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STUDIES ON EARLY DEATH IN EXPERIMENTAL ANIMAL RABIES

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Abstract: It has been shown that inadequately immunized humans dying of rabies succumb after an incubation period shorter than that of any unvaccinated human rabies cases. This report is concerned with the reproduction and analysis of this phenomenon called "early death" in experimental animals. A significant reduction of the incubation period was recognized in hamsters ($P<0.01$), and rabbits ($P<0.05$) immunized with potent anti-rabies vaccine after being challenged by rabies virus, while the reduction was not significant among animals that were treated with anti-rabies virus serum. The viral antigen in the central nervous system examined at the onset of disease by immunofluorescent staining rather decreased and inclusion bodies were less plentiful among animals that died earlier than in the controls, whereas no marked deposition of complement or immunoglobulin in nerve cells was proved in this study. The phenomenon could be reproduced in experimental animals vaccinated after exposure, although it could not be shown clearly that "early death" in experimental animals is mediated through anti-rabies antibodies.

INTRODUCTION

Rabies is a fatal disease which causes severe encephalomyelitis and death after a long incubation period. To prevent the onset of this disease, various kinds of rabies vaccines have been investigated and applied since the development of the first vaccine by Pasteur in 1885. Rabies is usually prevented successfully if people are vaccinated before being bitten by rabid animals and enough antibody titer is produced (Gerichter *et al.*, 1978; Anderson *et al.*, 1980; Ajjan *et al.*, 1980). However the effect of post-exposure treatment with vaccine is not complete. Moreover, it has been observed that among humans (Ohtani, 1959; Held *et al.*, 1967) or laboratory animals (Sikes *et al.*, 1971; Baer and Cleary, 1972; Blancou *et al.*, 1980) if vaccine was inoculated after infection with rabies virus, subjects could not be prevented from catching the diseases, and some vaccinated subjects died sooner than non-vaccinated ones. This phenomenon has been termed "early death", and the immunopathological mechanism, especially that mediated by antibodies rather than immune T cells, is suspected of causing this phenomenon (Prabhakar and Nathanson, 1981; Porterfield, 1981).

This phenomenon has been observed during some experiments examining the efficacy of vaccine on human cases, but few experimental trials reproducing or

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investigating the mechanism have been made. The present study was undertaken to reproduce this phenomenon using experimental animals with post-exposure treatment by vaccine or antiserum, and thereafter the author tried to examine the mechanism from a morphological point of view.

Materials and Methods

Animals: Japanese white rabbits and Syrian golden hamsters were used with initial weight of 2.0–2.3 kg and 25–32 g, respectively. Virus titrations were made in ddY suckling mice 3–7 days of age.

Virus: A 10 per cent emulsion of monkey brain infected with rabies virus was prepared in Eagle's minimal essential medium (MEM, pH 7.4). The supernatant after centrifuged at 3,000 rpm for 10 min was stored at −70°C until use. The titer of the virus in the preparation by intracerebral inoculation of suckling mice was $10^{4.8}LD_{50}$/0.01 m*l*.

Vaccine: Rabies vaccine prepared in chick-embryo cell cultures (TC vaccine, Chemo-Sero Therapeutic Research Institute, Kumamoto, Japan) was used. This vaccine was prepared by the tissue culture adapted strain of the HEP-Flury strain of rabies virus and inactivated with β-propiolactone. The lyophilized vaccine in single-does vials was reconstituted with sterile water just before being used. The infective rabies virus contained in this vaccine before inactivation was over $10^{7.0}LD_{50}$/m*l*.

Sera for postexposure treatment: Four kinds of sera were prepared to reproduce "early death" phenomenon; anti-rabies hamster serum with neutralizing antibody titer (NT) of $10^{2.6}$ (serum A), anti-rabies rabbit serum with antibody titer of $10^{2.2}$ (serum B), anti-rabies rabbit serum with antibody titer of $10^{4.2}$ (serum C), and anti-rabies monkey serum with antibody titer of $10^{3.5}$ (serum D). Neutralizing antibody titer of these sera were calculated as shown below. Serum A was obtained after daily injections of the vaccine for 14 days (0.2 m*l* in each), serum B after daily injections for 6 days (1.0 m*l* in each), serum C after 7 injections at an interval of one month (1.0 m*l* in each), and serum D after 4 injections at an interval of one month (1.0 m*l* in each).

Serum B was separated on a Sephadex G–200 (Pharmacia Co., Uppsala, Sweden) using phosphate buffered saline (PBS, pH 7.2) and absorbance of each elution fraction was read at 280 nm by a spectrophotometer. Three peaks of absorbance were obtained, and each peak was concentrated to the initial volume of the serum, then separated into two aliquots. The first aliquot was used to measure the serum neutralization antibody titer, and the second was mixed with 0.2 M 2-mercaptoethanol in PBS (2ME). Both samples were kept at room temperature for 18 hours and then titrated for anti-rabies antibody. The neutralizing antibody titers of peak I, peak II and peak III without 2ME treatment were $10^{2.0}$, $10^{1.8}$ and 0 respectively. Titers fell significantly in the sample at peak I treated with 2ME (from $10^{2.0}$ to less than $10^{0.2}$), but not in the sample at peak II. So it was thought that peak I contained primarily IgM, peak II primarily IgG, and peak III proteins such as albumin that are smaller than the immunoglobulins.

Serum neutralization tests for assaying each immune serum were performed as

follows: A tenfold dilution of heat-inactivated serum was prepared with MEM. Each dilution was mixed with an equal volume of 10 per cent suspension of rabbit brain infected with Nishigahara strain of rabies virus ($10^{2.0}LD_{50}$). The above mixtures were incubated for one hour at 37°C, and then inoculated into the brains of 4–5 suckling mice. Fifty per cent end-point titer for each specimen was determined by the Reed-Muench method.

Sera for immunofluorescent staining: Sera used for immunofluorescent (IF) staining in this experiment were as follows: FITC-conjugated anti-rabies rabbit serum prepared in our laboratory, FITC-conjugated goat anti-rabbit IgG (Eiken Co., Tokyo, Japan), and FITC-conjugated goat anti-rabbit IgM (Cappel Laboratories, Downington, U. S. A.) were used for direct IF staining. Rabbit anti-hamster IgG (MBL Co., Nagoya, Japan), rabbit anti-hamster C3 (Cappel Laboratories, Downington, U. S. A.), goat anti-rabbit C3 (Fujizoki Co., Tokyo, Japan), and FITC-conjugated anti-rabbit IgG (Eiken Co., Tokyo, Japan) were used for indirect IF staining.

Method of IF staining: IF staining was done as described previously (Burns *et al.*, 1978; Swoveland *et al.*, 1979; Johnson *et al.*, 1980). Formalin fixed tissues, embedded in paraffin were sectioned to 4 μ thickness. Those sections were deparaffinized by immersion in xylene for 20 minutes, rehydrated through 100, 90, 80 and 70 per cent ethanol, and washed with phosphate buffered saline (PBS, pH 7.2). Tissues were digested with 0.2 per cent trypsin solution (Merck Co., Darmstadt, Germany) with 0.01 per cent $CaCl_2$ for 90 minutes at 37°C, followed by washing with PBS for 15 minutes. Direct or indirect methods of IF staining were followed. Each section was covered with specific antiserum, then incubated overnight at 4°C. After incubation they were washed with PBS, mounted with glycerine phosphate buffer, and observed under a fluorescent microscope with substage illumination (Olympus, Tokyo, Japan).

Treatment: The vaccinated animals consisted of five groups of hamsters and one group of rabbits. In the hamster groups, there were 5–6 hamsters in each control group and 10–11 in each vaccinated group, while there were 3 rabbits in the control group and 6 rabbits in the vaccinated one. Virus was inoculated into the following tissues: the cornea of group I, the neck muscle of group II, the back muscle of group III, the fore footpad of group IV, the rear footpad of group V, and the cornea of group VI.

Each animal was anesthetized by an intraperitoneal injection of pentobarbital. The cornea of groups I and VI was scarified and 0.02 m*l* of virus suspension was administered. In groups II–V, 0.02 m*l* of stock virus was injected into the site indicated above. Groups I–V received 0.2 m*l* and group VI, 1.0 m*l* respectively, of TC vaccine intraperitoneally immediately after the inoculation of virus. TC vaccine was injected daily and totally 14 doses were given to each hamster and rabbit.

The serum-treated hamsters and rabbits were divided into 10 groups according to the serum injected. Serum A was used for group I, serum B for groups II and V, serum C for groups III and VI, rabbit IgM for group VII, rabbit IgG for group VIII, and serum D for group IX. Groups IV and X were used as controls. One m*l* and 10 m*l* of serum were injected intraperitoneally into hamsters (groups I–III) and

rabbits (groups V–IX) respectively, immediately after the inoculation of virus. The injections were given on three consecutive days, hence the total amount of serum for each hamster was 3 m*l*, and 30 m*l* for each rabbit.

Animals were observed for 50 days after challenge of the virus and killed when showing clear clinical signs of rabies. Specimens were collected from the inoculation site, spinal cord, trigeminal ganglion, cerebellum, cerebrum and pons. The specimens were fixed in 10 per cent buffered formalin solution (pH 7.2) for 14 days. After the process of paraffin embedding, 4 μ thick sections were cut and stained with immunofluorescent (IF), hematoxylin-eosin (HE) and luxol fast blue (LFB).

Results

Clinical signs: All rabid hamsters showed clinical signs as follows: weakness, elevation of body temperature, ataxia, and paralysis. Convulsion and tonic spasm were also observed and appeared more frequently among the vaccinated groups than in the controls. All rabbits showed similar clinical signs, i.e., weakness, elevation of body temperature and ataxia followed by paralysis. A few animals showed salivation or urinary incontinence.

Mortality rate and incubation period: The mortality rate among the vaccinated hamsters was lower than that of the control ones. All the control groups suffered 100 per cent mortality, while the vaccinated groups showed a mortality rate in the

Table 1 Effect of vaccination on incubation period and mortality rate

Group	Animal	Site of inoculation	Mortality		Incubation period					
			Control	Vaccinated	Control	M*	SD**	Vaccinated	M*	SD**
I	Hamster	Cornea	5/5 (100%)	8/10 (80%)	18, 19, 22 24, 27	22.0	3.3	10, 11, 15 15, 15, 16 16, 17	14.3	2.3
II	Hamster	Neck muscle	5/5 (100%)	8/11 (73%)	17, 20, 24 29, 32	24.4	5.5	13, 14, 14 15, 17, 17 17, 17	15.5	1.6
III	Hamster	Back muscle	5/5 (100%)	7/11 (64%)	25, 25, 27 28, 29	26.8	1.6	16, 16, 17 18, 19, 19 19	17.7	1.3
IV	Hamster	Fore footpad	5/5 (100%)	7/11 (64%)	25, 27, 29 33, 34	29.6	3.4	14, 16, 19 19, 19, 20 25	18.9	3.2
V	Hamster	Rear footpad	6/6 (100%)	7/11 (64%)	26, 29, 29 39, 39, 41	33.8	6.0	17, 18, 18 18, 19, 19 20	18.4	0.9
VI	Rabbit	Cornea	3/3 (100%)	6/6 (100%)	14, 16, 17	15.7	1.2	12, 13, 13 13, 14, 15	13.3	0.9

0.02 m*l* of stock virus ($10^{4.8}LD_{50}$) was inoculated into each site. Each animal was observed daily and was killed after showing clear clinical signs of rabies.

*M: mean, **SD: standard deviation.

range of 64–80 per cent. In rabbits, the mortality rate was 100 per cent in all groups including the control.

The range of incubation periods varied in each group. It was shorter when the inoculation site was closer to the head. Within the same group of animals inoculated at the same site the incubation periods also varied. In the vaccinated hamsters, the incubation periods were markedly shorter than those of the controls. The decrease in incubation period, compared with that of control, was 35.0 per cent in group I, 36.5 per cent in group II, 34.0 per cent in group III, 36.1 per cent in group IV, and 45.6 per cent in group V; all the values were statistically significant ($P<0.01$). In rabbits, the decrease was 15.3 per cent ($P<0.05$). The standard deviation (SD) of the incubation periods in all vaccinated groups were smaller than those of the controls; especially in group V, the SD of the vaccinated hamsters was 0.9, whereas that of controls was 6.0 (Table 1).

In all groups injected with serum, the mean incubation period differed from that of the control groups, but the difference was not statistically significant ($P>0.05$). When sera with low antibody titer (sera A and B) were used, the incubation periods were slightly shorter than those of the control groups. But when hamsters were treated with anti-rabies hyperimmune rabbit serum (serum C) for three consecutive days immediately following virus inoculation, the incubation periods were prolonged as long as 5.8 days. When injected with whole serum, however, regardless of its antibody titer, the incubation periods of rabbits were slightly shortened. The

Table 2 Effect of anti-rabies serum on incubation period and mortality rate

Group	Animal	anti-rabies serum (antibody titer)		Mortality	Incubation Period	Mean
I	Hamster	Serum A	($NT=10^{2.6}$)	3/5 (60%)	17, 20, 22	19.7
II	Hamster	Serum B	($NT=10^{2.2}$)	3/5 (60%)	19, 20, 22	20.3
III	Hamster	Serum C	($NT=10^{4.2}$)	2/5 (40%)	24, 32	28.0
IV	Hamster	—	(control)	5/5 (100%)	20, 21, 22 24, 24	22.2
V	Rabbit	Serum B	($NT=10^{2.2}$)	3/3 (100%)	13, 14, 14	13.7
VI	Rabbit	Serum C	($NT=10^{4.2}$)	3/3 (100%)	14, 15, 15	14.7
VII	Rabbit	Rabbit IgM	($NT=10^{2.0}$)	3/3 (100%)	15, 15, 15	15.0
VIII	Rabbit	Rabbit IgG	($NT=10^{1.8}$)	3/3 (100%)	14, 14, 14	14.0
IX	Rabbit	Serum D	($NT=10^{3.5}$)	3/3 (100%)	12, 14, 19	15.0
X	Rabbit	—	(control)	4/4 (100%)	15, 15, 16 16	15.5

Serum A: anti-rabies hamster serum, Serum B and C: anti-rabies rabbit serum, Serum D: anti-rabies monkey serum, NT: neutralizing antibody titer.

Serum A was produced after 10–14 consecutive injections of TC vaccine, serum B after 5–7 consecutive injections, serum C: after 6–7 injections at intervals of one month, and serum D after 4 injections at intervals of one month.

Group I–III, one m*l* and V–IX, 10 m*l* of serum was injected immediately after inoculation of virus. The injections were performed for three consecutive days.

decrease was largest when they were injected with serum B, although this decrease rate was only 1.8 days. The results of treatment with serum components showed that both IgM and IgG slightly reduced the incubation periods and the decrease was slightly larger (one day) when using IgG than using IgM but the difference was not statistically significant ($P>0.05$) (Table 2).

Results of IF staining: Direct IF staining for rabies virus in various parts of the CNS showed that in the vaccinated groups the intensity of fluorescence was generally weaker than in the controls, and some were almost negative. The fluorescence also differed in different parts of the nervous system in the same animal. Generally the fluorescence could be recognized in the cerebellum, cerebrum, pons, and cervical cord. However, no fluorescence was detected at the site of inoculation in all the groups. In the control of group I, the strongest fluorescence was shown in the cerebrum; the same was in the cervical cord in groups III–V. In vaccinated groups, the distribution of viral antigen was almost the same as that in the control groups but the intensity of fluorescence was weaker. The viral antigen was located in the cytoplasm of the nerve cells, and was completely negative in the nuclei. In the vaccinated groups, nerve cells did not show strongly positive reactions like those of the control ones.

Intravascular IgG, IgM, C3, and surface IgG of lymphocytes infiltrating around blood vessels or in meninges were stained, but those deposited in nerve cells could not

Table 3 Results of anti-rabies immunofluorescent (IF) staining (vaccinated animals)

Group	Treatment	Sites of specimen collection					
		Cerebrum	Cerebellum	Pons	Spinal cord	Trigeminal ganglion	Site of inoculation
I	Control	⫴	⫲	⫲	⫲	+	—
	Vaccination	+	+	+	+	+	—
II	Control	⫲	⫲	⫲	⫲	ND*	—
	Vaccination	+	+	+	+	ND	—
III	Control	⫲	+	⫲	⫴	ND	—
	Vaccnation	+	+	+	⫲	ND	—
IV	Control	⫲	⫲	⫲	⫴	ND	—
	Vaccination	+	+	+	⫲	ND	—
V	Control	⫲	⫲	⫲	⫲	ND	—
	Vaccination	+	+	+	+	ND	—
VI	Control	⫲	⫲	⫲	+	+	—
	Vaccination	+	+	+	+	+	—

Group I–V: Hamsters, Group VI: Rabbits.
In group I, rabies virus was inoculated into the cornea; in group II, into the neck muscle; in group III, into the back muscle; in group IV, into the fore footpad; in group V, into the rear footpad; and in group VI, into the cornea.
*ND: not done, Intensity of fluorescence: ⫴ strong, ⫲ intermediate, + weak.

Table 4 Results of anti-rabies immunofluorescent (IF) staining (serum-injected animals)

Group	Treatment	Sites of specimen collection					
		Cerebrum	Cerebellum	Pons	Spinal cord	Trigeminal ganglion	Site of inoculation
I	Serum A	+	+	+	+	+	−
II	Serum B	++	++	++	++	+	−
III	Serum C	+++	++	+++	++	+	−
IV	Control	++	++	++	++	+	−
V	Serum B	+	+	+	+	+	−
VI	Serum C	++	++	++	++	+	−
VII	Rabbit IgM	++	++	++	++	+	−
VII	Rabbit IgG	++	++	++	++	+	−
IX	Serum D	++	++	++	++	+	−
X	Control	++	++	++	++	+	−

Antibody titer: Serum A ($NT=10^{2.6}$), Serum B ($NT=10^{2.2}$), Serum C ($NT=10^{4.2}$), Serum D ($NT=10^{3.5}$).
Group I–IV: Hamsters, Group V–X: Rabbits *ND: not done.
Intensity of fluorescence: +++ strong, ++ intermediate, + weak.

be stained by the IF method. Deposition of C3 was recognized around some blood vessels of vaccinated animals but this finding was also observed in some control animals (Table 3).

The differences in the intensity of fluorescence among serum-injected groups were small. Generally the shorter the incubation period, the weaker the fluorescence. The fluorescence was the strongest in groups III in which the incubation period was the longest among the above groups. Like the vaccinated groups, no viral antigen was found in the cornea (Table 4).

The chronological spread of rabies virus in CNS was studied. Rabbits were divided into three groups (control, vaccinated, and serum-injected) and 0.02 m*l* of stock virus was inoculated into the cornea. One m*l* of TC vaccine or 10 m*l* of anti-rabies monkey serum (serum D) was injected intraperitoneally in each rabbit; the vaccine was injected every five days and the serum seven days after inoculation of virus. A rabbit of each group was killed each on day 6, 11, 12, 14, 18 and 19 after inoculation of virus, and the CNS of each hamster was examined by the IF and HE staining methods.

In all groups of rabbits, the viral antigen was recognized in the trigeminal ganglion six days after inoculation, and after 12 days in various parts of the brain in all the groups. The fluorescence in CNS increased day by day, the strongest fluorescence was observed in the brain of a control rabbit 19 days after inoculation. The intensity of fluorescence in the vaccinated group was slightly weaker than that of the control when these were examined at the same time after virus inoculation (Figure 1, Photos 1–4).

Results of HE and LFB staining: Examination of various parts of the CNS with HE staining indicated that the inflammatory findings in the meninges or perivascular

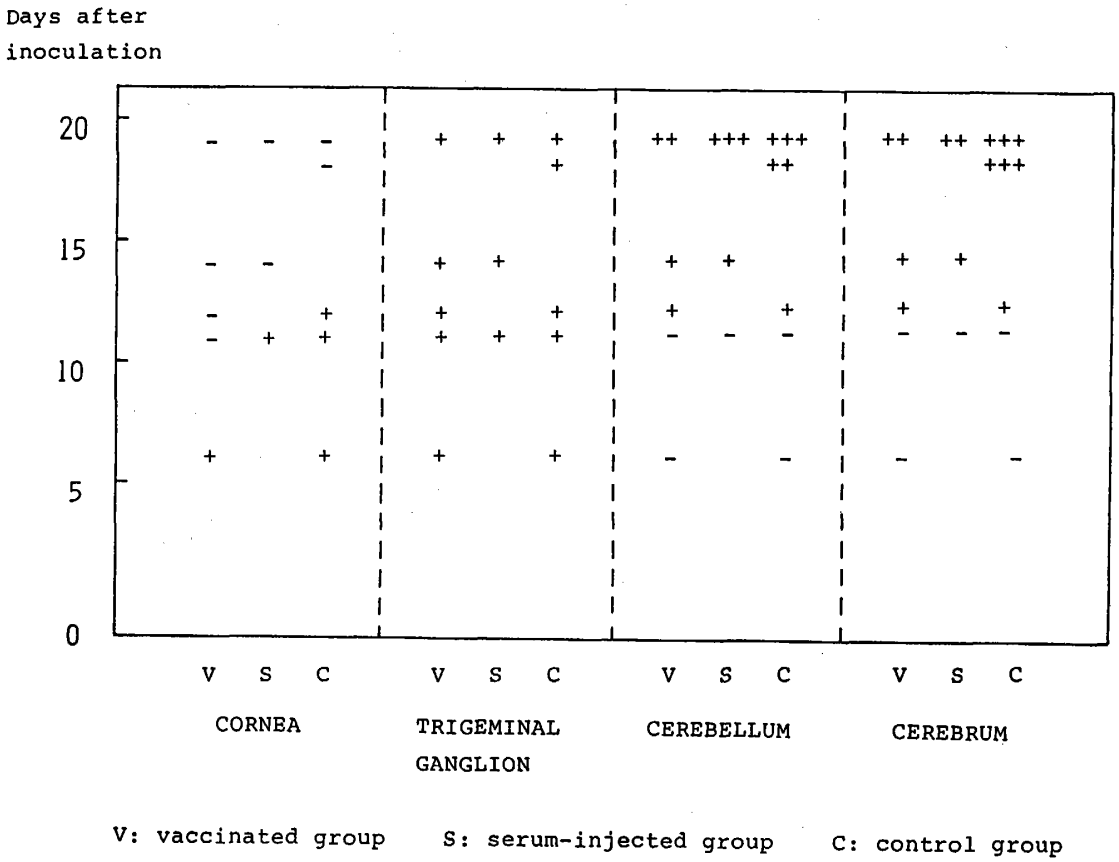

Figure 1 Chronological spread of rabies virus in the CNS of rabbits after inoculation into the cornea.

region were stronger in the vaccinated groups than in the contols when both were compared at the same time after virus challenge and also at the time of onset of rabies. The changes in nerve cells were rather weak in the vaccinated groups. Infiltration of mononuclear cells around blood vessels and nerve cells was observed in almost all sections, and in some of them vacuolation of cytoplasm was visible. Control animals revealed a number of inclusion bodies which, however, were few in the animals that died earlier after vaccination. No demyelinated lesion was seen in either group (Photos 5, 6).

Discussion

Many references have reported the efficacy of preexposure treatment against rabies with various types of vaccines (Nicholson *et al.*, 1978; Ajjan *et al.*, 1980; Anderson *et al.*, 1980), but the exact mechanism underlying the phenomenon is still poorly understood, although humoral immunity has been shown to play an important role in clearing rabies virus from the nervous tissues (Miller *et al.*, 1978; Moreno *et al.*, 1979). Weinmann *et al.* (1979) reported that administration of human leukocyte interferon could reduce the mortality rate in cynomolgus monkeys. Smith (1981) and other investigators (Wiktor *et al.*, 1974; Lagrance *et al.*, 1979; Nicholson *et al.*, 1979)

reported that T lymphocytes were critical for normal clearance of rabies virus infection and they stressed the importance of relationship between T lymphocytes and antibody responses.

According to Sikes *et al.* (1971) a single dose of TC vaccine gave effective protection to monkeys but "early death" was also observed in animals that died after challenge, if they had been treated with vaccine before or after the challenge. According to some other investigators (Baer *et al.*, 1972; Blancou *et al.*, 1980; Prabhaker and Nathanson, 1981), postexposure treatment with vaccine was often effective, but when the death could not be prevented, the incubation period was usually shortened. But different results have been obtained as to the effect of postexposure treatment with immune serum; in an experiment on mice, postexposure treatment with hyperimmune serum was unable to reduce the mortality rate, however the incubation period had been prolonged (Koprowski *et al.*, 1950).

In the present study, "early death" phenomenon was reproduced by postexposure treatment with TC vaccine, but the hamsters treated with the hyperimmune serum (serum C) succumbed after a longer incubation period. These results coincide with the previous reports described above, while treatment by sera with low antibody titers (sera A and B) caused animals to succumb slightly sooner than the control groups. The previous evidence that vaccination can produce "early death" but hyperimmune serum cannot, makes us suspect that this phenomenon is mediated by IgM rather than by IgG. But in the present study we could not confirm this anticipation, because no significant difference in the incubation period was obtained between IgM and IgG fractions.

Apart from rabies, there are some other infectious diseases in which antibodies appear to produce effects detrimental to the host. Dengue fever is usually a curable disease which produces fever and rash, but in some cases severe hemorrhagic syndromes are seen (Halstead, 1980). As a mechanism of this phenomenon, antibody-mediated enhancement of viral replication is suspected (Halstesd and O'rourke, 1977; Halstead, 1979; Peiris and Porterfield, 1979). According to this hypothesis, antibody combined with antigen can be taken by macrophages easily through Fc receptors and then viral replication is enhanced. This phenomenon was confirmed by *in vitro* and *in vivo* studies and has been regarded as one of the mechanisms causing dengue hemorrhagic fever and West Nile fever (Peiris and Porterfield, 1979).

The same mechanism may be applicable to explaining "early death" from rabies. But in the present study the CNS of animals that died earlier than controls for all vaccinations or serum injections showed decreased fluorescence for rabies viral antigen although the clinical signs of both vaccinated and control groups were almost the same. These results coincide with our unpublished data which were obtained by direct IF staining of fresh frozen tissues in suckling mice. The decrease of fluorescence may be only an apparent phenomenon, because antigenic sites may be covered with antibodies, leaving fewer antigenic sites which will bind fluorescence conjugated antibodies. Further studies are necessary to confirm whether viral antigen is truly decreased or not.

Immunolysis mediated by the immune complex and complement is also suspected as it is regarded as a causative factor of atypical measles and some types of glom-

erulonephritis. According to Wiktor *et al.* (1968), cultivated nerve cells infected with rabies virus could be fell into lysis by being added with antibody and complement. Data of electron microscopic studies suggested that virus clearance might require both neutralization of free virus and virus infected cells (Murphy *et al.*, 1973; Iwasaki and Clark, 1975), and the importance of participation of complement on neutralization of virus is suspected (Daniels *et al.*, 1970). These evidences, though still fragmentary, well suggest that "early death" might well be the result of tissue injury mediated by complement which became bound to antigen and antibody complex.

In the present study, complement deposited in nerve cells was not proved, although there were some areas where deposition of complement was seen around blood vessels. This finding may be one of the causative factors of "early death", though not yet affirmed by the present experiments. Detailed study using a more sensitive method is required to prove whether complement and immunoglobulins are deposited around blood vessels or nerve cells.

In the animals that died earlier than controls, inflammatory findings were mainly observed in the meninges and perivascular area rather than the gray matter without any demyelinated lesions. Considering the appearance of inclusion bodies in the cytoplasm of nerve cells and the decreased viral antigen in the nerve cells, not only direct injury by virus but also any immunopathological mechanism must be relevant to the phenomenon. It is true that this phenomenon is strongly suspected of being caused by inadequate immunization with subneutralizing antibody; at the same time, the participation of some other factors is also suspected. Although it was not proved clearly in the present study that this phenomenon is antibody mediated, experiments using this model may lead to further understanding of the contribution of host immunological responses to rabies pathogenesis and the mechanism of the "early death".

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実験的狂犬病における early death に関する研究

小　原　　博

狂犬病ワクチンの接種を受けてなお発症した例では，しばしば潜伏期が短いという現象が知られており early death といわれている。その機序解明のために，本研究では，実験動物を用いて感染後発病阻止実験を行い，この現象を再現し，形態学的検討を行った。

街上毒狂犬病ウイルス（$10^{4.8}LD_{50}$）を実験動物（ハムスター，ウサギ）に感染後，ワクチンあるいは各種免疫段階の血清を一定期間毎に接種し，明確な臨床症状を呈した時点で致死せしめ，ホルマリン固定後，中枢神経各部位について，螢光抗体法，HE 染色法，LFB 染色法による検索を行った。

その結果，ワクチン接種群では，ハムスター（$P<0.01$）ウサギ（$P<0.05$）ともに潜伏期の有意な短縮が認められ，この現象を再現することができた。免疫初期血清（抗体価 $NT=10^{2.2}$）接種群では潜伏期の僅かな短縮が，免疫後期血清（抗体価 $NT=10^{4.2}$）接種群では延長の傾向が認められた。発症時点で螢光抗体法により検索した脳内ウイルス抗原量は，early death を呈した動物では，対照群に比し著明に低下しており，ウイルスによる直接的細胞障害の他に，免疫学的機序による障害が関与していると考えられた。

組織学的検索の結果，early death 群では対照群に比し，灰白質の障害よりも，髄膜，血管周囲への炎症所見が強く認められたが，封入体は少なかった。脱髄巣は認められなかった。補体 C3 の血管周囲への沈着が認められるものもあったが，対照群に比し有意な差ではなかった。IgG, IgM, 補体 C3 の細胞内沈着は証明されなかった。

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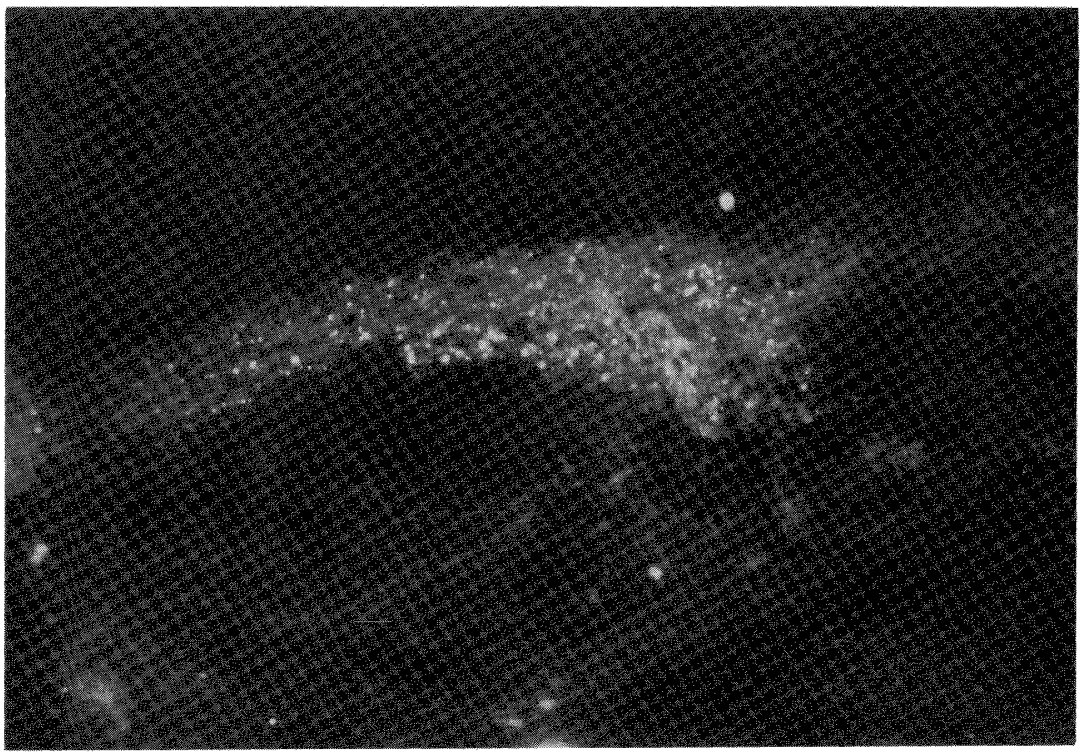

Photo. 1 Rabies virus antigen in epithelium of cornea 3 days after intracorneal inoculation as revealed by immunofluorescence. Control group. 400×.

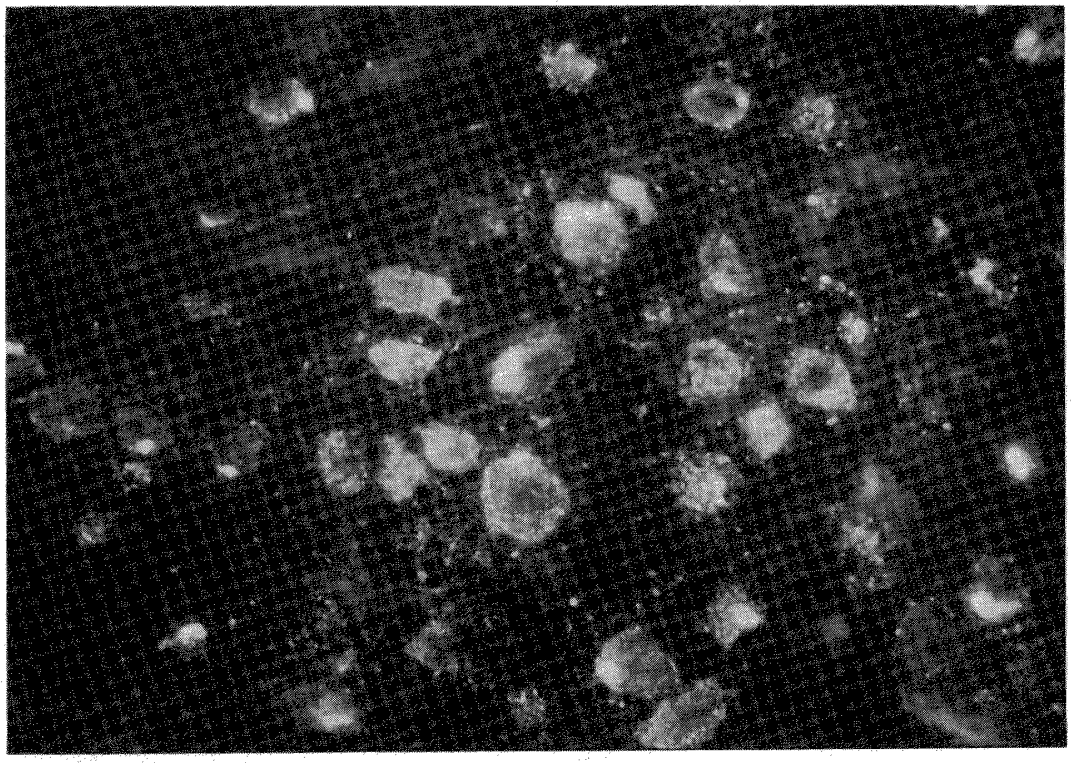

Photo. 2 Rabies virus antigen in trigeminal ganglion 7 days after intra-corneal inoculation as revealed by immunofluorescence. Control group. 200×.

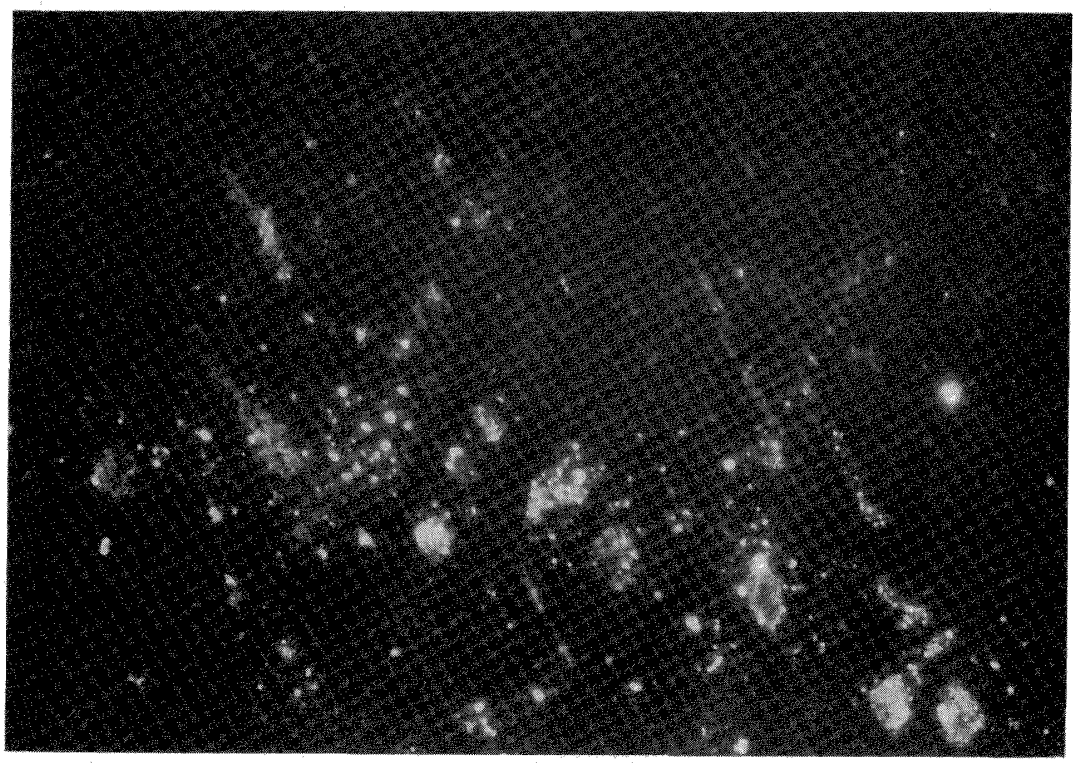

Photo. 3 Rabies virus antigen in cerebellum 14 days after intracorneal inoculation as revealed by immunofluorescence. Control group. 200×.

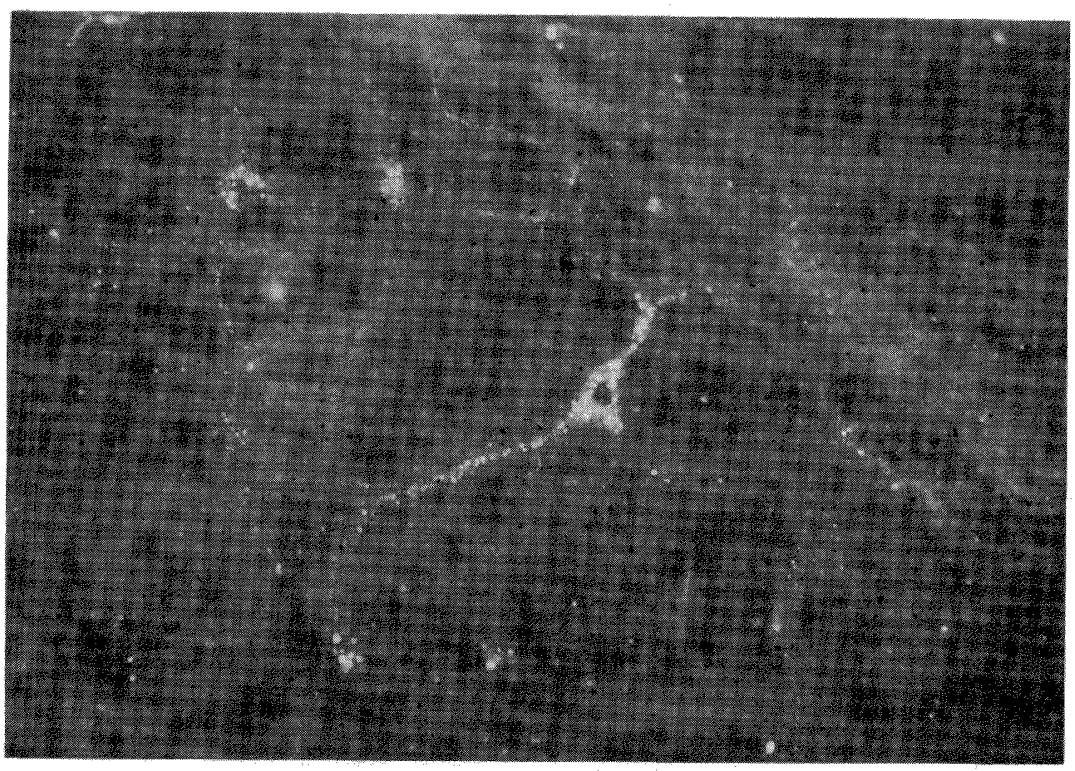

Photo. 4 Rabies virus antigen in pons 14 days after intracorneal inoculation as revealed by immunofluorescence. Control group. 200×.

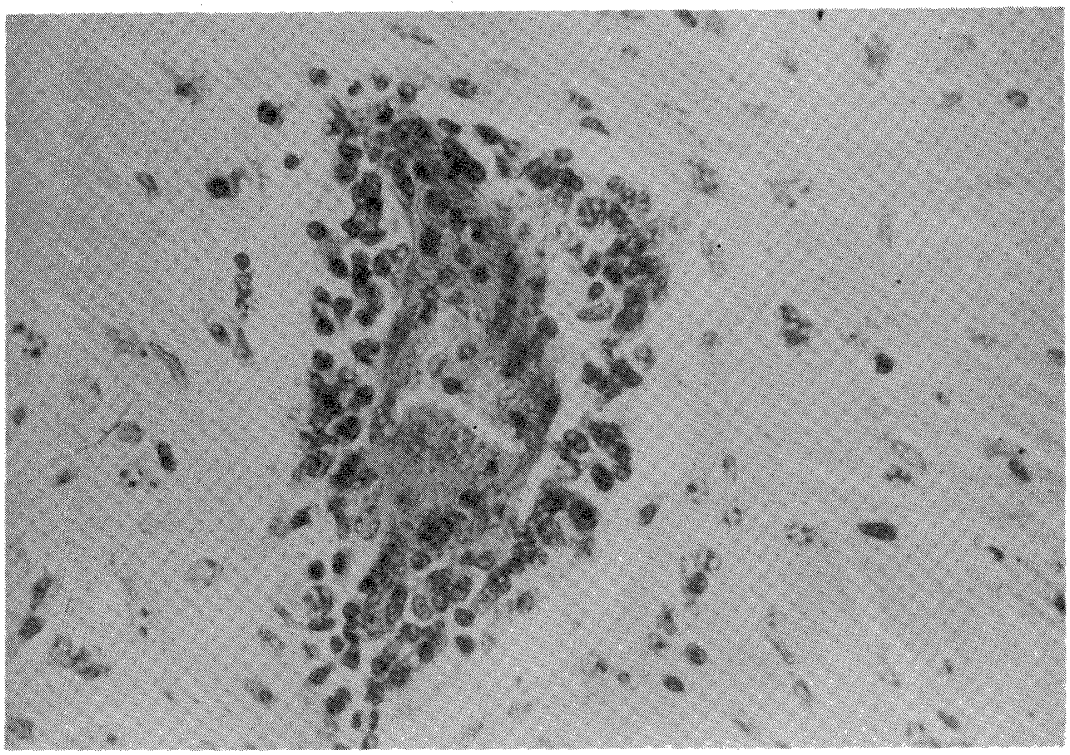

Photo. 5 Infiltration of mononuclear cells around a small blood vessel in cerebrum of vaccinated group. 400×.

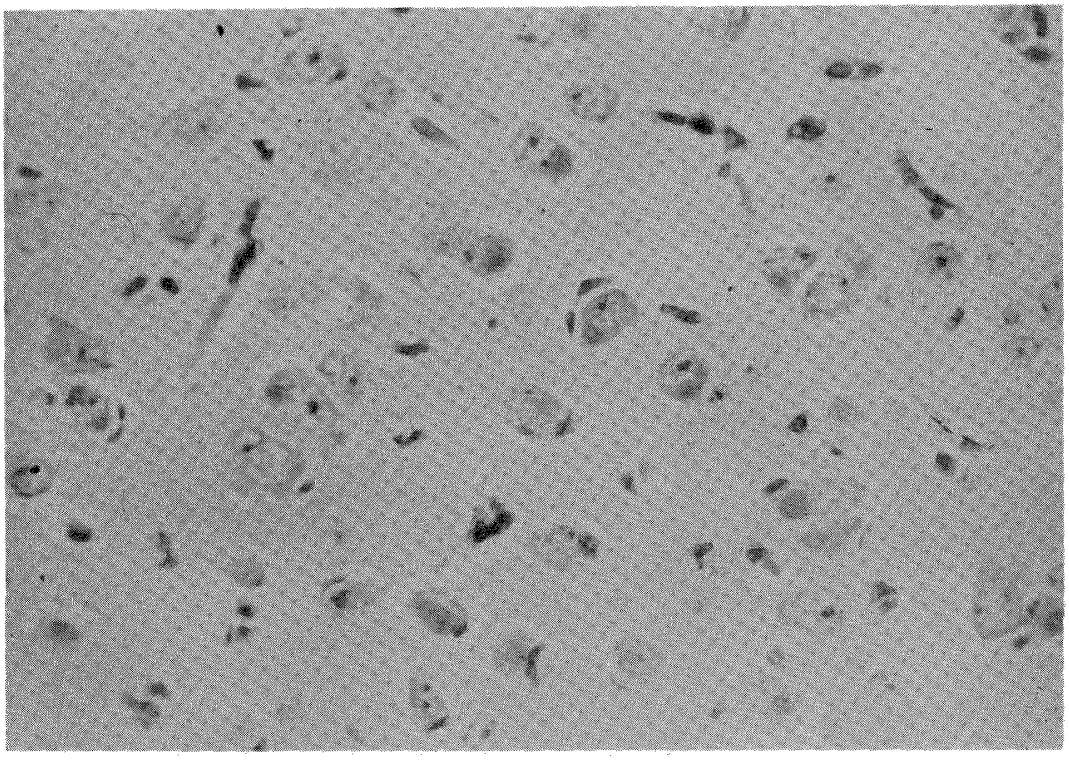

Photo. 6 Inclusion bodies are clearly seen in the cytoplasm of nerve cells in cerebrum of control group. 200×.

日本熱帯医学会雑誌　第12巻　第1号　1984　17-20頁

ケニア在住日本人およびその家庭で働く ケニア人の腸管寄生虫検査

井　関　基　弘

受付　昭和58年9月16日／受理　昭和59年2月6日

国際交流が進むなかで日本人の海外滞在者は年々増加する傾向にあり，家族連れ，長期滞在のケースが増えている。熱帯地をはじめ衛生状態の悪い発展途上国に長期間滞在する人達にとって健康管理には不安が多い。著者が医療協力プロジェクトで1980年4月から1年間ケニアのナイロビに滞在した際にも日本人学校や青年海外協力隊，その他長期滞在の人達から寄生虫疾患について種々相談を受けた。その内容は，個人からはアタマジラミや条虫など肉眼的に見える寄生虫感染に自分で気付いて，駆虫をどうすればよいかという相談や，発熱した人からのマラリア検査の依頼，マラリアの予防内服方法についての相談などであり，団体としては日本人学校，日本人会婦人部や留学生サークルなどからの，ケニアで注意すべき寄生虫病やその感染予防方法に関する講演依頼などであった。当時，ナイロビ日本人会には454人（成人：男168人，女142人；15歳未満の子供：男80人，女64人）が登録されていたが，これ以外に地方で働く青年海外協力隊員や建設関係者も多く，短期出張者や観光旅行者を除いて，ケニア全体に滞在する日本人の数は相当なものであった。在ケニア日本大使館には医務官（当時は大利昌久博士）が駐在しておられ，これらの人達の健康相談にも応じておられたが出張などで不在の際には私達に相談がもたらされるのである。

長期滞在者はこの国の社会慣習上，掃除・洗濯・皿洗いなどをするサーヴァントを家庭に雇わざるをえず，また，料理・子守りを手伝うメイド，運転手，アスカリと称する守衛など数人を雇っている日本人家庭も多い。この現地人使用人達は通いのこともあるが，その家や同一敷地内に住み込みで毎日働いている。従って，この人達からの感染にも不安を感じている人が多かった。

1981年3月，日本人会からの要望で大使館を通じて在ケニア日本人およびその家庭で働くケニア人の糞便検査の依頼があり，寄生虫検査と細菌学的検査を行った。著者は寄生虫検査を担当したので，その結果を報告する。

検査対象

ナイロビに在住する日本人と，主としてその家庭で働くケニア人。提出された検体数は日本人205（成人：男79，女64；子供：男33，女29）およびケニア人65（成人：男33，女28；子供4）であった。日本人検体提出者のケニア滞在期間については正確なデータは得られなかったが，3カ月位から10年以上になる人もあり，平均すると1年以上になると思われる。

検査方法

ホルマリン・エーテル法による集卵・集シストを行い，ヨード・ヨードカリ染色をして鏡検した。

検査結果

表に示したように，日本人からは大腸アメーバのシスト陽性者が1例（成人・女）と横川吸虫卵陽性者が1例（成人・男）検出されたのみであった。

一方，ケニア人においては赤痢アメーバと大腸アメーバのシスト陽性者がそれぞれ11例(16.9%)，

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The prevalence of intestinal parasites among 205 Japanese living in Nairobi and 65 Kenyans working in the Japanese homes

Parasites	Positive case number in	
	205 Japanese (%)	65 Kenyans (%)
Entamoeba histolytica		11 (16.9)
Entamoeba coli	1 (0.5)	11 (16.9)
Endolimax nana		17 (26.2)
Iodamoeba bütschlii		1 (1.5)
Giardia lamblia		1 (1.5)
Chilomastix mesnili		3 (4.6)
Ascaris lumbricoides		2 (3.1)
Hookworm		15 (23.1)
Trichuris trichiura		7 (10.8)
Strongyloides stercoralis (?)*		2 (3.1)
Metagonimus yokogawai	1 (0.5)	
Schistosoma mansoni		3 (4.6)
Taenia sp.		2 (3.1)

* Suspected from the morphology of the rhabditiform larvae.

小形アメーバは17例（26.2%）など原虫感染も多く認められ，また蠕虫類では鉤虫が15例（23.1%）で最も多く，鞭虫7例（10.8%）の他，マンソン住血吸虫，回虫，条虫（無鉤条虫と思われる）などの感染もみられた。2例からラブジチス型幼虫が検出された。培養によるフィラリア型の観察は行っていないが，食道の形態および生殖原基の大きさなどからおそらく糞線虫であろうと思われた。ケニア人65人のうち41人が何らかの腸管寄生虫に感染しており，総陽性率（総陽性者数/総検査数）は63.1%であった。このうち，1種感染は19例であり，2種混合感染が11例，3種混合が10例，4種混合が1例であった。

考　察

山浦ら（1976, 1981）によると青年海外協力隊で海外に長期滞在して帰国した日本人の消化器系寄生虫感染率は25～30%という高い値を示しているが，今回の調査では日本人の寄生虫感染者は予想以上に少なかった。横川吸虫はおそらく日本国内でアユを食べて感染したのであろうから，ケニアでの感染が疑われたのは205人中1例のみであった。これはナイロビで日本人が住んでいる生活環境は上水道・下水道・水洗便所が完備しており，他の熱帯地に比べると街全体の衛生状態が優れているし，2年以上も滞在している人の中には自分で定期的に駆虫薬を服用している人も多いと聴くので，その効果が出ているのかも知れない。また，青年海外協力隊員の場合は地方で働くことが多いので，その生活環境や生活様式とナイロビ在住者のそれとは大きく異なる点もあげられる。

それではナイロビで生活している日本人にとって寄生虫感染の恐れは無いのであろうか。著者は1年間のナイロビ滞在中に Naivasha, Machakos, Kitui などケニア各地の一般住民2,000人余の腸管寄生原虫感染状況を調査したが，その平均感染率は赤痢アメーバ31.8%，大腸アメーバ52.3%，原虫のみの総陽性率は75.1%にも達した (Iseki *et al.*, 1983)。また，Pamba and Mulega (1981) はナイロビのケニア人学童698人の糞便検査を行い，回虫47.7%，鞭虫12.2%，鉤虫7.9%，マンソン住血吸虫6.9%，小形条虫3.9%，テニア3.0%という陽性率を報告している。一見清潔そ

うにみえるナイロビも人口の急激な都市集中化で地方からの人口流入が多く，郊外に住みついている彼等の生活レベルは低いし，衛生状態はあまり良くないことがうかがえる。そして今回の調査で，日本人家庭で働くケニア人もその60%以上が何らかの腸管寄生虫に感染しており，その半数以上が2種混合感染あるいは3種混合感染であったことは，彼等の生活する環境の衛生状態がいかに悪いものであるかを如実に示しているといえる。ナイロビの日本人家庭で働いている間はまだ良いが，彼等の多くは田舎の出身であり，クリスマスなどの休暇には里帰りすることが多いので，そのような時に感染するのであろう。特に，17%近くが赤痢アメーバのシストを排出していることは，彼等が料理や，食器洗い，子守り，掃除，洗濯など家庭内で直接密接に日本人の生活に係わっている点で留意しなければならない。赤痢アメーバは，中間宿主を要する住血吸虫などと違って，手指などについたそのシストが食物や食器，飲料水などを汚染し，感染が容易に起こりうるからである。

これらのことから，ナイロビの都市部で生活している日本人にとっても寄生虫感染の機会は身近に存在する。現に，私の滞在期間中に日本人で条虫症3例，イソスポーラ症1例を経験した。また，大利（1983）によると，在ケニア日本大使館医務室では1980年に腸管寄生虫に関して，アメーバ症6例，蠕虫症7例があったことを報告している。

今回の調査では，日本人における感染者は少なかったが，長期滞在者はその家庭内使用人を含めて日常の感染予防に十分注意する必要がある。

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A SURVEY ON INTESTINAL PARASITE INFECTION AMONG JAPANESE LIVING IN NAIROBI AND KENYANS WORKING IN THE JAPANESE HOMES

MOTOHIRO ISEKI

(Received September 16 1983/Accepted February 6 1984)

The stool examinations on 205 Japanese living in Nairobi, Kenya, and 65 Kenyans working in the Japanese homes as an indoor- or outdoor servant, maid or driver were carried out in March, 1981. The Japanese group was composed of 143 adults (79 males and 64 females) and 62 children under 15 years old (33 males and 29 females). Most of them had been staying in Nairobi for 6 months or more. The Kenyan group was composed of 61 adults (33 males and 28 females) and 4 children. The stool specimens were examined by formol-ether sedimentation method followed by iodine-staining.

Out of 205 specimens from the Japanese group, only one case of *Entamoeba coli* infection and one case of *Metagonimus yokogawai* infection were detected. The latter case was suspected that the infection had occurred by ingestion of undercooked *Ayu*, a fresh water fish *Plecoglosus altivelis*, during his stay in Japan. On the other hand, out of 65 Kenyan persons 41 (63.1%) were infected with one or more intestinal parasite species. The parasite species detected and their infection rates were 16.9 per cent for *Entamoeba histolytica*, 16.9 per cent for *E. coli*, 26.2 per cent for *Endolimax nana*, 1.5 per cent for *Iodamoeba bütschlii*, 1.5 per cent for *Giardia lamblia*, 4.6 per cent for *Chilomastix mesnili*, 3.1 per cent for *Ascaris lumbricoides*, 23.1 per cent for hookworm, 10.8 per cent for *Trichuris trichiura*, 3.1 per cent for *Strongyloides stercoralis*, 4.6 per cent for *Schistosoma mansoni* and 3.1 per cent for *Taenia* sp., respectively.

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A PARASITIC SURVEY AND MASS-TREATMENT USING PYRANTEL PAMOATE ON NIGERIAN SCHOOL CHILDEN*

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(Received August 22 1983/Accepted February 15 1984)

Abstract: The survey and mass-teratment of helminthic infections with pyrantel pamoate was carried out on 140 school children in Ile-Ife, Western State of Nigeria. It was marked that 129 children were positive for *Ascaris lumbricoides* (*As. lumbricoides*), *Trichuris trichiura* (*Tr. trichiura*) or *Necator americanus* (*Ne. americanus*). Of the parasite-positive children, 82 could be allocated for the treatment with pyrantel pamoate. They were divided on the basis of having single, double or triple infections of the following intestinal nematodes; *As. lumbricoides*, *Tr. trichiura* and *Ne. americanus*.

The results demonstrate that pyrantel pamoate is effective in curing *As. lumbricoides* almost completely, but is less effective against *Tr. trichiura* and *Ne. americanus*.

INTRODUCTION

Pyrantel pamoate has been reported to be highly effective with less side or toxic effects in the treatment of infections with *As. lumbricoides* (Yokogawa *et al.*, 1970; Kobayashi *et al.*, 1970; Desowitz *et al.*, 1970; Bell and Nassif, 1971; Kojima *et al.*, 1978), *Ancylostoma duodenale* (Yokogawa *et al.*, 1970; Hori, 1971; Simwogerere, 1972; Nassif and Bell, 1972), and *Ne. americanus* (Yokogawa *et al.*, 1970; Ishizaki *et al.*, 1971; Kobayashi *et al.*, 1971; Simwogerere, 1972; Sato *et al.*, 1973; Bortero and Castaso, 1973). The effect of pyrantel pamoate on single or multiple infections with *As. lumbricoides*, *An. duodenale* and *Trichostrongylus* spp. in Arab Republic of Egypt has been reported by Nassif and Bell (1972).

The present investigation was to determine the effect of pyrantel pamoate on single and multiple infections with *As. lumbricoides*, *Ne. americanus* and *Tr. trichiura* in Nigeria.

MATERIALS AND METHODS

The present study was carried out in Ile-Ife which lies about 250 km north–east of Lagos, the capital city of Nigeria, with a population of about 150,000. The

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parasitological survey and mass-treatment of helminthic infections with pyrantel pamoate was made on 8–11 years old school children during the period from October 1978 to February 1979. A total of 140 school children were surveyed for parasitic infections by fecal examinations with the direct smear, the brine-flotation, MGL technics and the filter paper cultivation technics, and urinary examination for the detection of helminthic and protozoan infections. Of the 129 children positive for parasitic infection, 82 individuals suffering from single, double and triple infections with *As. lumbricoides*, *Ne. americanus* and *Tr. trichiura* were treated with pyrantel pamoate (Combantrin®). It was administered as tablets in a single oral dose after lunch according to the following dosage; age 10–11 years 400 mg, age 8–9 years 300 mg. These doses are expressed as pyrantel base. Evaluation was performed by the fecal examinations for the detection of intestinal helminths about four weeks after the treatment.

Results

The prevalence of intestinal helminths infection was 92.1 per cent (Table 1). *As. lumbricoides* was positive in 73.6 per cent, *Tr. trichiura* 72.9 per cent, *Ne. americanus* 73.6 per cent and *Strongiloides stercoralis* in 2.8 per cent. In intestinal protozoa, the prevalence of *Entamoeba histolytica* infection was 19.3 per cent, *Entamoeba coli* 25.7 per cent, *Endlimax nana* 15.7 per cent, *Iodamoeba bütschlii* 15.0 per cent and *Giardia lamblia* 9.3 per cent. The urinary examination of 140 individuals for parasites was carried out and the prevalence of *Schistosoma haematobium* infection was 30.0 per cent. A total of 140 school children was investigated, and distribution of these in single, double and triple infections is shown in Table 2. The infection with a single species was observed in 19 cases (13.6%), those with double species in 40 cases (28.6%), and with

Table 1 Prevalence of intestinal parasites in school children in Ile-Ife, Nigeria

Examinee	Male (%)	Female (%)	Total (%)
No. examined	83	57	140
Not infected	6 (7.2)	5 (8.8)	11 (7.9)
As. lumbricoides	60 (72.3)	43 (75.4)	103 (73.6)
Tr. trichiura	59 (71.1)	43 (75.4)	102 (72.9)
Ne. americanus	68 (81.9)	35 (61.4)	103 (73.6)
St. stercoralis	2 (2.4)	2 (3.5)	4 (2.8)
**Sc. haematobium*	31 (37.3)	11 (19.3)	42 (30.0)
En. histolytica	17 (20.5)	10 (17.5)	27 (19.3)
En. coli	25 (30.1)	11 (19.3)	36 (25.7)
En. nana	16 (19.3)	6 (10.5)	22 (15.7)
Io. bütschlii	15 (18.1)	6 (10.5)	21 (15.0)
Gi. lamblia	10 (12.0)	3 (5.2)	13 (9.3)

* identified in urine.

the triple species in 70 cases (50.0%).

Regarding single infections, in the six patients involving two with *As. lumbricoides*, one with *Tr. trichiura*, and three with *Ne. americanus* cure rate of 100 per cent was obtained (Table 3). Double infection, 23 in total, were composed of three patients

Table 2 Prevalence of single and multiple infections with three common intestinal nematodes, *Ascaris lumbricoides*, *Trichuris trichiura* and *Necator americanus* in school children, in Ile-Ife

Examinee	Male (%)	Female (%)	Total (%)
No. examined	83	57	140
Not infected	6 (7.2)	5 (8.8)	11 (7.9)
Single infection			
As. lumbricoides	3 (3.6)	4 (7.0)	7 (5.0)
Tr. trichiura	2 (2.4)	3 (5.3)	5 (3.6)
Ne. americanus	5 (6.0)	2 (3.5)	7 (5.0)
Double infection			
As. lumbricoides + *Tr. trichiura*	3 (3.6)	10 (17.5)	13 (9.3)
As. lumbricoides + *Ne. americanus*	10 (12.0)	3 (5.3)	13 (9.3)
Tr. trichiura + *Ne. americanus*	10 (12.0)	4 (7.0)	14 (10.0)
Triple infection			
As. lumbricoides + *Tr. trichiura* + *Ne. americanus*	44 (53.0)	26 (45.6)	70 (50.0)

Table 3 Cure rate using pyrantel pamoate in each combination of infection with *Ascaris lumbricoides*, *Trichuris trichiura* and *Necator americanus*

Combination of infection	*As. lumbricoides*		*Tr. trichiura*		*Ne. americanus*	
	No. treated	No. cured (%)	No. treated	No. cured (%)	No. treated	No. cured (%)
Single	2	2 (100)	1	1 (100)	3	3 (100)
Double	11	11 (100)	15	8 (53.3)	20	9 (45.0)
As. lumbricoides + *Tr. trichiura*	3	3 (100)	3	1 (33.3)		
As. lumbricoides + *Ne. americanus*	8	8 (100)			8	3 (37.5)
Tr. trichiura + *Ne. americanus*			12	7 (58.3)	12	6 (50.0)
Triple	53	51 (96.2)	53	12 (22.6)	53	18 (34.0)
Total	66	64 (97.0)	69	21 (30.4)	76	30 (39.5)

wiht a combination of *As. lumbricoides* and *Tr. trichiura*, eight with *As. lumbricoides* and *Ne. americanus*, and 12 with *Tr. trichiura* and *Ne. americanus*. Cure rates of 100 per cent, 33.3 to 58.3 per cent and 37.5 to 50.0 per cent were obtained for *As. lumbricoides*, *Tr. trichiura* and *Ne. americanus* infections, respectively. In the 53 triple infections, cure rates for *As. lumbricoides*, *Tr. trichiura* and *Ne. americanus*, were 96.2, 22.6 and 34.0 per cent respectively. Over all cure rates for the 66 patients with *As. lumbricoides*, 69 with *Tr. trichiura* and 76 with *Ne. americanus* were 97.0, 30.4 and 39.5 per cent respectively.

Discussion

Parasitological surveys, reported by Kaneko and Odiachi (1976) and Hori and Odiachi (1978) revealed high rates of infections with intestinal helminths and protozoans among school children in Ile-Ife. In this paper, the anthelmintic effect of pyrantel pamoate was examined in infected school children with one, two or three of the common nematode parasites, *As. lumbricoides*, *Tr. trichiura* and *Ne. americanus*.

For the anthelmintic effect of pyrantel pamoate, Kobayashi *et al.* (1970) reported that the cure rate of 98.1 per cent for *As. lumbricoides* infection was obtained by the administration of a single dose of 10 mg/kg body weight, and that of 97.7 per cent by 5 mg/kg. The effect of pyrantel pamoate on *Ne. americanus* has been reported by several investigators. Desowitz *et al.* (1970), Yokogawa *et al.* (1970) and Sato *et al.* (1973) exhibited pyrantel pamoate to be highly effective by a single administration of 10 or 20 mg/kg. On the other hand, Kobayashi *et al.* (1971) showed that only 45.5 per cent by 10 mg/kg and 52.2 per cent by 20 mg/kg were cured. Ishizaki *et al.* (1971) also reported that the cure rates were 68.8 per cent by single dose of 10 mg/kg and 89.3 per cent by the same dose of pyrantel pamoate for three consecutive days. There are few detailed reports on the anthelmintic effect on *Tr. trichiura*, Tani *et al.* (1975) reported that the cure rates for *Tr. trichiura* infection treated with pyrantel pamoate ranged between 15.6 and 52.2 per cent by a single dose of 10 mg/kg. We obtained the cure rate of 30.4 per cent with a single dose (approximately 10 mg/kg body weight) of pyrantel pamoate.

From these results, it can be concluded that pyrantel pamoate is less effective against *Ne. americanus* and *Tr. trichiura* than against *As. lumbricoides*. According to Ishizaki *et al.* (1971), Yokogawa and Niimura (1981), as well as manufacturer's literature it may be necessary to use twice the recommended dose for one to three consecutive days in the heavy or multiple infections of *Ne. americanus* and *Tr. trichiura*. Thus the anthelmintic effect of pyrantel pamoate was observed to differ depending upon the nematode species and, probably, upon the degree of infection.

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ナイジェリアにおける学童の寄生虫調査ならびに Pyrantel pamoate による集団駆虫成績

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ナイジェリア，イフェ地区の学童140名について糞便検査によって腸管寄生蠕虫類と原虫類を，尿沈査からビルハルツ住血吸虫卵の検出を行った。その結果，腸管内寄生蠕虫のうち回虫，鞭虫および鉤虫の寄生率はそれぞれ73.6%，72.9%，73.6%であり，それらの単種あるいは多種混合感染者は129名であった。これらの虫卵陽性者のうち，単種感染，2種あるいは3種混合感染者82名について pyrantel pamoate の1回投与により駆虫を行った。駆虫成績は，回虫に対しては全体の97.0%で，ほぼ完全に近い虫卵陰転率が得られたが，鞭虫では30.4%，鉤虫では39.5%と低率であった。

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HUMAN TUNGIASIS (*TUNGA PENETRANS*): A REPORT ON THREE JAPANESE INFECTED IN VENEZUELA

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(Received September 26 1983/Accepted February 1984)

Abstract: Tungiasis of authors' own, suffered during the stay in Parima mountains in the Federal Territory of Amazonas, Venezuela from 19th October to 2nd November, 1982, was reported. Of the authors, three were infected with *Tunga penetrans*, developing one, four and five lesions on feet, respectively. In all the cases, gravid fleas were removed by curettage and the antibiotic ointment was applied on the wounds. Pain disappeared soon and lesions healed smoothly after treatment.

The chigoe flea, *Tunga penetrans* Linnaeus, 1758, is widely distributed in tropical and sub-tropical regions of North and South Americas, the West Indies and Africa. It is called as *nigua* and *bicho do pê* in Latin America. The flea parasitizes on various vertebrate animals on the land. Although it is originally a tiny flea, after penetration into the skin, the gravid female eventually enlarges like a small pea. The burrowing into dermal tissue of man frequently causes serious inflammation of the affected feet and other portions, which can lead to ulceration and fibrosis. Further, tungiasis ulcer can easily be followed by secondary bacterial infections.

The authors stayed in Parima region of Amazonas state in Venezuela for the studies on onchocerciasis during the period between October and November, 1982. They walked around the field to collect larvae and adults of blackflies and the housing areas called "Shabono" of Yanomama Indian. During and after this survey, they found chigoe fleas in themselves. The report deals with this experience.

CASE REPORT

Case 1. S. N., 34 year old male. The patient was parasitized by five chigoe fleas. One of these burrowed into the skin of lateral portion of the first toe of the left foot. The remaining four were found all from the first toe of the right foot; one, aside the nail, two under the nail, and one in the sole. As he noticed the infection of fleas

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before leaving Parima, all fleas were still small and seemed like a white patch with a black dot in the center. They were removed easily by incision of the affected skin with an injection needle. Suppuration and bleeding were not observed thereafter.

Case 2. H. S., 43 year old male. The patient was parasitized by one flea alone. It burrowed into the skin under the nail of the fourth toe of the left foot. The size of flea was approximately 5 mm in diameter, when he became aware of the infection seven days after the departure from Parima. The lesion showed transluscent nodule with a central dark spot inside the skin and was suppurative. Bleeding was seen while the flea was enucleated.

Case 3. I. T., 47 year old male. Four chigoe fleas were found: two, aside and under the nail of the fourth toe of left foot (Photo. 1); one, between the first and second toes of the right foot; and one, under the nail of the third toe of the right foot. Within seven days after leaving there, the patient noticed the infection of the former three fleas which looked like transluscent subcutaneous nodule with a central dark spot on the surface. On the other hand, the fourth flea was found out 25 days after he left the Parima region when the peculiar sensation of affected region increased. As this lesion had been covered by a preceding subcutaneous bleeding, it was overlooked when other lesions were discovered before (Photo. 2).

In all the cases after enucleation of insects, broad-spectrum antibiotic ointment was applied. Peculiar sensation, itching and pain disappeared. The lesion healed rather quickly.

Description of Chigoe Flea

Flea specimens removed were microscopically examined for entomological identification. All the females were found to be gravid, harbored many ova (ca. 0.63 mm long by 0.32 mm wide), and were identified as *Tunga penetrans* by possessing no ctenidium on the pronotum, no suture from the base of the antennal groove to the vertex, sharply-angled frons, elongated mandibles with distinct serrations, and the anterior apical angle of the hind coxae projecting downwards as a triangular tooth (Photo. 4), as defined in the key of Smit (1973).

Discussion

Various tungiasis cases have been previously reported. Nishimoto and Nakajima (1975), Goldman (1976), Monti *et al.* (1980), Ott *et al.* (1980) and Zalar and Walther (1980) reported imported cases from Africa, and Scholten *et al.* (1977), Brothers (1979) and Soei and Van der Kaay (1980) reported similar cases who returned from South America. In these reported cases, the pulp and sides of the toes were the most affected regions rather than the sole or dorsum of the toes shown in author's cases.

The present report, the second case-report among Japanese, shows that in the infestation foci of chigoe flea, visitors would easily be parasitized even in a short term of stay.

Current treatment of human tungiasis is limited to the immersion of the affected limb into four per cent formalin solution or exposure to DDT spraying (WHO, 1979).

The flea can also be removed by incision. Ade-Serrano *et al.* (1982) tested niridazole against tungiasis, in which burrows were effectively dried up within 3 weeks. However, most reliable and simple way of treatment is to dig the flea out by a cleaned needle bloodlessly. This treatment procedure has been empirically adopted by native people using a wooden stick or needle.

The tourists who travel endemic areas of tungiasis should not walk barefooted and/or with sandals alone. Application of repellent is recommendable. Because it is said that even shoes are not effective barriers for chigoe fleas. If one noticed the infection with an early stage of flea, it should be digged out soon with a cleaned needle. Because, tetanus and gas gangrene cases have ever been reported hitherto as the complication of tungiasis in Central America.

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ベネズエラで経験したスナノミ症

野田伸一[1]・高岡宏行[2]・鈴木　博[3]・多田　功[4]

著者らはオンコセルカ症の調査のため，1982年に約2カ月間ベネズエラ国アマゾナス州と北部諸州とに滞在した。そのうち10月19日から11月2日までアマゾナス州パリマとその周辺に滞在し，オンコセルカ症を媒介するブユの採集と疫学調査を実施した。特に，シャボノと呼ばれるヤノママ族の家屋内を歩いた。その後，4人のうち3人がスナノミの寄生をうけた，寄生数はそれぞれ5，4，1匹であった。摘出された標本の形態的特徴から，これらはすべてスナノミ *Tunga penetrans* と同定された。皮膚を切開してスナノミを取り除き，抗生物質軟膏を塗布したが，その後，痛みと独特な異物感は消失し，創口は急速に治癒した。

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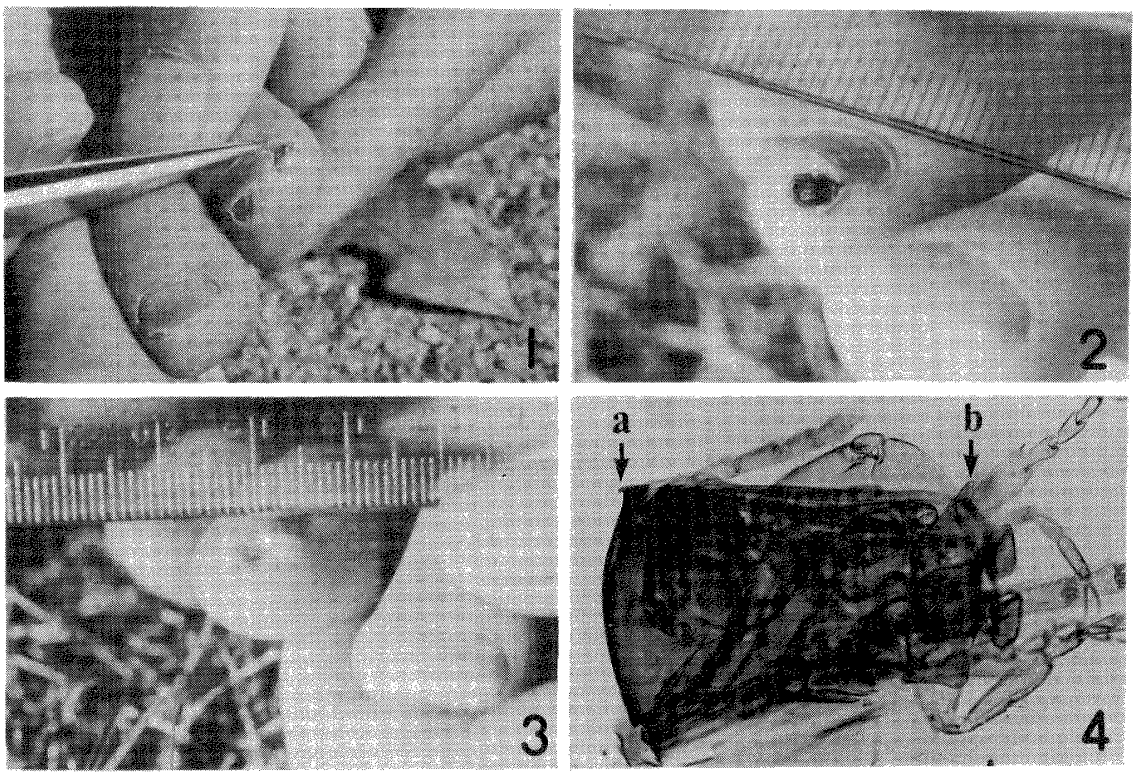

Photo. 1 Two lesions caused by infection with *Tunga penetrans* on the fourth toe of the left foot in the third case.

Photo. 2 Lesion caused by infection with *T. penetrans* on the third toe of the right foot in the third case. It was covered by a subcutaneous bleeding before enucleation.

Photo. 3 The same lesion shown in Photo. 2 after enucleation.

Photo. 4 Lateral view of gravid female of *T. penetrans* removed from the first case (showing only head and thorax). a, frons sharply angled. b, anterior apical-angle of hind coxa projecting downward as a triangular tooth.

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A NOTE ON THE SIPHON AND TRUMPET IN LARVAL AND PUPAL STAGES OF *MANSONIA UNIFORMIS* (THEOBALD) (DIPTERA, CULICIDAE)

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(Received March 2 1983/Accepted March 10 1984)

Abstract: From the observation on the larval and pupal behavior of *Mansonia uniformis* under laboratory conditions, it was proved that both larva and pupa obtained oxygen through the respiratory apparatus of the siphon of the larvae (larval respiratory gill, LRG), or of the trumpet of the pupae (pupal respiratory gill, PRG). This indicates the presence of respiratory "gills" in *Mansonia* spp. The mechanism of respiration is discussed and illustrated by black and white photographs.

INTRODUCTION

The Mansonini tribe includes epidemiologically a very important group of vectors of filariasis in several tropical regions, especially among the Southeast and East Asian countries.

Bionomics and ecological observations of Mansonini mosquitoes have been reported by several researchers indicating that the larvae and pupae of Mansonini mosquitoes take in oxygen through siphons and trumpets, which are inserted into the roots of water plants (Yamaguti and La Casse, 1950; Laurence and Smith, 1958; Laurence, 1960; Burton, 1964; Ramalingam *et al.*, 1968; Samarawickrema, 1968; Wharton, 1978) and morphological contributions were made by Harbach and Knight (1980).

The author has conducted ecological observations in the field and studied the rearing methods of Mansonini mosquitoes, *Mansonia* (*Mansonioides*) *uniformis* (Theobald) and *Coquillettidia* (*Coquillettidia*) *ochracea* (Theobald) in the laboratory (Iwaki 1982).

The respiratory system of larvae and pupae of Mansonini mosquitoes, with their morphological and physiological differences from *Aedes*, *Culex* and *Anopheles* mosquitoes were particularly investigated. The author, however, had not been able to confirm clearly the presence of larval respiratory "gills" (LRG) and pupal respiratory "gills" (PRG) in Mansonini larvae and pupae while breeding these mosquitoes in Japan.

The author had an opportunity to observe and study the biology of *Mansonia uniformis* in Southeast Asia from April 1 to September 25 in 1982, where he succeed in to observing "gills" on *Mansonia* larvae and pupae. These "gills" were demonstrated as respiratory apparatuses for the first time. The author wishes to emphasize in the present paper that the respiratory mechanism of Mansonini mosquitoes differ from that of the *Aedes*, *Culex* and *Anopheles* species.

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Materials and Methods

Adult *Mansonia uniformis* females were collected using a dry-ice trap at the Midorogaike-pond area in Kyoto City. In the laboratory the females after sucking blood were allowed to lay their eggs beneath the leaves of water plants (*Salvinia notans*).

In addition, the author used other larvae and pupae of *Mansonia uniformis* from the colony kept in the laboratory of the Department of Medical Entomology, Faculty of Tropical Medicine, Mahidol University in Thailand. In most of the studies the author used filter paper and styloform sheets for breeding of the larvae and pupae. The larvae and pupae were kept individually in water on a slide glass with a hole and were observed through a high optical microscope under free living conditions. The larval behavior could be well observed using the above mentioned method, especially when a human hair was attached to the tip of the siphon.

Results and Discussion

While the author colonized the 1st instar larvae of *Mansonia uniformis* immediately after they hatched out from eggs, he observed that many of the 1st instar larvae attached to the wall of the water tank. They were alive for over 20 days without floating to water surface, and some of them developed into 2nd instar larvae. The 1st instar larvae had no accessories on their siphonal tips during the swimming time (Photo. 1), their siphon being slender and cylindrical, therefore they closely resembled *Aedes* and *Culex* larvae morphologically. But when the larvae of *Mansonia uniformis* attached their bodies to the wall of the water tanks or to water plant roots, they protruded inner spiracular teeth (IST) and larval respiratory gill of thin membrane tissue were observed at their siphonal tips (Photo. 2). At the same time, their bodies were fixed in place with the open spiracular teeth (OST). The siphon of the 2nd instar larvae is triangular in shape with a saw on one side at its edge (Photo. 3).

The larvae fixed their bodies to the stems and roots of water plants by means of the OST in their siphon. The attachment of the body to the water plant in the larvae occurred in the following three steps: a) the tissue of the stem or root of the plant was injured by the saw, b) the IST was attached to the damaged tissue and its siphonal organ was inserted into the stem or root of the water plant, and c) the larvae protruded its IST to fix the body and also protruded its LRG into the water from the tip of the triangular siphon, in order to respire oxygen through the LRG from the water.

In the succeeding 3rd and 4th stages of larval development (i.e. 3rd and 4th instar), the siphon became shorter and wider. The form of the siphon varied at each stage of larval development in order to accommodate the different shapes of the larvae (Photos. 3, 4 and 5). The tip of the trumpet of the pupae was covered with PRG like a net which allows the pupae to take in oxygen (Photos. 6 and 7).

It was believed that in the Mansonini group, as against mosquitoes of the *Aedes*, *Culex* and *Anopheles* groups, the insertion of the siphon and trumpet into the stems or roots of plants (i.e. *Pistia* group) was a key element in respiration. In a recent text-

book on mosquitoes, Gillett (1971) explained that in the genus *Ficalbia* there is a complete series of species showing different degrees of adaptation for tapping air from plants. In *F. pallida* the tip of the siphon is fully adapted for piercing plant tissue and, although the modifications of the siphon tip of *F. perplexens* and *F. splendens* are not as advanced, they allow them to tap the air trapped on the underside of floating leaves.

Unlike the respiration systems of other mosquito groups, that of Mansonini larvae and pupae had not been clearly established. In a very recent study of *Mansonia* and *Coquillettidia*, Breeland *et al.* (1981) tried to breed these mosquitoes artificially, but they were unsuccessful, losing most of the larvae in the first instar stage.

The author was able to breed successive generations of *Mansonia uniformis* in the laboratory and carefully observed the respiratory mechanism of the larvae and pupae. The larvae and pupae attach their bodies to water tanks or water plants and took in oxygen from the water through the LRG or PRG surrounding the tip of the siphon or trumpet.

The blood of the *Mansonia* larvae flows through the cylindrical tube at the centre of the siphon tube in the direction indicated by the arrow in Figure 1. The muscular tube at the base of the cylindrical tube works as a pump or heart of the open circulation pulsating continually and the blood then flows through the tube to the LRG. Furthermore, this cylindrical tube functions as a valve at the entrance and exit of the LRG while the larvae are fixed to the roots. In this stage the blood passes through the LRG, enters into the trachea and then returns to the abdominal parts (Figure 1.) The trachea of *Mansonia* does not develop tracheoles as it does in *Aedes* and *Culex* mosquitoes. Instead there are many tiny pores in the walls of the trachea and the blood flows in and out through these pores. Under the author's observation it became clear that the respiratory mechanisms of the *Mansonia* larvae and pupae are quite different from respiration in other mosquito groups.

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アシマダラヌマカの幼虫期および蛹期の呼吸管と呼吸角についての考察

岩　城　　操

熱帯性ヌマカの幼虫および蛹は，水生植物の根や茎に呼吸管または呼吸角をさし込み酸素を吸収して生活すると考えられているが，実験室内でヌマカ（アシマダラヌマカ）を飼育して，孵化直後の幼虫から蛹までの呼吸管および呼吸角の形態と水中における呼吸法を詳細に観察した結果，つぎのような知見が得られた。

1) 1令幼虫の呼吸管は，細長い管状で，その先端に体を水中植物などに固定するための爪と非常に薄い膜の鰓状組織を持っている。
2) 2令から4令までの幼虫の呼吸管は，令の進行にしたがって巾広い扁平な三角形に変化する。また，呼吸管先端には，体を固定するための爪と1令幼虫のときと同様の鰓状組織がみられた。
3) 蛹の呼吸角先端部を包むように，ネット状の袋型を呈した鰓状組織のあることを多数の個体を調べることによって観察した。
4) 幼虫および蛹の体内を循環する血液は，血液を送り出すポンプから，薄い膜の鰓状組織内を通過し，水中の溶存酸素を吸収するものと考えられる。

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Photo. 1. The siphon of the 1st instar larva in the swimming time.

Photo. 2. The siphon of the 1st instar larva while hanging from the root of a water plant.

Photo. 3. The siphon of the 2nd instar larva.

Photo. 4. The siphon of the 3rd instar larva.

Photo. 5. The siphon of the 4th instar larva.

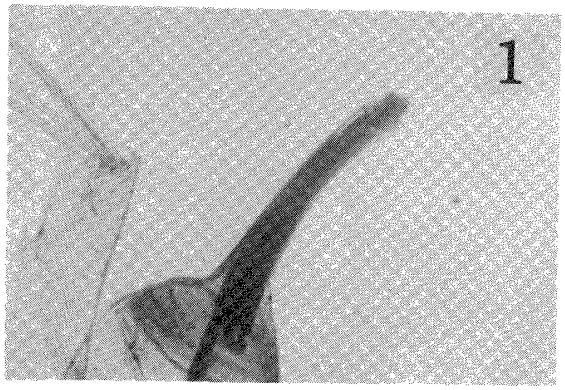
Photo. 6. A side view of the tip of the pupal trumpet and the PRG.

Photo. 7. A dorsal view of the tip of the pupal trumpet and the PRG.

Figure 1. The open blood system of the 4th instar larva.

Abbreviations

IST:	inner spiracular teeth
LRG:	larval respiratory "gill"
OST:	outer spiracular teeth
P:	pump (heart)
PRG:	pupal respiratory "gill"
S:	siphon
SAW:	saw
T:	trumpet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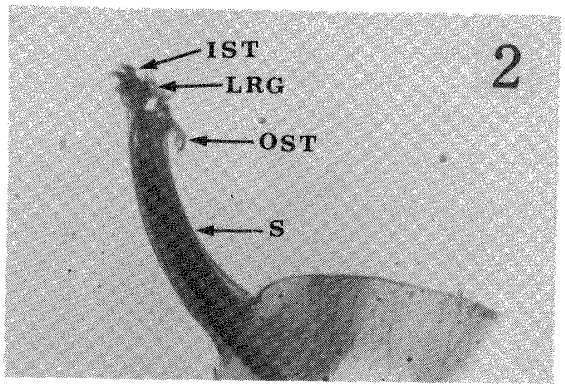
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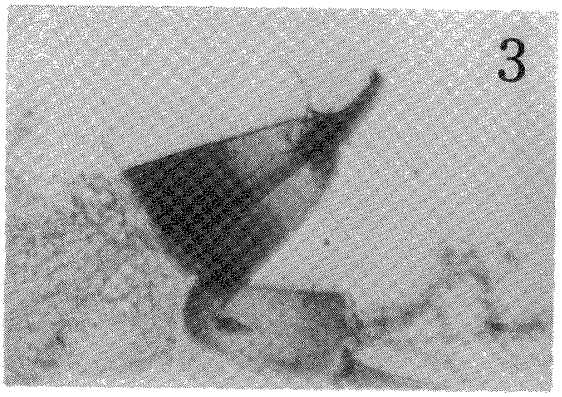
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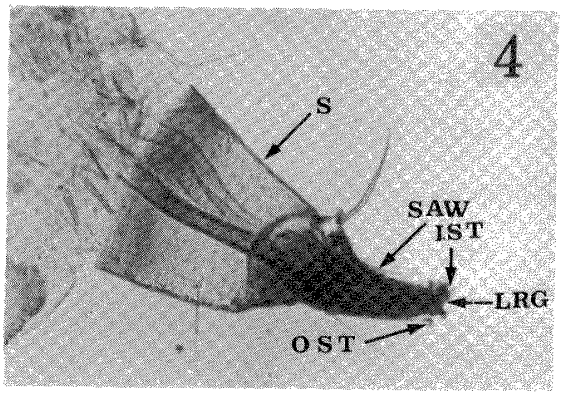
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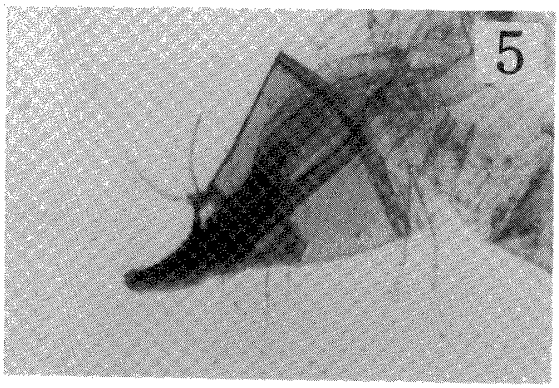
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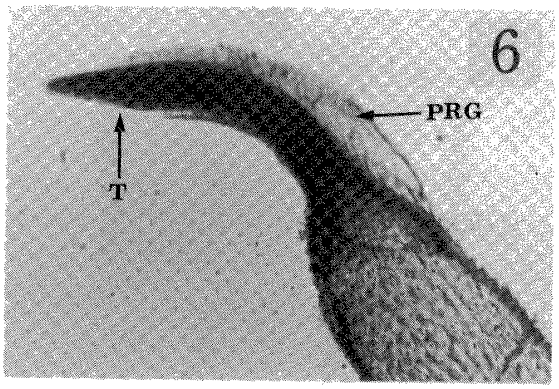
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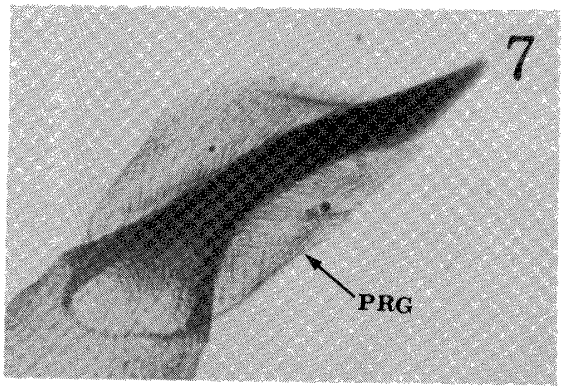
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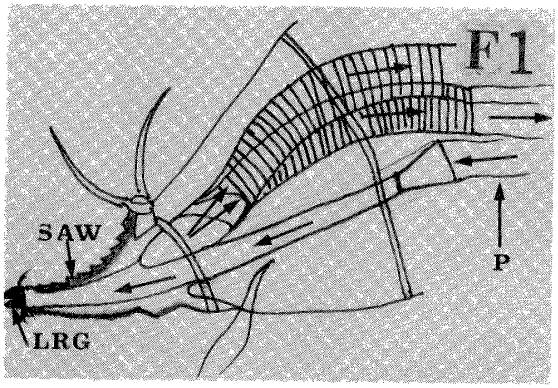
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