Anemia-Inducing Factor in the Early Stage of Hookworm Infection

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ABSTRACT

Hookworm anemia could start at a very early stage (even 3 days after infection) and that such an anemia was hardly thought to be induced from hemolysis or from dysfunction of bone marrow. Even in an improper host, the hookworm larvae could decrease the blood value hematologically, even though it was temporary. Hookworm larvae produced no anemia-inducing substance which might responsible for the early-stage anemia. The larvae taken from the intestine of the proper host on the 6th day of infection, showed ingestion of blood as in an adult worm even though no teeth had formed yet. The heavy infection group of improper host showed a decrease of circulating red cells on the 3rd day of infection but returned soon to its own normal level in the test with radioactive isotope. X-ray findings showed generalized densities in the lung field that might represent pneumonitis and hemorrhage and histopathological findings of the lungs also revealed hemorrhage in the early stage of hookworm infection in both the proper and the improper host.

The decrease of the hematological blood figure in the early stage of hookworm infection is considered to be induced mainly by the blood loss into the damaged tissue, especially in the lungs during larval migration in the case of heavy infection. Then the blood loss is continued by the blood sucking of immature worms in the intestinal canal of the proper host.

INTRODUCTION

Toxic substance, nutritional disturbance and blood sucking have been recognized as main causes of hookworm anemia. Delangen (1922), Kitayama (1950), Matsumoto (1952) and Hara (1956) found disfunction of hematopoietic organs or hemolytic substances in hookworm infection, while Whipple (1909), Rotter (1931), Darke (1959) believed the anemia originated by nutritional disturbances due to the pathological change of intestinal wall. However, above two theories met a considerable contradiction as a main factor to induce anemia. Looss (1911), Wells (1931), Nishi (1933) and Matsuji (1960) reported that hookworm adults sucked the blood from intestinal wall, which let to anemia on account of continuous blood loss.

Miyagawa (1943) Morishita (1951) found in the experiments with dogs that anemia appeared 3—4 days after the hookworm infection though the infected worms still were in migrating immature stage and not yet in intestinal phase. This indicated that there may be anemia-inducing factor even in larval stage of hookworm. Kitayama (1950) presumed that the factor would exist through the life history, regardless of the egg or adult. The present study has been conducted to confirm whether the larval or immature stage of hookworm have anemia inducing substance or any other factor which responsible for hookworm infection of the early-stage.

EXPERIMENTAL STUDY

A. Beginning time of hookworm anemia

The time and the intensity of anemia due to hookworm infestation were examined in proper host, dogs, infected with Ancylostoma caninum.

Material:

Puppies: About 1—3 months aged puppies were-
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used.
The same brethren were in the same group.
Hookworm larvae: Filariform larvae of Ancylostoma caninum cultured in the laboratory were used.

Method:
Heavy infection: 10,000 larvae per Kg of body weight of puppies were given for infection, 5,000 larvae by mouth and 5,000 larvae by skin. The puppies in a best general condition with the largest amount of hemoglobin content among the same group were selected as heavy infection group.
Light infection: About 400 larvae were given to per Kg of body weight by mouth and skin.
Hematological examination: Red blood cell count, white blood cell count, hemoglobin (cyanmethemoglobin method), hematocrit (capillary tube method) were tested with the blood taken in the balanced oxalate mixture in the morning.

| Table 1. Blood figure of the puppies infected with Ancylostoma caninum massively |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
| dog #   | wt. larve | 1.4kg/14,000          |                | 1.2kg/500        |                | 1.1kg/control   |                |
| day    | Hb* | Ht | RBC | WBC | Hb* | Ht | RBC | WBC | Hb* | Ht | RBC | WBC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 10 | 70 | 30 | 490 | 19.3 | 65 | 25 | 460 | 23.2 | 55 | 22 | 440 | 15.0 |
| 5  | 70 | 29 | 471 | 18.4 | 65 | 25 | 455 | 18.0 | 55 | 19 | 401 | 10.0 |
| 0  | 70 | 29 | 478 | 11.2 | 65 | 27 | 480 | 14.5 | 55 | 20 | 417 | 8.3 |
| 3  | 50 | 23 | 362 | 10.6 | 60 | 25 | 438 | 17.2 | 55 | 22 | 457 | 7.1 |
| 6  | 25 | 11 | 178 | 11.1 | 55 | 24 | 414 | 14.1 | 60 | 27 | 480 | 7.3 |
| 11 | expired |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|

* Done by Sahli’s method (%).

| dog #   | wt. larve | 2.8kg/28,000          |                | 2.5kg/1,000        |                | 2.5kg/control   |                |
| day    | Hb | Ht | RBC | WBC | Hb | Ht | RBC | WBC | Hb | Ht | RBC | WBC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 10 | 13.8 | 44 | 508 | 18.0 | 13.2 | 39 | 521 | 11.3 | 13.1 | 41 | 511 | 22.1 |
| 5  | 13.5 | 44 | 561 | 16.1 | 12.8 | 39 | 490 | 10.1 | 13.0 | 42 | 542 | 21.3 |
| 0  | 13.8 | 45 | 528 | 16.3 | 13.6 | 42 | 518 | 9.4 | 13.0 | 40 | 501 | 19.8 |
| 3  | 10.4 | 36 | 418 | 20.2 | 13.4 | 41 | 539 | 13.1 | 13.8 | 43 | 543 | 16.3 |
| 6  | 8.8 | 29 | 328 | 21.1 | 13.8 | 42 | 502 | 14.2 | 13.0 | 46 | 561 | 17.0 |
| 9  | 5.6 | 18 | 234 | 11.1 | 13.0 | 41 | 499 | 10.2 | 13.5 | 44 | 512 | 15.1 |

* Dog 4 expired nine days after infection.

| dog #   | wt. larve | 4.5kg/45,000          |                | 4.0kg/control        |                |
| day    | Hb | Ht | RBC | WBC | Serum bilirubin | Hb | Ht | RBC | WBC | Serum bilirubin |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 10 | 11.9 | 38 | 510 | 13.8 | 12.9 | 40 | 581 | 12.8 |
| 5  | 12.4 | 40 | 520 | 15.0 |
| 0  | 12.8 | 41 | 561 | 10.6 | 0.095 | 1.1 |
| 3  | 12.6 | 38 | 577 | 15.0 | 0.08 | 1.14 |
| 6  | 9.7 | 30 | 383 | 25.7 | 1.14 |
| 8  | Expired |

Hb: Hemoglobin(g%)  
RBC: red blood cells (million)  
WBC: white blood cells (thousand)  
Ht: Hematocrit(%)  
Serum bilirubin d. t.
before feeding.

Serum bilirubin: Serum bilirubin was tested before and after the infection in one group of puppies among three.

Pathological examination: Histopathological examination was tested with the puppies of heavy infection group which showed severe anemia and expired at the end.

Result:

Hematological change: The heavy infection group showed a decrease of blood figure (R.B.C. count, W.B.C. count, Hemoglobin, Hematocrit) at the early stage of infection and expired about 10 days after infection with marked anemia.

However, the light infection group showed no significant changes of blood figure and the control group generally showed a tendency of increasing in its blood figure (Table 1).

The findings of serum bilirubin showed no particular change before and after infection (Table 1).

Histopathological findings: The histopathological findings of the expired puppies of the heavy infection group showed generalized hemorrhagic foci and marked stasis in lungs due to the inflammatory process (Photo. 1), hyperplasia in bone marrow (humerus), slight inflammatory process in small intestine and slight stasis in liver.

But no particular changes were found in spleen, heart, and kidney.

Discussion:

The heavy infection group which had the higher value of hemoglobin content among the group at the beginning of infection generally showed a continuous decreasing of blood figure leading to marked anemia from as early as 3—4 days of infection. On the other hand, the light infection group and the control group which had lower values of hemoglobin among the same group showed no particular change or even an increase of blood figure. From this result it was considered that hookworm larvae also had the anemia-inducing factor. But such an anemia was believed to be derived neither from hemolysis nor from the dysfunction of hematopoietic organ, because of the findings of the serum bilirubin and the histopathological findings of bone marrow as stated above.

B. Blood figure of hookworm anemia in Improper host

The influence of larva migration of hookworm was mainly examined with puppies infected with human hookworm, Ancylostoma duodenale.

Material:

Puppies: Same brethren of puppies of 2 Kg of body weight, all male, were the same group.

Hookworm: Filariform larvae of Ancylostoma duodenale cultured in the laboratory.

Method:

Infection was done by skin, mouth, or vein. In the intravenous infection, clarified larvae were injected into the jugular vein of the puppy. Each animal was infected as follows:

Group 1, dog 1: 2 Kg of body weight, 20,000 larvae were given for infection
by skin and mouth, half by half.
dog 2: 2.5 Kg body weight, 10,000 larvae were given by skin and mouth.
dog 3: 2.3 Kg, control.
Group 2, dog 4: 2 Kg body weight, 20,000 larvae were given by skin and mouth.
dog 5: 2.2 Kg body weight, 5,000 larvae were given intravenously.
dog 6: 1.9 Kg body weight, control.
Hematological examination: The same as in Experiment A.

Result:
In the heavy infection group, blood figure began to decrease from the 3rd day (dog 4) and from the 6th day (dog 1) of infection. This lasted during the period of 14 days' observation without producing marked anemic condition as in the case of proper host, and they found to recover a month later (Fig. 1). The light infection group and the control group showed no particular findings. Here, even in the case of improper host, a decrease of blood figure was seen, though temporally and slightly. As for egg production in this case, eggs were created a month later of infection in both of the heavy and the light infection groups in the stool test. Among them, 299 adult worms were taken out from dog 4, and only 6 worms from dog 5 at the sacrifice of the dogs. But the other dogs of the heavy and the light infection groups turned out negative in the stool test three months later and no adult worms were taken out at their sacrifice.

Discussion:
As stated above in the test of egg production even in the case of improper host, Ancylostoma duodenale versus dogs, that the development to adult stage was difficult, a decrease of blood figure appeared in the early stage of infection though that was not severe as in the case of proper host. It seemed evidently that the hookworm larvae entity has any factors that cause a decrease of blood figure of its host.

C. Anemia-inducing substance
1. Dead larvae and the larval metabolism product

In order to examine the anemia-inducing substance which might be produced by the larvae, metabolic products of the larvae and the dead larvae were inoculated to puppies.

Material:
Dead larvae: The mixture of the clarified larvae that were killed half by freezing and half by 0.05% phenol solution.
Emulsion of larvae: Made with living larvae.
Larval metabolic product: The exsheathed larvae were kept in a normal saline solution at 37°C for 24 hours. The media was filtered through Seitz-filter. Ten ml of the media would contain the metabolite of 10,000 larvae for 24 hours.
Emulsion of Toxocara canis: About 7 cm long Toxocara canis was grinded and phenol solution was added to make it 0.05% solution for preservation.

Method:
The experimental animals, their breeding, the hematological examination were carried on with the same method employed in Experiment A. The experimental puppies were divided as follows.

a) The group of heavy inoculation with dead larvae.
Dog 1 (♀, 1.4 Kg): Total 700,000 larvae were inoculated intramuscularly. (The following dogs from 2 to 6 were inoculated with the same method as in dog 1.)
Dog 2 (♀, 1.2 Kg): Total 300,000 larvae.
Dog 3 (♀, 1.2 Kg): Total 400,000 larvae.
Dog 4 (♀, 1.2 Kg): Total 320,000 larvae.

b) The group of light inoculation with dead larvae.
Dog 5 (♀, 1.1 Kg): Total 200,000 larvae.
Dog 6 (♀, 1.2 Kg): Total 200,000 larvae.
Dog 7 (♀, 1.0 Kg): The emulsion of total 200,000 larvae was inoculated intramuscularly.
Dog 8 (♀, 1.1 Kg): The emulsion of 20,000 larvae intramuscularly.

C) The group of metabolism product injection
Dog 9 (♀, 1.1 Kg): Intraperitoneal injection of 11ml daily for 20 times.
Dog 10 (♀, 1.0 Kg): 10ml daily for 20 times.
Dog 11 (♀, 1.0 Kg): 10ml daily for 20 times.
Dog 12 (♀, 1.2kg): 12 ml daily for 20 times.

**D) The group of the inoculation with the emulsion of the Toxocara canis.**

Dog 13 (♀, 1.3kg): The emulsion of one worm was inoculated intramuscularly devided into 10 times.

**E) The group of the injection of normal saline**

Dog 14 (♀, 1.1kg): 11 ml of normal saline was injected daily into the peritoneal cavity for 20 times.

**Result:**

Two puppies (dog 1, 2) in the group of heavy inoculation with dead larvae showed anemia among those five groups. Dog 1 and 2 expired on the 13th and 20th day after infection respectively. Dog 3, dog 4 and all the puppies in the other groups, however, showed no particular decrease of blood figure. The histopathological findings of the dog 1 and 2 showed marked hyperplasia in the bone marrow (humerus).

**2. Red blood cell fragility**

The red blood cell fragility test was done with hypotonic saline solution before and after infection in order to examine any slight hemolysis in the early stage of hookworm infection.

**Material:**

Rabbits: About 2Kg body weight regardless of its sex were used.

Filariform larvae of Ancylostoma caninum.

**Method:**

Five rabbits were infected with 10,000 hookworm larvae per Kg of body weight half by mouth and half by skin to each rabbit. The infected rabbits and other five rabbits of the control group were tested for osmotic fragility of their red cells with hypotonic saline solution on the preceding day of infection and three days after infection.

**Result:**

The infection group as well as the control group showed no particular change before and after infection in the osmotic fragility test of the red blood cells.

**Discussion:**

Hemolysis had nothing to do with the causes of a decrease of the blood figure in the early stage of hookworm infection, judging from the above results of red cell fragility test.

The presence of anemia-inducing substance that Kitayama and Iwada insisted upon as a cause of anemia can not be thought responsible for the early stage anemia.

Metabolic products from 10,000 larvae per Kg body weight of the puppies were examined, which led to the thought that they could not be the cause of the anemia in the early stage of infection. The constituents of 200,000 larvae could not produce any decrease of blood figure in the puppies of about 1 Kg of body weight. The amount of the constituents of the expired larvae during the migration in the host of about 1 Kg body weight, infected with about 10,000 hookworm larvae, was much less and negligible compared with that of 200,000 larval body that induced no decrease of blood figure at all (Experiment C). Therefore the anemia-inducing factor contained in the constituents of the larval body hardly played a role as the factor investiget in this subject. And so the cause of the anemia shown in the group of heavy injection of dead larvae (700,000 larvae) has nothing to do with this subject. Whether it may happen that this anemia was hookworm origin entity, any anemia inducing substance reported by Kitayama is hardly agreed owing to the findings of hyperplasis of the bone marrow.

**D. Beginning of Blood Sucking**

The beginning of blood sucking after infection was examined to know whether it might contribute to the induction of anemia especially in the early stage of infection. The puppies were infected with hookworm larvae by skin only and its blood was labeled with radioisotope, then the larvae were taken out again by sacrificing the puppies at given intervals. The host's blood was detected by autoradiography of the worm taken from the host.

**Material:**

1. Puppies: About one month aged puppies free from hookworm infection.

2. Filariform larvae of Ancylostoma caninum.
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3. Autoradiographic emulsion: Radiographic dental film; Rinn Corporation, Chicago Stripping Film; Kodak Ltd., London.

Method:
In order to get the puppies free from hookworm infection particularly, the village in the countryside was selected at first, where was free from canine hookworm prevalence. The puppies bred in this village were used for experiments after convincing negative for hookworm infection in both the mother dog and her puppies. The puppies were infected with filariiform larvae of Ancylostoma caninum only by skin and the skin on which the larvae were applied was covered completely lest they should be infected through mouth.

The blood of each puppy was labeled with 200 uC Cr⁴⁺, 24 hours prior to each scheduled time of sacrificing of the puppies to be taken out the hookworm larvae.

The larvae were taken out from lung tissue by Baermann's method, then each larva was taken out one by one with the bamboo fiber under the microscope and put into the clear hemolyzed blood added with Cr⁴⁺ and then the larvae were put into distilled water one by one for 10 times in order to get clear larva without debris which was contaminated with radioisotope. The larvae taken from the intestinal canal were also washed with distilled water by the same method as above, needless to employ Baermann's method.

Sample A: The larva lump taken from lungs three days (two days 12 hours) after infection were dried in the slide glass.
Sample B: The larva taken from the intestinal canal 6 days after infection was put individually for the sampling for autoradiography.
Sample C: The internal tissue of the same larva as Sample B was squeezed out.
Sample D: The adult worm taken from another dog whose blood had labeled with Cr⁴⁺.
Sample E: The adult worm taken in the dog of Sample D of which internal tissue was squeezed out.
Sample F: Adult worm taken from the dog that no procedure was added.
Sample G: The individual larva taken from lungs of puppies three days after infection.
Sample H: The individual larva taken from intestinal canal 4 days after infection, whose host's blood was labeled with 250 uC of I¹³HSA.
Sample A-F were put to autoradiography by contact method with radiographic dental film at 4°C, and Sample G-H by stripping film method (Kodak Ltd.).

Result:
Those autoradiographic emulsions were developed and fixed with Kodak developer D-19 and Fixer P-10.
The results of each sample were as follows.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Days</th>
<th>Where</th>
<th>RI</th>
<th>Ex</th>
<th>Result</th>
<th>Figured</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>3</td>
<td>lung</td>
<td>Cr⁴⁺</td>
<td>100</td>
<td>no</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>6</td>
<td>intestine</td>
<td>Cr⁴⁺</td>
<td>yes photo</td>
<td>3-1</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>6</td>
<td>intestine</td>
<td>Cr⁴⁺</td>
<td>yes photo</td>
<td>3-2</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>adult</td>
<td>intestine</td>
<td>Cr⁴⁺</td>
<td>yes photo</td>
<td>3-3</td>
<td>(right)</td>
</tr>
<tr>
<td>E</td>
<td>adult</td>
<td>intestine</td>
<td>Cr⁴⁺</td>
<td>no photo</td>
<td>3-2</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>adult</td>
<td>intestine no</td>
<td>Cr⁴⁺</td>
<td>yes photo</td>
<td>3-3</td>
<td>(left)</td>
</tr>
<tr>
<td>G</td>
<td>3</td>
<td>lung I¹³HSA</td>
<td>32</td>
<td>no</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>4</td>
<td>intestine I¹³HSA</td>
<td>32</td>
<td>no</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Days after infection when the worms were taken out from the host.
** The locality where the worm taken out from.
*** Radioisotope given to the host
**** The time of exposure for autoradiography in days.

The larvae could be taken out of lungs even 9 days after infection, though the number was very few. The number of larvae that could be taken out of lungs was the most from the 3rd day after infection. The growing and developing
of the worm taken out of lungs were not appreciable until 8 days after infection. From the intestinal canal, no worm was found one day (25 hrs.) after infection and a few were found on the 3rd day, but the 4th day showed a sudden increase in number, and worms were found numerously from the 6th day. Among the worms taken out of the intestine on the 4th, 6th, and 9th day of infection many of them grew to the length of 1.6mm, 2.5mm and 3.5mm respectively.

Discussion:
The larvae were not believed to suck blood during migration in view of the fact that no remarkable development and growing could be seen in the time of migration in lungs and that autoradiographic findings showed no blood sucking during migration in lungs. Even if the larvae could suck blood during migration, its amount would be too small to induce a finding of a decrease of blood figure hematologically.

Therefore such a trifle of blood suck might not contribute as the factor of this subject.

The blood suck of the worm in the intestine, however, was seen as early as 6 days after infection, which was earlier date than 12 days of Morishita's report (1956).

E. The loss of the circulating red blood cells at the early stage of infection

The phase of loss of circulating red blood cells in the early stage of hookworm infection was examined by way of red blood cell survival time test.

Material:
1. Rabbits: About 2Kg body weight regardless of its sex.
2. Filariform larvae of Ancylostoma caninum.
3. Radioisotope: Sodium chromate(Na₂Cr₂O₇)

Method:
Ten ml. of heparinized blood sample of rabbits was labeled with 100 uC of Cr⁶¹, then the red blood cells were washed three times with normal saline by centrifuge.

In all cases the last fluid had no appreciable radioactivity. The washed red blood cells were floated in normal saline and transfused again into each of the rabbits of the respective blood.

Four days after labeling of the blood, five rabbits were infected with 10,000 larvae of Ancylostoma caninum per Kg of body weight half by skin and half by mouth as in heavy infection group, and another five rabbits were with 2,000 larvae as in light infection group. Five rabbits were used as the control group.

Two ml of blood sample were taken with heparinized syringe at given intervals. Of the

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**Fig. 2.** The change of the circulating red blood cells of the rabbits infected with Ancylostoma caninum.

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**Fig. 3.** The change of the blood figure of the rabbits infected with Ancylostoma caninum.
two ml of blood samples, exactly one ml was
taken out for the radioactivity count and the
rest for general hematological test (hemoglobin,
red blood cell count, white blood cell count,
hematocrit). X-ray test of the lungs of the heavy
infection group and the control group was exa-
mined before and after infection.

Result:
The radioactivity of blood samples taken out
two days after labeling was fixed as 100 and then
the measured radioactivity of samples in the
course of experiments was plotted semilogarith-
ically (Fig. 2). In the heavy infection group,
the ratio of decreasing radioactivity showed a
sharp down from the 3rd day of infection and
then had a tendency to return slightly towards
its original ratio for few days, and it recovered
completely after 6 days of infection paralleling
the prearranged course.

In the light infection group, no particular
change of ratio was observed throughout the
whole course (Fig. 2).

In the radiological examination of lungs no
pathological findings were detected at first in
both the heavy infection group and the control
group. In the heavy infection group, however,
generalized densities were seen in the picture 5
days after infection which represented the find-
ings of generalized pneumonitis and hemorrh-
age (photo. 2–3, 2–4).

In the hematological findings of the heavy in-
fec tion group, the decreasing of blood figure
began 3 days after infection as in the case of
Experiment B (Ancylostoma duodenale to dogs)
but it was not so severe as that in case of
proper host infection (Fig. 3).

Discussion:
In the heavy infection group, the ratio of de-
creasing radioactivity began to overrun on the
3rd day of infection and the actual course of the
radioactivity count was separated from the pre-
arranged course of it. It continued for a few days
with recovering tendency, then recovered com-
pletely after 6 days of infection with such findings
that the loss of the circulating red blood was
most severe three days after infection and gra-
dually came to a stop after six days of infection.

Summary and Discussion
In case of proper host infected with hookworm
massively, a severe anemic condition could be
seen within a few days and blood figure began
to fall even 3 days after infection. In case of
improper host, the blood figure began to decrease
three days after infection with the same manner
as in the proper host, though it never proceeded
to a severe anemic condition as in case of proper
host. Therefore, it is thought that the larva has
any anemia inducing factor (Experiment A and
B).

No hemolytic phenomenon appeared during
migration of the hookworm larvae (Experiment
A and C). The factor, if any, which was re-
ported to induce the dysfunction of hematopoietic
organ by Kitayama(1950) and Iwada(1960), was
believed to do little as the factor of the early-
stage anemia of the hookworm infection. The
metabolite of larvae (10,000 larvae/Kg body
weight of puppy/day) and 0.2 million of the
dead larval bodies did nothing to induce a de-
crease of blood figure (Experiment C).
Among the heavy inoculation group of the
dead larvae, 2 puppies showed anemia, and sup-
posing that this anemia might be originated by
hookworm infection, the factor that induced
anemic condition with such huge amount of
larvae as 0.7 million, 0.3 million, could never
have any significance for this subject, because
the larvae which were killed and absorbed by
the host during migration actually in a puppy
infected with 10,000 larvae were negligible in
comparison with 0.2 million of the dead larvae
(Experiment C), which made no change of the
blood figure. It was very hard to agree to Kitay-
ama’s report that the toxic substance to induce
hypofunction of hematopoietic organ was con-
tained in the larval body, because the histopatho-
logical findings of bone marrow showed hyper-
plasia in case of massive inoculation of dead
larvae. The cause of the anemia seen in the
heavy inoculation group, therefore, was not
believed to be hookworm origin itself, though
not certain. It had thus been concluded that
anemia-inducing toxic substance has nothing to do with this subject.

On hookworm infection, the invading larvae concentrate into the lungs suddenly from 3 to 4 days after infection. Though no blood sucking of the larvae had been detected during migration (Experiment D), the lungs of the host suffer considerably from severe damages due to the concentrated number of larvae migrating through lung tissue at a time in case of massive infection. Chandler (1929), Gradwohl (1948) and Craig et al (1953) stated that only slight hemorrhage was seen in the lung when small number of larvae were infected, but considerably large bleeding with hemorrhagic pool in the alveoli could be seen when a large number of larvae invaded at the same time. Yamasaki (1951) reported that such hemorrhagic catarrhal pneumonitis could be seen notably three days after infection but abated much six days after infection.

Also in this experiment, the generalized inflammatory process with marked stasis and hemorrhagic findings in lungs were seen in the early stage of infection.

The improper host that suffered from larval invasion only lost the circulating red blood cells markedly on the 3rd day of infection and had a tendency to recover after 6 days of infection (Experiment E).

It had been considered that the actual hemorrhage in lungs directly and the marked stasis due to inflammation indirectly contributed to the decrease of the blood figure in the early stage of infection. Such a hemorrhage in lungs occurred both in the proper host and in the improper host. And in case of proper host, intestinal bleeding by blood sucking of worms started as early as 6 days after infection when the teeth were not yet developed fully in the mouth capsule of worms (Experiment D). The initial blood loss due to lung invasion was followed directly by the progressively intense hemorrhage in the intestine with no chance of recovery of the decreasing blood figure due to the lung damage, which finally caused a severe anemic condition in case of proper host.

The anemia-inducing factor in the early stage of hookworm was, therefore, believed to be the blood loss due to the lung invasion by the migrating larvae in massive number following intense hemorrhage in the intestine.

**Conclusion:**

1) Hookworm anemia could start in very early stage, even three days after infection, and so any anemia-inducing factor is believed to be derived from larval stage in proper host.

2) In improper host also, the hookworm larvae could decrease the blood figure to some extent in the early stage (3–4 days) of infection temporarily but it recovered again.

3) Any anemia-inducing toxin contained in the larval body or larval metabolite is believed to have no connection with the factor related to the early-stage anemia of hookworm infection.

4) In improper host, a temporary loss of circulating red blood cells was seen corresponding to the invasion of the lungs during larval migration in the early stage of hookworm infection.

5) In the intestine, the blood suck by the worms started as early as 6 days after infection in proper host.

Therefore the anemia-inducing factors in the early stage of hookworm infection are believed to be the blood loss due to lung damage and early blood sucking in the intestine.

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Photograph 1: The lung and the bone marrow of the puppy infected with hookworm massively.

Photo 1—1: The lungs showing generalized inflammation and bleeding. Expired 9 days after infection (D-4).

Photo 1—2: The bone marrow showing hyperplasia (D-4, H-E stain, x 100).

Photograph 2: The lungs of the rabbits infected with Ancylostoma caninum massively.

Photo 2—1: The lungs showing severe inflammation, stasis and hemorrhage (left, R-9) and the normal (right, R-19).

Photo 2—2: The lung tissue showing hemorrhage and inflammatory process (R-9). H-E stain, x 100.
Photo 2-3: X-ray findings before the infection (R-6)

Photo 2-4: X-ray findings 4 days after infection, showing generalized densities (R-6)

Photo graph 3: The autoradiograph of the hookworm taken from the puppies whose blood was labeled with radioactive isotope.

Photo 3-1: The autoradiograph of the worm taken 6 days after infection in the intestinal canal (contact method).

6-day worm

Photo 3-2: The autoradiograph of the internal tissue showing positive sign on the emulsion (R). No sign on the part of the skin of the worm (dotted line).

Photo 3-3: The adult worm processed with (positive sign) and without (dotted line) radioactive isotope (contact method).