STUDIES IN FILARIASIS.

A thesis submitted for the degree of
Doctor of Medicine of the University of Glasgow.

by

JAMES ANDERSON McFADZEAN, M.B.,Ch.B.(Glas.)

Colonial Medical Research Student, 1949-1951.

Member of the Scientific Staff of the Medical Research Council.

From :-

The National Institute for Medical Research, London,
and
The Medical Research Council Laboratories, Gambia, B.W.A.

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

INTRODUCTION.

This thesis reports work undertaken by the candidate while studying filariasis in the department of chemotherapy-biological at the National Institute for Medical Research, London, and at the Medical Research Council laboratories, Gambia, British West Africa. For convenience, the experimental work is divided into three parts. Part 1 describes experiments on filariasis undertaken at the National Institute for Medical Research. Part 2 describes an investigation which was undertaken in the laboratory with monkeys, and also a similar investigation which was later undertaken with patients in West Africa. Part 3 describes investigations of filariasis in West Africa.

The work described in Part 1, Section B of the thesis is based on microscopical techniques developed by Mr.J. Smiles of the Optics department of the National Institute for Medical Research. The estimations of blood phosphorus described in Part 2, 1, were undertaken by Mr.S. Crowther.

NOTE.

Figures and tables are inserted after the summary in each section, and in certain cases they can be folded out and examined while reading the text.

CONTENTS.

CONTENTS (cont)

CONTENTS (cont)

P A R T 1.

Investigations of Experimental Filariasis in the Laboratory.

PART 1

SECTION A

Immunity in Filariasis of Experimental Animals.

Introduction.

This section describes an investigation of immune bodies in the filarial infections of experimental animals. A summary of the ways in which immune bodies may be detected, is given at the beginning of the discussion. For a number of reasons, all the points given in the summary were not investigated, and for simplicity the experimental work has been classified into

A. Experiments in vitro.

B. Experiments in vivo.

C. Skin tests.

The filarial infections which were available for study at the National Institute for Medical Research, were Dirofilaria repens in dogs, a filarial worm in monkeys, and Litomosoides carinii in cotton rats. For all practical purposes, these infections are non-pathogenic; as is pointed out in the discussion this non-pathogenicity probably had an important influence on the results obtained. Microfilariae of Dirofilaria repens and of the monkey parasite developed satisfactorily to infective larvae in Aedes aegypti mosquitoes and migrated eventually into the proboscis; microfilariae of Litomosoides carinii developed in the mite Liponyssus bacoti. Dried Dirofilaria immitis from dogs in Borneo were obtained

from Sir Neil H. Fairley for use as antigen.

Methods.

As a first step in this investigation rabbits were immunised with filarial antigen so as to provide sera known to contain large amounts of antibodies. Six rabbits of approximately the same age and weight were given a course of subcutaneous injections of Dirofilaria immitis antigen. The antigen was prepared by grinding the dried worms in a tissue grinder, and making a 1/500 suspension in sterile Ringer's solution. They were not extracted lest any fraction of immunological importance be removed. Rabbits 1 and 11 received 1 mg./Kg. body weight, rabbits 111 and 1V received 10 mgs./Kg., and rabbits V and V1 received 20 mgs./Kg. The injections were made subcutaneously on the inner sides of the thighs. The first two injections were made at an interval of 3 weeks, and subsequent injections were given every 10 days. The rabbits were bled at intervals, the blood allowed to clot at 37°C for 1 hour and then centrifuged, and the serum removed. The antibody titres

of the sera were estimated by means of the haemolytic complement fixation test, using the method described by Fulton and Dumbell (1949) with drops of the fluids on perspex plates. The dilutions of the sera employed were 1/4, 1/8 and 1/16. The antigen for the test was prepared by extraction of the dried worms with saline, the lipoids having previously been removed by acetone and ether. The complement was obtained from guinea pigs. Five weeks after the first injection, little complement was fixed by any of the rabbits. At seven weeks, the two rabbits given 10mgs./Kg. of antigen had high titres in a 1/4 dilution, and one of the rabbits given 20 mgs./Kg. had a similar titre. The rabbits given 1 mg./Kg., and one of the rabbits given 20 mg./Kg. fixed only small quantities of complement and their sera were not employed for further experimental work. The normal control rabbit serum fixed only insignificant amounts of complement. The sera were kept at a temperature of -2°C. until required, but were not stored for a long period.

Experiments were undertaken using these immune rabbit sera, D. immitis and L. carinii antigens, and the filarial infections which were available. It is regretted that homologous antigen and antiserum were not always available, e.g. D. immitis was the only antigen available in quantity and D. repens the only parasite available in dogs. As reactions in filarial infections

are of a group character (Rhodain and Dubois (1931, 1932); and Mohr and Lippelt (1940)), antigens as closely allied as the above would detect any antibodies present.

RESULTS.

A. EXPERIMENTS IN VITRO.

(1) With Infective Larvae. Infective larvae of D. repens and of the monkey filaria were obtained by allowing Aedes aegypti mosquitoes to feed on an infected animal anaesthetised with sodium pentobarbitone. When the larvae were mature, the mosquitoes were dissected in a drop of saline and the larvae were picked up with a fine capillary pipette and transferred to the serum to be tested for immune bodies, a drop or two of which had been placed on a sterile slide. The serum was then walled off with a ring of melted vaseline and a sterile cover glass was applied. Small quantities of penicillin and streptomycin were added to the preparations to inhibit growth of bacteria. The slides were kept at room temperature and examined at intervals.

In the first experiments fine granular precipitates formed on the larvae along their entire lengths with clumps at the head and tail. These were most marked at 18 hours, and were more abundant in immune rabbit serum than in normal serum. The amount of precipitate varied during each period of observation,

becoming detached from, and reattached to, the larvae with their writhing movements. In these early preparations, a varying amount of debris was present which could in the first place have become adherent to the larvae. Attempts were then made to obtain cleaner preparations. Each time a larva was removed from a mosquito it was transferred into two washes of sterile saline, before putting it into the serum to be tested. The preparations obtained in this manner were free from debris. The precipitates observed in the earlier prepartions were now absent in the majority of cases. Several series of experiments were performed using rabbit sera, and also dog and monkey sera with their respective infective larvae. No precipitates formed on the infective larvae in the immune sera. The larvae in these preparations survived for varying periods, but could live for approximately one week. The survival times of the larvae in immune and normal sera were not significantly different.

(11) With Adult Worms. The only adult filarial worms available were L. carinii. Males and females were incubated in Carrel flasks at 37°C. with immune and normal rabbit sera. They survived for 3-4 days just as well in serum from immune rabbits as in that from normal rabbits, and their motility was equally good in the two

cases. No precipitates were observed round the worms. This result was confirmed by repeated experiment.

(111) With Microfilariae. a). Microfilariae of L. carinii were incubated at 37°C. under sterile conditions with immune and normal rabbit sera, in small glass flasks sealed with sealing wax, and they were examined at intervals with the inverted microscope according to the technique described by Hawking, Sewell and Thurston (1950). The survival times of the microfilariae in different experiments varied considerably; in some cases they lived for 7-8 days, while in others they died within 2-3 days. The nature of the serum, whether from normal or immunised rabbits had no constant effect on the time of survival, nor were any other differences noted suggesting an immune reaction e.g. agglutination of the microfilariae in the immune serum compared to the control. The microfilariae were taken out of the tubes after death and examined with a higher magnification than was possible with the inverted microscope. No differences were noted between those incubated with immune and those with normal sera.

b). Adhesin reaction. An attempt was made to demonstrate this phenomenon which was described by Pandit et al. (1929). (The serum to be tested for immune bodies is added to diluted citrated

blood containing microfilariae; a positive test is shown by the white blood cells adhering to the microfilariae). Sera from a dog whose peripheral blood had become free from microfilariae, from a dog whose peripheral blood contained microfilariae, from a normal dog and from immunised and normal rabbits, were tested against blood from a dog with a high microfilaria count. Only one or two leucocytes were seen adhering to microfilariae in one preparation, and that had been made with the serum from the dog whose peripheral blood had once contained microfilariae, but the reaction was not sufficiently marked to be significant.

B. EXPERIMENTS IN VIVO.

(1) Passive Immunity. Two cotton rats infected with L. carinii were each given a total of 6 cc. of immune rabbit serum injected intraperitoneally in 9 and 10 daily doses respectively. Microfilaria counts were taken before and after the course of injections, and the rats were autopsied one week after the last injection. In both cases the microfilaria count decreased, but this could have been due to the variation found in the counts in L. carinii infections. In one rat which had a long standing infection, 55% of the adult female worms were dead, but in the second rat all the adults were alive at autopsy, so the result is not significant.

11). Active Immunity. a). Prophylactic value of D. immitis antigen. Ten normal cotton rats were given a course of injections of 20 mg./Kg. of D. immitis antigen as described previously for the rabbits. Eight weeks after the first injection, sera (obtained by heart puncture of the rats) were subjected to the complement fixation test. Insignificant amounts of complement were fixed compared to those fixed by the rabbits. The test was repeated three weeks later, and again only insignificant amounts of complement were fixed. The rats were then put into tanks along with normal untreated rats so as to expose them to infection by L. carinii as described by Hawking and Sewell (1948). After the incubation period, blood smears were taken from the immunised and control rats. Of the immunised rats, 7 out of 8 had microfilariae in their blood, and of the control rats, only 4 out of 10 had microfilariae, showing that the course of injections of antigen had had no prophylactic effect.

b). Therapeutic effect of D. immitis antigen. Three pairs of cotton rats infected with L. carinii, each pair having been infected at the same time and in the same tank, and all having microfilariae in their blood, were used for this experiment. One rat of each pair was given a course of subcutaneous injections of D. immitis antigen (20 mg./Kg.)

every ten days for a total of nine injections. Weekly microfilaria counts were taken and the rats were autopsied one week after the last injection. The number of adults found was recorded and their lengths measured - see Table I. The worms of the immunised rats did not differ in state or length from those of the control rats. There was a great weekly variation in the microfilarial counts in both groups of rats but there was no general trend in either group.

Skin Tests.

Skin tests were performed on the animals available, using D. immitis and L. carinii antigens. The dried worms were prepared as described above for the immunisation of rabbits. Ringer's solution was used as a control. Dogs, monkeys and rabbits were examined, but not cotton rats as their skin is not satisfactory for intradermal injection.

1. Dogs. Three dogs (3, 10 and 11) infected with D. repens were examined. Dogs 10 and 11 had microfilariae in their blood; both were light infections and both had been infected on the same date. Dog 3 was an old infection and at the time of the skin tests microfilariae were no longer present in the blood. Three normal dogs, A, B and C were used as controls. The dogs were anaesthetised with intravenous sodium pentobarbitone,

and the shaved skin of the abdomen was used for all tests. The dilutions of antigen used were 1/2,000, and 1/8,000 and 0.05 cc. of each was injected. The size of the wheal immediately after the injection was measured and if it varied greatly from the average figure, the injection was repeated at a different site. Each set of injections was repeated more than once in each animal. Table 11 shows the measurements of the wheals produced in the dogs with D. immitis antigen. The wheals produced by L. carinii antigen were of the same order. After 30 minutes the wheals gradually faded away. The wheals produced by Ringer's solution did not increase in size, but remained stationary at first and then gradually decreased. In all the dogs, infected and normal, the wheals produced by the antigen were well defined. Erythema is not recorded because of the difficulty of observing it on the dark skin of some of the dogs. In the three infected dogs, the wheals produced by the antigen were always larger than in any of the control animals; this was more marked with dogs 3 and 10, than with dog 11, As the smallest wheal in an infected animal (dog 11) approximates in size to the largest wheal in a normal animal (dog B), evaluation of the test is difficult. With such a small number of animals it is not possible to deduce any limiting figures. No delayed reactions were observed.

2. Monkeys. In monkeys with filariasis, it was

not possible to produce well defined wheals. The wheals which were produced rapidly disappeared, and no immediate or delayed reactions were produced with the above dilutions of antigens, or with a 1/500 dilution.

3. Rabbits. As with the monkeys, it was not possible to produce well defined wheals and the injection of antigen intradermally into immunised rabbits produced no significant reaction compared to normal animals.

Immune sera from rabbits III, IV and VI were used in an endeavour to demonstrate a Prausnitz-Kustner reaction. 0.1 cc. of immune serum was injected intradermally into a normal rabbit and 30 minutes later 0.1 cc. of 1/500 D. immitis antigen was injected into the same site. The controls consisted of normal rabbit serum with antigen injected into the same site half an hour later, of both sera alone, and of the antigen alone. All these immune rabbit sera had been shown to have a high titre of complement fixing antibodies. Serum from rabbit III + antigen produced no reaction, serum from rabbit IV + antigen produced a wheal with pseudopodiae of 3 x 4.5 cms. at 5½ hours which had disappeared at 23 hours, while the only control producing a reaction was the immune serum alone which produced a wheal 2 x 2 cms. Serum from rabbit VI + antigen produced a wheal measuring 3.5 x 2 cms. at 5 hours which disappeared at 22 hours while that produced by the normal serum and antigen measured 2.5 x 1.5 cms. These reactions

are not very striking, but they provide slight indication of the presence of antibodies in the sera of rabbits IV and VI.

Histamine Reactions in Dogs, Monkeys and Rabbits.

In view of the failure to demonstrate immediate skin reactions in monkeys and rabbits, their reactions to intradermal injection of histamine were investigated. 0.05 cc. of histamine acid phosphate in dilution of 1/1,000,000, 1/100,000, 1/10,000 and 1/1,000 were each injected intradermally into a dog, a monkey and a rabbit. In the dog, all dilutions produced wheals which rapidly increased in size and which were similar to those produced by the injection of antigen. In the monkey and rabbit the wheals produced by the injection of fluid gradually faded. The immediate response to antigen injected intradermally into an immune animal is believed to be due to the liberation of histamine. My results (described above) are in accordance with this belief, since responses were obtained to antigen in dogs, which react to histamine with wheal formation, but not in monkeys and rabbits, which do not react to histamine.

DISCUSSION.

It is almost certain that filarial infections

produce some kind of immune response in the host, since

1. Practically all kinds of infections produce immune responses.

2. In populations exposed to heavy filarial infections, the infection rate does not always increase pari passu with age, although the highest rates are often found in the older age groups.

3. There is much evidence that filarial lymphangitis and Calabar swellings are antigen-antibody reactions.

However, the demonstration of specific immune reactions in the laboratory has proved difficult.

The possible ways for demonstrating such reactions in the host-parasite relationship may be summarised as follows (after Culbertson, 1941):

1. <u>With the host</u>. a). Exposure of the host to reinfection and demonstration of its ability to resist infection.

b). Injection of antigen intradermally, and demonstration of immune reactions in the form of wheals, oedema and erythema.

11. <u>With serum from the host</u>.

a). Passive transfer of immunity by the injection of the host's serum into a non-immune animal.

b). Tests to detect any of the following immune responses:- agglutinins, precipitins, complement fixing antibodies, adhesins.

111. With the parasite. Demonstration in the immune host of :-

a). A smaller number or smaller size of parasites developing.

b). The failure of maturation of parasites.

c). A briefer persistence of the parasites.

Analysed according to the above summary the facts about immune responses to filarial infections, as known from the above experiments and from the literature, are as follows

1. REACTIONS WITH THE HOST.

a). Resistance to reinfection.

In unpublished experiments at the National Institute for Medical Research, Miss W.A.F.Webber treated cotton rats infected with L.carinii with cyanine and hetrazan to kill the adult worms and microfilariae, and subsequently re-exposed them to infection. There was no evidence of immunity since these rats became as heavily infected as the normal cotton rats.

Similarly in the experiments described above

cotton rats were immunised with D. immitis antigen. They received a course of injections similar to that given to the rabbits, which had produced high titres of complement fixing antibodies. For some obscure reason they did not produce any complement fixing antibodies. When exposed to infection with L. carinii, these rats developed a higher infection rate than the control rats, showing that the injections of antigen had had no protective value.

b). Skin tests.

The skin test as a diagnostic aid in filariasis was described by Taliaferro and Hoffman (1930) and Fairley (1931), using D. immitis antigen. Since then numerous papers have appeared on the subject. The dilutions of antigen, usually D. immitis, have varied from 1/200 (Taliaferro and Hoffman, 1930) to 1/100,000 (Wharton and Stelma, 1946). Saunders et al. (1946) gave a review of the literature to date. The specificity of the skin test is doubtful. Bozicevich and Hutter (1944) concluded that a dilution of 1/8,000 in 0.01 cc. amounts was fairly specific. Using this dilution, however, Wharton and Stelma (1946) and Behm and Hayman (1946) reported 6% false positive reactions, and the former authors subsequently used a dilution of 1/100,000 injecting 0.02 cc. which gave only 2% false positives in controls and 20% false negatives in cases of filariasis. Wharton (1947) gives further reports on 215 cases tested in

Georgetown using this high dilution. He reported 89.8% positive reactions, 5.1% negative and 5.1% indeterminate. Zarrow and Rifkin (1946) tested varying dilutions from 1/200 - 1/16,000 of D. immitis antigen. They found that 65 cases of hookworm infestation all gave negative skin responses in dilutions over 1/2,000. Microfilariae of D. immitis have also been used as antigen. Franks (1946) demonstrated two antibodies, one specifically directed against microfilariae and the other against the adult worms. Franks et al. (1947) examined 320 negatives in Okinawa and obtained 61% positive reactions, and noted that most of the negative reactions were in cases with microfilariae in the peripheral blood, and that more negative reactions were found in the older age groups. He concluded that the most successful case for testing was where the infection was slight without desensitisation of the host, and that antigen prepared from microfilariae was more specific than that prepared from the adult worm. False positives were noted with Trichinella spiralis antigen. Warren (1947) also recorded cross reaction with T. spiralis with the complement fixation test. Lampe (1950) after a careful investigation of the skin test with 1/4,000 dilution of D. immitis antigen concluded that there is a great overlap between negative and positive responses with a variety of dubious responses and that the test "cannot serve to arrive at a diagnosis of clinical wuchereriasis".

With the animals examined here, three dogs infected with D. repens gave mild positive reactions, infected monkeys gave no reaction, and hyperimmunised rabbits gave no direct reaction and only a mild Praunsnitz-Kustner reaction. Bruynoghe (1939) investigated the antigenic properties of microfilariae of D. immitis. He found that the microfilariae injected into rabbits and guinea pigs did not produce any appreciable skin sensitisation to specific extracts. Wharton et al. (1947) used 1/100 dilutions of D. immitis in sensitised rabbits, and obtained a wheal reaction with a maximum at 18 - 24 hours, but 1/1,000 gave little or no reaction. There was no cross reaction with ascaris; filarial antigen produced a strong reaction in rabbits sensitised with Trichinella antigen, but rabbits sensitised to filarial antigen did not react to Trichinella.

Calabar swellings in Loaiasis are generally considered to be due to an antigen-antibody reaction, but the exact antigen factor is not known.

Augustine and Lherisson (1946) have suggested that many of the false positives in human skin tests may be due to sensitisation to non-human filarial worms. Though filarial worms are very limited in the hosts in which they can develop it is conceivable that mosquitoes infected with non-human infective larvae may deposit the larvae

in the skin of man and this would be sufficient to produce sensitisation. I have recently allowed Aedes aegypti mosquitoes with mature infective larvae of D. repens to feed on 5 day old mice. Infective larvae have been found under the skin of the mice 3 days after the mosquitoes had fed and there was no reaction round them (see figure). As the infective larvae can thus survive for several days, some of the false positive reactions in man may well be due to the infective larvae of other species of Filariae which have failed to establish themselves.

Thus, the skin test can be of value in the examination of large groups to determine approximately the incidence of filariasis, but it is of little value as a diagnostic aid in an individual case.

11. REACTIONS WITH SERUM.

a). Passive Transfer of Immunity.

In the experiments performed above no evidence could be obtained that serum from an immunised animal had any action in killing the worms in vivo.

b). 1. Agglutinins. This reaction was investigated in the experiments with immune sera and microfilariae in vitro, but there was no indication of the presence of agglutinins.

2. Precipitins. A precipitate at the junction of antigenic fluid and immune serum has been demonstrated by Culbertson et al. (1944) who tested 81 patients living in an endemic area of filariasis for a year and obtained 73% positive reactions. Several papers have described precipitins in immune sera with nematode infective larvae. Sarles (1938) reported precipitates round the infective larvae of Nippostrongylus muris incubated with immune serum. He described four types of precipitates (1) cuticular (2) excretory (3) oral and (4) intestinal. He found no difference in the survival time of the larvae in immune and normal sera. In the present work with filarial infective larvae no precipitates were observed in immune sera and no differences were noted in the survival times of the larvae.

3. Complement fixing antibody. This test has been used in laboratories as an aid to the diagnosis of filarial infections since it was described by Fairley (1931). In the above experiments, it was shown that repeated injections of antigen produced high titres of complement fixing antibodies in rabbits but not in cotton rats. Tests for complement fixation have been employed for some time in this laboratory and have been successful in demonstrating complement fixing antibodies in cotton rats infected with L. carinii and also in a number of patients.

With monkeys and dogs with filarial infections difficulties were encountered, and the first attempts to demonstrate complement fixing antibodies in these animals were unsuccessful. At this point it was learned that Dr. W. Minning of the Tropeninstitut, Hamburg, was conducting a detailed investigation of the subject. To avoid duplication of his work, my experiments were discontinued and a collaboration was arranged by which sera from animals in this laboratory were supplied to him for study. The results, some of which have been confirmed by me, will be published by Dr. Minning in due course. Although complement fixing antibodies can be demonstrated in infected monkeys and dogs, the subject is ~~apparently~~ complicated and anomalous results are often obtained. Some animals with long standing and heavy infections may fail to show antibodies, while positive reactions may be given by uninfected animals. The explanation of these anomalies is not yet known.

Bruynoghe (1939) failed to demonstrate complement fixing antibodies in rabbits and guinea pigs immunised with microfilariae of D. immitis. Bozicevich and Hutter (1944) failed to demonstrate complement fixing antibodies in 25 suspected cases of filariasis which gave a positive skin test with D. immitis antigen. They then immunised rabbits with the residue of the antigen and demonstrated complement fixing antibodies in the rabbit

sera. Warren et al. (1946) reported that they could demonstrate complement fixation in cases of Wuchereria bancrofti using D. Immitis antigen, but not with an antigen prepared from microfilaria bancrofti. Both antigens were used to immunise rabbits and they were able to fix complement in the rabbits using both antigens. Warren (1947) immunised rabbits with D. immitis, W. bancrofti and Trichinella spiralis antigens; these three antigens and L. carinii fixed complement in the hyperimmune sera from all three rabbits.

As regards clinical results, Fairley (1931) examined the sera of 70 patients. He reported no false positives although some cases with a negative result were infected with other helminths, but he observed negative reactions in three old cases of W. bancrofti infection. Fairley (1932) reported that all cases of Loa loa infections examined to date had given a positive result. Lloyd and Chandra (1933) reported 23 positive reactions out of 89 cases using D. immitis antigen, and three false positives in guinea worm infections. Bozicevich and Hutter (1944) reported negative results in 25 suspected cases of filariasis which gave positive skin reactions. Culbertson et al. (1944) reported 76.6% positive complement fixation results using L. carinii antigen in a series of 77 patients resident in a filarial area for one year. Goodman et al. (1945) using D. immitis antigen obtained a

positive result in 66% of 143 cases of filariasis among American soldiers evacuated from the South Pacific, but also 16 - 25% positive reactions in control subjects. Bozicevich et al. (1947) reported positive results in 60 cases of Onchocerca volvulus using a homologous antigen and in 49 out of 50 cases with D. immitis antigen.

Thus, while ~~never demonstrable~~ complement fixing antibodies can be demonstrated in a number of cases of filariasis, there is a similar overlap of positive and negative resilts as in the skin test.

4. Adhesins. The adhesin phenomenon was described in filariasis by Pandit et al. (1929). An attempt to demonstrate it here was not successful. Yokogawa et al. (1939) stated that the reaction is dependent on the blood group of the serum to be tested and of the serum containing microfilariae which is used as the reagent. They believe that where the reaction occurs in less than 2 days, it is due to a difference in blood groups, and where the blood groups are the same the reaction did not occur before the 5th day. This reaction has also been described in trypanosomiasis (Duke and Wallace, 1930).

5. There may also be added to this group observations on the survival time of the parasites in serum in vitro with or without any of the above reactions.

Arnold and Duggan (1937) immunised rabbits by injecting macerated D. immitis parasites weekly for three weeks (dose not stated) and found that the sera from these animals were markedly lethal to microfilariae of D. immitis. Murata (1939) also immunised rabbits with D. immitis antigen and found that the survival time of microfilariae transferred into the immunised rabbits was somewhat shorter than the control. This is difficult to assess as 90% of microfilariae transfused into an animal quickly disappear (Hawking 1940).

The results of the present series of experiments show that immune sera have no significant effect on the length of survival in vitro as compared with normal sera of adult filarial worms, microfilariae and infective larvae.

III.

Experiments with the development of filarial worms within the host are not practical, as their cycle in the host is usually not fully known and it is difficult to locate most species adult filarial worms at autopsy.

General Conclusions.

The results described in this paper and those reviewed above can now be considered in their general bearing on the host parasite relationship. The ideal of

the parasite is to live and let live and the more closely this state of mutual toleration is reached, the more successful is the parasite. In the same way, the more perfect the adaptation of the parasite, the less is the immune response which it provokes. Other helminths e.g. Nippostrongylus, Ancylostoma or Schistosoma cause appreciable lesions, and the immunological reactions which they provoke, are readily manifested. In the case of filarial infections (as is shown by the above work) immune responses are difficult to demonstrate and are slight in extent. On the other hand, the filarial worms are known to be highly successful in their adaptation to their hosts since most species of these helminths produce only mild and infrequent pathological lesions. The particular filariae studied in the present work are especially well-adapted as is shown by the absence of harmful effects. Thus D. repens in the dog and the new dirofilarial worm in the monkey cause no appreciable reaction either to the adult worm or to the microfilariae; and L. carinii lives in the pleural cavities of the cotton rat without producing more than a mild cellular exudate. Accordingly the failure to demonstrate well-marked immunological reactions to filarial infections, although at first sight disappointing, is really in accordance with the general principle described above, viz. that the more perfect the adaptation of the parasite, the less is the immune response of the host.

SUMMARY.

1. An investigation was made of the immune responses produced in three laboratory filarial infections, viz. Dirofilaria repens in dogs, a species of filarial infection in monkeys and Litomosoides carinii in cotton rats.

2. Immune sera with a high titre of complement fixing antibodies were obtained by immunising rabbits with Dirofilaria immitis antigen.

3. These immune sera and sera from infected animals showed only a slight or no immune reactions in vitro against filarial infective larvae, against adults of L. carinii and against microfilariae. Immune serum had no therapeutic action upon infections by L. carinii.

4. Agglutinins, precipitins and adhesins could not be demonstrated.

5. D. immitis antigen had no prophylactic or therapeutic action upon infections by L. carinii.

6. Weak immediate skin reactions were obtained in dogs infected with D. repens, but not in any of the other animals. A doubtful Prausnitz-Hustner reaction was observed with immune rabbit sera.

7. A review is given of the available information

about immunity in filarial infections. It is concluded that filarial infections do produce a certain immune response but this is small in extent and difficult to demonstrate. The smallness of the immune response may be correlated with the great success of filarial parasites in adapting themselves to their hosts, and it is a good example of the general principle that the more perfect the adaptation of the parasite, the less is the immune response of the host.

TABLE I

The effects of subcutaneous injections of *Dirofilaria immitis* antigen on *Litomosoides carinii* infections in cotton rats.

Number of cotton rat.	Number of adult worms.		Average length of adult worms (cms)	
	Male	Female	Male	Female.
No. 2701 (Immunised)	21	18	3·3	8·2.
No. 2700 (Control)	3	2	3.4	6·9.
No. 2706 (Immunised)	27	21	2·6	9·4
No. 2704 (Control)	17	17	2·7	7·8.
No. 2695. (Immunised)	61	41	2.1	7.5
Control died.	—	—	—	—

TABLE 2.

TABLE II.

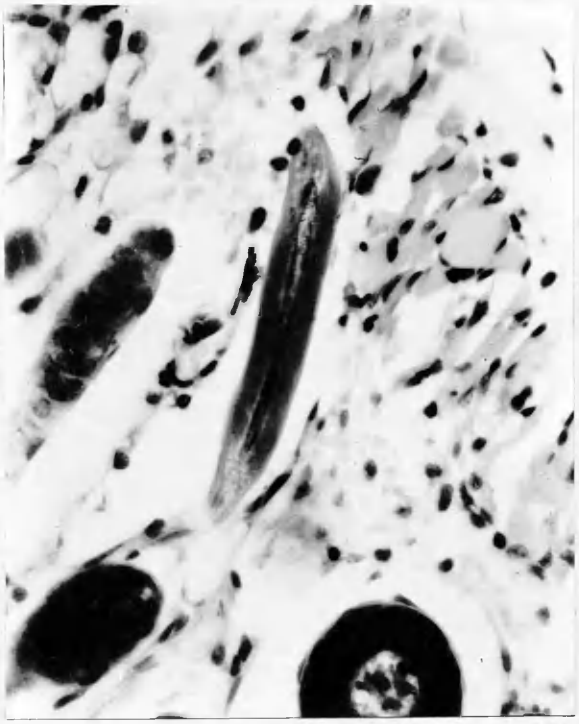
The size of wheals produced in dogs with intradermal injection of 0.05cc of filarial antigen.

Number of dog.	Size of wheals in millimetres.								
	10 minutes.			20 minutes.			30 minutes.		
	D. immitis 1/2,000.	D. immitis 1/8,000.	Ringer.	D. immitis 1/2,000.	D. immitis 1/8,000.	Ringer.	D. immitis 1/2,000.	D. immitis 1/8,000.	Ringer.
3	10×15	12×13	7×8	19×19	15×16	7×7	20×20	17×18	6×8.
10	14×16	12×15	7×8	18×18	17×17.	7×9	19×20	18×18	8×8.
11	10×12	10×12.	9×11	13×13	13×13	10×10	13×13	13×15.	10×10.
A	8×10	9×10	8×10	9×9	10×10	7×11	9×9	9×10.	6×6
B	8×8	8×8	6×6	11×11	11×11	6×7	12×12.	11×12.	5×6
C.	8×10.	7×10.	5×7	9×12	8×11.	5×7.	9×12.	9×12	5×7.

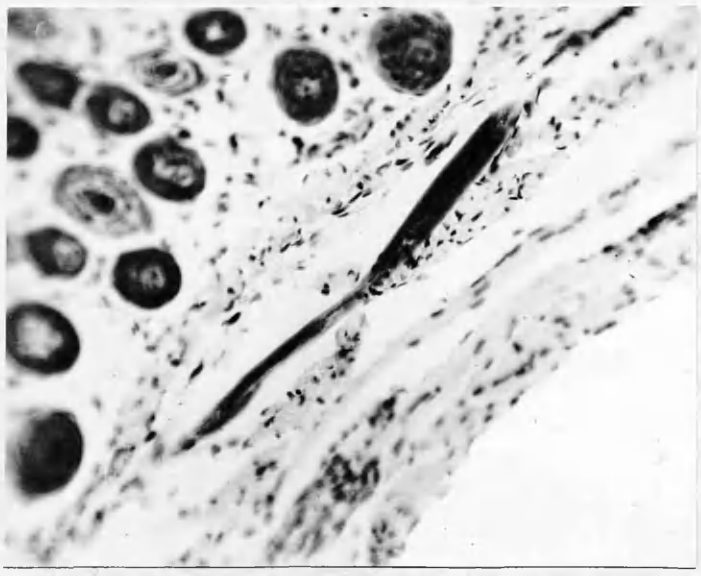
Note. All the wheals with Ringers were indistinct at 30 minutes.

FIGURE

Showing infective larvae of D. repens under the skin of baby mice 3 days after having been bitten by infected mosquitoes.



x 450



x 200

REFERENCES.

Arnold, Jr., J.G. & Duggan, T.L. (1937). J.Parasit.,23,561.

Augustine, D.L. & Lherisson, C.(1946). Amer.J.Hyg., 43, 38.

Behm,A.W.,& Hayman, Jr.,J.M.(1946). Amer.J.Med.Sci.,211,285.

Bozicevich,J.& Hutter,A.M.(1944). Amer.J.trop.Med., 24, 203.

Bozicevich,J.,Donovan,A.,Mazzotti,L.,Diaz,A.F.,& Padilla,E. (1947). Amer.J.trop.Med., 27, 51.

Bruynoghe,G. (1939). Ann.Soc.belge Med.trop., 19, 335.

Culbertson,J.T. (1941). Immunity Against Animal Parasites. New York: Columbia University Press.

Culbertson,J.T.,Rose,H.M.,& Demarest,C.R. (1944). Amer.J.Hyg., 39, 156.

Duke,H.L.,& Wallace,J.M. (1930). Parasitology, 22, 414.

Fairley,N.H. (1931). Trans.R.Soc.trop.Med.Hyg., 24, 635.

Fairley,N.H. (1932). Trans.R.Soc.trop.Med.Hyg., 25, 220.

Franks,M.B. (1946). J.Parasit., 32, 400.

Franks,M.B.,Chenoweth,Jr.,B.M.,& Stoll,N.R. (1947). Amer.J.trop.Med., 27, 617.

Fulton,F.,& Dumbell,K.R., (1949). J.gen.Microbiol., 3, 97.

Goodman,A.A.,Weinberger,E.M.,Lippincott,S.W.,Marble,A., & Wright,W.H. (1945). Ann.intern.Med., 23, 832.

Hawking,F., (1940). Ann.trop.Med.Parasit., 34, 121.

Hawking,F.,& Sewell,P. (1948). Brit.J.Pharmacol., 3, 285.

Hawking,F.,Sewell,P.,& Thurston,J.P. (1950). Brit.J.Pharmacol 5, 217.

Lampe,P.H.J., (1950) Docum.neerl.et Indones.morbis.trop., ~~1950~~, 2, 193.

Lloyd,R.B.,& Chandra,S.N. (1933). Ind.J.med.Red., 20, 1197.

Mohr,W.,& Lippelt,H. (1940). Klin.Wschr., 19, 157.

Murata,H. (1939). Fukuoka Acta med., 32, 54.

Pandit,C.G.,Pandit,S.R.,& Iyer,P.V.S. (1929). Ind.J.med.Res., 16, 946.

Rodhain,J.,& Dubois,A. (1931). Rev.belge Sci.med., 3, 613.

Rodhain,J.,& Dubois,A. (1932). Trans.R.Soc.trop.Med.Hyg., 25, 377.

Sarles,M.P. (1938) J.infect.Dis., 62, 337.

Saunders,G.M.,Bianco,A.A.,& Jordan,W.S. (1946). Nav.med.Bull., Wash., 46, 1242.

Taliaferro,W.H.,& Hoffman,W.A. (1930). J.prev.Med., 4, 261.

Warren,V.G.,Warren,J.,& Hunter,G.W. (1946). Amer.J.Hyg., 43, 164.

Warren,V.G. (1947). Amer.J.Hyg., 45, 299.

Wharton,D.R.A.,& Stelma,T. (1946). J.infect.Dis., 78, 49.

Wharton,D.R.A. (1947). J.infect.Dis., 80, 117.

Wharton,D.R.A.,Cuervo,C.,& Moyer,A.W. (1947). J.infect.Dis., 81, 254.

Yokogawa,S.,Kobayasi,H.,& Yosino,T. (1939). Acta.Jap.Med. trop., 1, 185.

Zarrow,M.,& Rifkin,H. (1946). Amer.J.med.Sci., 211, 97.

P A R T 1 (continued)

SECTION B

Phase-contrast Microscopy and Ultra-violet Micrography in the Investigation of the Biology of Filarial Worms

Introduction.

The introduction into the laboratory of the filarial infection Litomosoides carinii in cotton rats for screening drugs for antifilarial activity (Culbertson and Rose, 1944) constituted a major advance in the experimental study of filariasis. Prior to this, the laboratory filarial infections employed were Dirofilaria immitis and Dirofilaria repens in dogs, both of which have the disadvantagesof long incubation periods, and that relatively few dogs can be housed in one institution. By the technique described by Hawking and Sewell (1948) several hundred cotton rats may be housed in one room and a constant supply of infected animals made available by the trahsmission of the infection through the mite Liponyssus bacoti. The incubation period of this infection is 51 days. The adult worms live in the pleural cavities and mediastinum of the cotton rat producing little effect on the host. The females give birth to microfilariae which find their way into the peripheral blood stream.

It is of fundamental importance that the biology of the parasite be properly understood occupying as it does a major role in the search for antifilarial drugs. A gap in the knowledge of all filarial infections is the mode of development of the microfilariae. Penel (1904) described the stages in development in rough outline with simple diagrams. Further investigation of the subject could have been made with modern microscopical technique using fixed and stained material but the results obtained are not satisfactory due

largely to the thickness of the specimens. Preliminary examination of a number of specimens by phase-contrast microscopy revealed that this method could be of value, and a series of investigations were therefore undertaken.

The phase-contrast equipment used throughout was the standard equipment as supplied by Cooke-Troughton and Simms Ltd.

METHODS.

Cotton rats infected with L.carinii with microfilariae in the peripheral blood, were killed at different times by coal gas. The skin and pectoral muscles of the rats were reflected from the chest wall, the thorax opened and the adult worms removed from the pleural cavities and mediastinum and placed in Ringer's solution. Washing in Ringer was essential to obtain clean preparations, otherwise the cells of the plearal fluid of the cotton rat and other debris impaired the image and did not allow adequate flattening of the specimens for examination. An adult female worm was stretched out on a glass slide with a few drops of Ringer and examined with a binocular dissecting microscope.

P A R T 1 (continued)

SECTION B

1. The development of the microfilariae of Litomosoides carinii as observed by phase-contrast microscopy.

PREPARATIONS

Under a binocular dissecting microscope the adult female worm was incised at intervals along its length with a dissecting needle and the contents of the uterus which emerged from each incision were immediately picked up with fine capillary pipettes using only capillary traction. There was no necessity to dissect out the uterus. By this method large numbers of larvae at each stage of development from the ova to microfilariae were obtained, the earliest forms from the region of the tail and the most mature from near the head. The technique of preparing the specimens for examination consisted of pouring a thin layer of melted agar in Ringer's solution on a glass slide, and when the agar had set, placing a drop of the suspension of the organisms onto its surface. A cover glass was gently lowered on to the drop, and in the case of the earlier larvae the weight of the cover-glass supplied sufficient pressure to flatten the cells to obtain adequate representation of the structures. The strength of the agar may be varied by dilution with Ringer's solution and since it acts as a soft pad, the degree of flattening may thus be controlled. For this investigation 1 per cent agar in Ringer's solution was used. From the observation that red blood corpuscles placed on this agar did not undergo any changes over a considerable

period, it was possible to exclude any morphological changes occurring in the larvae, due to the agar. A number of preparations were also made on ordinary slides to ensure further that no changes were produced by the agar.

Please fold out in turn
pages 52, 54, and 56.

OBSERVATIONS

Fig.1 shows a single cell which has been flattened to show detail. The oval nucleus lies parallel to the long axis of the cell and contains diffuse nuclear material in clear nuclear sap. The cytoplasm contains large dark granules at the periphery, between them and the nucleus are short filamentous structures, and immediately round the nucleus is a narrow zone of densely packed granules.

After the first cleavage (Fig.2) the cytoplasmic division is very distinct, and the two daughter cells are always unequal in size. The nuclei are round and lie towards the opposite poles of the cells. The cytoplasmic organellae are similar to the above. In the majority of specimens the large granules are concentrated in one of the cells, probably indicating cellular differentiation at this early stage.

With subsequent cleavages the decrease in the relative amount of cytoplasm to nuclear structure was a marked feature. This is evident in Figs. 3 to 6.
In Fig.3 - four cell stage - the specimen is not flattened and the external membrane is clearly seen as is the "vitelline" space, whereas in Fig.5 because of flattening, the cells occupy the whole of the "vitelline" space as happens with mammalian eggs (Austin and Smiles, 1948).

Organisation of the developing larvae is seen in the appearance of large vacuole-like bodies of low refractive index which contain a small highly refractile irregularly shaped structure. Each of these bodies usually three in number, resembles the excretory cell seen in more mature larvae. About the same time two or three large dark granules appear, scattered in the structure of the larva. These are illustrated in Fig.7. Subsequently the larva begins to take shape as shown by an indentation, seen in Fig.8 on the right side of the larva. The vacuoles noted in the previous figure are still present and there is an increase in the numbers of large dark bodies, usually to four or five but there may be many more. The head and tail of the larva are now differentiated as a broad and a narrow end of the cellular structure (Figs.9 and 10). At the same time the first sign of a hook like structure is seen on the head. This is described in detail later.

The next stage in development is the formation of a body cavity in the larva which commences dorsally near the tail, and which gradually extends the length of the body. This is illustrated in Figs.11 to 15. During the formation of the body cavity, the larva increases in length and becomes a much slimmer structure. The formation of the cavity appears to be a reorganisation of the original cells, associated with the formation of

highly refractile subcuticular cells. The latter appear as the body cavity is being formed and are shown on the dorsal surface near the tail in Fig.11. This process continues until the larva appears as a hollow shell with an outer framework of the highly refractile subcuticular cells. When the process is complete (Fig.15) corrugations are visible on the surface of the larva, which subsequently become more distinct and more delicate. The hook structure also gradually develops as shown in Figs.11 and 13. Movement of the larva is noted first of all at the stage of development shown in Fig.11 and becomes more marked as the larva matures.

Further development is characterised by the body cavity becoming filled with other cellular structures, commencing at the head and extending towards the tail until the cavity is completely filled. (Figs.16 to 18). These cells have a lower refractive index than the subcuticular cells and appear to be of several types. The subcuticular cells themselves gradually become more rounded and are connected to one another by strands of cytoplasm. (Fig.17). Later they diminish in size.

From the stage shown in Fig.16, the larva is actively wriggling about inside the external membrane. Eventually it succeeds in stretching the membrane. This stretching takes a considerable period of time and the

process has been watched for several hours, during which the larva made only slight progress. The sequence of events is shown in Figs.19 to 22. A few specimens were observed where the membrane had been elongated, and where the larva had also made a pocket on one side. The excretory pore and excretory cell became clearly visible as the larva extended, and are shown in Fig.21. The excretory pore is surrounded by a dark refractile area, which in turn is surrounded by a clear area. This arrangement is only seen in a proportion of the larvae, and must be related to the functioning of the excretory apparatus.

Finally, the larva extends itself fully within the external membrane which now becomes the sheath of the microfilaria (Fig.22) and which at first is rounded at both ends. Later, the tail end becomes tapered with the movement of the larva up and down within the sheath.

In the microfilariae under phase-contrast (Fig.22), three constant vacuole-like structures are usually seen. The one in the middle of the larva is the excretory cell with the excretory pore lying anterior to it and surrounded with the structures described in Fig.21, and the other two structures are probably the nerve ring and the first genital cell respectively. A small low refractile spot behind the head is noted and which was visible in a number of the earlier specimens.

A microfilaria from the pleural fluid is shown in Fig.23. There are no significant differences between it and the microfilariae obtained from the uterus. The cells of the pleural fluid are also visible in this figure.

Microfilariae in the peripheral blood.

Because of the red blood cells, satisfactory photomicrographs could not be obtained of the microfilariae in the blood. However, no significant differences were noted between them and the microfilariae which were found in the uterus of the female and in the pleural fluid.

DISCUSSION

It has been possible to observe all the stages in the development of the larvae from a single cell. A photographic record has been obtained which portrays the natural state in a much superior manner to fixed and stained specimens. This photographic record is of value, as observations recorded as line drawings are often impressionist, and vary with the observer. Cf. the observations on the structure of the head of microfilariae as reviewed by Fulleborn (1929).

The general development of the larvae of Litomosoides is along the lines outlined briefly for Loa loa by Penel (1904) whose illustrations have been reproduced in recent publications (Chandler et al. 1940). Penel shows diagrammatically the development of the single cell to a larva. He states that the developing larva gradually stretched the egg-membrane to form the sheath of the microfilaria. Since then the origin of the sheaths of microfilariae and their absence in unsheathed species has been a subject of much discussion and theorising with few constructive observations. Penel's results were accepted for a time and are still believed by many, but unjust criticism of his observations have been made by modern authorities (Christenson, 1940) because he did not attempt a "critical study of the membranes", and he is

misquoted as stating that the vitelline membrane forms the sheath of the microfilaria. Penel's observation was that the "membrane ovulaire" was stretched to form the sheath. The observations made by phase-contrast microscopy confirm this early observation that the entire membrane goes to form the sheath. Chitwood and his colleagues believe that there is a chitinous shell within which there is a delicate vitelline membrane, visible only "by careful study with oil immersion lenses in formalin preserved materials". Many specimens have been observed in the present study including membranes from which the larvae have been removed, and no indication whatever has been seen of more than one structure in the membrane, which appears to have the same structure throughout development from the two cell stage. The whole membrane certainly enters into the formation of the sheath of the mature larva. Augustine (1937) suggested that the sheath of the microfilaria was derived from the shedding of the cuticle. He found in the adult worm of Vagrifilaria columbigallinae that the external membrane was present round coiled larvae but not round extended microfilariae. Further, he found what he took to be quantities of discarded membranes. Cross and Scott (1947) found no positive evidence that the larvae left their membranes at that point and they observed detritus in the uterus which they

presume is the material Augustine took to be discarded membranes. More recently, Kershaw (1948) stated that the microfilariae of Litomosoides carinii shed their sheaths shortly after emergence from the adult worm, and that the sheath "is always cast before the larva reaches the general spaces of the pleura". Kershaw used a rapid fixation technique on smears made either from the thoracic organs or from the adult female worm pulled across a slide. If the worm has been pulled lightly across the slide, the microfilariae are seen by phase-contrast to have sheaths, but if the adult has been subjected to greater trauma in the process, some of the microfilariae lose their sheaths. In specimens of pleural fluid all microfilariae possess sheaths when examined with the phase-contrast microscope. Similarly, all microfilariae in the peripheral blood have sheaths although Kershaw stated that sheaths could be demonstrated only "in more than 50 per cent" of the larvae. No evidence of the casting of the membrane was found, such as collections of empty membranes as described by Kershaw.

In the general cellular development of the larvae the outstanding feature was the formation of a body cavity with the simultaneous appearance of highly refractile subcuticular cells. The cavity gradually enlarged until it occupied the whole length of the developing

larva and the main cellular structures present were the subcuticular cells. The cavity then became filled with a number of different cells, the function and organisation of which could not be elucidated. As far as can be determined, this body cavity does not correspond with any cavity formed during the development of other nematode larvae which have been studied in detail e.g. Ascaris. This is to be expected as microfilariae have no gut, while the larvae of other nematodes have a gut at the corresponding stage of development. In the fully developed microfilariae, three round vacuole-like bodies, usually containing a small granule, were found. One of these was near the middle of the microfilaria and was obviously the excretory cell, from which a small connection could be seen running anteriorly to the excretory pore. The latter was visible only when the microfilaria was properly orientated. Another of these bodies lay mid-way between the excretory cell and the head, which suggested that it was the nerve ring. The third body lay mid-way between the excretory cell and the tail and was probably the genital cell. The first indication of these bodies was seen in fairly early forms - see Fig.7 - and they were present throughout subsequent development. There were no gaps in the nuclear column visible by phase-contrast, as described by Kershaw in fixed and stained preparations.

Cuticular corrugations were easily observed in the specimens. When the larva was coiled within the external membrane the corrugations on the inner side were close together and on the outer side were widely spaced. Foshay (1947) employing a silver deposition technique observed corrugations on the commoner blood microfilariae, but he was less certain of them in Litomosoides carinii. Stefanopoulo et al. (1949) with phase-contrast were able to see them in Litomosoides carinii and stressed that the sheaths of microfilariae in the blood were easily seen.

The movements of the microfilariae in the pleural fluid were striking. They were able to pass rapidly through packed masses of cells in the pleural fluid with little or no difficulty, in contrast to some species of microfilariae which move actively only on one area.

It is clear from the above observations and the photomicrographs that phase-contrast microscopy is an excellent method for the study of the development of the nematode larvae.

SUMMARY

The development of the microfilariae of Litomosoides carinii from the ova, has been studied by phase-contrast microscopy, and recorded with photomicrographs. Details are given of the methods of preparation which give the best results. All stages in the development were obtained by puncturing the uterus of adult female worms at intervals along their lengths and mounting the larvae on agar coated slides.

A striking feature was the formation of a body cavity in the developing larvae which does not correspond with any cavity formed by other nematode larvae. It is shown conclusively that the sheaths of the microfilariae are derived from its whole external membrane or egg shell.

EXPLANATION OF FIGURES 1 - 9.

Figure 1. Single cell stage. This specimen is flattened to show detail in the cytoplasm. There is an outer zone of large granules with an inner zone of fine granules, with short filaments between.

Figure 2. Two cell stage. The specimen is again flattened and shows large granules concentrated in the upper cell.

Figure 3. Four cell stage. This specimen is not flattened. The external membrane and "vitelline space" are clearly visible. Note the decrease in the relative amount of cytoplasm to nuclear structure.

Figure 4. Multiple cell stage.

Figure 5. A later stage. The specimen is flattened Nuclear structures, barely resolved, are visible.

Figure 6. This specimen shows an increase in size with the increase in the number of nuclei.

Figure 7. Commencing organisation. Large vacuole-like bodies containing a small dark structure are visible and also two or three large dark granules.

Figure 8. The outline of the larva is irregular and there is an indentation on the right side, which is the first stage in the shaping of the larva.

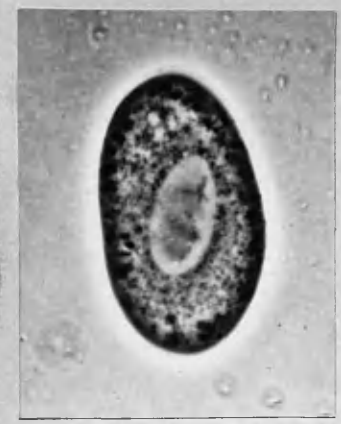
Figure 9. This shows a further decrease in size and the curvature of the larva is becoming more marked. A broad end and a narrow end can be distinguished, the former going to form the head and the latter the tail.

PLEASE

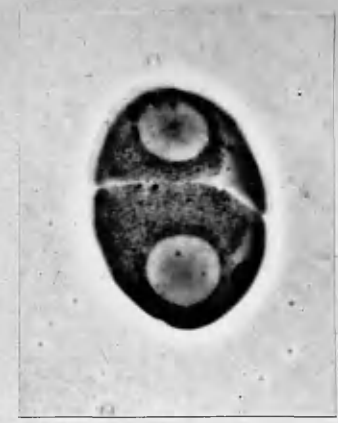
FOLD OUT

FIGURES 1-9

FIGURES 1-9

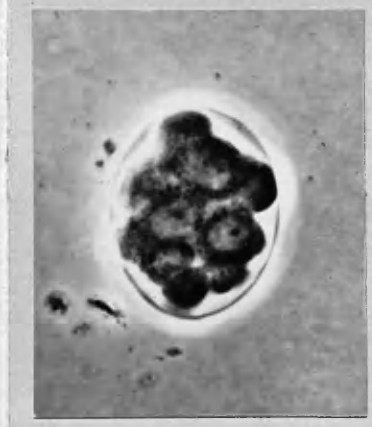


1

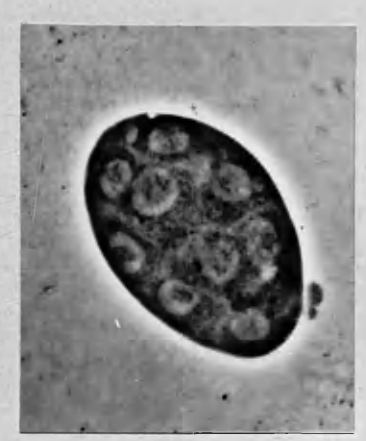


2

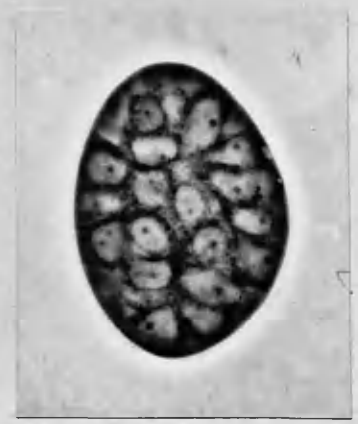
3



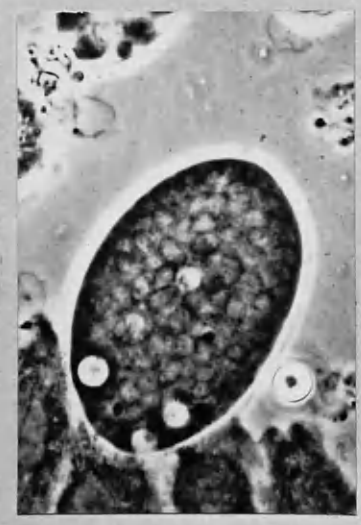
4



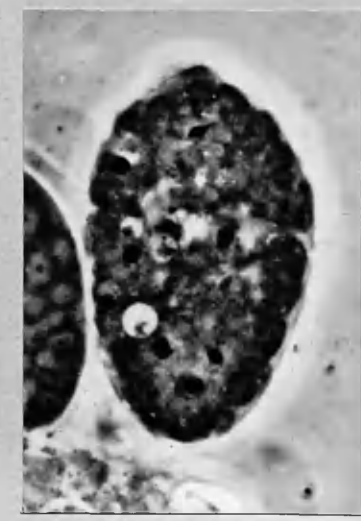
5



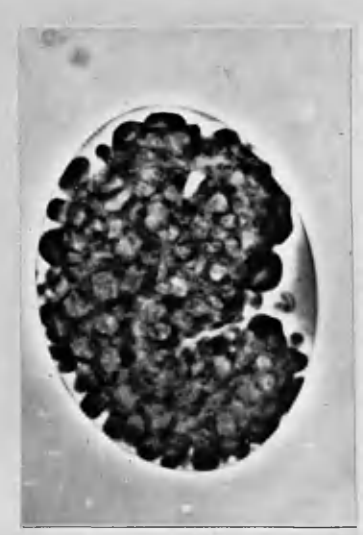
6



7



8



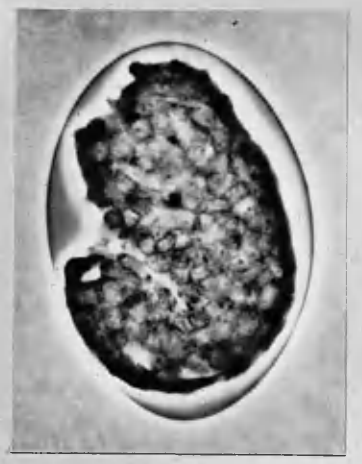
9

PLEASE

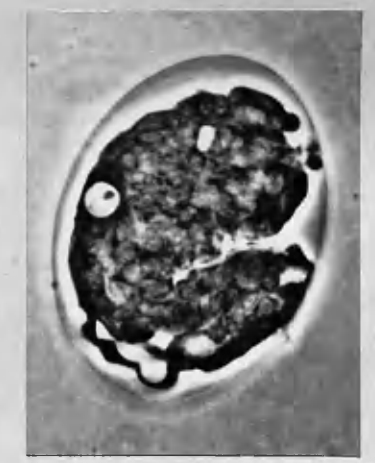
FOLD OUT

FIGURES 10-18

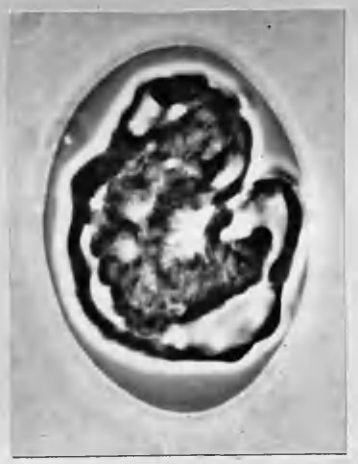
FIGURES 10-18



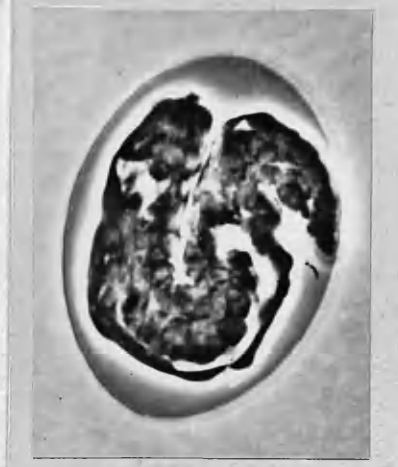
10



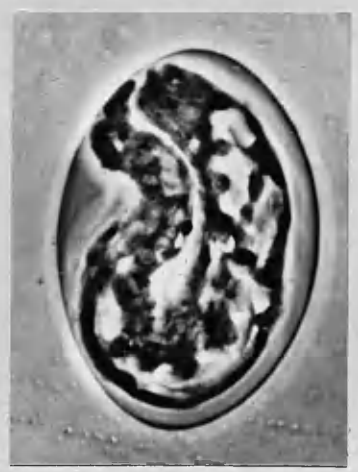
11



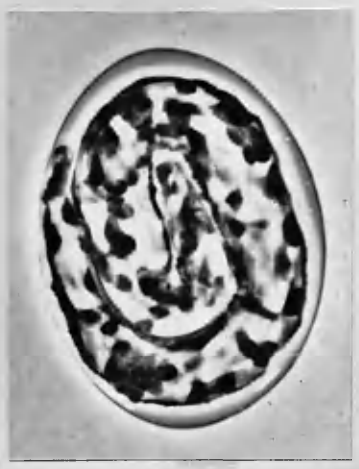
12



13

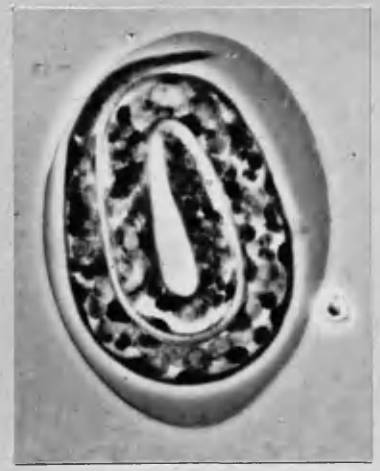


14

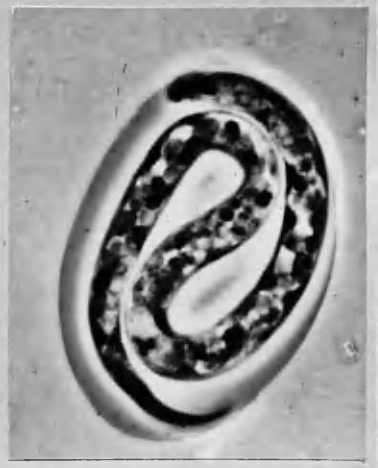


15

16



17



18

EXPLANATION OF FIGURES 19 - 23.

Figures 19 - 21. The external membrane is gradually being stretched by the movements of the larva. The hook on the head is seen in Figures 19-20. The excretory pore is visible in Figure 21, surrounded by a dark area and then a light area.

Figure 22. The larva has become fully extended within the external membrane. Three clear areas are seen in the body, the anterior being probably the nerve ring, the one in the middle the excretory cell and the posterior the genital cell. Just behind the head a small clear spot is noted which is visible in a percentage of the microfilariae.

Figure 23. A microfilaria from the pleural fluid. The clear area behind the head noted in Figure 22 is much larger in this specimen. Cells from the pleural fluid are visible.

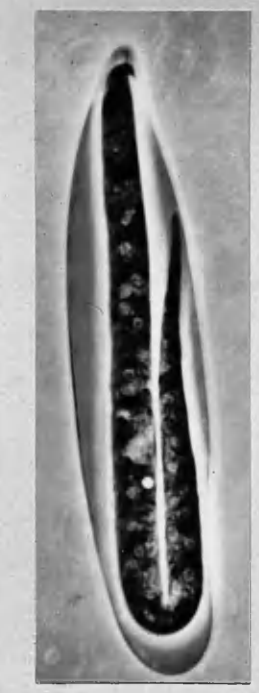
PLEASE

FOLD OUT

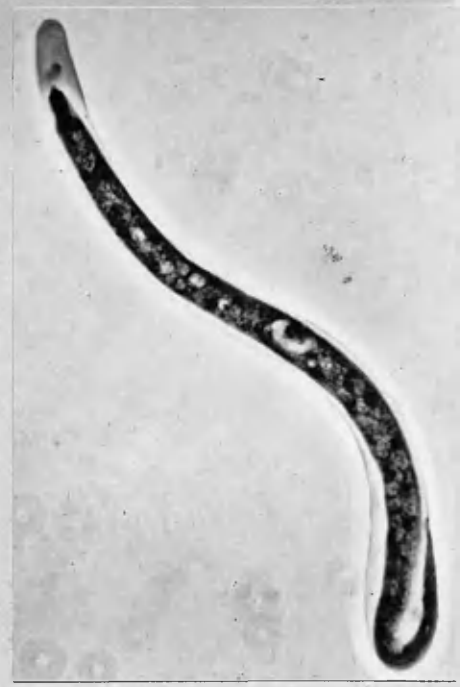
FIGURES 19-23

FIGURES 19-23

19

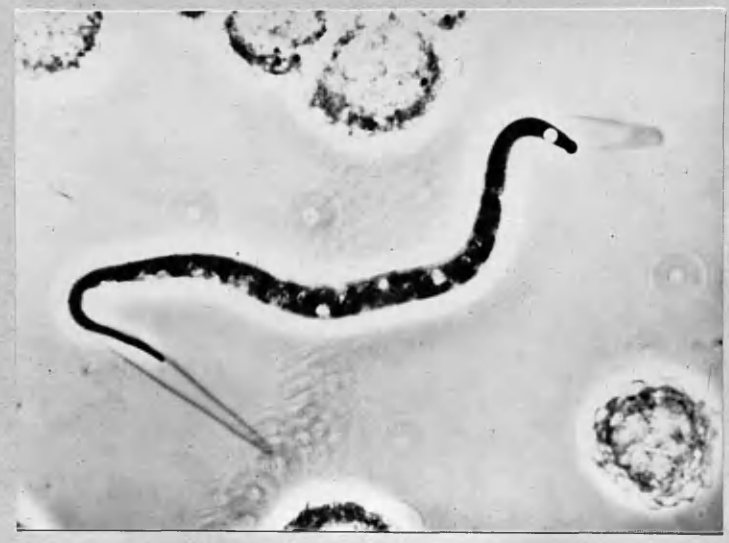


20



21

22



23

All photomicrographs are X 1300 except Fig.23 which is X 800, and were taken with the standard C. T. & S. phase-contrast equipment.

REFERENCES

Augustine, D. L. (1937). Trans.R.Soc.trop.Med.& Hyg., 31, 55.

Austin, C. R. & Smiles, J. (1948), J.Roy.Micros.Soc., 68, 13.

Chandler, A. C., Alicata, J. E. & Chitwood, M.B. (1940) in An Introduction to Nematology, Babylon, N.Y., Section II, Part II, Chapt.VI, 289.

Christenson, R. O., (1940), in An Introduction to Nematology, Babylon, N.Y., Section I, Part III, Chapt.XII, 180.

Cross, J. B. & Scott, J. A. (1947), Trans.Amer.Micros.Soc. 66, 1.

Culbertson, J. T. & Rose, H. M., (1944), Science, 99, 245.

Foshay, L. (1947), Amer.J.trop.Med., 27, 233.

Fulleborn, F., (1929), in Kolle and Wassermann's Handbuch der pathogen Mikroorganismen 3rd. Ed., 6, 1043, Jena: Fischer; Berlin, Wien; Urban u. Schwarzenberg.

Hawking, F. & Sewell, P. (1948), Brit.J.Pharmacol.& Chemotherapy, 3, 285.

Kershaw, W. E. (1948), Ann.trop.Med.& Parasitol. 42, 377.

Penel, R. (1904), Les Filaires du Sang de l'homme, Paris.

Stefanopoulo, G. J., Ovazza, M. & Bessis, M. (1949), Com.R.de Se de la Soc.de Biol., 143, 767.

PART 1 (continued)

SECTION B (continued)

II. The development and fertilisation of the ova of *Litomosoides carinii* as observed by phase-contrast microscopy.

PREPARATIONS

Female adult worms were prepared for dissection as described previously, and using a very fine sharp scalpel the tip of the tail was cut off thus permitting the extrusion of the ovaries. Small portions of the ovaries, the oviducts, and the first parts of the uteri were dissected out and mounted on agar and a coverglass applied. The weight of the coverglass was usually sufficient to cause extrusion of some cells at either end of the section, and this permitted detailed examination.

Please fold out in turn
pages 69 and 71.

OBSERVATIONS

The earliest cells were found near the tip of the ovary and had nuclei of low refractive index and cytoplasm containing numerous highly refractile granules (Figs.1 and 2). The ovarian cells were not easily detached from one another and later stages were seen to be attached to a central core of tissue (Fig.3). When the individual cells became detached in the process of preparation, a small projection was seen at one pole which had been the connecting band of tissue. As the ovaries were dissected towards the uteri, the cells increased in size and before the commencement of the maturation divisions they became detached and lay free (Fig.5). Subsequently, the nucleus moved to one pole of the cell and the first maturation division took place, as is shown in Figs.6 and 7, the latter showing late anaphase with a marked chromosome bridge. Unfortunately, it was not possible to obtain an exact chromosome count. After the first maturation division the ova came into contact with spermatozoa, as shown in Fig.8, in which the ova are intermingled with numerous spermatozoa. This occurs in the region of the oviducts, which are seen under the binocular dissecting microscope as narrow tubes between the ovaries and the uteri. Detailed structure of the spermatozoa was difficult to elucidate, and an adult male

worm was dissected in order to compare the spermatozoa from this source with those found in the female. Those obtained from the adult male had fine pseudopodia which were rapidly thrust out and withdrawn, and some of which had club shaped ends (Fig.9), while those found in the female were usually rounded off. In the cytoplasm of the spermatozoa a variable number of granules were visible.

Ova, each containing a spermatozoon were found only after a long search (Fig.10). It seems likely that rapid changes ensue in the spermatozoon on penetration of the ovum, as all other stages of development can be found relatively easily. Fig.11 shows a constant arrangement of structures subsequently seen, namely two bodies of a low refractive index similar to that of the nuclei of previous stages. They are of unequal size with the larger at the pole away from the polar bodies. These structures are probably the pronuclei, and ~~these~~ the smaller is the female pronucleus which has moved towards the larger which is the male pronucleus.

The fusion of the pronuclei was not observed and the stage next seen was the mitotic division of the zygote. Again it was not possible to obtain a chromosome count. Three stages of division are shown in Figs.12, 13, and 14, namely metaphase, and early and late anaphase. In all these figures smaller granules are scattered through-

out the cytoplasm, but larger structures have become concentrated towards one pole of the cell. After the formation of the two cell stage in which the division of the cytoplasm is very distinct, all these structures are usually to be found in one of the cells (Fig.15). The stages of division from the two cell stage to the four cell stage were also observed and are shown in Figs.16 and 17.

The development of the unfertilised ova.

Two rats were dissected a short time before microfilariae were due to appear in the peripheral blood. An adult female worm was found in each rat but no males. No microfilariae were found in the blood or pleural fluid and careful dissection of the worms did not reveal any spermatozoa, but the ovaries were mature and normal. In the first parts of the uteri, there were forms which contained up to 8 to 16 nuclei, but the cytoplasmic division was indefinite. A number of the specimens, however, appeared normal. The uterine contents were examined up to the vulva, and Fig.18 depicts the typical forms found. They appear to be degenerate 8 to 16 cell stages.

Two other observations made in the course of this investigation are worth recording. The first was noted at a level near the oviduct of an adult female worm.

A mature wriggling microfilaria was found in the centre of the mass of spermatozoa with its head towards the tail of the worm. It had certainly not been introduced by the technique of dissection. It was wriggling towards the tail, and the only possible explanation was that it had migrated backwards instead of towards the vulva. A second unusual finding was noted in the dissection of the uterus of another adult female worm. In the uterus near the head there were only a few mature actively wriggling microfilariae and these were embedded in a mass of spermatozoa which extended the entire length of the uterus. Apparently the worm had become so tightly packed with spermatozoa that the normal development and maturation of the larvae were interrupted.

DISCUSSION

The arrangement of the developing ova was striking, with the individual cells attached to the central core of the tissue. As they developed and separated from this tissue a small piece of the latter remained attached to the cell. It was not possible to determine if there was a micropyle formed at the site of attachment.

Cross and Scott (1947) stated that the "oviducts merge imperceptibly into the ovaries". In this investigation it has been found that the oviducts can be identified as narrow tubes broadening anteriorly into the uteri, and posteriorly into the ovaries, and also that the ovaries themselves narrow towards the tail. In all the fertile adult female worms dissected, spermatozoa were found in the region of the oviducts. Thus on fertilisation of the adult, the spermatozoa must make their way to the oviducts, and fertilise the ova there or in the first part of the ovaries. An estimate of the duration of one effective fertilisation of the female worm would be interesting, but it is too complex to be determined at present. No special storage compartment for the spermatozoa was found.

The fact that it took so long to find a spermatozoon inside an ovum and that one cannot be certain of observing it in each worm dissected, is strong evidence of a rapid change in the spermatozoon on penetration of the

ovum. It has not been possible to determine accurately at which point the second polar body is formed in relation to fertilisation.

As shown in Figs.12 to 15, the large granules within the cytoplasm of the zygote aggregate and are found in one of the daughter cells. These large granules may be a food store and it is not known why they should become concentrated in one cell.

SUMMARY

The development, fertilisation, and early divisions of the ova of Litomosoides carinii have been investigated by phase-contrast microscopy, and recorded with photomicrographs.

Fertilisation takes place in the region of the oviducts, where masses of spermatozoa are to be found.

Rapid changes probably ensue in the spermatozoon after it has entered the ovum.

Unfertilised ova were observed developing into abnormal 8 to 16 cell stages, but no further.

EXPLANATION OF FIGURES 1 - 9.

Figures 1 - 5. Ovarian cells from near the tip of the ovary, showing stages of development. Figure 3 shows the characteristic anatomy, the cells being attached to a central core of tissue (the rachis).

Figures 6 and 7. The first maturation division. Late anaphase is shown in Figure 7 with a marked chromosome bridge.

Figure 8. The ova are surrounded by masses of spermatozoa most of which have become rounded off.

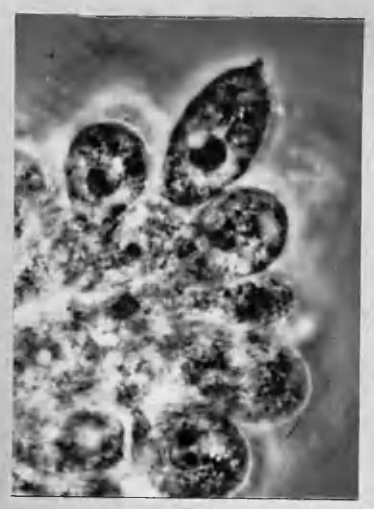
Figure 9. Spermatozoa from an adult male worm. Note the fine club shaped pseudopodia.

PLEASE

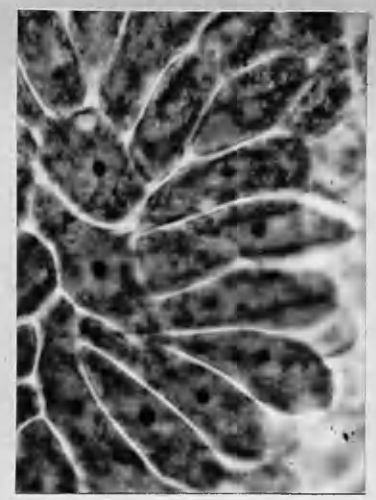
FOLD OUT

FIGURES 1-9

FIGURES 1-9



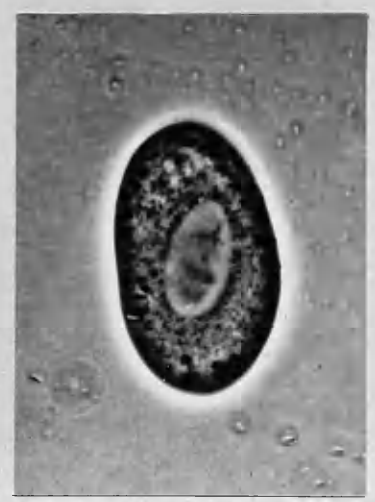
1



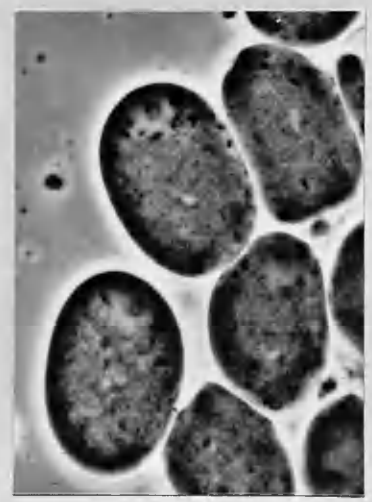
2

3

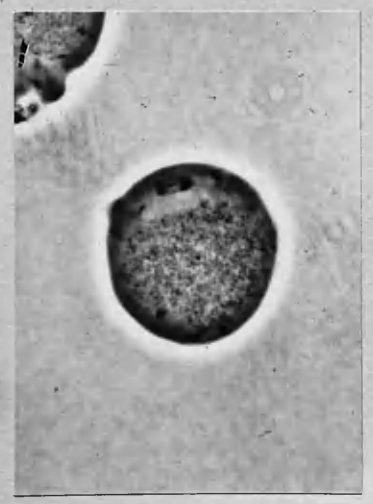
4



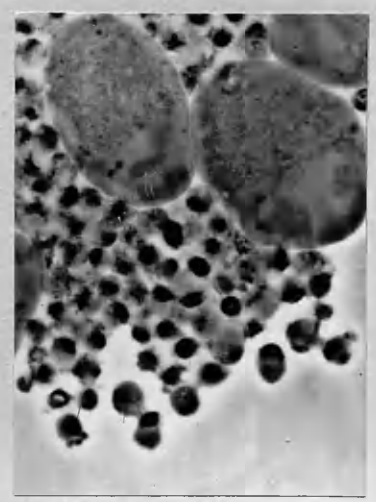
5



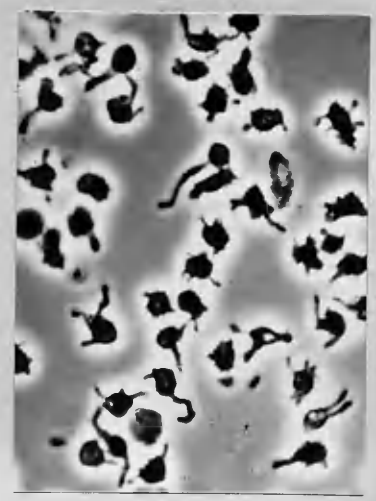
6



7



8



9

EXPLANATION OF FIGURES 10 - 18.

Figure 10. A spermatozoon is seen within the ovum at the lower right hand corner.

Figure 11. The structures in this specimen are probably pronuclei, the smaller being the female pronucleus as it is always nearer the polar bodies and moves towards the larger, the male pronucleus.

Figures 12 - 15. These figures show the division of the zygote into two cells, Figure 12 showing a metaphase plate, and Figures 13 and 14, early and late anaphase respectively.

Figures 16 and 17. Division into 4 cells. The polar bodies are seen on the left side of both specimens. In Figure 16, the left cell is in early anaphase and the right in metaphase. In Figure 17, the left cell is in metaphase and the right in late anaphase

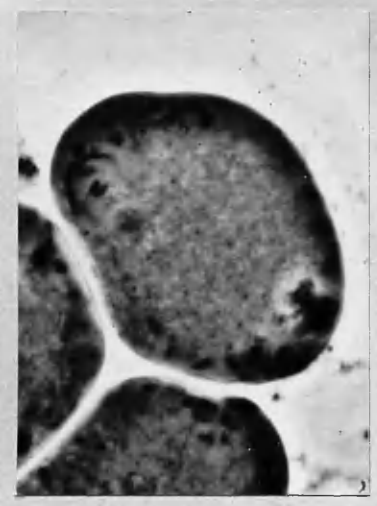
Figure 18. The typical forms found in the uterus of a mature unfertilised adult worm. They appear to be degenerate 8 - 16 cell stages.

PLEASE

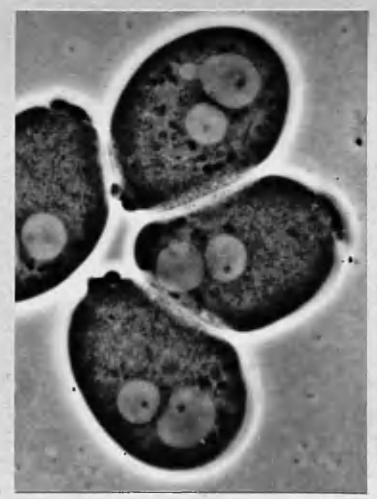
FOLD OUT

FIGURES 10-18

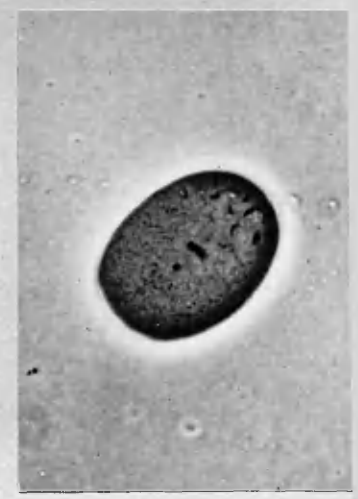
FIGURES 10–18



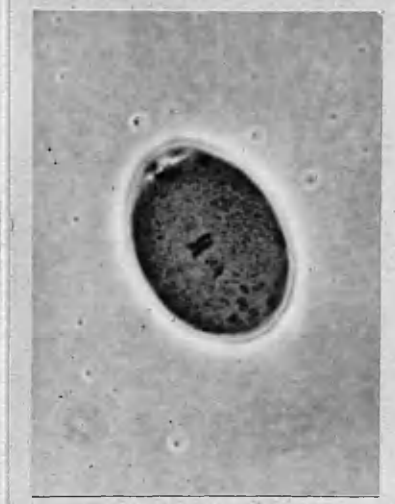
10



11

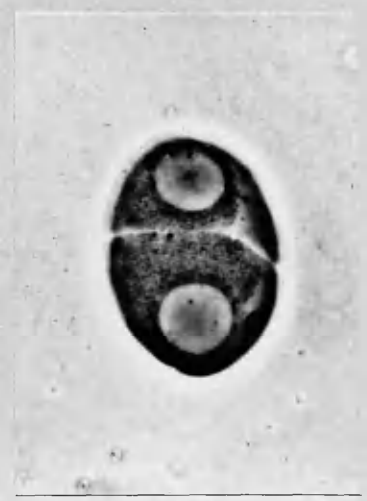


12

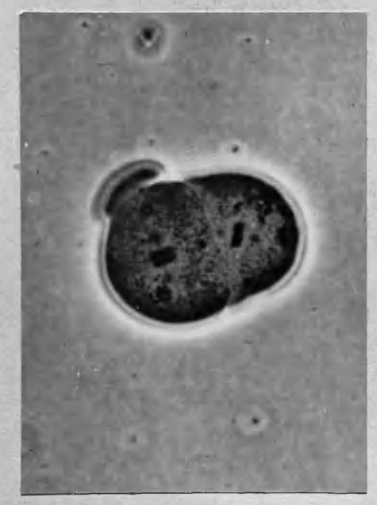


13

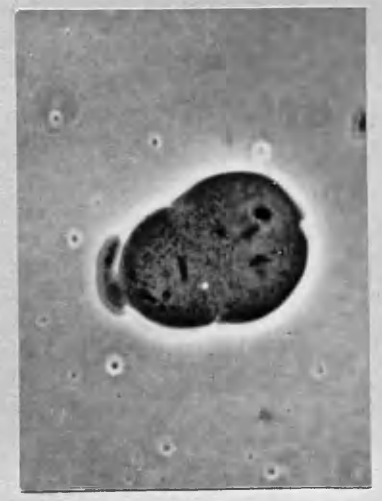
14



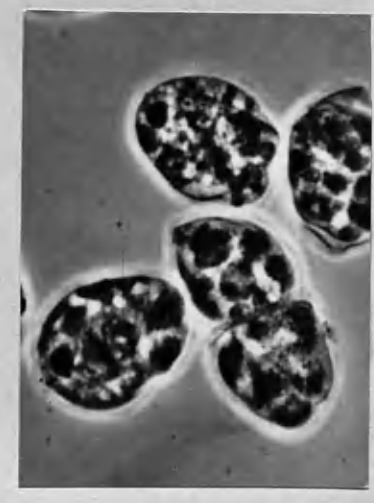
15



16



17



18

All photographs are X 1300 except Fig.10 which is X 1850, and were taken with the standard C. T. & S. phase-contrast equipment.

REFERENCE

Cross, J. B. & Scott, J. A. (1947), Trans.Amer. Micros.Soc., 66, 1.

P A R T 1 (continued)

SECTION B (continued)

111. Observations on the structure of microfilariae of Litomosoides carinii by phase-contrast microscopy and ultra-violet micrography.

PREPARATIONS

Drops of fluid from the pleural cavities of cotton rats infected with Litomosoides carinii were mounted on agar slides as described previously for examination by visual phase-contrast. Microfilariae from the peripheral blood were also examined, but the red blood cells interfered with the quality of the picture and prevented proper immobilisation of the microfilariae. To get rid of the cells, the blood obtained by cardiac puncture of the rat was mixed with 0.1 per cent sodium citrate to lyse the cells, centrifuged, and the microfilariae thus obtained were resuspended in Ringer's solution (after the method described by Knott, 1935). By comparison of the microfilariae treated in this fashion with those in fresh blood, it was found that no apparent changes were produced by this treatment. Further, the microfilariae, thus treated were capable of surviving for a period of days.

The movements of the head were observed when the weight of the coverglass had partially or fully immobilised the microfilariae. Because of the small size of the head, and of its highly refractive nature, no internal structures could be seen, but its shape was clearly defined and its movements were easily followed.

Micrographs of microfilariae were obtained by

means of direct and phase-contrast ultra-violet micography using the wave length 2749A of the cadmium spark, the specimens being mounted between agar spread on quartz slides and quartz covers. The microfilariae were more easily immobilised when very small drops of a suspension were used than when large drops were used. While in visual microscopy positive phase-contrast systems are used in which objects which retard the light passing through them are transformed in the image into amplitude differences, and the objects appear dark on a bright background, the system used in ultraviolet is a negative one and objects which retard the wave length used are represented by areas which are brighter than the surrounding medium. Thus the appearance of the head of the microfilaria, which in visual phase-contrast is depicted as a dark body, is reversed in the phase-contrast ultra-violet micrograph.

Please fold out in turn
pages 85 and 87.

OBSERVATIONS

Structure of the head.

As was recorded previously, there is a hook structure on the heads of the microfilariae. This is shaped like a rose thorn, and is clearly visible only when the hook is thrust forward and lying horizontally (see Fig.1). In fresh preparations, and even after several hours, the hook was constantly being protruded and retracted too rapidly to follow clearly in most microfilariae, but the details of the movement were seen in a number of specimens. When the hook was retracted it was visible only as a highly refractile pointed projection in the centre of the head flanked on either side by two clear projections (see Fig.5). When protruded, the central portion was thrust forward and at the same time was flexed (see Fig.6), and the hook itself was only seen when the head was rotated through 90° (see Fig.7). In Figs.5 and 6 it can be seen from the position of the clear area behind the head that the hook is thrust forward a considerable distance. When the hook was protruded, the two side projections disappeared and became visible again when it was retracted. After examining many specimens it was seen that the side projections were the result of the invagination of the cuticle produced when the hook was retracted, and the

invaginations straightened out when the hook was protruded. Proof of this was given in a number of specimens which died or were killed with the ultra-violet irradiation, in which the hook was found protruded various distances, and the further it was thrust out the smaller were the side projections (see Fig.2). The hook itself had a mass of highly refractile tissue as a base (Fig.1), and there is a less refractile area behind the base.

It was hoped that micrographs taken with transmitted and phase-contrast ultra-violet would help to elucidate the structures of the head, especially those involved in the movements of the hook. Evidently there are complicated structures present, but ultra-violet micrography is not capable of revealing them clearly. By the direct method of illumination, the head structures do not absorb the wave length 2749 A and the picture is made up of diffraction patterns, from which it can be inferred that fibres run from the base of the head through the body of the microfilaria (see Fig.3). By ultra-violet phase-contrast, fibres were more easily depicted, but it was not possible to determine their distal points of attachment (see Figs.1 and 2).

Other observations

The cellular structure of the microfilariae was recorded by both ultra-violet methods (see Figs.1

and 3). The direct picture brings out clearly the nuclei which absorb the ultra violet rays strongly. The nuclei vary in size, and are closely packed, and therefore the amount of cytoplasm present must be very small. In Fig.2 the cuticle is in focus, the corrugated structure of which is clearly revealed by the ultra-violet phase-contrast method. The variation in contrast of this periodic structure is due to out of focus images of structures lying below the cuticle. The band seen nearest the head in Fig.2 does not encircle the body. This thicker band is seen in all the microfilariae examined by this method, and in some micrographs there were two shorter bands nearer to the head.

Another interesting feature which was observed in the majority of microfilariae was a clear area at the tip of the tail. This is illustrated diagrammatically in Fig.8. This did not appear as a structure but rather as the commencement of a moult, as the cellular structure of the microfilariae ended proximal to it. This was observed in microfilariae in the pleural fluid, in the blood, and also in those taken from the intermediate host, the mite Liponyssus bacoti, shortly after feeding on an infected cotton rat.

An unusual appearance was observed in a high proportion of the microfilariae in one infected rat, and in a few microfilariae from one other rat. Within the

sheaths, there was a considerable amount of debris consisting of bodies of varying sizes, and it seemed at first as if there were two larvae within the same sheath. Some of these bodies appeared to be of a cellular nature. The larvae appeared normal in every other way and were quite as active as those with no debris within the sheaths.

The final observation was that sometimes embryos develop abnormally, as happen in most types of living creatures. Fig.4 illustrates an actively moving microfilaria found in the pleural fluid, in which the head is abnormal. None of the normal structures of the head described above were visible and the larvae appeared as a two headed monster. A few other specimens like this have been seen during the course of these investigations.

DISCUSSION

The main feature of the heads of the microfilariae is the presence of a hook. This appears at a fairly early stage in the development of the microfilariae, as shown previously. The movements of the hook which were observed, the active protrusion and flexion and then retraction, suggests that this is the mechanism used by the microfilariae to penetrate the tissues of the intermediate host. A similar hook has been described in other species, notable in microfilariae recondita (Fulleborn, 1929, after Noe). In most other species of microfilariae some form of projection on the head has been described (Fulleborn, 1929).

It is impossible at present to interpret in detail, the internal structures of the microfilariae as seen by the ultra-violet methods.

The clear area which was observed at the tail of the microfilariae, would require to be carefully traced through the development in the mite, to determine its nature. Its apparent lack of structure suggests that it may be the beginning of the first moult but the absence of refractile material at the tip of the tail, does not necessarily mean that there is no structure present.

The debris observed within the sheaths of a number of microfilariae is difficult to understand.

The fact that it was seen only in two rats out of some forty examined, suggests that there was some factor responsible for it in these two animals. The microfilariae appeared otherwise healthy, but the debris must have arisen from within the sheath.

SUMMARY

The head structure of the microfilariae of Litomosoides carinii is described as observed by phase-contrast microscopy, and by direct and phase contrast ultra-violet micrography. There is a hook which is constantly being protruded and retracted. When it is retracted, the cuticle near the head is invaginated.

A clear area at the tip of the tail of the microfilariae was observed, and this may be the beginning of the first moult.

In microfilariae from two rats, a considerable amount of debris was observed within the sheaths.

An illustration is given of a double-headed microfilaria.

EXPLANATION OF FIGURES 1 - 4.

Figure 1. Ultra-violet phase-contrast micrograph x 2350. The head of a microfilaria surrounded by cells of the pleural fluid. The hook is clearly seen and fibres are visible running backwards from the head.

Figure 2. Ultra-violet phase-contrast micrograph x 2350. The head of a microfilaria with the hook not quite fully protruded. The striations are shown clearly, with a thick band near the head.

Figure 3. Direct ultra-violet micrograph x 2350. The nuclear structures are shown absorbing the ultra-violet rays strongly.

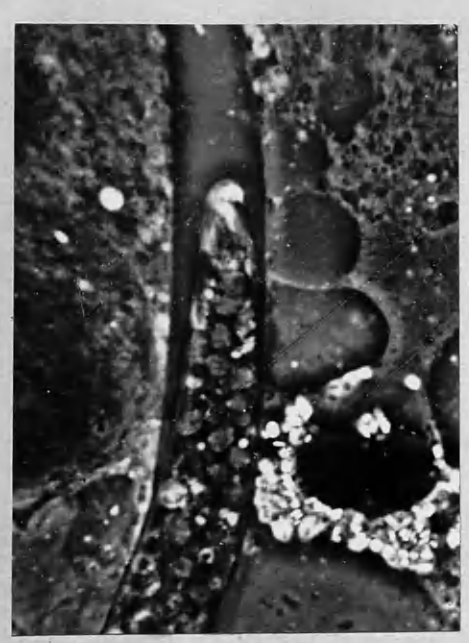
Figure 4. Visual phase-contrast micrograph x 800. A microfilaria with 2 heads with none of the normal head structures present.

PLEASE

FOLD OUT

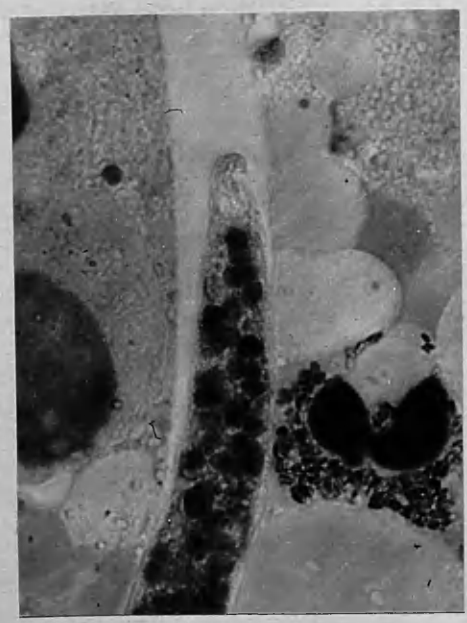
FIGURES 1-4

FIGURES· 1-4

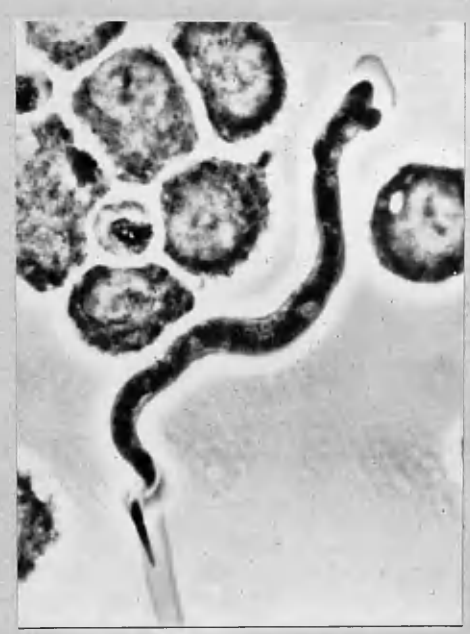


1

2



3



4

EXPLANATION OF FIGURES 5 - 8.

Stages in the movements of the head of the microfilaria.

Figures 5 - 7. Figure 5 shows the hook retracted with the cuticle invaginated.

Figures 6 and 7 show the hook fully protruded in two different planes.

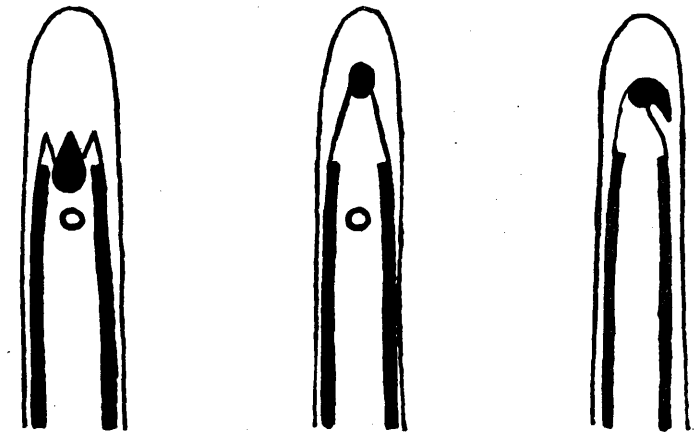
Figure 8. This shows the clear area near the tip of the tail which is seen in many microfilariae.

PLEASE

FOLD OUT

FIGURES 5-8

FIGURES 5-8



5

6

7

8

REFERENCES

Fulleborn, F. (1929) in Kolle and Wassermann's Handbuch der pathogen Mikroorganismen 3rd. Ed., 6, 1043, Jena: Fischer; Berlin, Wien: Urban u. Schwarzenberg.

Knott, J. (1935), Trans.R.Soc.trop.Med. & Hyg., 29, 59.

P A R T 2.

Investigations of the Phenomenon of Microfilarial Periodicity.

Introduction.

In 1879 ~~1897~~ Manson described the phenomenon of the periodicity of the larvae of the nematode parasite Filaria nocturna (Wuchereria bancrofti). He found that the larvae, or microfilariae, were largely absent from the blood of the host during the day, but in the evening they appeared in the blood in increasing numbers, reaching a maximum about midnight, and gradually decreasing again till morning. Figure 1 shows an example of this periodicity. It is now known that in a single host, namely man, there are ~~was~~ species of filarial infection, the microfilariae of which exhibit three types of periodicity. The first type was that described by Manson in which the microfilariae show nocturnal periodicity; secondly, there is the complete reverse of this in Loa Loa infections in which the microfilariae have a diurnal periodicity; and finally there is the non-periodic variety, W. bancrofti var. pacifica, in which the microfilariae are present in the blood stream throughout the twenty four hours with no regular fluctuation in their numbers. The cause of these phenomena is still unknown, but three important observations have been made. McKenzie (1881) showed that the cycle is orientated to the waking and sleeping habits of the host; reversal of the sleeping habits gradually produced reversal of the microfilarial periodicity. Manson (1899) made a post mortem of a patient who died during the day from cyanide poisoning, and found that the majority of

microfilariae were in the lungs and great vessels at this time. Hawking and Thurston (1951) took biopsies of the lungs during the day and during the night from monkeys and dogs infected with filarial worms with nocturnally periodic microfilariae, and showed that the increased number of microfilariae in the lungs during the day time is sufficient to account for the decrease in numbers in the blood during the same period. It is now clear that the periodic appearance of microfilariae in the peripheral blood is due to their migration backwards and forwards between this site and the samll vessels of the lungs, and these migrations are stimulated by some obscure cyclic change or changes within the host. (Incidentally although it is always assumed that the mechanism responsible for the periodicity in Loa loa is the same as that for W. bamcrofti, very few investigations in support of this assumption have ever been carried out. Further, nothing is known of the distribution of the microfilariae of L. loa in the body, and it is significant that while the periodicity of the microfilariae of W. bancrofti is reversed within a few days by reversal of the host's sleeping habits, Low (1921) failed to reverse the periodicity in a patient infected with L. loa, and the only record of a successful attempt is that by Kulz (1914) who recorded that travelling round the globe for two months changed the peak of the microfilarial tide by nine hours.)

Factors influencing the microfilarial cycle.

Many factors have been investigated in the past from darkness to the inhalation of turpentine spray, but a significant influence on the microfilarial cycle has rarely been demonstrated. Pyrexia can upset the cycle, and considerable numbers of microfilariae may be found in the blood during the day in W. bancrofti infections (Manson, 1882); but this finding is not supported by the present experiments. Sodium pentobarbitone injected intravenously increases the numbers of microfilariae in the peripheral blood to a varying degree (Hawking and Thurston, 1951)

Cyclical changes in man.

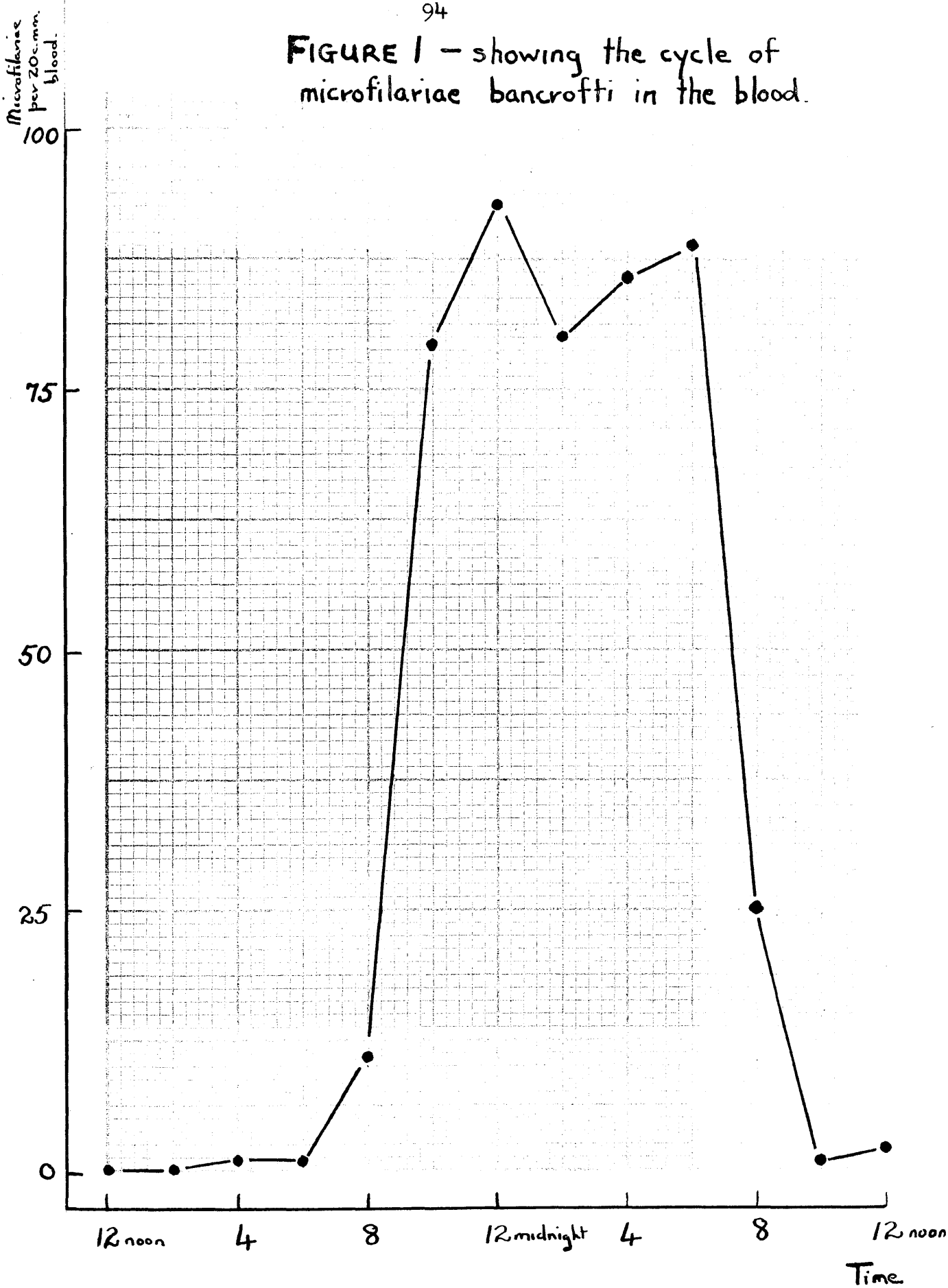
Since the microfilariae of nocturnally periodic species of filariae are in the lungs during the day, much of the present investigation deals with the effects of varying gas concentrations breathed by the host. According to the literature, there is an increase in the partial pressure of carbon dioxide in the alveoli in the early morning (Bass and Herr, 1922), and during sleep there is an increase in the carbon dioxide in the blood (Endres, 1923). Mills (1953) states that the alveolar carbon dioxide tension sometimes shows a diurnal rhythm. There is also a decrease in the oxygen saturation of the blood during sleep (Lundsgaard, 1918) (Doust and Schneider, 1952). Furthermore, a cycle like the microfilarial cycle is exhibited by the amount of phosphorus in the urine. According to Kleitman (1925): (1) Greater quantities of phosphorus are excreted per hour during sleep

than during waking, independent of the volume of urine. (2) On different dietary regimes and during fasting, most persons excreted the greatest amount of phosphorus during the first half of the night. (3) The rate of phosphorus excretion during the day is greater in the afternoon than in the morning, and there is a definite curve in some cases, with the excretion falling to its lowest point around noon. (4). The total acid in the urine does not always follow the phosphorus closely, but there appears to be some relationship.

Campbell and Webster (1922) reversed the normal routine of waking and sleeping for 4 days. At the end of that time the urinary cycle of the amounts of urine excreted between day and night remained the same, but there was an absolute increase in the phosphorus excreted during the day, so that more phosphorus was excreted then than during the night. They could disassociate the level of phosphorus excretion from every factor (including acidity of the urine) except sleep. (confirmed by Kleitman, 1923)

Stanbury and Thompson (1951) have shown that in the diurnal cycle of excretion of sodium, potassium, chloride and bicarbonate ions, a maximum was reached at mid-day and a minimum during sleep, and in some cases the fall began in the afternoon. There was however no parallel changes in the plasma levels of these substances during the 24 hours.

FIGURE 1 – showing the cycle of microfilariae bancrofti in the blood.



P A R T 2.

1. Investigations in Monkeys.

Materials and Methods.

These investigations were carried out on African monkeys (Cercopithecus aethiops johnstoni) infected with a filarial nematode Dirofilaria aethiops. The adult worms live in the fascial planes of the hamstring muscles and give birth to unsheathed microfilariae which exhibit a nocturnal periodicity very similar to W. bancrofti (Hawking and Thurston, 1951).

To enumerate the microfilariae, two or more quantities of 20c.mm. of blood were taken from the ear and spread evenly on a slide, dried, dehaemoglobinised, fixed with methyl alcohol and stained with Mayer's haemalum. All the counts were performed by myself, and the results are recorded as the average number of microfilariae per 20c.mm. The monkeys were exposed to various gas concentrations in a metal tank measuring approximately 3'x2'x2' with a lid fitting into a water moat thus rendering it airtight. The gas being investigated was introduced through a hole at one side of the tank, and an outlet at the other side allowed the mixed gases to escape. A water manometer was attached to ensure that the pressure within the tank did not rise above 1 atmosphere.

Results.

Increased oxygen concentration.

A standard set of conditions was evolved in order to make the experiments reproducible. An unanaesthetised monkey in a cage was placed in the tank and oxygen introduced at a rate of 4 litres per minute. Samples of the atmosphere within the tank were taken every 5 minutes, and the concentration of oxygen was estimated with a Beckman oxygen analyser. At the end of 30 minutes, the oxygen concentration had risen to 60%. Blood samples were taken from the ear before the animal was put in the tank and after the lid was taken off at the end of 30 minutes. Three monkeys with filariasis were investigated in this way, each monkey being placed in the tank for approximately 30 minutes around mid-day when there are few microfilariae in the peripheral blood. Examples of the changes in the number of microfilariae in the peripheral blood are as follows (their bloods at 10p.m. contained 150-200 Mf. per 20c.mm.):

Monkey 185. The number of microfilariae in the blood rose from 8 per 20c.mm. to 161 per 20c.mm.

Monkey 187. The number of microfilariae in the blood rose from 14 per20c.mm. to 232 per 20 c.mm.

Monkey 192. The number of microfilariae in the blood rose from 7 per 20c.mm. to 29 per 20c.mm.

Detailed results of microfilaria counts on monkey 185 are given in Figure 1.

To eliminate the possibility that there might have been some factor other than the increase in oxygen concentration which was responsible for the increase in the number of microfilariae in the blood, control experiments were performed in which monkey 185 was put in the tank for 30 minutes and air introduced at 4 litres per minute. The monkey was then removed from the tank for blood samples to be taken, and later exposed to oxygen under the standard conditions. There was no increase in the number of microfilariae after the monkey had been exposed to air, but a great increase after exposure to oxygen.

Reduced oxygen concentration.

Lowered concentrations of oxygen were produced in a similar manner to that in which increased oxygen concentrations were obtained, by introducing nitrogen into the tank at a rate of 4 litres per minute as described above. A standard set of readings of the oxygen concentration was obtained with the Beckman oxygen analyser. It was found that a monkey could easily withstand this treatment for 30 minutes, with yawning as the only sign of anoxia. After 40 minutes the animal vomited and tended to faint. Thus again 30 minutes was selected as the time of exposure, at the end of which time the oxygen concentration was 10%. Examples of the results obtained with the same three monkeys are as follows:

Monkey 185. The number of microfilariae in the blood rose from 1 per 20c.mm. to 109 per 20c.mm.

Monkey 187. The number of microfilariae in the blood rose from 13 per 20c..mm..to 102 per 20c.mm.

Monkey 192. The number of microfilariae in the blood rose from 2 per 20c..mm. to 42 per 20c.mm.

Details of the results obtained with monkey 185 are recorded in Figure 2, and shows a much more rapid fall in the number of microfilariae in the blood on re-exposure to the air, than was recorded on re-exposure to the air after increased oxygen. In the case of monkey 187 there was some delay in collecting the blood samples when the monkey was removed from the atmosphere of low oxygen concentration; and this delay may account for the smaller rise in the microfilarial count on this occasion compared with that of the previous experiment when the monkey had been exposed to high oxygen concentrations.

In order to obtain blood samples while the oxygen concentrations were changing, further experiments were performed in which a monkey anaesthetised with intravenous sodium pentobarbitone was put in the tank with its tail through a hole in the side. To eliminate as far as possible the effects of the anaesthetic which might confuse the picture, the monkey was left for approximately one hour after it was anaesthetised; when this was done, the number of microfilariae in the blood, which had increased with the anaesthetic, fell again to a reasonably steady level and so permitted the start of the experiment.

Blood obtained from the tail is usually arterial, and as was expected, samples taken while the microfilariae were being liberated from the vessels of the lungs contained greater numbers than capillary blood. The result of one such experiment is shown in Figure 3. (Monkey 187). When the concentration of oxygen was 38% the count had started to rise, and it reached a maximum when the concentration of oxygen was 70%. Figure 4 shows the result of a similar experiment in which the oxygen concentration was lowered. At a concentration of 17% oxygen the count had not risen, but at a concentration of 13% it had risen by 200 per 20c.mm. The last blood sample from the tail, just before bringing the monkey out of the tank, had 516 microfilariae per 20c.mm; at this point the blood was very blue. The next sample was taken 4 minutes later, after the blood had become oxygenated and the tail blood contained only 176 microfilariae per 20c.mm. This shows an extremely rapid removal of the microfilariae from the peripheral circulation. The capillary blood contained approximately the number present before the oxygen concentration was lowered, because the second blood sample was not taken until the blood was oxygenated.

Increased concentration of carbon dioxide.

For safety purposes, these experiments were not performed in the tank. A monkey was anaesthetised with intravenous sodium pentobarbitone and left for approximately one hour to allow the first liberation of microfilariae to

settle. Then pure carbon dioxide was administered by a tube placed near the monkey's face. The concentration of carbon dioxide was not measured accurately, but the dosage was estimated by the increase in the respiratory rate, which was raised from 16 per minute to 30-40 per minute for half an hour. This was repeated a number of times on different occasions and no significant alteration was produced in the number of microfilariae in the blood.

Hyperventilation. i.e. Decreased concentration of carbon dioxide in the alveolar air.

Again a monkey was anaesthetised with intravenous sodium pentobarbitone, and a tracheal tube inserted. After one hour, hyperventilation was commenced, using a pump which delivered 250cc. of air 44 times a minute. The hyperventilation was continued for a period of one hour; on disconnecting the pump there was apnoea for 2½ minutes. Blood samples taken at intervals revealed no significant change in the number of microfilariae present.

Night Experiments.

All the experiments described above were performed during the day to study factors which might liberate the microfilariae from the lungs, since it is simpler to investigate the problem from this aspect, rather than the factors which might remove the microfilariae from the blood at night time. However, the experiments were repeated shortly before midnight at an hour when the microfilariae are gradually increasing in

number. Exposure to carbon dioxide had no effect upon the number of microfilariae in the blood, while exposure to oxygen or to hypoxia caused an increaae in their number just as they did in the day time.

The Possible Role of the Phosphate Cycle.

It was shown above during the review of the literature that there is a 24 hour cycle in the excretion of phosphates in the urine, which closely resembles the cycle of nocturnally periodic microfilariae. Moreover, the urinary phosphates parallel the blood phosphates; and certain changes in gas concentrations influence phosphate levels, viz :-

(1). Increased concentration of carbon dioxide in the lungs increases the concentration of phosphates in the blood and urine.

(2). Hyperventilation decreases the concentration of phosphates in the blood and urine (Haldane, Wigglesworth and Woodrow, 1924). Thus if the phosphate and microfilarial cycles were directly related, increased carbon dioxide should increase the number of microfilariae during the day, and hyperventilation should decrease the number during the night. It has been shown above that this does not happen. Campbell and Webster (1922) showed that 35 to 40 per cent oxygen concentration did not influence the urinary phosphates. To determine whether 60 per cent oxygen influenced the acid soluble phosphorus of the blood, six unifected monkeys were in turn

placed in the tank and oxygen administered in the standard manner. Samples of venous blood were taken before and after being placed in the tank and the concentration of acid soluble phosphorus in the plasma was estimated. No significant changes were detected. Monkey 192 was transfused with a total of 621 mg. phosphorus (94 mg. per kg.) in the form of neutral phosphates as described by Addis et al. (1925). This quantity was more than adequate to produce a change in the blood level of phosphorus, but no change was produced in the number of microfilariae in the blood. From these findings there appears to be no direct relation between the phosphate cycle and the microfilarial cycle.

The Effect of Vaso-dilatation.

General vaso-dilatation can be readily produced by the injection of hexamethonium iodide (C.6.). This was done in two monkeys with filariasis, both of which were given slightly less than 1 mg. per kg. body weight intravenously. No significant change was recorded in the number of microfilariae in the blood.

The Effect of Varying the Temperature, Pulse Rate and Respiratory Rate.

In a series of experiments, the temperature of a monkey with filariasis was made to vary over the range of 92.3°F (rectal) to 104.6°F without producing any significant change in the number of microfilariae in the blood. Similarly the pulse rate was made to vary from 88 to 180 beats per minute,

and the respiratory rate from 16 to 36 per minute by the administration of carbon dioxide again without materially influencing the number of microfilariae in the blood. One of the monkeys (192) was examined after it had taken energetic exercise and the blood was found to contain considerably more than the usual number of microfilariae. The monkey was then put into the tank, exposed to air, and the number of microfilariae in the blood fell to the low figure which is usual at midday. Apparently the energetic exercise had promoted the migration of microfilariae into the peripheral blood.

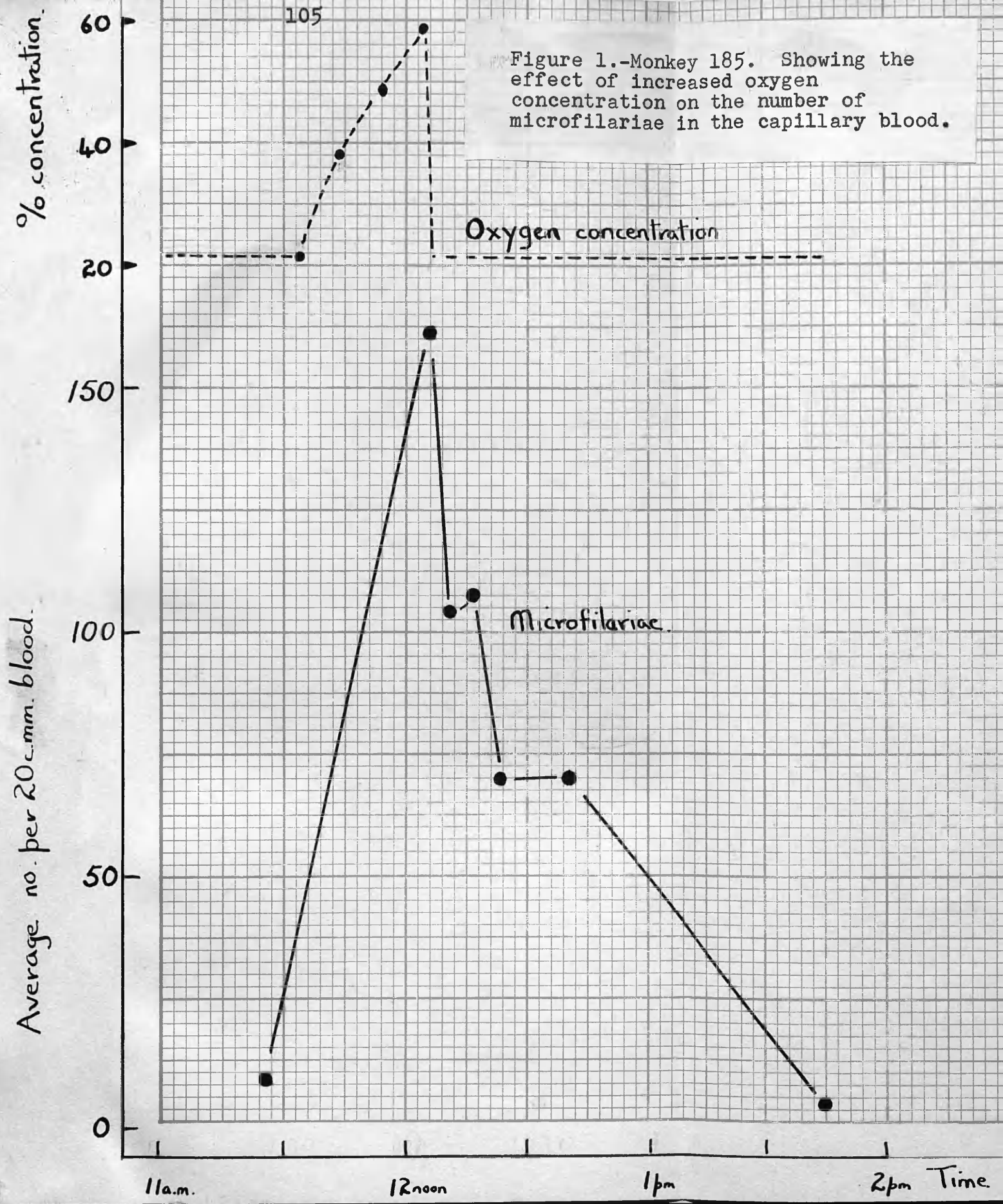


Figure 1.-Monkey 185. Showing the effect of increased oxygen concentration on the number of microfilariae in the capillary blood.

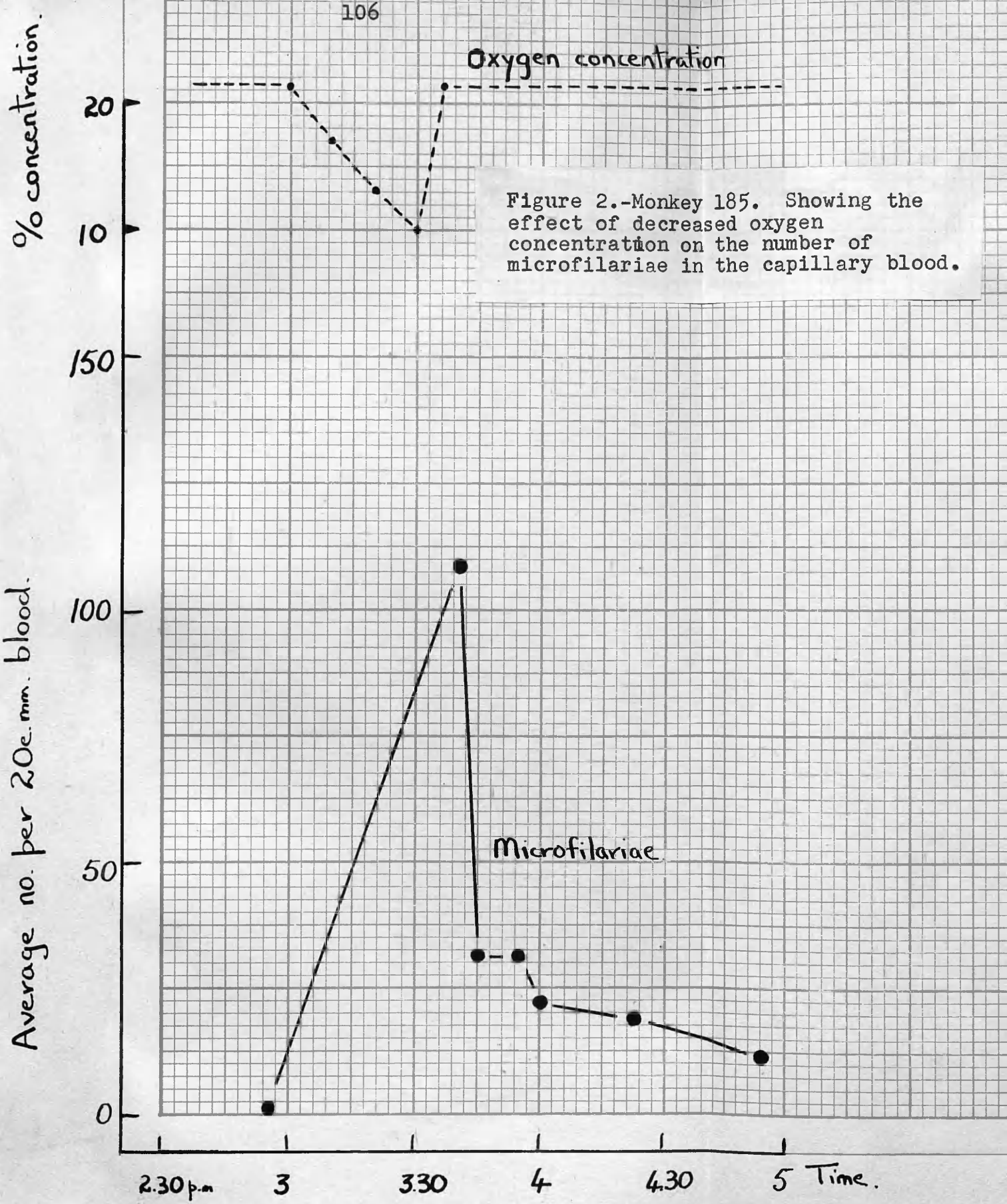
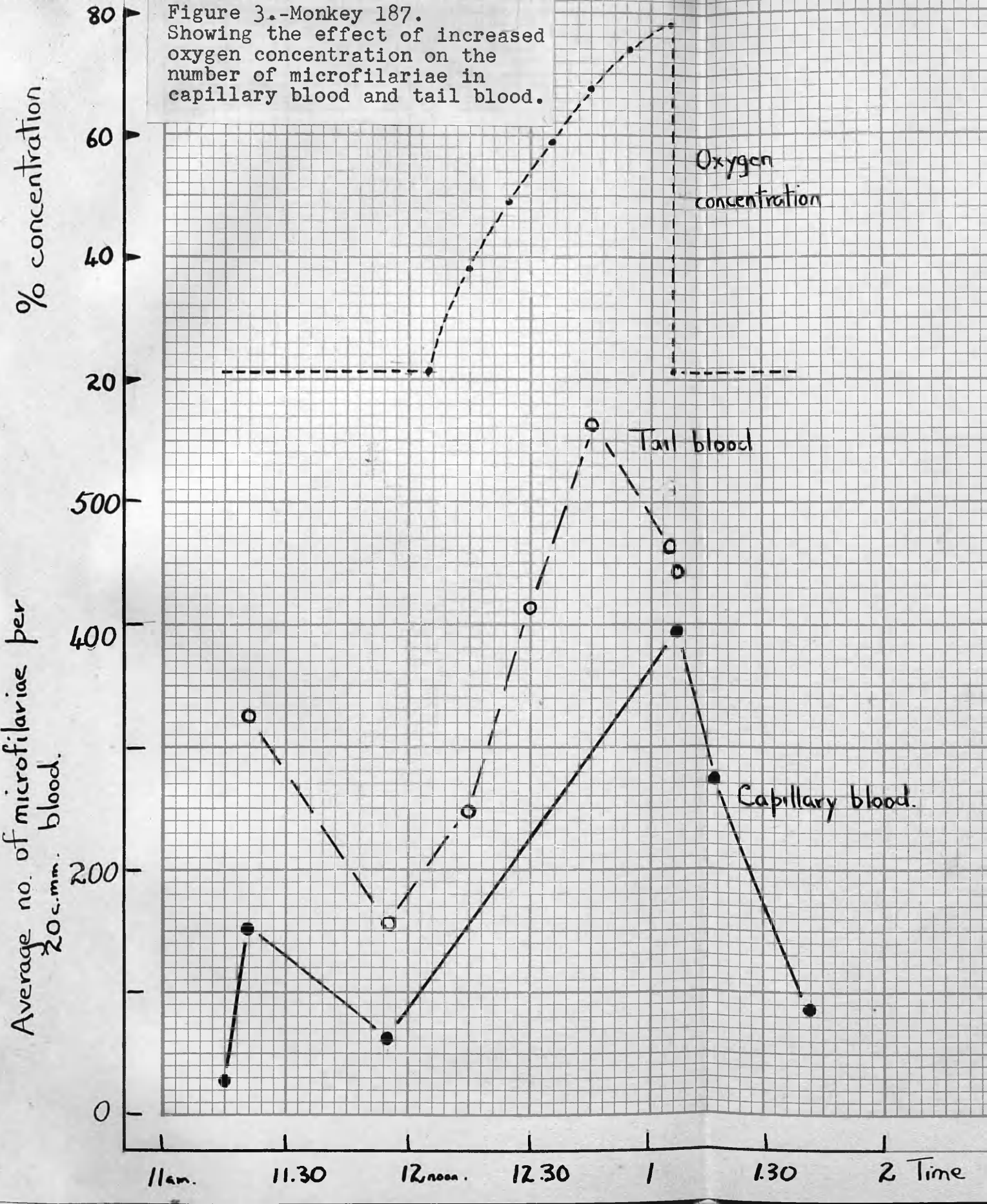


Figure 2.-Monkey 185. Showing the effect of decreased oxygen concentration on the number of microfilariae in the capillary blood.

Figure 3.-Monkey 187.
Showing the effect of increased oxygen concentration on the number of microfilariae in capillary blood and tail blood.



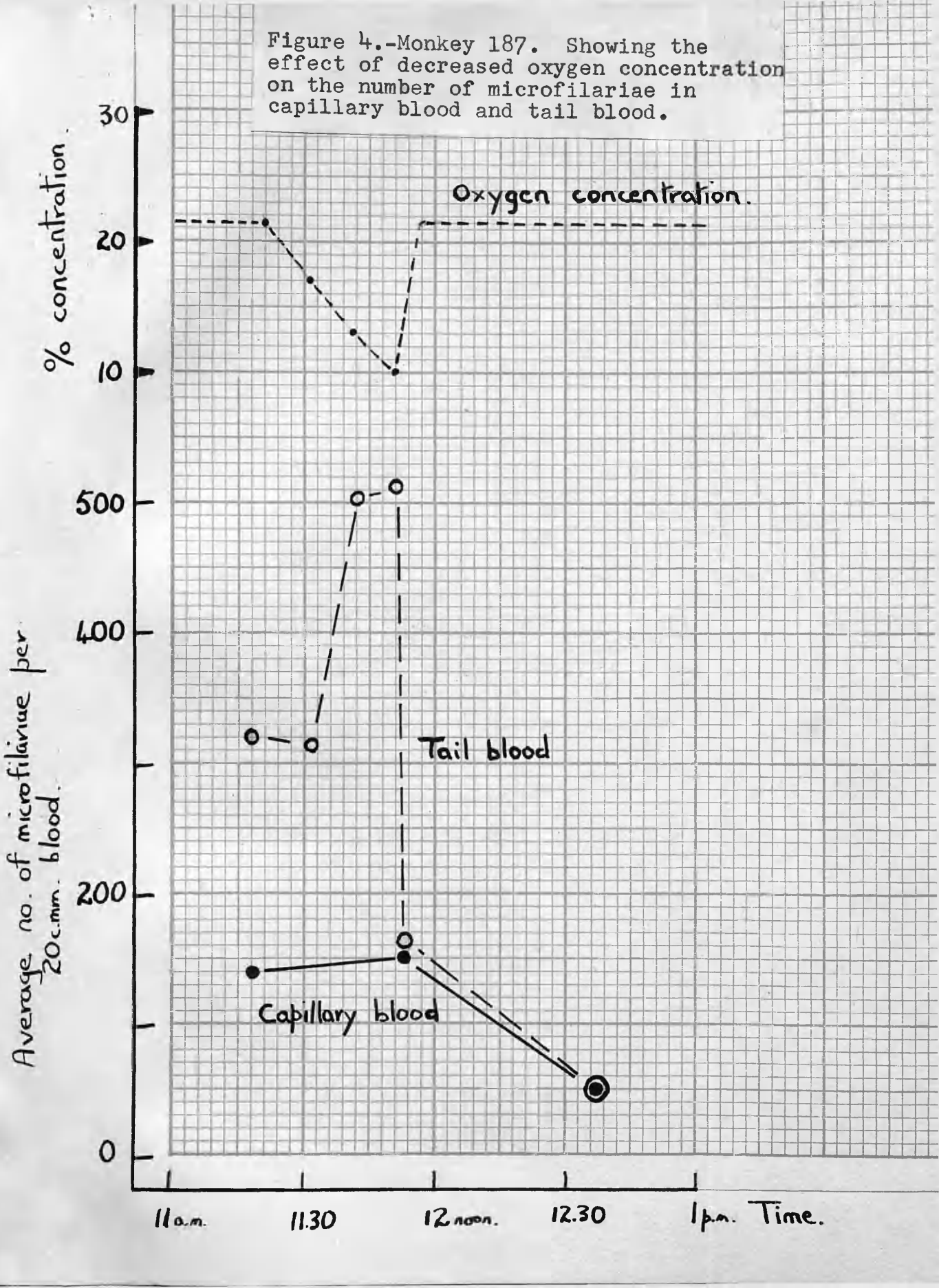


Figure 4.-Monkey 187. Showing the effect of decreased oxygen concentration on the number of microfilariae in capillary blood and tail blood.
% concentration.
30
20
10
Oxygen concentration.
500
400
200
0
Average no. of microfilariae per 20c.mm. blood.
Tail blood
Capillary blood
11 a.m.
11.30
12 noon.
12.30
1 p.m.
Time.

P A R T 2 (continued)

2. Investigations in Man.

Materials and Methods.

The following investigations were undertaken in Gambia, to determine the effects of breathing different gas concentrations, particularly different concentrations of oxygen, on the periodicity of microfilariae bancrofti in man. The subjects employed were mostly healthy young adult males infected with Wuchereria bancrofti.

The microfilariae of W. bancrofti exhibit a nocturnal periodicity as described previously. The microfilariae were enumerated as before by taking measured quantities of blood from a finger prick, and the results are expressed as the average number of microfilariae per 20c.mm. of blood.

Different gas mixtures were prepared and administered as follows:-

1. Different oxygen concentrations. Mixtures containing different concentrations of oxygen were prepared in a Douglas bag. Oxygen and nitrogen were passed through a gas meter in predetermined proportions, and finally the oxygen concentration in the bag was estimated by the simple syringe technique with pyrogallic acid. The patient lay on a bed and a mouthpiece which had an inlet and an outlet valve, was inserted between the teeth. A rubber nose clip was also applied to occlude the nostrils. When the patient was comfortable, a blood sample was taken and the Douglas bag connected to the mouthpiece. Owing to the limited capacity of the bag, the gas was usually

administered for a period of 30 minutes. In this way, patients were given different concentrations of oxygen varying from 10% to 100%.

2. Carbon dioxide. This was administered direct from a cylinder of carbon dioxide by means of a tube and funnel. The funnel was placed near the patient's face, and the amount of gas administered was controlled by observing the changes in the depth and rate of the respirations.

Experiments in the day-time were performed around mid-day, and experiments at night between 9p.m. and 12 midnight, during which time the microfilariae are increasing in number in the peripheral blood.

RESULTS.

1. Experiments in the Day-time.

A. Increased oxygen concentrations. Patients were given increased oxygen concentrations up to 100%. In most experiments, this produced a slight increase in the numbers of microfilariae in the blood e.g. the count might rise from 0 mf. per 20c.mm. to 6 mf. per 20c.mm. in a patient whose night blood contained several hundred microfilariae per 20c.mm. However, there was never an increase in numbers resembling that produced in monkeys by giving them increased oxygen concentrations in the day-time.

B. Reduced oxygen concentrations. Gas mixtures containing 10% or 13% oxygen were employed, and given for periods up to 30 minutes. Again , there was a slight increase in the numbers of microfilariae in the blood, but never an increase resembling that produced in monkeys with the same concentrations of oxygen,

C. Carbon dioxide. Increased concentrations of carbon dioxide were administered which were sufficient to produce a marked increase in the depth of the patient's respirations and in the respiratory rate, but no effect was produced in the numbers of microfilariae in the peripheral blood.

2. Experiments at Night-time.

A. Increased oxygen concentrations. A range of different concentrations of oxygen above that of air was administered to a number of patients in the late evening. Breathing 25%

oxygen for 30 minutes had no effect, but concentrations of 30% and over, produced a marked fall in the numbers of microfilariae in the peripheral blood. Experiments were repeated many times on different patients, and examples of the results obtained with the different concentrations of oxygen employed are shown in Table 1. The percentage fall in the numbers of microfilariae in the peripheral blood was not directly proportional to the concentration of oxygen breathed by the host i.e. 30% oxygen could produce as great a fall as 100% oxygen. The fall in the numbers of microfilariae did not vary greatly from patient. Table 2 shows the effect of breathing 100% oxygen on a group of patients, and the fall in the microfilaria count was of the order of 60-70% in all cases. A number of experiments were performed to determine how quickly breathing an increased concentration of oxygen produced an effect on the numbers of microfilariae in the blood. Up to 5 minutes after commencing to breath 100% oxygen, there was little effect, but at 10 minutes the microfilariae had fallen by 55% of the number originally present in the blood.

B. Reduced oxygen concentrations. No constant significant effect was produced on the numbers of microfilariae in the peripheral blood when low concentrations of oxygen were administered as described above.

C. Carbon dioxide. Increased concentrations of carbon dioxide did not produce any significant change in the numbers of microfilariae in the blood.

Possible Role of Phosphate Cycle.

As was shown in the previous section, there is a similarity between the cycle of the microfilariae and the cycle of the level of the phosphorus in the urine. The amount of phosphorus in the urine is said to parallel the amount in the blood, but no record could be found of frequent estimations of blood phosphorus over a 24 hour period. Thus venous blood samples were taken at 2 hourly intervals during 24 hours, from a patient whose blood contained microfilariae bancrofti. At the same time measured quantities of blood were taken from a finger prick for enumeration of microfilariae. The inorganic phosphorus in the whole blood was estimated by the method described by King (1946) immediately the samples were taken. The results are shown in Figure 1. There is a striking parallel between the numbers of microfilariae in the blood and the level of the inorganic phosphorus. As it is not known how much phosphorus the microfilariae contain, it was possible that the increase in the phosphorus in the whole blood was due to the increase in the numbers of microfilariae present. Therefore a similar series of estimations were undertaken at 4 hourly intervals, on a patient whose blood did not contain any microfilariae. The results are given in Figure 2, and show a cycle of the blood phosphorus similar to that recorded in the previous experiment.

TABLE 1

The effect of increased concentrations of oxygen at night-time on the numbers of microfilariae in the peripheral blood.

Oxygen concentration.	Patient.	Microfilariae /20c.mm. Before	After	% fall.
25% for ½ hour	No. 5.	200	217	-
30% " "	No. 2.	104	42	60
50% " "	No. 2.	62	19	69
100% " "	No. 4.	87	34	61

TABLE 2.

The effect of breathing 100% oxygen at night-time on the numbers of microfilariae in the peripheral blood.

Length of exposure to oxygen.	Patient.	Microfilariae /20c.mm. Before	After	% fall.
30 minutes	No. 1.	68	28	60
60 "	No. 1.	64	22	66
10 "	No. 2.	74	33	55
45 "	No. 3.	57	18	68
30 "	No. 4.	87	34	61

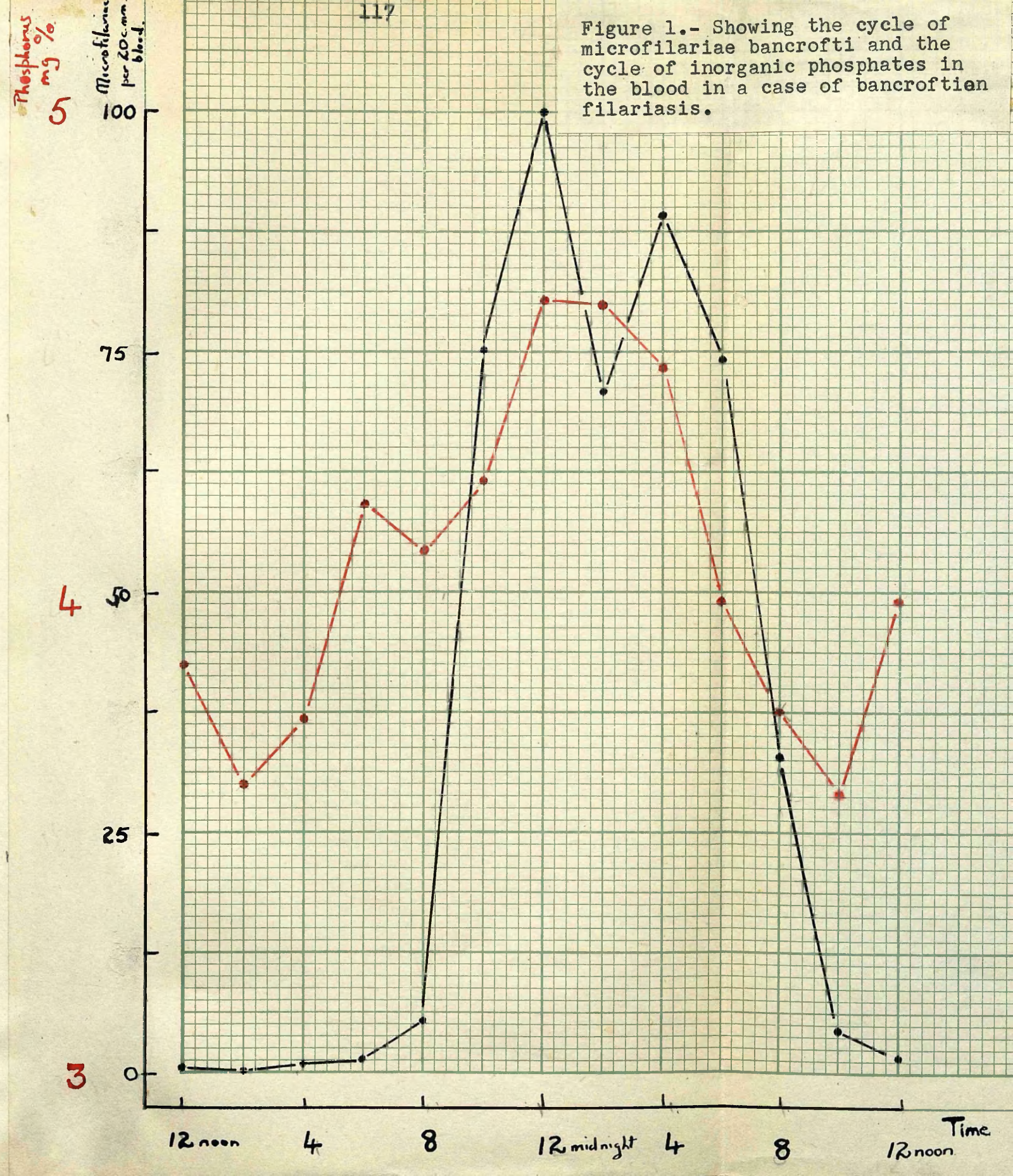


Figure 1.- Showing the cycle of microfilariae bancrofti and the cycle of inorganic phosphates in the blood in a case of bancroftian filariasis.

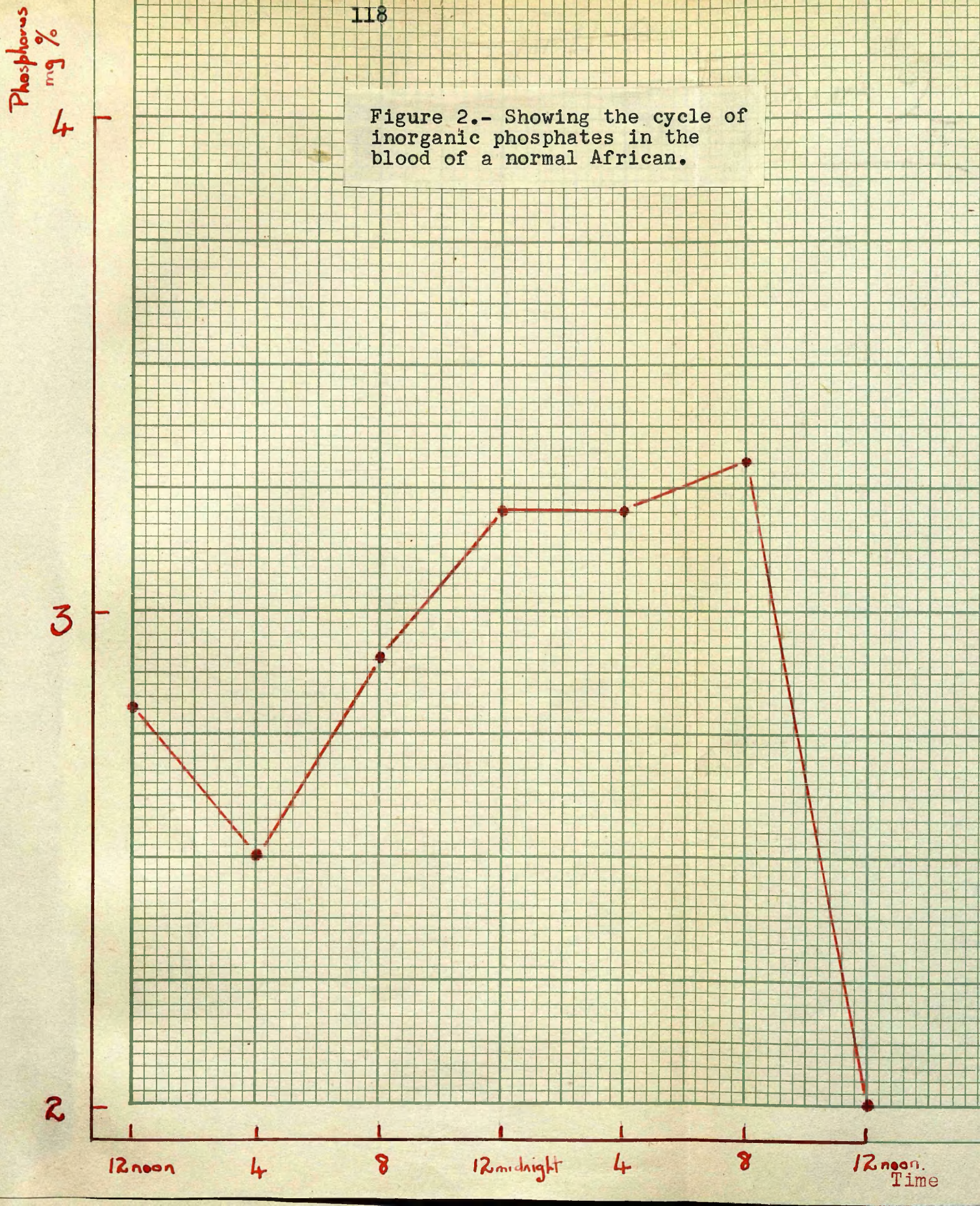


Figure 2.- Showing the cycle of inorganic phosphates in the blood of a normal African.

DISCUSSION.

The characteristic cycle of nocturnally periodic microfilariae has been shown by Hawking and Thurston (1951) to be due to their migration into the vessels of the lungs during the day-time and into the peripheral blood at night-time. It has been shown above that the migrations of the microfilariae can be influenced by changes in the oxygen concentration in the alveoli. This is the first factor which has been found to have any constant significant effect on microfilarial periodicity.

Two filarial parasites have been investigated, the microfilariae of which are nocturnally periodic, with similar cycles. In both, the cycles of the microfilariae were influenced by changes in the concentration of oxygen in the lungs but in vastly different ways. With the monkey parasite, increased or decreased oxygen concentrations liberated the microfilariae into the blood during the day. These measures had no significant effect on microfilariae bancrofti in man. Changes in oxygen concentration at night had little affect on the monkey microfilariae, but increased oxygen concentrations given to man at night, markedly reduced the numbers of microfilariae in the peripheral blood. These different effects may be due to different responses by the different hosts to changes in the oxygen concentration in the alveoli, or to different responses by the different species of the parasite.

One is unable to correlate these experimental findings with changes in gas concentrations known to occur in man in the waking and sleeping states. Further, of the many factors in the body which undergo cyclic changes, only the cycle of the blood phosphorus closely parallels the microfilarial cycle. However it has been shown above that the two cycles are not directly related to one another, as transfusion of phosphates in monkeys in the day-time did not affect the number of microfilariae in the peripheral blood. Similarly, increased oxygen concentrations in the day-time, which liberated the microfilariae of monkeys into the blood, had no effect on the blood level of phosphorus.

Further experimental investigations of the known cycles in man may reveal that one or more of them resemble the microfilarial cycle more closely than is thought at present, especially regarding a change occuring in the evening and reaching a maximum around midnight. It is more than likely however, that there is a major cycle in man governing the changes in the waking and sleeping states. Further investigations of the periodicity of microfilariae may not only supply the solution to an interesting phenomenon, but may also assist in the investigation of the still obscure phenomenon of sleep.

SUMMARY.

Investigations have been made into the effect of variation of the concentration of inspired gases on the periodicity of nocturnally periodic microfilariae in monkeys, and microfilariae bancrofti in man.

1. With monkeys.

Increase in the concentration of oxygen to above 38% or decrease below 13%, both at atmospheric pressure, produced great increases in the number of microfilariae in the blood in the day-time when the number is normally low; the same stimuli also produced a small increase when applied at night, when the number of microfilariae in the blood is normally high.

Variation of the concentration of carbon dioxide in the alveolar air had no significant effect on the microfilarial count during either day or night

Vasodilatation produced by the intravenous injection of hexamethonium iodide had no significant effect on the microfilarial count, but severe exercise in one monkey produced an increase during the day-time.

Variation of temperature, pulse rate and respiratory rate had no effect.

2. In man.

Increase or decrease in the concentration of oxygen breathed in the day-time had no significant effect on the numbers of microfilariae bancrofti in the peripheral blood.

Increased concentration of oxygen at night-time - 30% or over - produced a marked fall in the number of microfilariae in the blood after 10 minutes. Decrease in the concentration of oxygen at night-time had no effect.

Increased carbon dioxide had no effect either in the day-time or at night-time.

There is much resemblance between the cycle of phosphates in the blood and the urine, and the periodicity of nocturnally periodic microfilariae, but no direct relationship could be established.

REFERENCES.

Addis,T.,Meyers,B.A.,&Baker,L. (1925) Amer.J.Physiol., 72, 125

Bass,E.,&Herr,K. (1922) Z.Biol., 75, 279.

Campbell,J.A.,& Webster,T.A. (1922) Biochem.,J., 16, 507.

Doust,J.W.L.,& Schneider,R.A. (1952) Brit.Med.J., 1, 449

Endres,G. (1923) Biochem.Z., 142, 53.

Haldane, J.B.S., Wigglesworth,V.B.,& Woodrow,C.E. (1924) Proc.roy.Soc.B., 96, 1.

Hawking,F.,& Thurston,J.P. (1951) Trans.roy.Soc.trop.Med. Hyg. 45, 307.

King,E.J. (1946) Microanalysis in Medical Biochemistry, Churchill, p.14.

Kleitman,N. (1923) Amer.J.Physiol., 66, 67.

Kleitman,N. (1925) Ibid., 74, 225.

Kulz,L. (1914) Arch.Schiffs. u.Tropenhyg., 18, 248

Low,G.C.,& O'Driscoll,E.J. (1921) Lancet, 1, 798.

Lundsgaard,C. (1918) J.biol.Chem., 33, 133.

McKenzie,S. (1881) Lancet,2, 722.

Manson,P. (1879) Med.Rep.Shanghai,Spec.Ser., 18, 31.

Manson,P. (1882) Ibid., 23, 1.

Manson, P. (1899) Brit.Med.J., 2, 644.

Mills,J.N. (1953) J.Physiol., 122,66.

Stanbury, S.W.,&Thomson,A.E. (1951) Clin.Sci., 10, 267.

PART 3

Investigations of Filariasis in the Field.

PART 3

SECTION A

Filariasis in Gambia and Casamance, West Africa.

This section describes an investigation of the incidence of filariasis in Gambia and the results of a survey of a village in the neighbouring French territory of Casamance. The surveys in Gambia were undertaken as the first stage in a research programme in filariasis and were also necessary to establish contact with villages to obtain material for future work. It was found that few lesions were produced by Wuchereria bancrofti infections in Gambians, and subsequently a village was surveyed in Casamance where this infection was known to have a high morbidity.

FILARIASIS IN GAMBIA

Gambia is a narrow stretch of territory on either bank of the river Gambia, extending inland about 200 miles. There is a broad area south of the mouth of the river and it was in this area that most of the investigations were undertaken. A survey was made of each of three differently sited villages in Gambia i.e. a coastal village, an inland village and a village near the river in close association with swamp. A survey on a larger scale might have been desirable, but this small survey, which was as large as could be undertaken alone in the time available, probably gives a fair indication of the existing conditions. All blood smears were examined by myself.

The map shows the location of the villages selected. The nearest points to these villages for which meteorological data were available are also shown.

Coastal village - Kololi. This village is approximately $\frac{3}{4}$ mile from the sea. There is a small stream $1\frac{1}{2}$ miles from the village, and during the rainy season rice is grown about 1 mile away.

Inland village - Jiboroh. This village is 14 miles from the sea. There is a small river 1 mile away and again rice is grown during the rains less than 1 mile from the village.

Swamp village - Mandinari. This village lies at the edge of the mangrove swamp of the river and has swamp round other two sides.

Meteorological data.

Meteorological records for a 5 year period were available for the places shown on the map, and are given in Table 1. The nearest point to the coastal village for which records were available was at Cape St. Mary, which is on the coast 4 miles from the village. The nearest point to the swamp village where observations were made was at Bathurst, at the edge of the river, 5 miles away. There was no meteorological post near the inland village, but the main meteorological office was 12½ miles from the village, and some distance away from the coast and from the river. It is thought therefore that climatic conditions there are similar to those in the inland village.

(a) Rainfall. There is one rainy season in Gambia and most of the rain falls during the months of July, August and September. On the coast the annual rainfall is the highest of the fhree places - 54.4 inches per annum averaged over a 5 year period. Inland the rainfall was 48.2 inches per annum and at Bathurst near the river it was the lowest of the three places with 44.7 inches per annum.

(b) Temperature. The maximum average temperatures do

not vary greatly during the year at these three points, but the minimum temperatures were lowest during the months of December, January and February. There was little difference in temperature between the three points, the maximum being of the order of 87°F and the minimum of the order of 70°F, the greatest extremes being recorded at Yundum.

(c) Humidity. The relative humidity is of course highest during the rainy season, but did not vary greatly between the three points. At 09.00 hours, it varied between 50 per cent in the dry season and 90 per cent during the rainy season.

Microfilariae in the blood.

The inhabitants of the villages were examined compound by compound, as this is the only way in which one can be reasonably certain of seeing all the people in the village. Even then, however, the head of a large compound might forget one of his many relatives. Another difficulty encountered was that the people would bring their friends from neighbouring villages, in the hope that having given a few drops of their blood they would then become eligible to receive medicine. In the first village surveyed, almost half the people whose bloods were taken on the first night were strangers.

The ages of the patients in the villages were

assessed with the help of an African assistant. Blood smears were made between the hours of 9 p.m. and midnight. A thick film of blood was taken from the tip of the finger and spread evenly between two grease pencil lines on a slide. The smears, which consisted of approximately 20 c.mm. blood, were dried, dehaemoglobinised, fixed with methyl alcohol and stained with Mayer's haemalum. There was no advantage to be gained by taking more accurately measured volumes of blood as the time of taking the samples was spread over 3 hours, during which the numbers of microfilariae in the blood increase greatly.

Clinical signs of filariasis

Elephantiasis of the limbs was easily observed, but the other manifestations of the disease were more difficult to enumerate, as the villagers were bashful. The technique employed was to make it known that I would treat scrotal enlargements, and in this way cases could report quietly on their own. This is obviously not as satisfactory as examining the whole population, but was the best means possible in the circumstances.

RESULTS

The only filarial parasites found in the survey were Microfilaria bancrofti and Mf.perstans. No evidence was found to suggest the presence of Loa loa or of Onchocerca volvulus.

The age incidence of microfilariae in the blood of the inhabitants of the three villages is recorded in Table 2, (which also gives the figures for the village in Casamance), and the sex incidence in the populations over the age of 10 years is recorded in Table 3.

1. Microfilaria bancrofti.

(a) Incidence in the villages. The highest incidence of Mf.bancrofti in the blood was 38.3 per cent, and that was in the coastal village. This figure, by statistical analysis, is significantly higher than those in the inland village (19.2 per cent) and in the swamp village (25.9 per cent). There is no significant difference between the last two figures.

(b) Sex incidence. The incidence of parasitaemia in males over the age of 10 years in the inland village (31.1 per cent) is significantly higher than in females (13.8 per cent), but there is no significant difference in incidence between the sexes in the other villages.

(c) Age incidence. Only one child under the age of 5 years was found to have Mf.bancrofti in the blood. Above this age, the incidence in all the villages increased up to the age of 40 years after which there was only a small increase.

2. Microfilaria perstans.

(a) Incidence in the villages. The highest incidence

of Mf.perstans was found in the inland village (68.6 per cent) and this is significantly higher than the incidence in the coastal village (10.2 per cent) and in the swamp village (12.9 per cent). There is no significant difference between the last two figures. It is also noted that there is an inverse relationship between the incidence of W.bancrofti and A.perstans, but it is difficult to determine the significance of this observation.

(b) Sex incidence. In all three villages, males have a significantly higher incidence of Mf.perstans than females.

(c) Age incidence. As with Mf.bancrofti, the incidence of Mf.perstans in the villages increased up to the age of 40 years after which there was no marked change.

3. Lesions due to infestation with Wuchereria bancrofti.

The lesions found in the villages were as follows:-

(a) Coastal village - Kololi. Four cases of elephantiasis of the lower limb and 1 case of hydrocele. The 4 patients with elephantiasis were newcomers to the village, and the lesions were not the result of infections contracted in the village.

(b) Inland village - Jiboroh. Two cases of elephantiasis of the lower limb and 2 cases of hydrocele. Again the 2 cases of elephantiasis had recently come to the village.

(c) Swamp village - Mandinari. One case of elephan-

tiasis of the lower limb and 1 case of hydrocele.

Total Seven cases of elephantiasis of the lower limb and 4 cases of hydrocele. The latter figure is not accurate as explained above, but is sufficient to indicate a low incidence. With only one exception, the cases of elephantiasis occurred in people who had immigrated to Gambia from French territory. It is also the experience of medical officers in the Gambia that most cases of elephantiasis occur in such people and that cases in Gambians are relatively rare. Lymphadenopathy in the groins is common, but it may be due to chronic ulceration of the legs which is also common. Very few cases of acute lymphangitis and lymphadenitis were observed.

4. Transmission of filariasis.

No investigations were made on the transmission of filariasis, as this was to be investigated by an entomologist at a later date. In view of the very high incidence of infection by A.perstans in the inland village a survey was made of the banana population in the three villages, to determine if there was any correlation between incidence of perstans and the numbers of banana trees in the village. Hopkins (1952) in the British Cameroons found that Culicoides austeni which transmits A.perstans there, breeds in the decaying stems of banana

trees. In the three villages investigated in Gambia there were banana groves, but there was no correlation between the banana population and the incidence of perstans.

A striking feature of the results obtained is the low morbidity caused by W.bancrofti infections. As stated above, the cases of elephantiasis in Gambia usually occur in people who have come from French territory; accordingly, through the kindness and co-operation of the French Government, arrangements were made to survey a village in Casamance, which is the home of one of the immigrant tribes - the Manjargoes.

FILARIASIS IN A VILLAGE IN CASAMANCE

The village selected was Elana, which straggles along the edge of mangrove swamp by the river Casamance. Some of the compounds were in close proximity to the water while others were up to 1 mile away. The direct distance to this village from the villages surveyed in Gambia was of the order of 40 - 60 miles. The people of the village were more primitive than the tribes in Gambia and the great majority had lived all their lives there. It was interesting to find that 2 of the cases of elephantiasis which had been recorded in Kololi in Gambia, were brothers who had left this village after developing the lesions. The people were heavier than Gambians, spent more time working their farms, and grew more food stuffs thus having a bigger and better diet. They also drank considerable amounts of milk. While taking the blood smears a marked feature was the almost complete absence of gross anaemia which is commonly encountered in Gambia, particularly among the younger age groups. The village was surveyed in the same manner as employed in the Gambian villages.

The age incidence and sex incidence of microfilariae in the blood, are recorded in Tables 2 and 3. The incidence of Mf.bancrofti was found to be 39.5 per cent, and the incidence of Mf.perstans 9.5 per cent. Except for 8.3 per cent of children under the age of 5

years being infected, these figures are very similar to those obtained in Kololi (coastal village) in Gambia. (38.3 per cent with Mf.bancrofti and 10.2 per cent with Mf.perstans). The average density of Mf.bancrofti in the positive blood slides in Elana was less than in Kololi (109.6 compared to 167.1). Thus, parasitologically these villages are very similar, but in the village in Casamance there was the following morbidity - out of a population of 390 persons, there were 22 cases of elephantiasis of the limbs (5.6 per cent) and there were also a large number of cases of either hydrocele or elephantiasis of the scrotum.

Sex incidence of microfilaraemia. There was no significant difference in the sex incidence of Mf.bancrofti, but males had a significantly higher incidence of Mf.perstans than females.

Sex incidence of elephantiasis. Three quarters of the cases of elephantiasis occurred between the years of 30 and 50. The youngest patient with elephantiasis was aged 15 years.

Anatomical distribution. The lower limbs were always affected, with one exception, where there was also a mild degree of swelling of the forearm. In half the cases of elephantiasis both legs were affected.

It also became apparent during the survey, that cases of elephantiasis had a familial grouping. Thus, 2 or 3 members of the same family had the lesion while other families were completely free from it. Unfortunately an accurate investigation of this point was not possible, but lay observers in the district had also commented on the same fact. O'Connor and Hulse (1935) recorded a familial incidence of filariasis in Puerto Rico.

Incidence of Mf.bancrofti in cases of elephantiasis. Out of a total number of 31 cases of elephantiasis observed in Gambia and Casamance, including 2 from other villages not surveyed, 26 cases had no Mf.bancrofti in the blood, and 5 cases had Mf.bancrofti.

DISCUSSION

Wuchereria bancrofti

The results described above may be considered according to (1) the incidence of microfilaraemia in the different groups of the population and (2) the morbidity produced by the infection.

1. Incidence of microfilaraemia.

(a) General incidence. The incidences of infection with W.bancrofti were found to be 19.2 per cent, 25.9 per cent and 38.3 per cent in the populations of the three villages surveyed in Gambia, and 39.5 per cent in a village in Casamance 50 miles away. The population in the coastal village in Gambia had a significantly higher incidence of infection compared to the other 2 villages surveyed in Gambia. The reason for this is not known at present. A survey of another village in Gambia which is 100 miles inland and close to the river (McGregor and Smith, 1952) showed an incidence of 36 per cent of the population with Mf.bancrofti. No other reliable figures for Gambia have been discovered. As regards other figures available for West Africa, Blacklock (1922) reported from Sierra Leone that 19.6 per cent of 240 people there, harboured Mf.bancrofti, and Pinto and de Almeido (1947) reported that 49.2 per cent of 485 people in Portuguese Guinea had microfilariae in the blood. Results obtained

in other parts of the world vary considerably, but microfilaria rates of greater than 40 per cent of the total population are rarely reported. The microfilaria rate is, of course, greater if the adult population is considered alone. In general, therefore, the population of the Gambia has a fairly high incidence of microfilaraemia.

(b) Age incidence. The youngest child in the Gambian villages which was found to have Mf.bancrofti in the blood was aet. 5 years and infections under the age of 10 years were not common. There is no evidence that children are less exposed to infection than adults. Therefore this implies that microfilariae do not appear in the blood until after long and repeated exposure to infected mosquitoes. Probably the majority of infective larvae fail to reach maturity or fail to encounter a worm of the opposite sex.

(c) Sex incidence. In all the villages, males were more frequently infected with W.bancrofti than females; in the coastal village this difference was significant by statistical analysis. The difference may be explained by the fact that the men sit about outside the huts in the evening exposed to mosquitoes, while the women are cooking. The smoke from the cooking fires, many of which are inside the huts, will tend to protect the women from being bitten. The evening is probably the most important period of

transmission as many of the villagers use mosquito nets over their beds at night.

2. Morbidity of W.bancrofti.

A striking feature in the three Gambian villages which were surveyed, was the small number of lesions produced by W.bancrofti. Similarly in the village surveyed by McGregor and Smith (1952), the total incidence of lesions produced by W.bancrofti was less than 1 per cent. By contrast, in the village in Casamance (Elana), 5.6 per cent of the population had elephantiasis of the legs. Why is it there was such a high incidence of elephantiasis in this village? There was not a higher microfilaria rate in the total population in Elana compared to the village of Kololi in Gambia, in which there were no cases of elephantiasis. In both villages the microfilaria rates were of the order of 40 per cent, and as stated previously the microfilaria rate in any population is seldom greater than 40 per cent. There was, however, in Elana a higher microfilaria rate under the age of 10 years. It is suggested that the early onset of microfilaraemia and the high morbidity in the village in Casamance are both the results of exposure to more intense infection, due either to the presence of larger numbers of mosquitoes in the area, a longer transmission season, or a better vector of the parasite.

A.perstans.

The incidence of Mf.perstans was significantly higher in the inland village compared to the other 2 Gambian villages. The reason for this is not known, and as it has been shown that certain species of Culicoides which can transmit perstans breed in decaying banana trees (Hopkins, 1952), a survey was made of the banana trees in the villages. There was no correlation between the numbers of banana trees in the villages and the incidence of perstans.

In all the villages, males had a significantly higher incidence of Mf.perstans than females, and again the explanation of this is probably the same as that suggested for W.bancrofti, namely that in the evening the males sit outside their huts exposed to Culicoides, while the females are cooking.

SUMMARY

(1) A coastal village, a swamp village and an inland village in Gambia, British West Africa, were surveyed for filariasis. The filarial parasites found were Wuchereria bancrofti and Acanthocheilonema perstans.

(2) The incidence of Mf.bancrofti in the blood varied from 19.2 per cent to 38.3 per cent of the population, the highest incidence being found in the coastal village. Few cases of microfilaraemia were found under the age of 10 years.

In all the villages males were more commonly infected with W.bancrofti than females.

(3) The incidence of Mf.perstans in the blood varied from 10.2 per cent to 68.6 per cent of the population, the highest incidence being found in the inland village. There was no correlation between the incidence of Mf.perstans and the number of banana trees in the villages. Again males were more commonly infected than females.

(4) The number of lesions e.g. elephantiasis of the leg, produced by W.bancrofti infections in Gambia was extremely small.

(5) In a village which was subsequently surveyed in the neighbouring French territory of Casamance (about 50 miles distant) the incidence of Mf.bancrofti was 39.5

per cent. This is similar to the incidence found in one of the Gambian villages (38.3 per cent). In the Casamance village, there was, however, a greater incidence in the younger age groups and a high incidence of elephantiasis of the legs. It is suggested that this is due to exposure to more intense infection, which is not reflected in the microfilaria rates of the total population.

MAP - showing the locations of the villages in Gambia which were surveyed. Places for which meteorological data were available are shown in brackets.

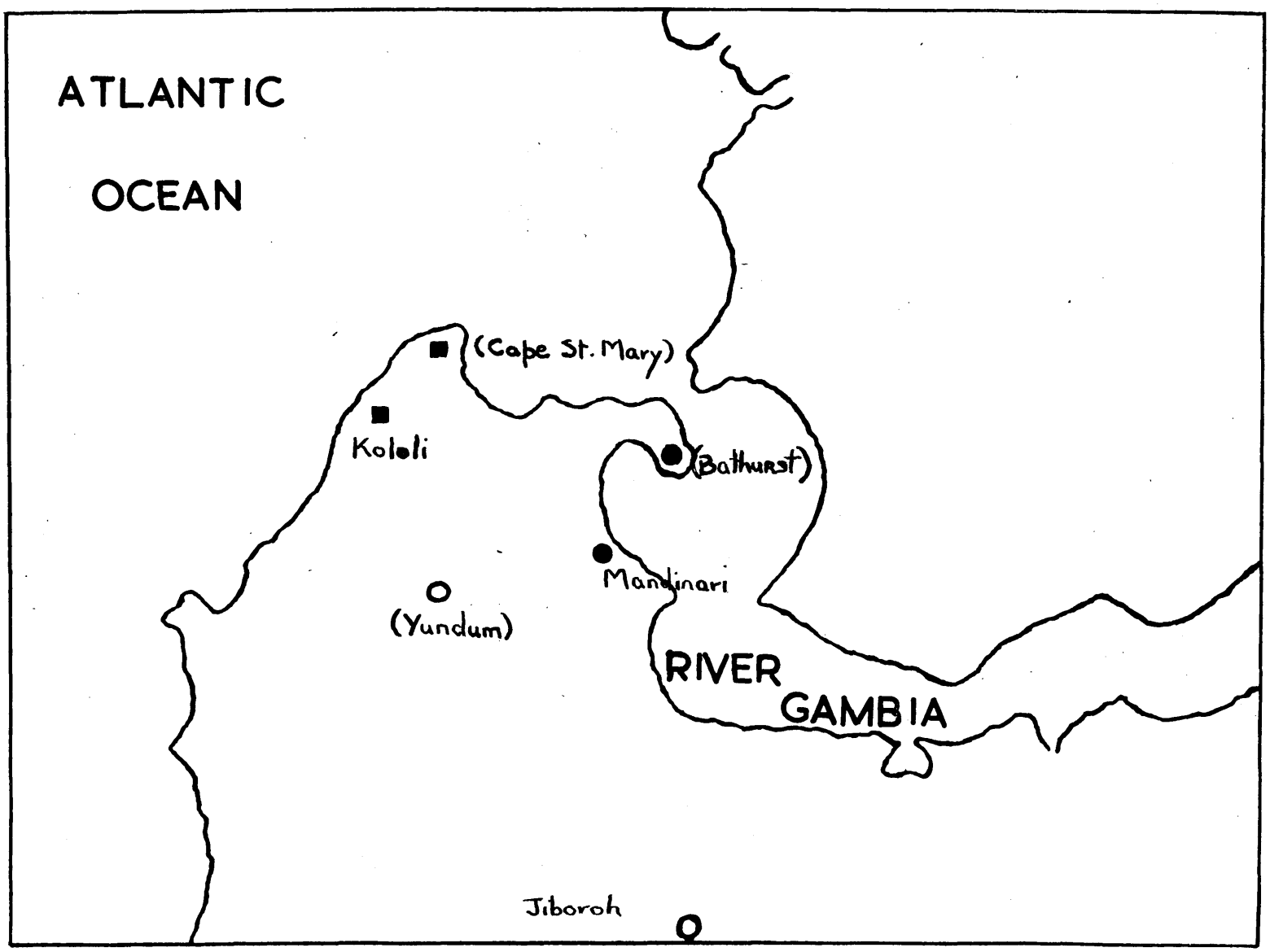


TABLE I

TABLE I.

METEOROLOGICAL DATA AT POINTS NEAR TO THE VILLAGES SURVEYED AND WITH SIMILAR CLIMATIC CONDITIONS.

	RAINFALL			MAXIMUM °F.			MINIMUM °F.			Rel. Humidity - % at 0.900 hrs.		
MONTHS	Bath.	Yund.	Cape	Bath.	Yund.	Cape	Bath.	Yund.	Cape	Bath.	Yund.	Cape
Jan.	-	-	-	85.6	87.8	82.3	65.0	57.0	64.3	47	53	55
Feb.	0.010	0.006	-	88.8	92.0	82.5	67.6	61.0	64.5	48	50	59
Mar.	-	-	-	90.8	95.4	83.0	68.8	63.8	67.0	64	61	68
Apr.	-	-	0.002	86.4	90.8	83.5	68.8	65.4	68.5	72	68	67
May	0.056	0.280	0.248	86.4	89.6	82.8	70.8	67.0	70.3	74	69	75
June	1.842	2.328	2.397	87.8	88.6	87.3	75.0	72.8	74.5	79	80	80
July	9.468	9.916	13.437	87.0	86.6	87.3	76.0	74.0	75.3	84	86	82
Aug.	19.046	21.676	17.643	86.2	85.4	86.3	75.6	73.4	74.8	88	90	85
Sept.	11.212	10.280	13.313	87.4	87.2	88.0	76.0	73.2	74.8	83	90	87
Oct.	2.956	3.894	5.647	89.6	89.2	87.3	76.6	72.0	74.8	79	86	83
Nov.	0.064	0.058	1.645	90.8	89.4	87.7	73.6	64.6	73.0	64	76	72
Dec.	o.086	0.108	0.128	86.8	87.4	83.5	67.8	60.8	67.7	55	66	64
Annual	44.740	48.294	54.460	87.8	89.1	85.1	71.8	67.1	70.8	69.7	72.9	73.3

TABLE 2

TABLE 2. Age incidence of Microfilaria bancrofti and Microfilaria perstans in the populations of the 4 villages surveyed.

Age Group (years)	Kololi - coastal village in Gambia			Jiboroh - inland village in Gambia			Mandinari - swamp village in Gambia			Elana - village in Casamance		
	Number in group	% with Mf.bancrofti	% with Mf.perstans	Number in Group	% with Mf.bancrofti	% with Mf.perstans	Number in Group	% with Mf.bancrofti	% with Mf.perstans	Number in Group	% with Mf.bancrofti	% with Mf.perstans
0 - 5	38	2.6	2.6	32	0	18.7	57	0	0	72	8.3	0
6 - 10	27	29.6	0	24	4.1	62.5	65	18.5	0	64	31.3	3.1
11 - 20	45	44.4	4.4	52	5.7	63.4	85	29.4	10.6	78	46.2	3.8
21 - 30	31	54.8	22.5	70	18.5	75.7	50	28	28	49	42.9	10.2
31 - 40	43	51.1	11.6	47	38.2	89.3	32	46.9	28.8	78	51.3	14.1
41 - 50	22	50.	27.2	22	45.4	86.3	15	66.7	33.3	36	61.1	33.3
51 - 60	9	55.5	22.2	4	50	100	9	55.6	33.3	11	63.6	18.2
60 +	9	22.2	0	4	50	75	3	33.3	33.3	2	100	100
Total	224	38.3	10.2	255	19.2	68.6	316	25.9	12.9	390	39.5	9.5

TABLE 3

TABLE 3. Sex incidence of Microfilaraemia in the populations over the age of 10 years in the 4 villages surveyed.

	Kololi - coastal village		Jiboroh - inland village		Mandinari - swamp village		Elana - Casamance village	
	Males	Females	Males	Females	Males	Females	Males	Females
Total number examined	89	70	119	80	97	97	130	124
Percentage with Microfilaria bancrofti	50.6	45.7	31.1	13.8	40.2	32	51.5	49.2
Percentage with Microfilaria perstans	16.9	10	84	67.5	33	9.3	21.5	5.6

REFERENCES

Blacklock, B. (1922). Ann.trop.Med.Parasit., 16, 107.

Hopkins, C. A. (1952). Ann.trop.Med.Parasit., 46, 165.

McGregor, I. A. & Smith, D. A. (1952). Trans.R.Soc.trop. Med.Hyg., 46, 403.

O'Connor, F. W. & Hulse, C. R. (1935). Puerto Rico J. publ.Hlth., 11, 167.

Pinto, A. R. & de Almeido, C. L. (1947). Ann.Inst.Med. trop.Lisboa., 4, 59.

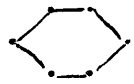
P A R T 3 (continued)

SECTION B

The Treatment of Filariasis due to *Wuchereria bancrofti* and *Acantho-cheilonema perstans* with Arsenamide.

THE TREATMENT OF FILARIASIS DUE TO W.BANCROFTI AND A.PERSTANS WITH ARSENAMIDE.

INTRODUCTION

Arsenamide is a trivalent arsenical and has the formula: $(COOH.CH_2.S)_2$ As  $CO.NH_2$.

It was first employed in the treatment of Wuchereria bancrofti infections by Thetford et al. (1948), when they reported the treatment of six cases. Since then, the compound has been used by various other workers as summarised in the Discussion below. This paper describes the results obtained in the treatment of 37 cases of filariasis in Gambia, British West Africa.

METHODS

Four groups of cases were investigated.

(1) Two cases of yaws were treated under close observation in the ward, as a preliminary test for tolerance. (2) Eleven cases of filariasis were treated in the ward under similar close observation. (3) Twenty-one cases were treated in a village; among these there was one fatality due to acute hepatic necrosis. (4) Five cases were treated in the ward with low doses and special precautions against toxic effects. For the present work the drug was supplied in 5 c.c. ampoules of 2.5 per cent buffered solution.

CLINICAL RESULTS

Group I. Two cases of yaws

(a) An adult male aet. 46 years, who complained of pain all over the body. Weight 60 kg. He was given one intravenous injection of 5 c.c. arsenamide, followed the next day by one injection of 7.5 c.c. and then 10 c.c. daily for 10 days. (2 mg./kg. arsenamide, 3 mg./kg. and then 4 mg./kg.). He vomited after the fifth and sixth injections; and occasionally a trace of albumin was found in the urine. During treatment he lost 3 kg. in weight which he subsequently regained. His previous symptoms cleared up.

(b) An adult male aet. 25 years, who had ulcers on the legs and on the arm. Weight 66 kg. He was given the same injections as Case (a). (1.9 mg./kg. arsenamide, 2.8 mg./kg. and then 3.8 mg./kg.). He did not vomit at all, but he lost 2 kg. in weight which was regained; and occasionally a faint trace of albumin was found in the urine. The ulcers cleared up rapidly.

These cases suggested that 10 c.c. of the solution (approximately 4 mg./kg.) daily would be tolerated. The dose level which was adopted for the trial against W.bancrofti was half this, viz. 5 c.c. (about 2 mg./kg.) daily for 12 days.

Group 2. Cases of filariasis treated in hospital.

Eleven cases were treated in the research ward at Fajara.

On admission, cases were examined clinically for any contraindication to treatment, and then 2 or 3 days were allowed to elapse in order that the microfilaria cycle could settle after the change in habits. At 10 p.m. on the evening before the injections of the drug were to commence, 2 lots of 20 c.mm. blood were taken from the finger tip and spread on a slide for enumeration of microfilariae in the usual way. Throughout the trial all the slides were examined and the microfilariae enumerated by myself. All subsequent measured quantities of blood in the cases treated in the ward were taken within a few minutes of 10 p.m. Conditions were further standardised by all the injections being given in most cases within a few minutes of 10 a.m. The urines from these cases were examined daily for albumin, each patient was weighed daily and a watch kept for any signs of toxicity of the drugs, particularly vomiting.

Table I records the microfilariae counts of the patients treated in the ward. Four to 5 days after the first injection, the total number of microfilariae (*bancrofti*) had decreased by 22 per cent; at 9 days it had decreased by 86 per cent; and at 12 days the total

reduction was 98 per cent. Subsequently, only occasional microfilariae were found in the bloods of these patients. Finally, when the last blood samples were taken (at 6 months) all the patients were free from microfilariae except for 1 who still had 2 microfilariae per 40 c.mm., and 1 patient who still had 1 microfilaria per 40 c.mm. (at 3 months).

In 8 of the patients Microfilaria perstans were also present initially; following treatment their total numbers were reduced by 72 per cent, and in 4 of the patients the blood became negative.

Toxic features observed in Group 2.

1. Vomiting. Three patients vomited occasionally. One other patient vomited 1/4 - 3 hours after each injection; this patient (Case 10) was receiving the largest dose per kg. of body weight and treatment was stopped.

2. Headache, (4 cases). This is difficult to assess as it is a common complaint among Africans.

3. Signs of death of adult worms or allergic reactions. Two patients had pyrexia. One other patient developed enlarged tender glands in the axilla and 1 patient developed a tender epididymis.

4. Loss of weight (1 case). This was the patient who vomited considerably.

5. Damage to liver (1 case). In 1 patient (No.6) the temperature rose finally reaching 104°F. after the 10th injection; 2 days later he vomited repeatedly; and the next day he developed jaundice with bile and albumin in the urine. He made a satisfactory recovery, and 1 month later he was much improved. It was difficult to assess the cause of the jaundice as it was possible that there was either previous liver damage or a coincidental infective hepatitis both of which are fairly common in Gambia. Since the patients previously treated had showed no other toxic features, the trial was continued; and subsequently 5 more cases were treated satisfactorily in the ward.

6. No albuminuria occurred in any of the patients, apart from the 1 case noted above.

Group 3. Cases treated in a village

Twenty-one cases of filariasis were selected for treatment in a village which had been previously surveyed. Heavy infections were chosen. Each patient was examined clinically. The projected dose schedule was a daily injection of 5 c.c. for 12 days. Since the lowest weight of any patient selected was 50 kg. no patient received a dose greater than 2.5 mg./kg. arsenamide daily. After the group had received 10 injections, however, treatment was discontinued because one of the patients died (see below).

The injections were given between 6 p.m. and 7.30 p.m. after the villagers had returned from their work in the fields. They were given intravenously and slowly with the patients lying down. Urine specimens were examined for albumin before treatment, and 3, 6, 9, and 15 days after the first injection. All patients were weighed before and after the course of injections. On the evening before the injections were commenced, measured quantities of blood were taken as described above, between the hours of 10 and 11 p.m. Any information on toxic features volunteered by the villagers was recorded, and direct questions were asked only after the course was completed.

Results

Table 2 records the microfilariae counts of

these patients. In all the blood samples taken 2 weeks after the first injection, only a single microfilaria bancrofti was found. Sixteen of these patients had received 10 injections of arsenamide, but 2 cases had received only 9 injections, 1 had received 8, and 1 had received only 5. Seven months after treatment the bloods of all the patients were free from microfilariae bancrofti.

Two of these patients also contained appreciable numbers of microfilariae perstans in the blood; 1 became negative.

Toxic features observed in the cases treated in the village.

1. Vomiting (7 cases, 33 per cent). One patient vomited after each injection, while the others vomited only occasionally.

2. Diarrhoea. Four people complained of occasional loose stools during the course of injections. This is difficult to assess; it was not noted in the cases treated in the ward.

3. Dizziness (2 cases).

4. Generalised pain. One person complained of pains all over the body. This is a common complaint in Africans and was probably associated with yaws.

5. Signs of death of adult worms or allergic reactions. One person developed a filarial abscess on the medial aspect of the thigh which subsequently broke down

and discharged thick creamy pus. One person developed a bilateral hydrocoele. One person had a transient enlargement of the scrotum. One person had an enlargement of the epididymis. Several days after the injections had ceased one person developed wheals on the skin associated with pruritis which cleared satisfactorily with "Anthisan".

6. Loss of weight. One person lost 3 kg. (associated with vomiting) and 1 person lost 3.5 kg. (not associated with vomiting).

7. Signs of liver damage. One man died from liver damage after receiving only 3 injections of the drug; this case is described in detail below. Another man developed jaundice 4½ weeks after the last injection; a fresh dry sterilised syringe and needle had been used for each injection, so this jaundice could not have been due to the passage of a virus.

8. There were no cases of albuminuria. The drug did not leak from a vein on any occasion.

Clinical and post-mortem report on the man who died after 3 injections of arsenamide.

The patient, aet. 55 years; weighed 53 kg. He was given 3 daily injections of 5 c.c. (2.4 mg./kg.). After the third injection he vomited and complained of vague retrosternal pains. The symptoms were mild and

hardly seemed sufficient to contraindicate further treatment; but no more injections were given. Two days afterwards he felt well; but later that night he developed pain in the back of the neck, staggered and was unable to stand or sit up. This was reported next day when the investigator returned to the village and found that the patient was comatose.

On admission to the ward. Patient comatose but able to move his limbs. Reflexes present and normal. Lumbar puncture - no increased pressure, Queckenstedt's test normal, Pandy's test positive, 60 lymphocytes per c.mm. No trypanosomes seen. Blood pressure - 230/120 mm. Hg. Urine contained no albumin or bile.

There was some doubt as to whether or not the patient had a slight icteric tinge but African observers considered that the colour of his conjunctivae was normal. The patient was treated with BAL and penicillin; but he developed the signs of basal pneumonia and died 4 days later.

Post-mortem findings. Liver weighed 880 gm. and was stained yellow. Kidneys weighed 170 gm. each. Heart weighed 284 gm.; some atheroma of heart and aorta. Urine contained no appreciable amounts of bile salts or pigments.

Histology. In the liver there were numerous areas

of recent focal damage from which most of the parenchyma cells had disappeared; there was considerable proliferation of fibroblasts, but no evidence of pre-existing cirrhosis. In the kidney, the cells of the cortical nephrons showed the appearance of massive necrosis (part of this appearance could have been due to post-mortem autolysis); many of the medium and small arterioles showed medial fibrosis and irregular intimal thickening.

It is concluded that death was due to widespread necrosis of the liver.

Group 4. Cases treated with low doses in hospital.

The experience with the third group of patients indicated that although large doses of arsenamide were therapeutically effective, they might also cause dangerous toxic effects. Accordingly an investigation was made to discover whether a much lower dose, which would be small enough to be safe, might still be sufficient to produce cure. For this purpose a limited group of patients was treated with strict precautions against toxic effects, viz. the dose was 0.3 mg./kg. daily (i.e. only one eighth of that previously used), the patients were kept under careful supervision in hospital, and they were given 50 gm. of dry skim milk daily (i.e. protein rich supplement) in addition to good hospital diet, so as to fortify the liver against the toxic effects of arsenic. No toxic effects were observed, and the therapeutic results are shown in Table 3. Reduction of the microfilaria count was much slower than it had been with large doses. In the 4 patients who could be followed for 5 months, the count was greatly reduced in 2 cases and probably the microfilariae will eventually disappear; in 1 case the count fell to a quarter of its original level, and in 1 case there has been no appreciable change. Thus the filaricidal effect of 0.3 mg./kg. daily although considerable is not completely satisfactory.

From this small group of patients, it might be deduced that 0.6 mg./kg. would be the minimum effective dose.

DISCUSSION

The action of a chemotherapeutic drug must be considered from two aspects, that of the parasite and that of the host, i.e. therapeutic action and toxicity respectively.

Therapeutic action - Arsenamide in high doses (2 mg./kg. daily) causes disappearance of the microfilariae of W.bancrofti from the blood within 2 weeks. Since this period is much less than their normal life span, the disappearance of the microfilariae must be directly due to a lethal action of the drug upon them. Such an action has been demonstrated in vitro in concentrations of approximately one in a million (Hawking, 1950). (The gradual disappearance of the microfilariae after small doses (0.3 mg./kg.) was presumably due to failure of replenishment after death of the adult worms).

Since the disappearance of microfilariae caused by arsenamide is permanent (more than 7 months, at least) there is evidence that death or permanent sterilization of the adult female worms has been produced; on general grounds and by analogy with the experimental findings with Litomosoides in cotton rats, it is almost certain that the adult worms have been killed. This effect is produced by doses of about 1 mg./kg. to 2.5 mg./kg.

usually given daily for 15 days. Thus Thetford et al. (1948) and Otto et al. (1952 and 1953) gave 1 mg./kg. for 15 days. In the present series the number of injections varied from 5 - 12; (9 patients received 12 injections, 17 received 10, 3 received 9, 1 received 8, and 1 received 5). Since the daily amount given was not calculated on a weight basis, the actual dose varied from 1.8 - 2.5 mg./kg. according to the weight of the patient. All the courses of injection were effective in removing microfilariae from the blood. With much smaller doses (0.3 mg./kg.) the antifilarial effect although considerable, is not complete. Perhaps the minimum curative dose is about 0.6 mg./kg.

Arsenamide also causes considerable reduction in the number of Mf.perstans in the blood. It is possible that arsenamide kills many of the adult worms of A.perstans, although it might require a long period of observation to obtain evidence of this since these microfilariae may live over $2\frac{1}{2}$ years in the absence of the adult worm. (Gonnert, 1942). As this parasite is nonpathogenic, however, such action is of academic interest only.

Toxic action - As described above, intensive treatment with arsenamide may produce various minor toxic effects e.g. vomiting, dizziness, headache, loss of weight and

(possibly) diarrhoea. Localised inflammation or allergic reactions due to the death of adult worms may also develop during treatment. All these, however, are overshadowed by the tendency of arsenamide to cause damage to the liver which may proceed (as in the case described above) to fatal necrosis. In the present series of 35 patients treated with doses of 1.8 - 2.5 mg./kg. per day 1 patient developed pyrexia after the tenth dose, and jaundice appeared 3 days later; 1 man developed jaundice about 4½ weeks after the last of 10 injections, and the relation of this to the treatment is not clear; and 1 man suffered fatal necrosis of the liver 3 days after receiving 3 doses of 2.4 mg./kg., total 7.2 mg./kg. Ampoules from this batch of material were returned to the manufacturers. Chemical examination revealed no alteration of composition, and biological assay showed no increase of toxicity. Therefore, the toxic effect on the liver must be due to the unchanged compound, probably to the arsenic part of the molecule; there is no reason for believing that the benzamide part of the molecule has any significant toxic action.

There had been considerable previous experience with this compound, and many patients had tolerated much greater total dosages without ill effects. Thus Murgatroyd (1937) administered it (under the name K.324)

to 8 patients with sleeping sickness in the Gambia in doses as high as 35 mg./kg.; doses greater than 2.5 mg. produced vomiting, abdominal pain, or diarrhoea and in 1 case there was severe jaundice. Thetford et al. (1948) and Otto et al. (1952), treated 18 cases of filariasis with doses of 1.0 mg./kg. daily intravenously for 15 days and observed only minor toxic reactions. Similarly, Otto et al. (1953) treated a further 15 cases with the same dose schedule. The main toxic features observed were diarrhoea and nausea; 1 case had severe lymphadenitis. Hawking (1950) treated 4 patients with daily injections of 100 or 150 mg. for 7 days (i.e. 1.7 - 2.5 mg./kg.). The larger doses were followed by vomiting and diarrhoea, but there was no suggestion of liver damage. In East Africa, 36 cases have been treated with doses of 1 mg./kg. daily for about 14 days; 1 case of jaundice occurred. (Filariasis Research Annual Report, 1952). Two cases of non-fatal severe hepatitis with jaundice, which occurred in the West Indies after treatment with arsenamide, have been heard of privately. No evidence of special toxic action upon the liver of rabbits, dogs, or monkeys was seen by Otto and Maren (1950); or by Drudge (1952) who gave dogs 2.2 mg. arsenamide per kg. daily i.v. for 15 days.

Apparently the death described above was another

example of the tendency of arsenic compounds to cause extensive necrosis of the liver in a very small proportion of the cases treated with it. Such subjects must be predisposed to this damage by accessory factors, but the nature of such factors and the causation of widespread liver necrosis in man are still largely unknown. The possible accessory factors include:-

(a) A virus infection, latent or incipient. Hepatitis, presumably infective, certainly occurs in the Gambia, since several Europeans developed it recently. In the present investigation a clean sterile syringe and needle were used for each injection, so there was no possibility of passage of infection by this means; moreover, the incubation period was much too short for viral hepatitis to develop from the patients' injections.

(b) Lack of essential nutrients especially tocopherol and cysteine. This food deficiency causes hepatic necrosis in rats (Himsworth, 1950). Many Africans are certainly malnourished, but there is no evidence that the state of nutrition of the present patient was worse than that of the other people in the same village who were treated. However, in any further trials with arsenical compounds it would be advisable to take the precaution of fortifying the liver against such toxic action by giving a

protein rich supplement e.g. 50 g. dried milk daily in addition to the usual food.

It is concluded that arsenamide, in ~~daily~~ doses of 1 mg. or more per kg. daily for 10 - 14 days, is an effective drug for the sterilisation of bancroftian infection in patients, but that its use is not devoid of risk. Since filariasis is not a fatal disease, it is accordingly not justified to use arsenamide for general treatment. In any further investigation of this drug careful precautions should be taken to avoid damage to the liver viz. the dose should be kept low, preferably below 0.6 mg./kg.; the patients should be treated in hospital under strict medical supervision; and the protein content of the patient's diet should be increased (e.g. 50 g. skim milk powder daily in addition to a good general diet) in order to fortify the liver, the protein supplement being given for 3 days before, during, and 3 days after treatment.

SUMMARY

(1) Thirty two cases of filariasis due to W.bancrofti were treated with arsenamide (p-bis(carboxymethyl mercapto)-arsino-benzamide). The dose was 125 mg. (i.e. 1.8 - 2.5 mg./kg.) daily by intravenous injection usually for 10 - 12 doses.

(2) This treatment removed most of the microfilariae of W.bancrofti from the blood within 2 weeks after the first dose, and all but 2 of the patients were free from microfilariae 6 months later. It is concluded that the treatment killed or sterilized the adult worms; it also killed many of the microfilariae (and the rest disappeared in the absence of adult worms to replenish their numbers). The treatment also caused a significant reduction in the number of Mf.perstans present, indicating a filaricidal action on this worm also.

(3) The drug undoubtedly has a toxic effect upon the liver. In 2 cases the treatment was followed by jaundice, 3 days and 4 weeks respectively, after the last injection; and 1 patient developed fatal necrosis of the liver after having received only 3 injections. Other minor toxic effects included vomiting, dizziness, headache, and loss of weight. A few patients showed localised inflammation or allergic reactions, apparently around worms which had been killed.

(4) Five more patients were treated with much lower doses (0.3 mg./kg.) daily for 14 days under strict medical supervision, and with a protein rich supplement to fortify the liver. No toxic effects were observed. Among the 4 patients who were followed for 5 months, the microfilaria count was reduced almost to zero in 2, it fell to a quarter in 1, and it remained unchanged in 1. Perhaps the minimum curative dose is about 0.6 mg./kg.

(5) Arsenamide in doses of 1 - 2 mg./kg. daily is a potent filaricidal drug against W.bancrofti but the danger of severe toxic action upon the liver is a contraindication to its use except under special conditions.

TABLE I

TABLE I. The treatment of 11 cases of bancroftian filariasis (Group 2) in hospital by Arsenamide, 125 mg. per person daily.

Case No.	Age	Weight kg.	No. of injections	Microfilariae per 40 c.mm. (Mf.perstans in brackets)							
				Start	Days 4 - 5	9	12	26	40 - 60	70 - 100	200 - 250
1	28	55	12	67(115)	48(67	9(58)	4(62)	4(53)	0(23)	0(39)	-
2	50	55	12	40(124)	26(125)	6(125)	5(98)	1(167)	0(46)	0(50)	-
3	30	65	12	399(4)	461(8)	72(7)	7(11)	1(8)	4(4)	1(4)	-
4	40	58	12	73(63)	63(70)	10(73)	0(30)	0(39)	0(37)	-	0(10)
5	17	42	12	302(7)	336(10)	31(4)	0(5)	0(0)	0(0)	0(1)	0(0)
6	40	62	10	459(41)	-	24(49)	0(25)	-	1(11)	0(6)	0(0)
7	18	61	12	580(19)	454(14)	107(10)	24(6)	-	4(3)	0(1)	0(0)
8	16	49	12	808	518	75	3	-	1	1	0
9	50	48	12	231(1)	107(0)	76(2)	9(0)	-	0(0)	2(0)	2(0)
10	50	41	9	117	22	-	0	0	-	0	0
11	25	54	12	103	75	-	1	0	0	-	-

The days are recorded after the first injection. Cases 5 and 10 were females, the rest males.

TABLE 2

TABLE II. The treatment of 21 cases of bancroftian filariasis (Group 3) in a village.

Case No.	Age	Weight kg.	No. of injections	Microfilariae per 40 c.mm. (Mf. perstans in brackets). Start	Weeks 2	5	12	17	30
14	35	65	10	228	0	1	0	0	0
16	38	56	10	52	0	0	0	0	0
17	40	70	10	65	0(1)	0	0	0	0
18	37	67	10	185	0	0	0	0	-
19	21	51	10	132	0	0	0	0	0
20	60	62	10	141	0	0	0	0(1)	0
21	45	68	10	284(1)	0(2)	-	-	-	-
22	50	61	10	35	0	0	0	0	0
23	40	51	10	116	0	0	0	0	-
24	35	61	10	166	0	0	0	0	0
25	35	50	10	141	0	0	0	0	0
26	25	64	10	21(67)	0(3)	0(7)	0(2)	0(6)	0(0)
27	28	65	10	263(9)	1(4)	1(4)	0(3)	0(2)	0(3)
31	50	67	10	59	0	0	0	0	0
32	35	52	10	652	0	0	0	0	0
28	36	58	10	1,337	0	0	-	0	0
29	55	50	9	64	0	0	0	0	0
30	18	63	9	132	0	0	0	-	-
13	36	61	8	230	0	0	0	0	0
15	35	50	5	834	0	0	0	0	0
12	55	53	3	19	Dead				

The weeks are recorded after the first injection. Cases 15, 19, 20, 23, 25, and 32 were females, the rest males. The dose was 125 mg. Arsenamide per patient daily.

TABLE 3

TABLE III. The treatment of 5 cases of bancroftian filariasis (Group 4) with low doses of Arsenamide, viz. 0.3 mg./kg. daily.

Case No.	Age Years	Weight kg.	No. of injections	Microfilariae per 40 c.mm. (*Mf. perstans* in brackets).			
				Start	Days - 14	80	155
33	26	54	14	83	112	26	17
34	24	51	14	52	66	66	5
35	15	37	14	13	12	6	1
36	9	25	14	111	151	120	102
37	40	48	14	104(59)	54(21)	-	-

Case 35 was male, the rest females.

REFERENCES

Drudge, J. H. (1952). Amer.J.vet.Res. 13, 220.

Filariasis Research Annual Report (1952), East African High Commission, Nairobi.

Gonnert, R. (1942) Zbl.Bakt.(Orig.). 149, 75.

Hawking, F. (1950). Trans.R.Soc.trop.Med.Hyg., 44, 153.

Himsworth, H. (1950). Lectures on the Liver and its Diseases, 2nd ed. Oxford. Blackwell Scientific Publications.

Murgatroyd, F. (1937). Ann.trop.Med.Parasit., 31, 473.

Otto, G. F., & Maren, T. H. (1950). Amer.J.Hyg., 51, 353.

Otto, G. F., Brown, H. W., Bell, S. D., Jr., & Thetford, N. D., (1952). Amer.J.trop.Med.& Hyg., 1, 470.

Otto, G. F., Jachowski, L. A., Jr., & Wharton, J. D. (1953). Amer.J.trop.Med.& Hyg., 2, 495.

Thetford, N. D., Otto, G. F., Brown, H. W., & Maren, T. H., (1948). Amer.J.trop.Med.& Hyg., 25, 577.

~~Watson, M. (1950). Ann.Soc.belge Med.trop., 30, 667.~~

PART 3 (continued)

SECTION C

The Effect of Adrenocorticotrophic Hormone on Elephantiasis of the Lower Limb.

Introduction.

This section describes the results of treatment by adrenocorticotropic hormone, of cases of elephantiasis of the lower limb in Gambia, Elephantiasis in the tropics is associated with infestation by the filarial parasite Wuchereria bancrofti and occurs where this parasite is endemic. It is thought that the adult worms, which inhabit the lymphatics of the groin, produce oedema and later elephantiasis by obstruction of the lymph flow. Possibly lymphatic obstruction is produced either by a foreign body reaction round dead worms or by an antigen antibody reaction round living worms, and it was thought firstly, that A.C.T.H. might interfere with any reaction round the worms in the lymphatics of the groin. Secondly, A.C.T.H. might reduce the amount of elephantoid tissue in the limb. This elephantoid tissue is connective tissue much hypertrophied by its being constantly bathed in lymph.

MATERIALS AND METHODS

Measurement of the limbs.

Direct measurement of the circumferences of the legs by a tape measure at fixed points was found to be inaccurate. Therefore a method of measurement by water displacement was used. A bucket of approximately 12 inches diameter by 16½ inches deep was filled with water. The patient inserted his leg, and when movement had stopped, the water level was raised until it overflowed. The leg was then gently withdrawn and the excess water shaken into the bucket. Measured volumes of water were added to the bucket from a 1 litre measuring cylinder until a drop or two of water began to overflow. See Fig.1. The patient always stood in the same place and inserted his leg in the same fashion, and the bucket was not moved during the investigations. Ten readings were made on a normal leg in this manner and the standard deviation of a single observation found to be 25 c.c. This figure could probably be decreased by the addition of a wetting agent to the water.

Great care has to be observed in assessing the value of treatment as the size of an elephantoid limb varies greatly depending on the amount of standing and exercise undertaken by the patient. Thus a limb measured immediately on rising in the morning will be

considerably smaller than when measured after working in the fields. A standard set of conditions ~~were~~ was evolved to eliminate as far as possible a false result being obtained. On admission, each patient was kept moving about during the day for 2 or 3 days and he was not allowed to elevate his legs at all during the day time. The limb was measured at the same time daily until a fairly constant maximum figure was obtained. The patient then had complete rest in bed and a crepe bandage was applied to the affected limb and renewed daily at the same time and by the same person. The patient was only allowed up for daily weighing and measurement of the limb. When a steady minimum reading was obtained A.C.T.H. therapy was commenced. When the administration of A.C.T.H. had ceased, the patient remained in bed for approximately one week, and then returned to leading an active life about the ward. The limb was subsequently measured at intervals.

A.C.T.H. Dosage

The dose schedule employed was as follows:- 12.5 mg. were injected at 6 hourly intervals for 2 days followed by 15 mg. 6 hourly for 2 days, followed by 20 mg. 6 hourly for 4 days. Subsequently, if no improvement was observed at any stage during this treatment, the doses were tapered off.

The patients were weighed daily, given a high protein salt restricted diet, restricted fluid intake, and 0.5 gm. of potassium acetate four times daily, for some time before commencing A.C.T.H. dosage and during treatment.

Estimation of eosinophiles.

The total number of white cells was estimated in the standard fashion, and the number of eosinophiles per 300 - 400 white cells was counted in a thick film stained by Field's rapid method. This was done 4 hours after the morning injection of A.C.T.H. - usually about 11 a.m. This technique was simple, speedy and accurate.

RESULTS

Case 1. An adult male, aet. 25 years, weighing 64 kg., with elephantiasis of the right leg below the knee of 11 years duration. A night blood film contained no microfilariae bancrofti although microfilariae perstans were present. Graph 1 records the water displacement of the affected limb before, during and after treatment with A.C.T.H. During the preceding rest in bed, the leg decreased by 1,175 c.c. (23.6%), during A.C.T.H. therapy it decreased by 380 c.c. (7.6%) in the first 24 hours, and subsequently showed only a very gradual tendency to decrease. After the patient became active again, the displacement of the leg increased until when he was examined 3½ months after therapy it was only 105 c.c. less than the original maximum figure. The patient had a high eosinophilia and A.C.T.H. had little effect on the number of eosinophiles.

Case 2. An adult male, aet. 30 years, weighing 53 kg. with elephantiasis of the left leg ~~about~~ above and below the knee of unknown duration. A night blood film contained no microfilariae. Graph 2 records the water displacement of both the affected limb and the normal right leg before, during , and after treatment with A.C.T.H. During the preceding rest in bed the leg decreased by 1,570 c.c.

(30.5%), but during A.C.T.H. therapy it did not alter significantly. After A.C.T.H. was discontinued the limb decreased by 350 c.c. After the patient became active the limb increased in size until the displacement was only 290 c.c. less than the original maximum figure. The apparently normal leg decreased with rest in bed by 255 c.c. (10.2%) and rose again when the patient became active to 75 c.c. less than the original maximum figure. The total white cells showed a gradual tendency to rise during treatment with A.C.T.H., and the eosinophiles decreased to nil at one point.

Case 3. An adult male, aet. ?30 years, weighing 80 kg., with gross elephantiasis of the left leg below the knee, of 10 years duration, and mild elephantiasis of the right leg below the knee of 2 years duration. A night blood film contained no microfilariae bancrofti although microfilariae perstans were present. Graph 3 records the water displacement of both limbs before, during, and after treatment with A.C.T.H. During the preceding red in bed, the left leg decreased by 5,370 c.c. (53.4%) but during A.C.T.H. therapy it did not alter significantly. In this case there was a long interval during which the volume of the left leg gradually fell and it was longer than one month before A.C.T.H. was exhibited. Fig.2 illustrates

the marked changes produced in the legs of cases 2 and 3 with rest in bed. After the patient became active again, the displacement of the leg increased rapidly but as the patient wished to return home, the last measurement obtained showed the left leg to be 1,630 c.c. smaller than the original measurement. Similarly the right leg fell by 1,550 c.c. (29.6%) during the preceding rest in bed, but no significant change took place during treatment by A.C.T.H. When the patient became active again the right leg increased in size until it was actually 110 c.c. greater than the original maximum measurement. The eosinophiles only gradually decreased in number but no change occurred in the total number of white cells.

Cases 4 and 5. These cases were controls and were treated in exactly the same manner as the above cases with the exception that they did not receive A.C.T.H.

Case 4. An adult male, aet. 25 years, weighing 77 kg., with elephantiasis of the left leg above and below the knee, and a milder degree of elephantiasis of the right leg below the knee. A night blood film contained no microfilariae. Graph 4 records the water displacements of both legs at all stages of treatment. With rest in bed the left leg decreased by 2,980 c.c. (36.9%) and the right leg by 1,805 c.c. (31.6%). When the patient became

active again the left leg increased in size until the displacement was only 720 c.c. less than the original maximum, and similarly the right leg increased until the displacement was only 305 c.c. less than the original maximum.

Case 5. An adult male, aet. 28 years, weighing 60 kg., with elephantiasis of the left leg below the knee. A night blood film contained no microfilariae. Graph 5 records the water displacement of both legs at all stages of treatment. With rest in bed the left leg decreased by 1,750 c.c. (37.4%). The changes in size of limbs of cases 4 and 5 are shown in Fig.3. After the patient became active the limb increased in size until the displacement was only 350 c.c. less than the original maximum. With rest in bed the apparently normal leg decreased by 520 c.c. (16.1%) and when the patient became active again it increased in size until its displacement was 230 c.c. greater than the original maximum.

DISCUSSION

In all five cases of elephantiasis investigated there were very marked reductions in the water displacements of the elephantoid legs with rest in bed and the application of a crepe bandage (See Table). Even in two apparently normal legs the displacements decreased by 10.2 per cent and 16.1 per cent respectively. It is difficult to say whether these were early cases of elephantiasis or if there was a mild degree of nutritional oedema superimposed. All the legs, including those just mentioned more or less returned to the original measurements when the patients became active again. In view of the marked reduction in size of the elephantoid legs with rest in bed - up to 53.4 per cent - it is likely that many of the successes claimed for unorthodox treatments in the past have been the result of rest in bed alone.

It is clear from the results obtained that A.C.T.H. has no effect on elephantiasis of the leg associated with infestation with W.bancrofti. The fall in water displacement 24 hours after the first injection with case 1, and the fall in displacement recorded in case 2 after the A.C.T.H. dosage had ceased are thought to have been due to further loss of fluid from the leg with rest in bed.

In only one case out of three, did A.C.T.H.

have any marked effect on the number of eosinophiles in the blood. (See Graph VI). All three cases had initially high eosinophile counts and it was the lowest of the three which fell on the administration of A.C.T.H. The highest count of all (3,000 - 4,000/c.mm.) showed no response, and the next highest count (1,000 - 2,000/c.mm.) showed a gradual tendency to fall. The total white count in one case gradually rose, but in the other two cases there was no significant change. (See Graph VII).

Thick films for malaria parasites were examined daily during treatment by A.C.T.H., as relapses of malaria have been reported during treatment (Schmidt & Squires, 1951). Plasmodium falciparum is hyperendemic in Gambia, and P.malariae and P.ovale also occur. Parasites were not found in any of the cases during treatment with A.C.T.H.

At present there is no satisfactory treatment for elephantiasis of the limbs. One of the above cases, however, was visited in his own village several months after treatment, and it is interesting to record that he had experienced marked psychological improvement. For the first time in a number of years, he had been able to work on his farm. This was in spite of the fact that there had been no sustained physical improvement.

SUMMARY

(1) Five cases of elephantiasis of the lower limb were investigated in Gambia, British West Africa. Three cases were treated with adrenocorticotropic hormone and 2 cases observed as controls.

(2) The limbs were reduced in size as far as possible with rest in bed and the application of crepe bandages as a preliminary to treatment. This produced a decrease in the water displacement of the limbs of up to 53.4 per cent. It is suggested that many of the successes claimed for unorthodox treatments are the result of rest in bed alone. Two apparently normal limbs were reduced by rest in bed, but it was not possible to say if these were early cases of elephantiasis or cases of mild nutritional oedema.

(3) A.C.T.H. was administered subsequently and no significant effect was produced.

(4) Two of the cases treated had very high eosinophilias and the total number of eosinophiles in the blood was only slightly affected by A.C.T.H.

Figure 1.

The water displacement technique of measuring legs.

A. Inserting the leg.

B. Replacing the displaced water.

Figure 2.

Cases 2 and 3. Showing the changes in size of the elephantoid limbs produced by rest in bed and the application of crepe bandages.

Case 2 - before.

Case 2 - after.

Case 3 - before.

Case 3 - after.

Figure 3.

Cases 4 and 5. Showing the changes in size of the elephantoid limbs produced by rest in bed and the application of crepe bandages.

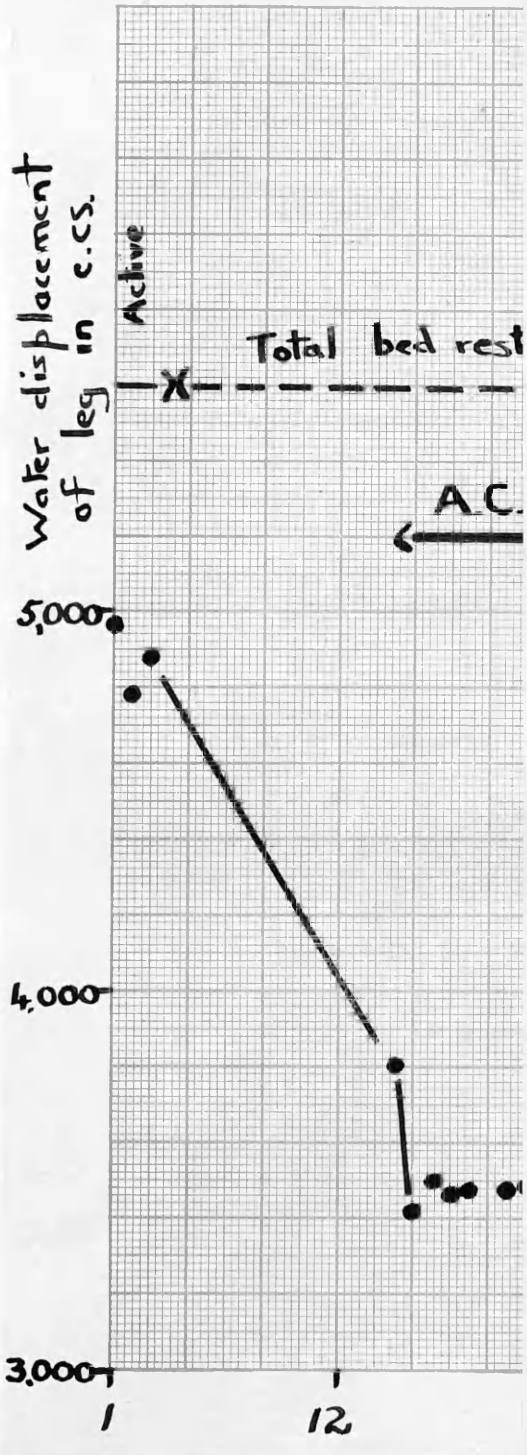
Case 4 - before.

Case 4 - after.

Case 5 - before.

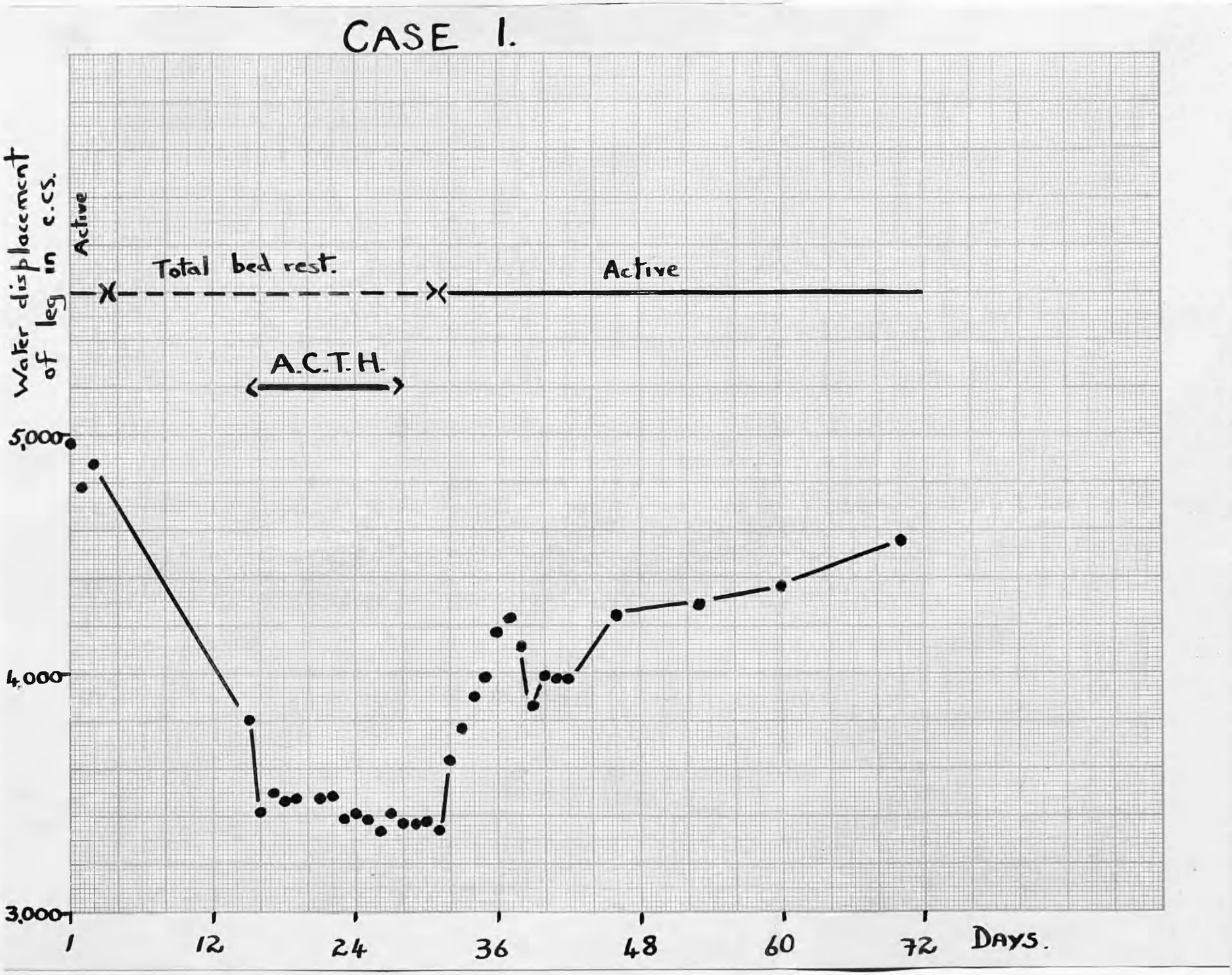
Case 5 - after.

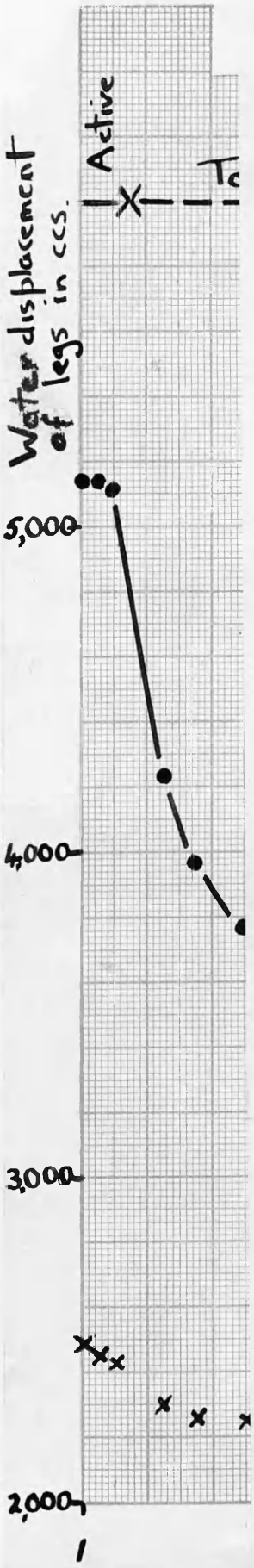
Graph 1 - showing the effect of rest in bed and A.C.T.H. on the water displacement of the elephantoid leg in Case 1.



GRAPH 1.

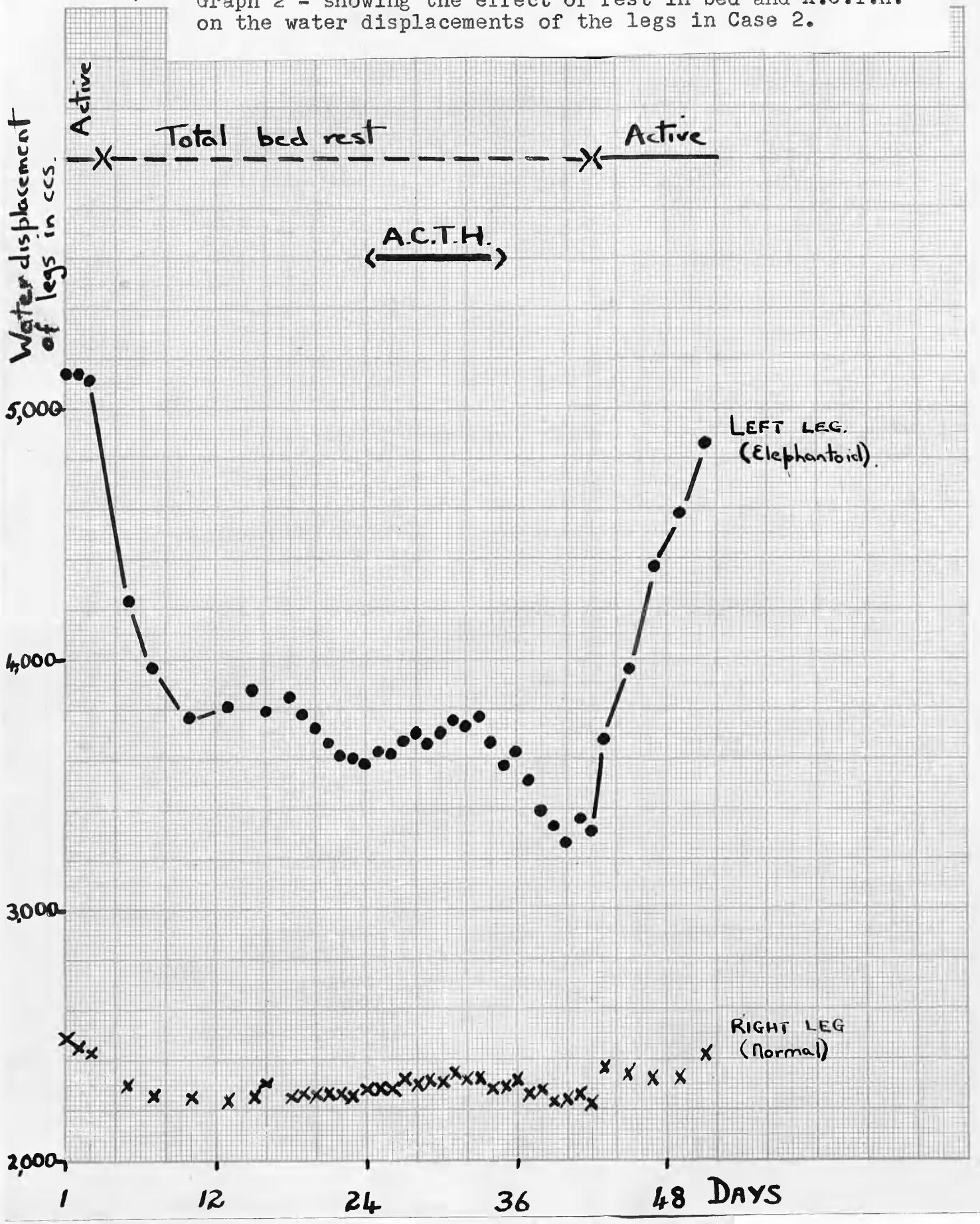
Graph 1 - showing the effect of rest in bed and A.C.T.H. on the water displacement of the elephantoid leg in Case 1.



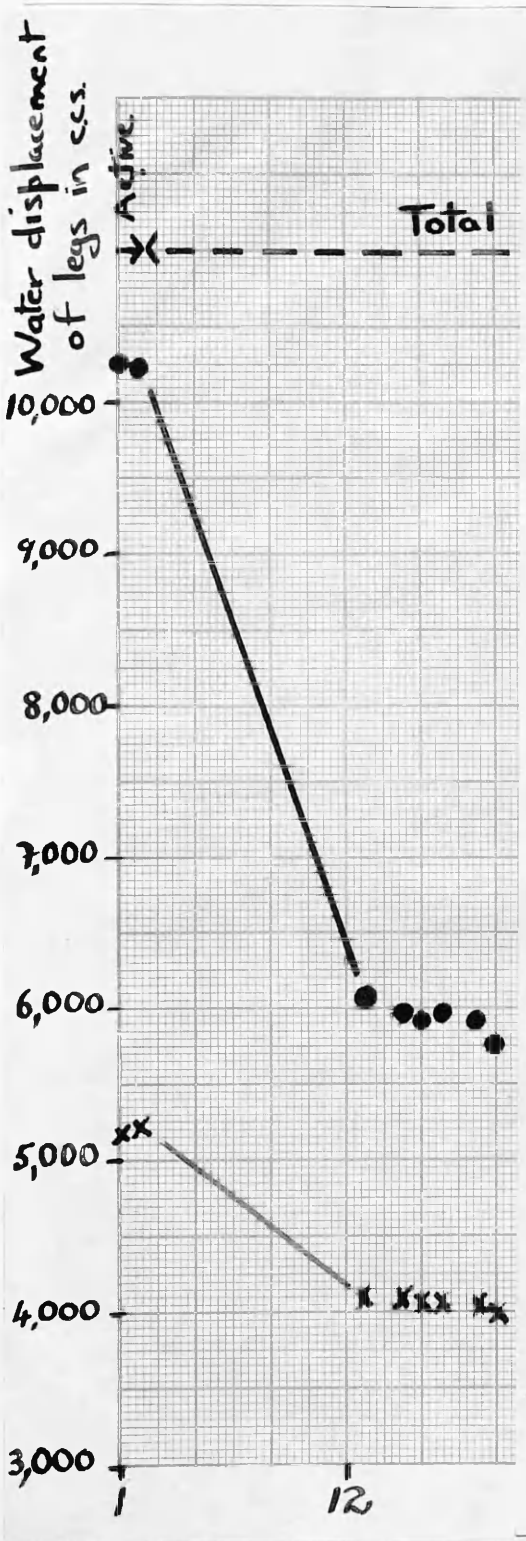

Water displacement of legs in ccs.
Active
T_c
5,000
4,000
3,000
2,000
1

GRAPH 2.

Graph 2 - showing the effect of rest in bed and A.C.T.H. on the water displacements of the legs in Case 2.

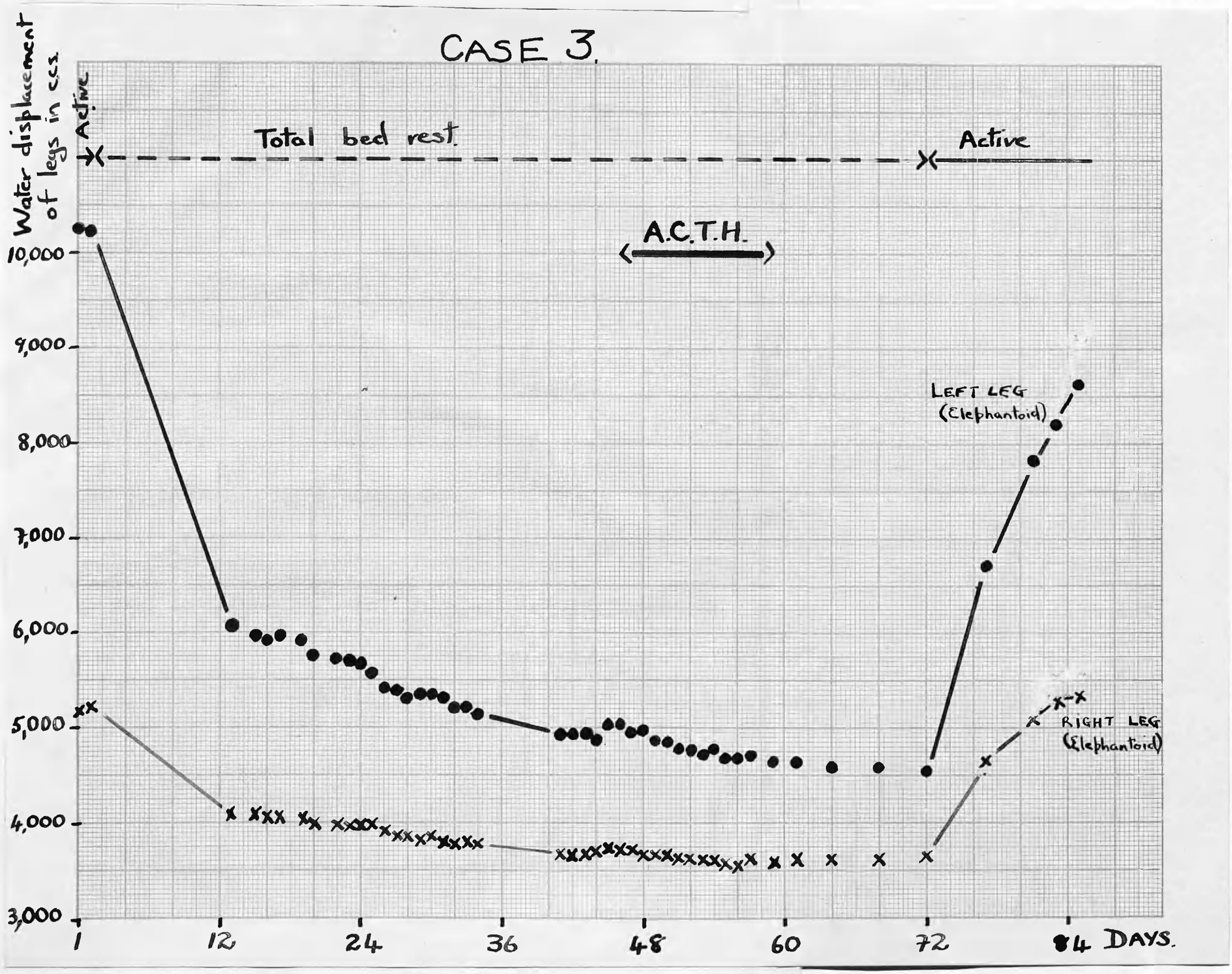


Graph 3 - showing the effect of rest in bed and A.C.T.H. on the water displacements of the legs in Case3.

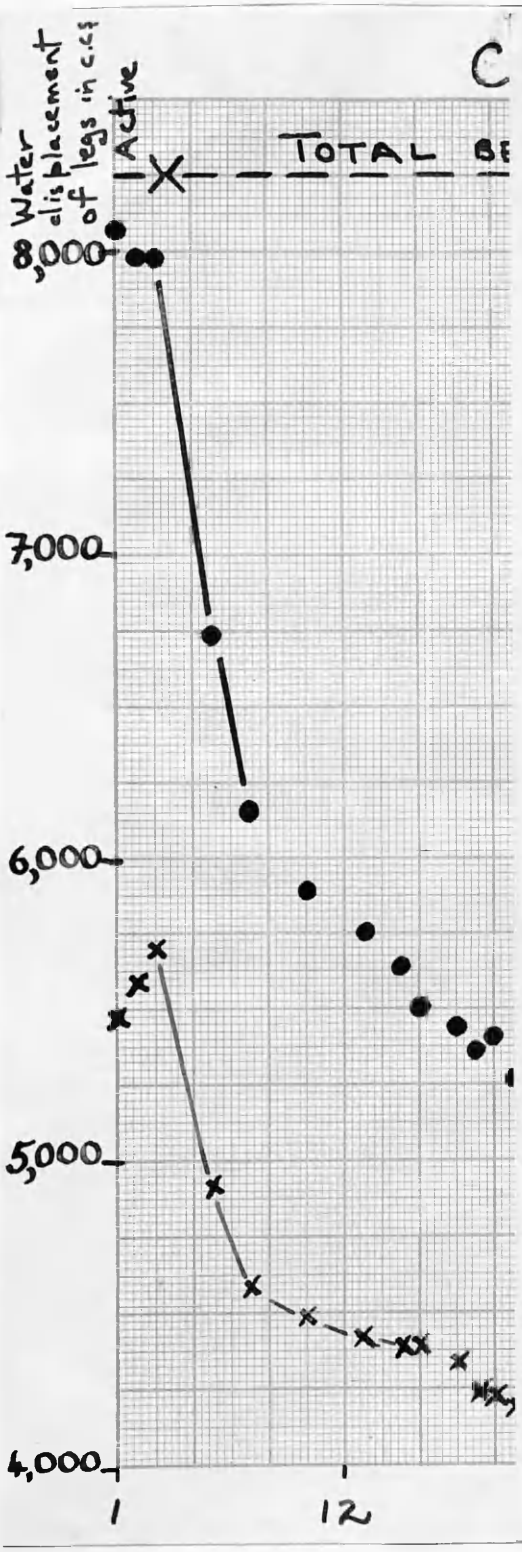


GRAPH 3

Graph 3 - showing the effect of rest in bed and A.C.T.H. on the water displacements of the legs in Case3.

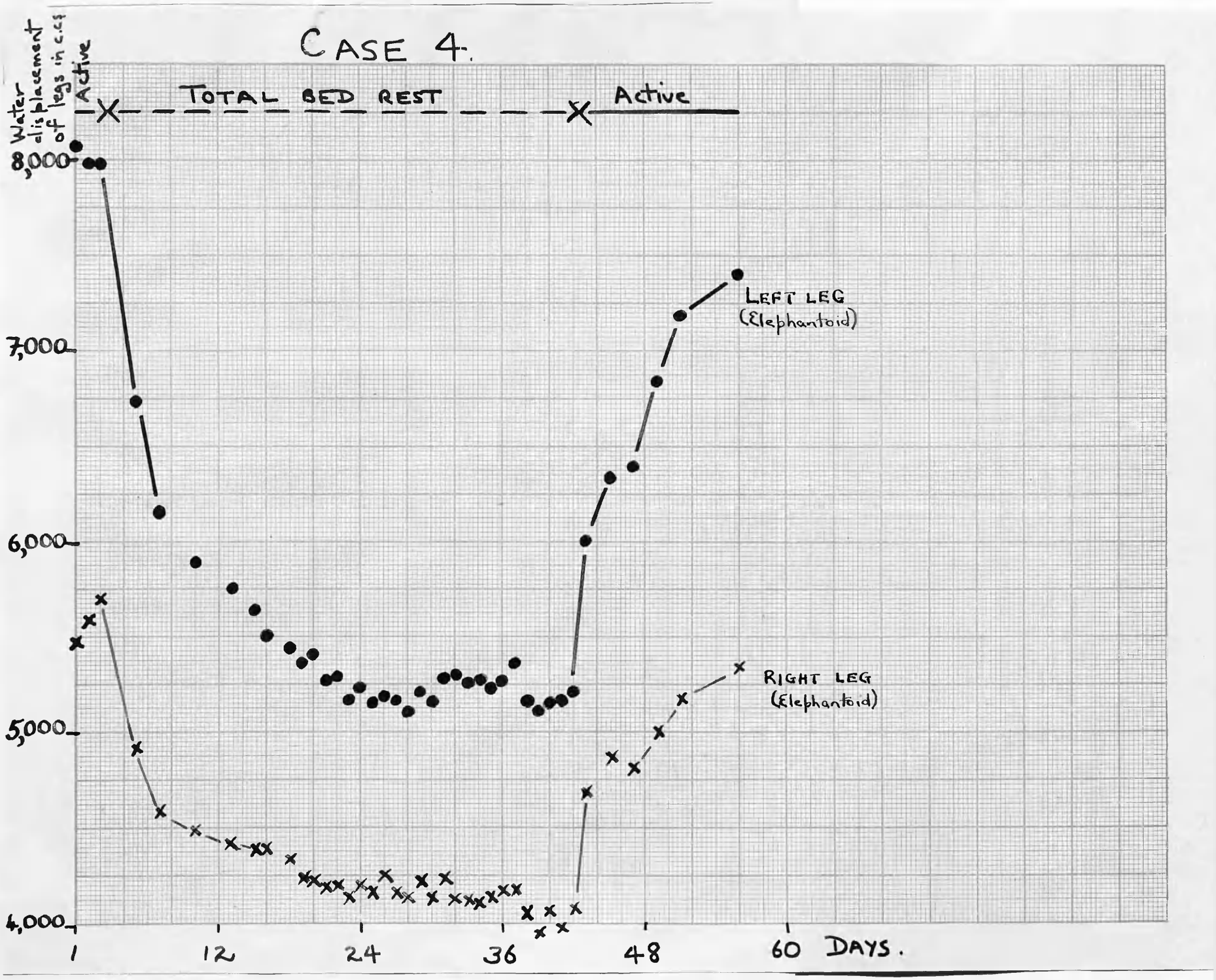


Graph 4 - showing the effect of rest in bed on the water displacements of the legs in Case 4.

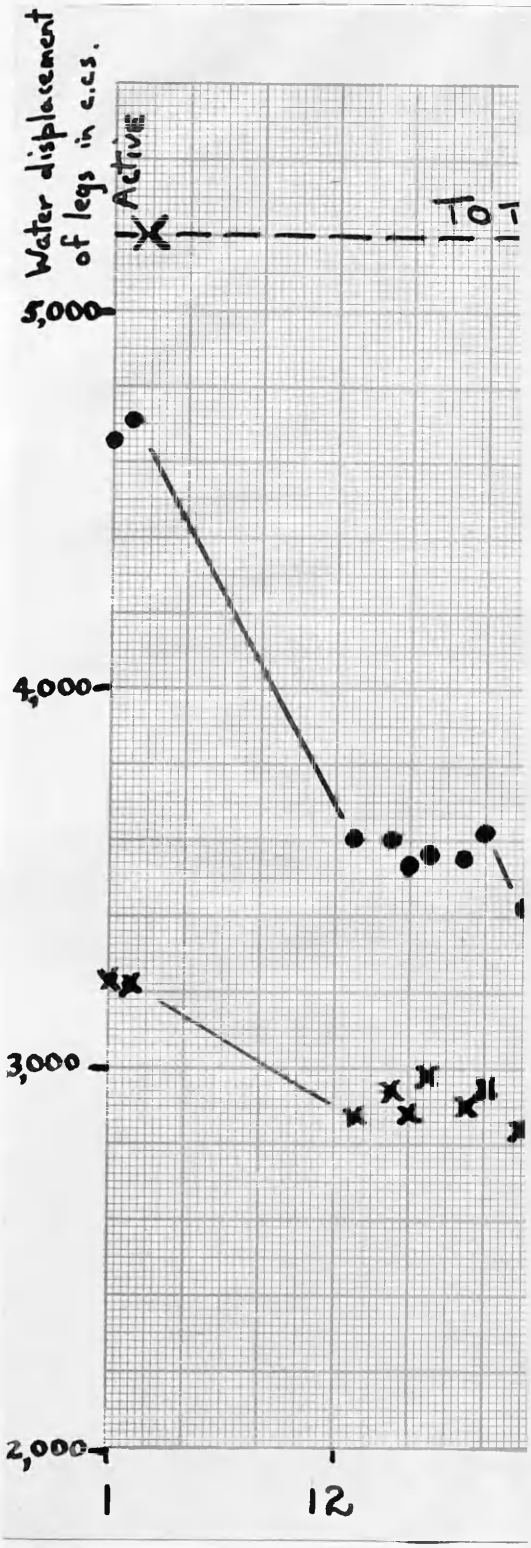


GRAPH 4

Graph 4 - showing the effect of rest in bed on the water displacements of the legs in Case 4.

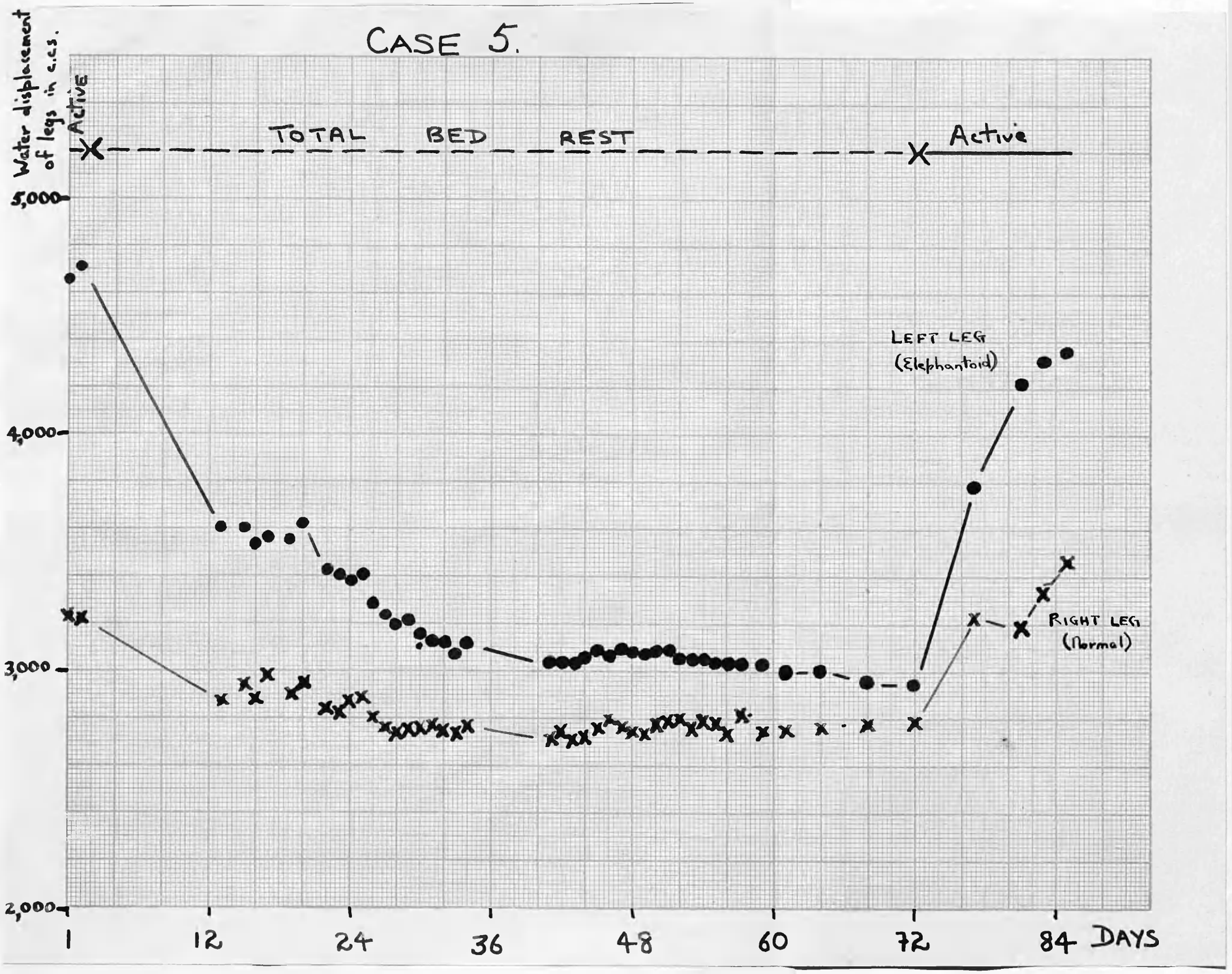


Graph 5 - showing the effect of rest in bed on the water displacements of the legs in Case 5.

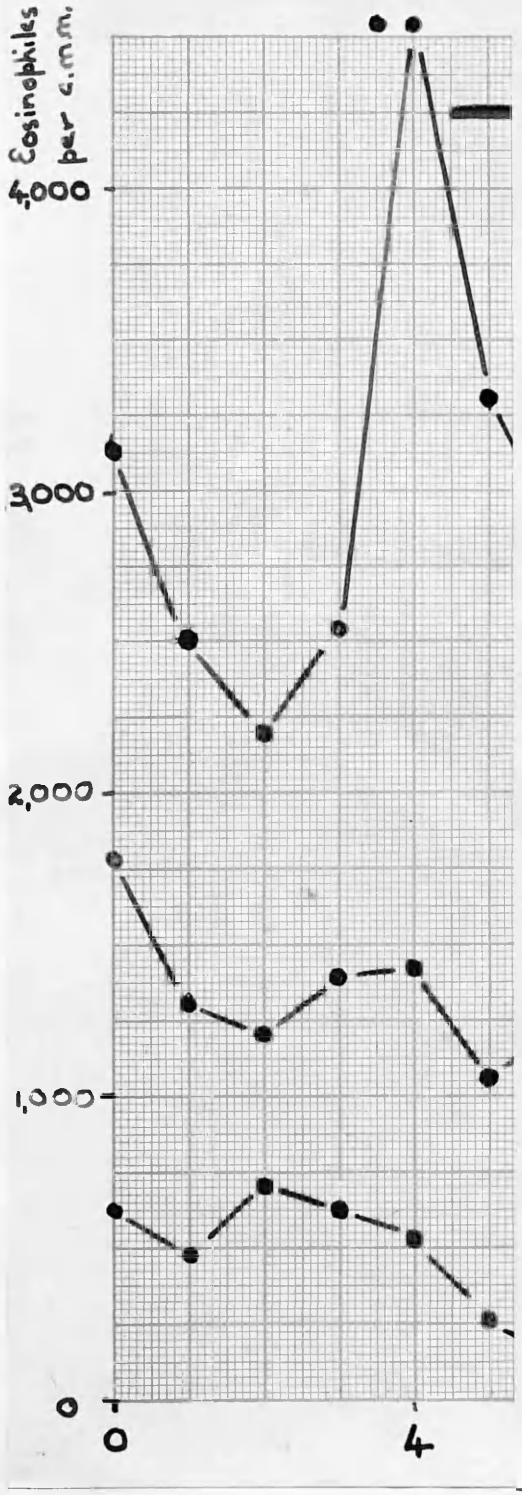


GRAPH 5.

Graph 5 - showing the effect of rest in bed on the water displacements of the legs in Case 5.

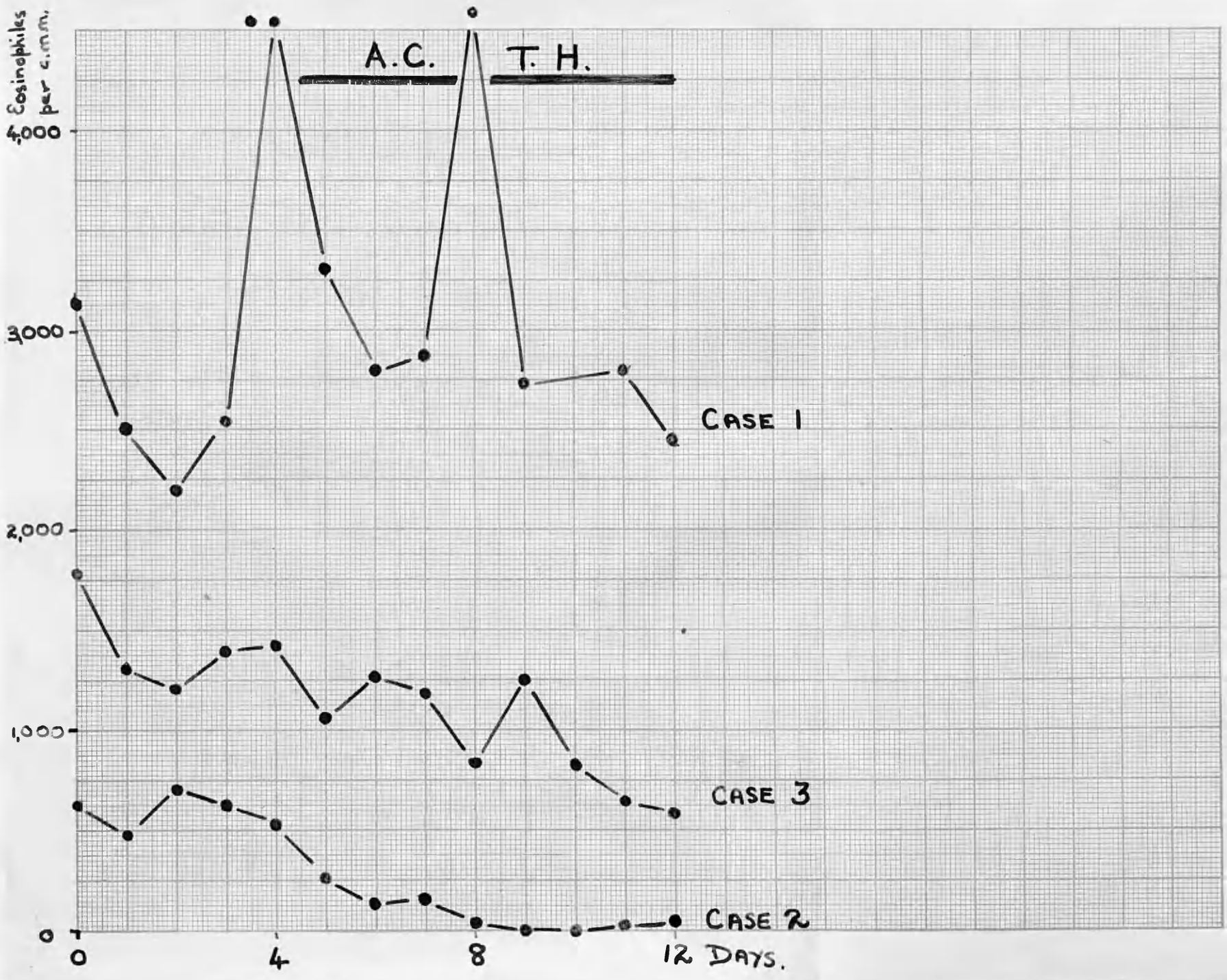


Graph 6 - showing the effect of A.C.T.H. on the eosinophile counts in the three cases of elephantiasis.

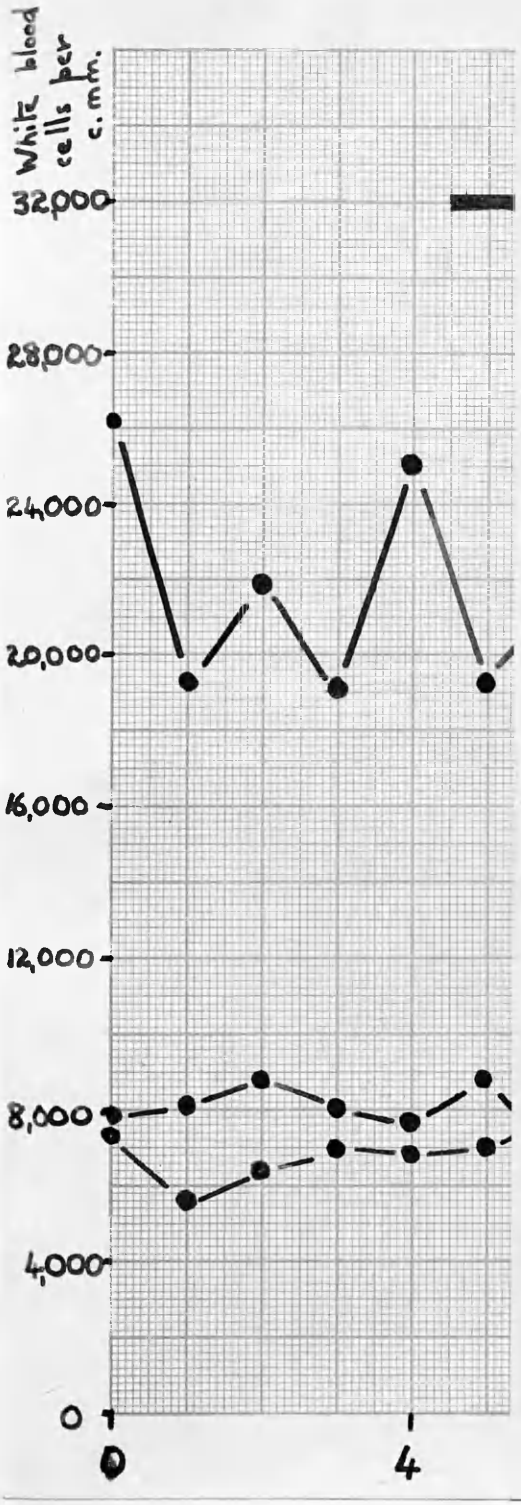


GRAPH 6

Graph 6 - showing the effect of A.C.T.H. on the eosinophile counts in the three cases of elephantiasis.

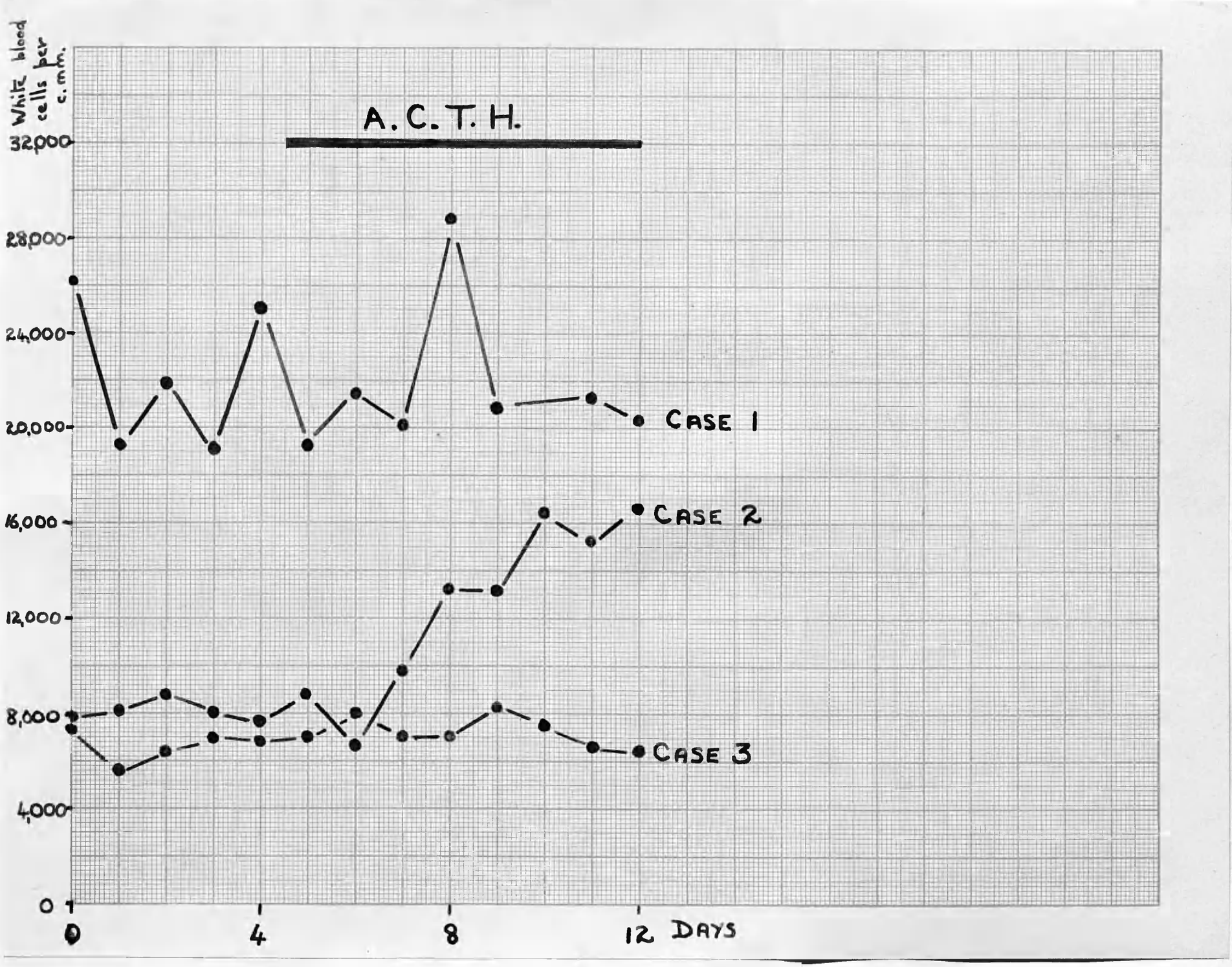


Graph 7 - showing the effect of A.C.T.H. on the white cell counts in the three cases of elephantiasis.



GRAPH 7.

Graph 7 - showing the effect of A.C.T.H. on the white cell counts in the three cases of elephantiasis.



T A B L E.

The percentage decrease observed in the water displacements of the legs between the maximum and minimum readings.

Case number.	Leg.	Maximum volume(cc.)	Minimum volume(cc.)	% decrease.
1	Elephantoid	4970	3355	32.6
2	Elephantoid	5150	3260	36.7
	Normal	2495	2240	10.2
3	Elephantoid (left)	10250	4530	55.8
	Elephantoid (right)	5240	3550	32.3
4	Elephantoid (left)	8080	5100	36.9
	Elephantoid (right)	5705	3900	31.6
5	Elephantoid	4710	2950	37.4
	Normal	3230	2710	16.1

Cases 1-3 were given A.C.T.H. Cases 4 & 5 were controls.

REFERENCE.

Schmidt, L.H. & Squires, W.L., (1951). J.Exp.Med., 94, 501.