infected monkeys, Nos. 1 to 3, 5, 6 and 9, which suffered from a mild invasion of the CNS by larvae of *T. canis*, showed no clinical abnormality, except mild or moderate inappetence and depression for several weeks together with occasional mild coughing. The remaining three infected monkeys, Nos. 4, 7 and 8, harbored considerable numbers of viable larvae not only in the thoracic and abdominal viscera but also in the CNS, and began to develop such clinical signs as anorexia, weakness and coughing 4 to 7 days after infection. Severe neurological signs that terminated in paralysis were also noted in these three animals on the 8th to 16th days. The postmortem examination of them revealed parasitic granuloma formation and focal tissue destruction with hemorrhage at numerous sites on the cut surfaces of the brain and spinal cord. From these results, it should be emphasized that the severe neurological disorders resulted from the CNS damage which had been caused by the invasion of *T. canis* larvae in the brain and spinal cord.

Hematological findings of the infected animals included transiently reduced erythrocyte counts and marked eosinophilia. The latter appeared soon after inoculation, increased to a high degree between the 2nd and the 4th week, and declined as the infection progressed. Analysis of their blood plasma also showed increased globulin concentrations, reduced albumin values, and decreased albumin/globulin ratio, in addition to elevated plasma transaminase levels. No important changes were found in any other parameter.

In 1952, Beaver et al. [5] introduced the term "visceral larva migrans" to denote a clinical syndrome resulting from the invasion of human viscera by the larvae of nematodes normally parasitic to lower animals. Since then, numerous cases of human infection with larvae of the common ascarid of dogs, *Toxocara canis*, have been reported in the world [2, 6, 26, 27].

This syndrome occurs primarily in children and is characterized by a pronounced and prolonged eosinophilia with or without constitutional symptoms, such as varying degrees of hepatomegaly, hypergammaglobulinemia, and some degree of pulmonary infiltration [4, 12-18, 26]. A variety of neurological symptoms and signs, including convulsions, delirium, paralysis and

meningism, have also been observed in some cases of visceral larva migrans in human beings [3, 6, 8, 11, 12, 15, 19, 21-23, 25]. The relationship of the neurologic manifestations to cerebral involvement by the larvae of *T. canis*, however, remains obscure. The present work was undertaken to ascertain whether or not severe neurological disorders occurred in monkeys inoculated with a large number of embryonated eggs of *T. canis*.

This report describes briefly the salient clinical, hematological, biochemical and pathological features of experimental toxocariasis in monkeys. The histopathological changes associated with the disease reported here will be presented soon elsewhere.

Materials and Methods

1. Experimental animals

Nine monkeys of the genus *Macaca* were obtained from some zoological gardens in Osaka Prefecture for the present experiment. Of them, four belonged to *Macaca fascicularis*, one to *M. nemestrina*, two to *M. cyclopis*, and two to *M. fuscata*. Upon their arrival, they were isolated individually and fed pelleted commercial monkey feed with fruit supplement. When they adjusted themselves to the new environment and became free from eggs of parasites, they were subjected to the experiment (Table 1).

2. Preparation of infective *Toxocara canis* eggs

Eggs were obtained by dissection from the uteri of gravid female worms of *T. canis* which had been collected from naturally infected dogs in a locality. Batches of fertile eggs were treated with 1% NaOH to remove the albuminous coating, and incubated in tap water with 0.5% formalin. The culture was kept at 25°C in large Petri dishes and agitated slightly every day. Infective eggs developed in approximately 28 days. Prior to inoculation the eggs were washed several times with tap water.

3. Infection procedure

All the monkeys were infected by feeding a small piece of banana in which a proper volume of suspension containing a calculated number of infective embryonated eggs had been embedded. Two monkeys (Nos. 1 and 2) were inoculated with 100,000 eggs each, another two (Nos. 3 and 4) with 200,000 and 300,000 eggs, respectively, and the remaining

five (Nos. 5 to 9) with 400,000 eggs each.

4. Clinical examination

All the monkeys were regularly observed for clinical signs throughout the course of experiment. They were agitated to facilitate the detection of such signs as coughing, dyspnea, and neurological disorders, including posterior paresis and ataxia.

5. Hematological examination

Blood samples were taken into a sterile syringe from the saphenous vein before and at weekly intervals after infection. Two ml of sample was then transferred to each glass vial containing disodium salt of ethylenediaminetetraacetic acid (sodium EDTA).

Total erythrocyte (RBC) and leucocyte (WBC) counts were performed using a "Toa" microcell counter. Packed cell volume (PCV) was determined in a high-speed microhematocrit centrifuge. Hemoglobin (Hb) concentration was measured by a standard cyanmethemoglobin procedure. Coverslip smears, prepared immediately after sampling, were stained by the Wright-Giemsa method. Two hundred leucocytes were examined for their differential counts.

6. Blood chemistry

To collect blood plasma, four ml of oxalated blood obtained from each monkey was kept at room temperature for 30 minutes, and then centrifuged at 3,000 rpm for 15 minutes. Total plasma protein values were determined by the micro-Kjeldahl technique. The plasma protein fractions were determined by the disc electrophoretic method [10] and subsequent scanning of amid black stained gel columns on a "Fuji" recording densitometer (FD-A IV type).

The activities of plasma glutamate oxaloacetate (PGOT) and plasma glutamate pyruvate (PGPT) transaminases and alkaline phosphatase (ALP) were estimated with a rapid blood analyzer (RaBA) system (Chugai Pharmaceutical Co., Ltd.). These enzyme activities were expressed in the following units; PGOT and PGPT in Karmen units (K-U) and ALP in King-Armstrong units (K-A-U).

7. Autopsy procedure

Autopsy was performed on the monkeys shortly after lethal intravenous injection with barbiturate at the end of experiment. The gross lesions found were identified.

8. Recovery of larvae from tissues

At necropsy, approximately one-tenth portions of the brain, spinal cord, lungs, heart, liver, kidneys, pancreas, diaphragm, and skeletal muscle masses from different sites of each organ, and the left eye were removed and examined for the presence of *Toxocara* larvae. To recover the larvae from these

Fig. 1. Hematological changes and clinical features in monkeys inoculated with embryonated eggs of *T. canis*

Remarks.

- : Severe clinical signs. ◐ : Moderate clinical signs.
 ○ : Mild clinical signs. ○ : No clinical sign

organs and tissues, except the central nervous system (CNS), the digestion technique was employed. Larvae in the CNS were counted directly by squashing the tissues between two glass plates. The total number of larvae in each organ was estimated by multiplying the number of larvae recovered per gram of organ by the weight of the organ. The entire content of the bowel was also examined for the presence of helminths, by using standard techniques.

Results

1. Clinical signs

The onset and development of visible clinical symptoms were observed in the nine monkeys inoculated with 100,000 to 400,000 embryonated eggs. The results obtained from four monkeys are shown in Fig. 1. The clinical signs in the animals varied in severity, considerably depending upon the number of larvae migrating in various organs and tissues, including the CNS.

Six monkeys, Nos. 1, 2, 3, 5, 6 and 9, which suffered less parasitic invasion of the CNS showed no clinical abnormal changes, except mild or moderate inappetence, depression for several weeks, and occasional mild coughing.

The remaining three monkeys, Nos. 4, 7 and 8, which harbored considerable numbers of viable larvae in the CNS and viscera began to develop such clinical signs as anorexia, weakness and coughing 4 to 7 days after infection. Severe neurologic signs, terminating in paralysis, were also noted in these three animals on the 8th to 16th days after inoculation.

Monkey No. 4, from which 96,765 larvae were recovered, exhibited indistinct weakness or incoordination of both hind legs 11 days after inoculation. The following day the animal was listless and reluctant to move about in the cage even when he was agitated. On the 13th day the monkey seemed unable to keep his balance,

and fell to his right side when rising or walking. On the 17th day both hind legs were paralyzed almost perfectly and the animal moved by using the forelegs and dragging the hind legs behind the body. Reduction in food consumption, which led to emaciation, was also evident during this period. The following day he could not rise, because prominent paralysis of the hind quarters occurred (Fig. 3). Autopsy was performed on the 18th day after inoculation.

Monkey No. 7, from which 48,693 larvae were recovered, showed a slight weakness of the left hind leg 8 to 9 days after inoculation. The following day the animal was limping in his left hind limb, which was often held up so that the foot might not touch the floor. On the 11th day after inoculation, he tended to turn counter-clockwise on the left hind leg when he was moving. From the 12th to 16th days the above-mentioned signs became more pronounced. In addition, coughing was noted occasionally. On the 20th day the hind quarters were paralyzed. He was autopsied on the following day.

Monkey No. 8, from which 83,692 larvae were recovered, displayed weakness and incoordination of the right hind leg on the 16th day after inoculation. Thereafter the above-mentioned signs became more pronounced day after day. On the 20th day the right hind limb was paralyzed and he was reluctant to rise and walk even when he was stimulated. His right hind leg was dragged behind when he moved with difficulty. On the 23rd day paralysis of the rear quarters occurred and the animal almost always sat with both hind legs extended to the front. On the following day the animal moved by using the forelegs and dragging the hind legs behind when taken from the cage to be examined his

Table 1. Results of experimental inoculation of monkeys with various numbers of embryonated eggs of *T. canis*

Monkey			No. of eggs inoculated			No. of larvae per gram of organ weight													Total No. of larvae recovered
No.	Species	Sex	Body weight (kg)	Per monkey ($\times 10^4$)	Per gram of body weight	Interval in days between infection and necropsy	Cerebrum	Cerebellum	Spinal cord	Liver	Lungs	Heart	Kidneys	Pancreas	Intestinal wall	Skeletal muscles	Diaphragm	Eyes	
1	<i>M. fascicularis</i>	M	5.0	10	20	42	0 (59)	3 (6)	0 (10)	2 (102)	0 (31)	0 (16)	0 (16)	0 (7)	0 (255)	2 (3,330)	0 (20)	0 (7.6)	6,882
2	<i>M. fascicularis</i>	M	5.0	10	20	49	2 (66)	0 (7)	0 (9)	2 (110)	0 (25)	0 (20)	0 (22)	0 (15)	0 (330)	2 (3,330)	0 (22)	0 (7)	7,012
3	<i>M. fascicularis</i>	M	6.2	20	32	56	14 (60)	24 (5)	1 (10)	124 (104)	0 (35)	0 (22)	3 (24)	0 (11)	0 (316)	4 (5,328)	0 (27)	0 (9)	35,250
4*	<i>M. nemestrina</i>	M	3.2	30	93	18	97 (82)	107 (8)	25 (7)	208 (86)	28 (38)	15 (10)	40 (14)	0 (7)	0 (164)	32 (2,110)	46 (13)	0 (6)	96,765
5	<i>M. cyclopis</i>	M	5.0	40	80	7	14 (83)	14 (7)	2 (11)	90 (102)	26 (70)	5 (24)	20 (16)	2 (14)	0 (230)	3 (3,580)	0 (22)	1 (8)	23,498
6	<i>M. cyclopis</i>	M	5.4	40	74	14	1 (80)	8 (8)	0 (11)	137 (106)	79 (43)	10 (28)	1 (20)	2 (9)	0 (230)	6 (3,900)	3 (18)	0 (8)	41,835
7*	<i>M. fascicularis</i>	M	2.8	40	142	21	58 (57)	22 (6)	0 (5)	278 (74)	42 (30)	80 (12)	20 (30)	1 (6)	4 (130)	11 (1,930)	10 (10)	1 (7)	48,693
8*	<i>M. fuscata</i>	M	8.0	40	50	28	16 (100)	28 (10)	4 (12)	400 (178)	0 (56)	0 (34)	3 (38)	0 (20)	0 (408)	2 (5,200)	2 (25)	0 (10)	83,692
9	<i>M. fuscata</i>	M	9.2	40	43	35	3 (92)	4 (10)	2 (13)	104 (170)	24 (55)	4 (42)	7 (48)	0 (18)	0 (440)	2 (6,500)	4 (27)	0 (10)	32,954

Remarks. In parentheses is shown the weight of organ in grams.

*: These monkeys showed severe motor paralysis in the course of experiment.

gait (Fig. 4). He remained in this state until the 28th day, when he was autopsied.

2. Hematological changes

Seven monkeys infected with 100,000 to 400,000 infective *T. canis* eggs were examined for changes in hematological values. The results obtained from four of them are shown in Fig. 1. Over the period of observation, there was no evidence of anemia in two monkeys, Nos. 1 and 2, from which were recovered 6,882 and 7,012 larvae, respectively. Five monkeys, Nos. 3, 4, 7, 8 and 9, from which 32,954 to 96,765 larvae were recovered, developed a transient decline in the RBC count between the 2nd and the 5th week after inoculation. During this period the count reached the lowest value, or 3.67 millions per mm³, in monkey No. 3, 4.17 millions per mm³ in monkey No. 4, and 3.72 millions per mm³ in monkey No. 9.

PCV and Hb values in the above-mentioned five monkeys generally followed the decline in RBC count, i.e., they began to show a slight to moderate transient decrease 1 to 3 weeks after inoculation, reaching the lowest value of 28.5% (in monkey No. 4) and of 7.8 g per 100 ml of blood (in monkey No. 7), respectively.

Total and differential WBC counts were recorded in seven monkeys inoculated with 100,000 to 400,000 embryonated eggs. The WBC counts ranged from 7.6 to 16.7 thousand per mm³ in these animals before inoculation. They increased rapidly to a range of 15.5 to 26.1 thousand per mm³ between the 2nd and the 3rd week after inoculation. During this period the curves representing eosinophil percentage obtained from the differential cell counts showed almost similar trends to those of the WBC counts; a maximum eosinophilia of 46% occurred in monkey No. 9 in the 4th week after inoculation. Subsequently,

the WBC counts showed a tendency to return gradually to the normal range in all the monkeys, except Nos. 2 and 3 in which the counts showed a secondary rise around 7 and 6 weeks after inoculation, respectively. Although the differential eosinophil counts also showed a decline as the infection progressed, they remained at considerably high levels in most of the animals until the end of experiment.

3. Blood chemistry

The changes in total plasma protein, plasma protein fractions, PGPT, PGOT and ALP were serially evaluated in six infected monkeys. The results of plasma protein and plasma enzyme estimation in four of these monkeys are shown in Table 2 and Fig. 2, respectively.

a. Changes in total plasma protein and plasma protein fractions

In two monkeys, Nos. 1 and 2, from which 6,882 and 7,012 larvae, respectively, were recovered the concentration of total plasma protein was within the normal range throughout the experimental period. In four monkeys, Nos. 3, 4, 8 and 9, from which were recovered 35,250, 96,765, 83,692 and 32,954 larvae, respectively, it dropped transitorily within a week after inoculation, exceeded the preinoculation value about the 3rd week, and then remained at a level a little higher than the preinoculation level until the end of the experimental period. It reached 8.4 g per 100 ml in Nos. 8 and 9.

When the plasma protein fractions were examined, albumin decreased in concentration in four monkeys, Nos. 3, 4, 8 and 9, between the 8th and the 15th day after inoculation. It reached 1.9 and 1.8 g per 100 ml in two monkeys, Nos. 4 and 9, respectively. Then, it remained at levels a little lower than the preinoculation levels until the end of experiment. There were

Table 2. Changes in plasma proteins (g/100 ml) in monkeys inoculated with embryonated eggs of *T. canis*

Monkey No.	Weeks p.i.	Total protein	Albumin	Alpha-lipoprotein	Alpha ₁ -globulin	Alpha ₂ -globulin	Transferrin C	Beta'-globulin	Beta-and gamma-globulin	Slow alpha ₂ -globulin	Slow beta-globulin	Albumin/globulin ratio
2	0	7.4	2.7	0.3	0.1	0.1	0.9	0.7	2.0	0.2	0.4	0.6
	1	7.2	2.8	0.2	0.2	0.1	0.6	0.6	2.1	0.2	0.4	0.6
	2	7.5	3.0	0.2	0.2	—	0.7	0.5	2.2	0.3	0.4	0.7
	3	7.3	2.3	0.2	—	0.2	0.6	0.5	2.9	0.2	0.4	0.5
	4	7.5	2.9	0.3	0.1	0.1	0.7	0.6	2.5	—	0.3	0.6
	5	7.0	3.4	0.2	0.1	—	0.7	0.3	1.8	0.2	0.3	0.9
	6	7.5	2.8	0.2	0.1	0.2	0.7	0.3	2.6	0.2	0.4	0.6
	7	7.5	2.8	0.2	0.1	0.2	0.5	0.6	2.5	0.2	0.4	0.6
3	0	7.1	3.1	0.3	—	0.2	0.7	0.5	1.7	0.2	0.4	0.8
	1	5.8	3.0	0.2	—	0.1	0.7	0.3	1.1	0.1	0.3	1.1
	2	6.7	2.1	0.3	—	0.2	0.7	0.4	2.0	0.5	0.5	0.5
	3	7.1	2.1	0.3	—	0.2	0.7	0.5	2.8	0.1	0.4	0.4
	4	6.9	2.6	0.3	0.1	—	0.7	0.4	2.3	0.2	0.3	0.6
	5	7.9	3.3	0.2	0.1	0.2	0.7	0.5	2.4	0.2	0.3	0.7
	6	7.3	2.6	0.2	0.1	0.2	0.7	0.5	2.5	0.2	0.3	0.6
	7	7.5	2.5	0.3	—	0.1	0.7	0.5	2.6	0.3	0.5	0.5
8*	0	7.3	3.1	0.3	0.2	0.1	0.6	0.4	2.1	0.2	0.3	0.8
	1	6.7	2.4	0.2	0.1	0.1	0.7	0.3	2.3	0.3	0.3	0.6
	2	8.4	2.7	0.3	0.1	0.2	0.6	0.9	3.2	0.1	0.3	0.5
	3	8.1	2.5	0.4	0.3	—	0.7	1.0	2.8	0.1	0.3	0.5
	4	7.7	2.5	0.3	0.1	0.1	0.7	0.8	2.6	0.2	0.4	0.5
9	0	7.3	3.0	0.3	0.1	0.1	0.8	0.4	2.1	0.1	0.3	0.7
	1	6.7	2.1	0.5	0.2	0.1	0.6	0.6	1.8	0.2	0.6	0.5
	2	6.9	1.8	0.4	0.2	—	0.6	0.4	3.1	0.1	0.3	0.4
	3	7.6	2.0	0.4	0.2	0.1	0.8	0.4	3.0	0.2	0.4	0.4
	4	7.8	2.6	0.2	0.2	—	0.7	0.5	3.1	0.2	0.3	0.5
	5	8.4	2.5	0.4	0.2	0.1	0.7	0.5	3.2	0.2	0.5	0.4

Remarks. p. i.: Postinfection.

*: This monkey showed severe motor paralysis in the course of experiment.

no consistent or significant changes in alpha-lipoprotein, alpha₁-globulin, transferrin C, beta'-globulin, slow alpha₂-globulin and slow beta-globulin levels in the course of experiment. Beta- and gamma-globulin, however, showed a significant increase which was accounted for the increase in total plasma proteins.

The greatest increase in the concentration of beta- and gamma-globulin occurred in three monkeys, Nos. 3, 8 and 9, from which were recovered 35,250, 83,692 and 32,954 viable larvae, respectively. Two to 3 weeks after infection, the concentrations

of these globulins were 1.0 to 1.1 g per 100 ml higher than their preinoculation levels in these three monkeys. They were maintained at relatively high levels until the end of experiment. As a result of hypoalbuminemia with an increase of beta- and gamma-globulin levels, an apparent decrease in the albumin/globulin ratio occurred in four monkeys, Nos. 3, 4, 8 and 9.

b. Changes in plasma transaminases and alkaline phosphatase

PGPT and PGOT began to increase in the infected monkeys soon after inoculation. In four monkeys, Nos. 3, 4, 8 and 9,

Fig. 2. Changes in plasma GOT and GPT activities in monkeys inoculated with embryonated eggs of *T. canis*

from which were recovered 35,250, 96,765, 83,629 and 32,954 larvae, respectively, PGPT activity showed significantly high levels, reaching 153 to 408 K-U at maximum, 5 to 7 days after infection. In the other two monkeys, Nos. 1 and 2, from which 6,882 and 7,012 larvae, respectively, were recovered, the blood plasma showed relatively low levels of PGPT activity, or less than 100 K-U. Thereafter, PGPT activity fell gradually to a normal level in all the infected monkeys. PGOT activity fluctuated in essentially the same manner as PGPT activity, and reached a maximum value of 90 to 120 K-U in four monkeys, Nos. 3, 4, 8 and 9, and of 37 to 52 K-U in two monkeys, Nos. 1 and 2. When both transaminase activities were examined, PGPT tended to be higher in level than

PGOT in all the monkeys. No significant changes were observed in the ALP level throughout the experimental period.

4. Distribution of larvae in monkeys

The organs examined and the number of larvae recovered from the nine infected monkeys are shown in Table 1. In two monkeys, Nos. 1 and 2, from which were recovered 6,882 and 7,012 larvae, respectively, a small number of larvae were found only in the liver, CNS, and skeletal muscle masses. In the other seven monkeys, Nos. 3 to 9, from which 32,954 to 96,765 larvae were recovered, more organs and tissues were invaded by larvae, although there were differences in the number of larvae found among them. For instance, three monkeys, Nos. 4, 7 and 8, from which were recovered 96,765, 48,693 and 83,692

Table 3. Summary of the most important necropsy findings in monkeys inoculated with embryonated eggs of *T. canis*

Monkey No.	Duration of infection (days)	Gross pathological changes																		
		Liver			Swelling of hepatic lymph nodes	Lungs			Swelling of tracheo-bronchial lymph nodes	Fibrinous pleurisy	Kidneys (cortex)		Cerebrum		Cerebellum		Spinal cord		Granulomatous lesions in retina	
		Enlargement	Petechiae	Granulomatous lesions		Petechiae	Granulomatous lesions	Hemosiderosis			Petechiae	Granulomatous lesions	Petechiae	Granulomatous lesions	Petechiae	Granulomatous lesions	Petechiae	Granulomatous lesions		
1	42	—	—	+	+	+	+	—	+	—	—	++	—	—	—	—	—	—	—	—
2	49	—	—	+	+	—	+	—	—	+	—	+	—	—	—	—	—	—	—	—
3	56	—	++	++	+	—	++	—	++	—	—	+	—	+	—	+	—	—	—	—
4*	18	++	++	++	++	—	++	++	++	—	+	++	—	++	—	++	—	+	—	+
5	7	—	++	++	++	++	—	—	++	—	++	—	+	—	—	—	—	—	—	—
6	14	—	++	++	++	+	++	—	++	—	+	++	—	+	+	+	—	—	—	—
7*	21	+	—	++	+	—	++	++	++	++	—	++	—	++	—	++	—	—	—	—
8*	28	+	—	++	++	—	++	—	++	—	—	++	+	++	—	++	+	+	—	—
9	35	—	—	++	+	—	++	—	++	—	—	++	+	++	+	++	—	—	—	+

Remarks.

+++: Extremely severe, ++: severe, +: moderate, +: mild, —: absent.

*: These monkeys showed severe motor paralysis in the course of experiment.

larvae, respectively, showed severe neurologic signs 8 to 16 days after inoculation. In them, a considerable number of larvae were seen in tissues of the liver and brain. These organs were followed by the diaphragm, kidneys, skeletal muscle masses, lungs, spinal cord and heart in monkey No. 4, by the heart, lungs, kidneys, skeletal muscle masses, diaphragm, eyes and pancreas in monkey No. 7, and by the spinal cord, kidneys, diaphragm and skeletal muscle masses in monkey No. 8. No larvae were found in the spleen and intestinal contents. In these three monkeys, the total recovery rate of larvae was 20.9%, ranging from 12.2 to 32.3%.

5. Gross pathology

The most important necropsy findings are summarized in Table 3. Gross pathological changes varied in intensity and distribution. They consisted primarily of granuloma formation and focal tissue destruction with hemorrhage caused by

migrating larvae. With a few exceptions, these lesions were apparently related to the surviving period of monkeys and the number of larvae recovered.

In two monkeys, Nos. 1 and 2, from which 6,882 and 7,012 larvae, respectively, were recovered, discrete verminous lesions were limited to the liver, lungs and kidneys. In four monkeys, Nos. 3, 5, 6 and 9, from which were recovered 35,250, 23,498, 41,835 and 32,954 larvae, respectively, more or less severe involvement was noted in various organs and tissues, including the liver (Fig. 7), lungs (Figs. 11 and 12), kidneys (Fig. 10), pancreas, heart, diaphragm, CNS and eyes (No. 9). The postmortem examination of the remaining three monkeys, Nos. 4, 7 and 8, from which were recovered 96,765, 48,693 and 83,695 larvae, respectively, revealed very severe alterations in most of the organs (Figs. 8 and 9) and tissues. Parasitic granuloma formation and focal tissue destruction with hemor-

rhage were also found at numerous sites on the cut surfaces of the brain (Figs. 5 and 6) and spinal cord in these three animals.

Discussion

In visceral larva migrans caused by *T. canis* in human beings, the clinical picture varies from an asymptomatic state, except persistent eosinophilia, to the syndrome of chronic hyper-eosinophilia, hepatomegaly, moderate pulmonary infiltration, fever, coughing and hyperglobulinemia. Numerous human cases, including some fatal ones, involved with the eye, brain, heart and other organs have been reported from most parts of the world. It is also well known that a variety of neurological symptoms, including convulsions, delirium, paralysis and meningism develop in some patients. The pathogenesis of these manifestations, however, has remained unknown. It has only been suggested by some workers [3, 6, 8, 11, 15, 19, 21, 23, 25] that there may be some correlation between the symptoms and the occurrence of *Toxocara* larvae and granulomas in the CNS.

Done et al. [13] showed that, in experimental inoculation of pigs with variable numbers of embryonated eggs of *T. canis*, the presence of larvae in the brain and spinal cord was associated with nervous signs which were apparently provoked by the host reaction to static larvae rather than by the damage resulting from active migration.

In the present experiment, six infected monkeys, Nos. 1, 2, 3, 5, 6 and 9, which suffered from a mild *T. canis* invasion of the CNS showed no clinical abnormality, except mild or moderate inappetence, depression for several weeks, and occasional mild coughing. The remaining three monkeys, Nos. 4, 7 and 8, harbored consider-

able numbers of viable larvae not only in the thoracic and abdominal viscera but also in the CNS. They began to develop such clinical signs as anorexia, weakness and coughing 4 to 7 days after inoculation. Such severe neurologic signs as terminating in paralysis were also found in these three animals on the 8th to 16th days after inoculation. The postmortem examination of these animals revealed parasitic granuloma formation and focal tissue destruction with hemorrhage at numerous sites on the cut surfaces of the brain and spinal cord. Therefore, it should be emphasized that severe neurologic disorders resulted from CNS damage caused by the occurrence of *Toxocara* larvae in the brain and spinal cord.

The most significant hematological finding after experimental *T. canis* infection was marked leucocytosis due primarily to a dramatic increase in the number of eosinophils. Such pattern of a peak eosinophilic response 2 to 3 weeks after inoculation followed by a rapid decline in the eosinophil count as seen in this study with Macaca monkeys has been reported in mice [7], guinea pigs [9, 20], baboons [1] and Macaca monkeys [7, 24]. In contrast, in human beings experimentally infected with *T. canis* [9, 22], leucocytosis and eosinophilia persisted for at least a year. In naturally occurring infection [5, 12, 14-16, 26, 27], the number of eosinophils may remain at an elevated level for many years. Unfortunately, little is known at present about the reason why there is a difference in eosinophilic response between man and any other animal species infected with *T. canis*. Further studies should be undertaken to clarify the function of eosinophil leucocytes in the pathophysiologic and immune mechanisms involved in these host responses to *T. canis* and other parasitic

infections.

In the present experiment, there was no evidence of anemia in two monkeys, Nos. 1 and 2, from which 6,882 and 7,012 larvae, respectively, were recovered. In five monkeys, Nos. 3, 4, 7, 8 and 9, from which 32,954 to 96,765 larvae were recovered, a transient fall was observed in RBC, Hb and PCV values 2 to 5 weeks after inoculation. These values began to increase slowly to a normal level in all the animals 5 weeks after inoculation with a dose of infective *T. canis* eggs. Therefore, loss of blood in various organs of monkeys caused by invasion of a large number of *T. canis* larvae is suggested to be the cause of the anemia of this disease. It is also possible that stress and inappetence which supervene after infection may exert influence on these values.

In four of six *Macaca* monkeys inoculated with different numbers of infective *T. canis* eggs, varying degrees of hyperproteinemia, hypergammaglobulinemia with hypoalbuminemia and elevated plasma transaminase levels were noted. These changes are in agreement with observations reported by Huntley et al. [15] and Heiner et al. [14] for human beings and Aljeboori et al. [1] for baboons.

In the present study, the greatest increase in the concentration of beta- and gamma-globulin occurred in three monkeys, Nos. 3, 8 and 9, from which 35,250, 83,692 and 32,954 larvae, respectively, were recovered. In these three monkeys, the values 2 to 3 weeks after inoculation were 1.0 to 1.1 g per 100 ml larger than the pre-inoculation values and remained at relatively high levels until the end of experiment. Simultaneously with a general increase in beta- and gamma-globulin, there was a slight decrease in the albumin fraction that reached its lowest level between

the 8th and the 14th day after inoculation in all the animals, except two, Nos. 1 and 2, from which 7,012 or less larvae were recovered.

It has been reported in natural human cases of *T. canis* infection [15] that the liver damage causes changes in the serum protein fractions and induces a decrease of albumin and an increase of the globulins, depending upon the degree of liver involvement. Both hepatic involvement and antigenic stimulation by the parasite are presumed to be significant factors in the present study.

Furthermore, an increase in PGPT and PGOT activities was observed 5 to 7 days after inoculation. Although the significance of this change is not yet fully understood, the change may probably be related mainly to damage of the liver.

There were no important changes in alpha-lipoprotein, alpha₁-globulin, transferrin C, slow alpha₂-globulin, slow beta-globulin or ALP of any infected monkey.

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Explanation of Figures

- Fig. 3. Monkey No. 4 showing prominent paralysis of hind quarters 18 days after inoculation with 300,000 embryonated *T. canis* eggs.
- Fig. 4. Monkey No. 8 showing difficulty in rising as a result of extreme weakness of rear quarters 24 days after inoculation with 400,000 infective *T. canis* eggs.
- Fig. 5. Coronal section of brain through mammillary body in monkey No. 8. It shows hemorrhagic and granulomatous (arrows) lesions caused by larvae of *T. canis*.
- Fig. 6. Coronal section of brain through anterior portion of posterior horn of lateral ventricle in monkey No. 8. It shows hemorrhagic and granulomatous (arrows) lesions caused by larvae of *T. canis*.
- Fig. 7. Liver of monkey No. 6 inoculated with 400,000 worm eggs. It shows a large number of hemorrhagic spots and whitish nodules on its visceral surface.
- Fig. 8. Liver of monkey No. 8 showing numerous whitish nodules on its visceral surface.
- Fig. 9. Kidneys of monkey No. 4 showing numerous granulomatous lesions in their cortex.
- Fig. 10. Kidneys of monkey No. 5 inoculated with 400,000 worm eggs. It shows numerous petechiae on their surfaces.
- Fig. 11. Lungs of monkey No. 5 showing severe hemorrhagic lesions on their dorsal surfaces.
- Fig. 12. Lungs of monkey No. 6 showing a large number of granulomatous lesions (arrows) scattered on their dorsal surfaces.