RECENT INVESTIGATION ON FILARIASIS

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INTRODUCTION

Two years ago I had the pleasure of visiting your Institute and of making the acquaintance of the distinguished scientists whose papers I had already read in your Annals. It is a great privilege to be permitted to speak to you again today. Filariasis, like other branches of tropical medicine is a subject which has received much attention from Portuguese doctors, so you will pardon me if I do not attempt to give a general review of the whole matter. Instead, if I may I will describe some of the problems which my collaborators and I have tried to study during the past six years, some of the difficulties we have met, and some of the ways by which we have tried to overcome them. If I seem to speak almost exclusively of our own work, I hope that you will understand that this is not because I wish to boast about it. Some parts of it we are proud about, but most of it we feel that other workers would have been more clever, and would have succeeded where we have made only little progress. It is hoped, however, that a description of the experiments which I know by personal experience, will be more vivid and interesting to you, than a review of things which are known only by hearsay and by the report of others.

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DESCRIPTION OF EXPERIMENTAL FACILITIES

In the first place, I should explain that I work in London at the National Institute for Medical Research, which is maintained by the British Government to carry out fundamental research in physiology, biochemistry, biophysics, virus diseases and similar subjects which may ultimately help the progress of medicine. My department of this Institute is planned to study chemotherapy. After the war we decided that the chemotherapy of filariasis was a promising field for work because firstly the filarial worm is a large organism and therefore it ought to have many weak points which can be attacked by drugs; and secondly because there was no drug known at that time which was effective in curing filariasis, so that anything we found would be valuable. This lack of progress in the treatment of filariasis had been due to the fact that new drugs could be tested only on human patients, which was very slow; it required a great deal of the chemical compound for each test; and one was very limited as to the compounds one could try, in case they were toxic to the patient. Accordingly the first thing to do was to find a small experimental animal which contained a filarial worm something like the human ones and which could be bred and infected in the laboratory at a reasonable expense. Fortunately such an animal had just become available in the cotton rat *(Sigmodon hispidus)* which is infected by the worm called *Litomosoides carinii.* To study the chemotherapeutic effect of a drug, one injects it into the rat for six days. Then one waits a week to give the drug time to act on the worms and then one kills the rat, opens the thorax and looks to see if the worms are alive or dead. The microfilaria is rather short and fat; it swims round in the blood of the rats and is transmitted to other rats by the mite called *Liponyssus bacoti.* In order to transmit the infection on a large scale we have many metal tanks about 50 × 100 cm large by about 25cm deep. There is a gutter round the outside filled with oil to prevent the mites crawling out, and a lid on top to prevent forage mites and other strange insects from getting in and spoiling our cultures. On the bottom of the tank there is a layer of sawdust in which the Liponyssus mites breed. First we put into the tank a cage containing a heavily infected cotton rat. The mites feed on this rat and so take up microfilariae which develop in them in the usual way. After about two weeks we take out the single infected rat and put in 4 cages containing 20 small uninfected rats. The mites come back to feed on them, and as they do so, they transmit the larvae of the filarial worms

to the new rats. After a week, the new rats are removed from the tank and are stored in a separate room for 50 days, while the filarial worms develop and microfilariae appear in the blood. The rats are then ready for experiment (1,2).

The filariasis of cotton rats is useful for chemotherapy and similar research; but the microfilariae of cotton rats are non-periodic, being present in the blood in equal numbers by day and by night. This may be correlated with the feeding habits of the mites, which bite equally by day and by night. In order to study questions of periodicity we have to use experimental infections in dogs and in monkeys. The infections in dogs are *Dirofilaria repens* and *Dirofilaria immitis*, which are transmitted by mosquitoes such as *Aedes aegypti* or *Anopheles maculipennis*. The infection in monkeys was found in East African monkeys, *Cercopithecus aethiops johnstoni*. They are greyish monkeys and the males have a bright blue scrotum. The worm is a species of *Dirofilaria* which it is proposed to call *Dirofilaria aethiops* after its host. The adult worms live between the muscles at the back of the thigh and seem to cause no obvious harm. The microfilaria is unsheathed and it has a well marked nocturnal periodicity just like *Mf. bancrofti*. It is transmitted in the laboratory with some difficulty by *Aedes aegypti* mosquitoes, the larvae developing in the subcuticular tissues of the thorax of the mosquito.

INSECTARIUM

For the maintenance of these experimental infections, we require large numbers of insects, chiefly mosquitoes and mites. To handle them we have erected a small building, which we call an Insectarium. It was placed *outside* the main Institute because other people in the Institute were afraid that our insects might escape and attack them, and still more because we were afraid that our insects would be harmed by the other people putting down DDT or gammexane. There is a corridor along the front of the building, a small laboratory at one end, a room for mosquito larvae, a room of adult mosquitoes, a room for the tanks containing mites and cotton rats, and an animal room for storing the cotton rats while the infection is developing. The temperature and humidity of the three rooms for insects are controlled by simple but effective mechanisms. The laboratory presents no special features. In the larva room, the mosquito larvae breed

in shallow pans on long shelves. When the pupae appear in the pans they are picked off and transferred to the adult mosquito room next door. The mosquitoes are kept in large cages. When we want them to feed on a monkey or a dog we anaesthetise the animal by injecting pentobarbitone and push it inside the cage. The next room contains the tanks for cotton rats and mites. And the last is where the cotton rats are stored until they are ready for experiment. During the last six years we have infected about 5,000 rats.

EXPERIMENTAL RESULTS

So much for our equiment and facilities for experimental work on filariasis. Now let me show you of our results. Many of these have already been published in the Transactions of the Royal Society of Tropical Medicine and in the British Journal of Pharmacology and Chemotherapy, so I will not weary you with tables and masses of data. Instead, if I may, I will describe in broad outlines some of the subjects which have been studied.

PERIODICITY OF MICROFILARIAE

As you know, *Mf. bancrofti* is numerous in the peripheral blood by night but scarce or absent by day. It was this curious behaviour of the larval worms which attracted Manson's attention 70 years ago. It suggested to him that the microfilariae might come into the blood every evening in order to be taken up by some insect which sucked blood at night, such as the mosquito. And so he was led on to the transmission by mosquitoes of filariasis, and from that to the transmission by mosquitoes of malaria. Since then there has been much speculation as to how this periodic appearance and disappearance of microfilariae is brought about. Manson believed that during the day the microfilariae retired to the heart, great vessels and lungs and reappeared the next evening, that is the same brood of microfilariae reappeared each night. But most people could not understand how such small weak things as microfilariae could hold themselves in the heart and aorta against the stream of blood rushing past them at high speed. Accordingly CLAYTON LANE and others suggested that when the microfilariae disappeared every morning, they were destroyed by the reticulo-endothelial system of the body; and the next day the female adult worms produced a completely new batch of larvae. According to this theory

all the female worms discharged their larvae into the lymphatics at the same hour of the day, probably somewhere about 2.0 p.m. CLAYTON LANE tried to show by careful study of histological specimens that all the female worms in any one pathological specimen were all at the same stage of parturition. But this demonstration was not very convincing. According to his theory enormous numbers of microfilariae had to be produced and killed every twenty four hours; but no one could find any clear evidence as to the place in the body where these microfilariae were destroyed. Several workers, including KNOTT, and myself, tried transfusing microfilariae from one patient into another. Over 90 per cent of the microfilariae disappeared at once (which seemed to support Lane's theory) but a few persisted in the blood for one or more weeks (which seemed to support Manson's view). Thus the evidence for deciding between these two theories was conflicting.

Animal experiments — When we began to study the question three years ago it seemed to us that the main reason for the difficulty was the fact that people had had to rely exclusively upon *human* material. And human material, although of course very important, is very intractable for experimentation. With living patients, one can rarely cut out the specimens one would like to examine. And if one tries to get post mortem examinations, one finds that men heavily infected with microfilariae seldom die in a sudden way at midday or at midnight. With so many difficulties in the way of observation and experiment, it was almost impossible to make further progress. But if one turned to experimental animals, most of these difficulties disappeared. In the infected monkeys, which I have previously described, the microfilariae show a nocturnal periodicity, as well marked as that of *Mf. bancrofti*. From these animals, we could take out specimens of almost any organ at midday and count the microfilariae in it; and then after a few days we could take out a similar piece at midnight and count the microfilariae again for comparison. Finally we could kill the animal at midday or at midnight, when we knew what the cycle of the microfilariae had been; we could do a post mortem examination immediately; and we could map out the distribution of the microfilariae throughout the whole body. By post mortem examinations made in this way, we found that most of the microfilariae were usually in the circulating blood (about — 80% at night). The liver contained a few (e.g. about 4% of the total). In the skin and the muscles the microfilariae were distributed very thinly; but as these tissues are so large, their total content of microfilariae might be considerable (up to 30% of the whole; usually much less). The

other organs (apart from the lungs) contained very few. But the *lungs* always contained very large numbers, e.g. from 30 to 80 per cent of the total in the whole body by day. (Incidentally the microfilariae were almost all inside the blood vessels or capillaries; only a few were present in the tissues outside.) When we examined pieces of the organs, taken by biopsy at midday and then again at midnight, the same picture was found. The liver, spleen, and other organs contained few microfilariae, and these were less numerous by day than by night i.e. they passively followed the number of microfilariae in the blood. But the *lungs* always contained great numbers of microfilariae, which were *more* numerous by day than by night. Moreover the numbers disappearing from the lungs at night were sufficent to account for the numbers which appeared in the blood at that time. So it is clear that the periodic disappearance and reappearance of the microfilariae in the peripheral blood is due to their accumulation during the day in the capillaries of the lung and to their liberation from thence at night. When a patient dies or an animal is killed, as the circulation slows down for death, many microfilariae leave the capillaries of the lung and pour out into the left side of the heart and into the aorta; and this explains the confusing picture found at post mortem examinations by Manson and others (3).

SUBSIDIARY QUESTIONS

Granted that this is the explanation of microfilarial periodicity, many subsidiary questions at once arise:

1. *Why do the microfilariae accumulate in the lungs during the day?* It is clear that the microfilariae must be in the blood at night time when the mosquito bites, otherwise they would never be transmitted. But why should they not be there during the daytime as well? We must postulate that for some physiological reason, the lung capillaries are the most favourable place for the microfilariae to survive. But if they stayed there all the 24 hours, they would never meet the mosquito and so would never be transmitted. According to this conception, periodicity is a compromise, which enables the microfilaria to make the best of both worlds. It spends the daytime in the lungs enjoying the favourable conditions there; it comes out at night into the peripheral blood hoping to meet the mosquito; and in the morning, if it has been disappointed, it goes back to the lung to rest and recuperate until the nex night.

2. *Pathological effect of Mf. on the lung.* It might have been expected that the accumulation of so many microfilariae in the capillaries of the lung would cause pathological changes in the surrounding tissue, but as far as we could see in monkeys and dogs, this is not usually the case. It would be valuable to study the lungs carefully for such lesions in post mortem examinations of men dying with many microfilariae in their blood. It would also be interesting to know what happens when men with many microfilariae get acute infections of the lungs, such as lobar pneumonia.

3. *How do the Mf. hold themselves in the lung?* Another unsolved problem is presented by the question — How do the microfilariae hold themselves in the lungs? Normally microfilariae cause no resistance when they go through capillaries. When watched under the microscope they rush past as quickly as the red blood corpuscles do, so that it is difficult or impossible, to recognise them as they go through the small blood vessels. We might imagine that when they come to the lung capillaries, they suddenly contract into a curved position like a cork screw, and in this way hold themselves against the walls of the capillary. But nothing is yet known on this subject.

4. *Finally how do the microfilariae know when to stay in the lung* e.g. by day, or when to come out e.g. by night? This behaviour cannot depend upon any opening or shutting of capillaries by the host, because *Mf. loa* and *Mf. bancrofti* of the Pacific area have cycles quite different from the ordinary nocturnal one of typical *Mf. bancrofti.* I believe that it depends on the microfilaria giving an active response to some stimulus supplied by the host. This stimulus is connected with the diurnal rhythms of the host. Many of the functions of the body show cyclic fluctuations during the 24 hours, e.g. the temperature, the alkalinity of the body, the tension of carbon dioxide in the lungs, the amount and composition of the urine etc. The most conspicuous of these diurnal fluctuations is sleeping and waking but this is not the primary cause of the other ones. If a person stays awake at night and sleeps at midday, it does not alter the other cycles, nor does it affect the behaviour of the microfilariae. It is only when the person changes his whole mode of life over a long period e.g. by becoming a night watchman, that the other cycles (including the periodicity of the microfilariae) undergo a similar change in their timing. Presumably the microfilariae are sensitive to one or more of these cyclic fluctuations of the body and respond to it as a stimulus for their accumulation in, or emigration out of, the lungs. But which of the cycles is thus responsible

for the microfilarial one is still unknown. We have done much work on this subject but have not been able to obtain any clear results.

Attempts to link the movements of the microfilariae with the acidalkali, or the phosphate cycles of the body have been unsuccessful; but we have found that the movements of *Mf. bancrofti* at night can be inverted (i.e. they can be caused to leave the peripheral blood and to accumulate in the lungs) by violent exercise, or by hyperventilation of the lungs. These observations may provide a clue as to the stimuli responsible for the periodicity of the microfilariae, but the full explanation is not yet available.

CHEMOTHERAPY OF FILARIASIS

Let us now consider the chemotherapy of filariasis, which as I said at the beginning was the initial reason for our study of filariasis. It is still the subject which occupies a great deal of our time and attention. Various compounds have been reported to be effective in curing the infections of Litomosoides in cotton rats, such as the cyanine compounds discovered by PETERS, BUEDING and others (4), and methylene violet studied by ourselves (5); but they have no action on *W. bancrofti*. Pentavalent antimony compounds such as neostibosan are effective in man if given in large, almost toxic, doses; but they require too long treatment and are too toxic for practical use. Arsenic compounds are also active, and there is one compound named «Arsenamide» which I should like to mention in particular. This compound has the formula O As <⎯⎯> $CONH_2$ and it was discovered by OTTO and MAREN about three years ago (6). It is given intravenously as 5cc of a 2.5% solution daily for about fifteen days. In this dose, it is certainly effective in killing the adult worms of *W. bancrofti*, and my colleague, Dr. J. A. MCFADZEAN has cured 32 patients in this way. Unfortunately in the thirty third case toxic effects appeared after the third dose and the patient died of acute necrosis of the liver. Therefore until this matter has been further investigated, I would recommend great caution in the use of Arsenamide.

HETRAZAN

The only compound which is of practical importance at present for the treatment of filariasis is *hetrazan* which is also called Banocide and Notézine. It is a very remarkable and interesting compound. When a large dose e.g. 10mg per kg is given by mouth, the concentration in the blood

rapidly rises to a peak of about 3-4 microgm per cc. and then gradually falls during the next 24 hours; the excretion in the urine persists for about two days. When it is used experimentally in cotton rats, being given by intravenous injection (so as to avoid any time being lost while the drug is absorded from the intestine) most of the microfilariae disappear from the blood very quickly; in fact 95 per cent of them disappear within 2 minutes. This is an extremely rapid disappearance. When rats are killed at various periods after treatment with hetrazan, and sections of the different organs are examined to see where the microfilariae have gone, it is found that most of them have collected in the liver. Before hetrazan is given, the number of microfilariae in the liver is very low. Directly after hetrazan, the number in the liver increases greatly being maximal after about two hours. Then it gradually falls again until 24 hours when it is back to the normal low figure again. In a neutral organ, like the kidney, which takes no part in the action of hetrazan, the number of microfilariae remains low throughout. When we examined the sections of the liver more closely, the fate of the microfilariae in the liver became clear. Before hetrazan had been given, the microfilariae were few and they lay in the sinusoids without the cells of the liver taking any notice of them. Five hours after hetrazan had been given, the microfilariae were numerous and they were surrounded by phagocytes which destroyed them. Apparently hetrazan acts on the microfilariae by changing them in some way so that they become attractive to the macrophages of the body which seize hold of them and then destroy them. Thus hetrazan acts as a kind of *opsonin.* In rats, most of the macrophages of the reticulo-endothelial system occur in the liver, while the spleen and bone marrow are quite small. Accordingly in rats, most of the destruction of the microfilariae occurs in the liver. In man, the bone marrow and spleen may be more important (7,8).

So much for the action of hetrazan upon the microfilariae. Its action upon the adult worms seems to differ according to the species. It has practically no action upon the adult worms of *Litomosoides* in cotton rats, or upon those of *Onchocerca volvulus* in man. On the other hand, it is usually quite effective in destroying the adult worms of *Loa loa.* We will consider its action upon the adult worms of *Wuchereria bancrofti* after I have described a recent field trial of hetrazan.

Field trial of Hetrazan — This field trial was carried out by Dr. I. McGregor and myself in the colony of the Gambia, West Africa (9). The British Medical Research Council has a small Field Station there at

Fajara. A hundred miles inland there is a small isolated primitive village called Kenaba. There Dr. McGREGOR has carried out intensive studies during the past two years on the results of controlling parasitic infections such as malaria and trypanosomiasis, and examining the effect on the general health of the people. There are about 700 people in the whole village. In February 1951 blood films were collected at night from everyone in the village and the number of people carrying *Mf. bancrofti* and *Mf. perstans* was determined. For simplicity I will deal only with the 326 people who were over 10 years old. All the people who had *Mf. bancrofti* in their blood were treated. They numbered 155. The dosage of hetrazan was 5 mg base per kg body weight, given by mouth once daily for 5 days. The dosage was chosen in the light of experience in East Africa as the minimum dose which would probably be effective and which would still be small enough to be tolerated. It was given once daily so as to make it possible for Dr. McGREGOR to administer each dose himself and make sure that it had actually been swallowed. And it was restricted to the people with microfilariae so as to save the labour of administration and also to save the cost of the drug itself which is quite expensive.

Ten months later, in December 1951 we re-examined all the people in the village. Of the 155 people who had been treated 64 per cent had become free from microfilariae; while among the 171 persons not treated, about 6.2 per cent had now developed microfilariae. However, the total number of microfilariae in the blood of the whole group was reduced by 94 per cent, i.e. there were only 6 per cent as many microfilariae as before left to infect mosquitoes and so transmit the worms to new persons. Since these results were obtained 10 months after the treatment, they presumably indicate the *permanent* effects of hetrazan; and it is clear that hetrazan must have killed or sterilised the great majority of the adult female worms in this village, otherwise the number of microfilariae would soon have increased again.

Effect on D. perstans — So far we have spoken only of *bancrofti*, but many of these people were also infected with *Dipetalonema perstans*. There were 69 persons who contained *Mf. perstans* and who were treated with hetrazan. Ten months later 72 per cent of these persons were negative and the total number of the microfilariae in the group had been reduced by 92 per cent. Meanwhile in a similar group of 71 persons who had not been treated, the number of infections had also dropped by 32 per cent, but

the total number of microfilariae in the group had increased by almost 50 per cent. So it is clear that hetrazan also has some effect in destroying or sterilising the adult worms of *Dipetalonema perstans*. However, as perstans is not pathogenic, this action of hetrazan is of academic interest rather than of practical importance.

Toxic effects — So far this seems a very good result. Most of the microfilariae in the village have been abolished by a five day course of treatment. Unfortunately, there is another side to the picture, namely the toxic effects of the treatment. Twenty five per cent of the people treated complained spontaneously of unpleasant effects which were attributed to the drug; and many who did not actually complain looked unwell and miserable. The chief complaints were headache (in 17 per cent), anorexia (10 per cent), nausea (8 per cent), vomiting (4 per cent), abdominal pain like colic (4 per cent), and diarrhoea (2 per cent). Five persons (2.8 per cent) had cough, pain in the chest, and fever like acute bronchitis. The condition was severe, but it lasted only about 1 day. All these five patients had heavy infections of microfilariae, and the syndrome probably represents an allergic reaction to the sudden destruction in the body of great numbers of larvae. Whether it has anything to do with microfilariae being destroyed in the lungs is not known. One patient had a papular rash which was widely distributed over the limbs and trunk and which disappeared in 2 days. Six people (3 per cent) had localised painful swellings, which probably represented an inflammatory reaction round adult filarial worms.

A few of these symptoms e.g. anorexia, nausea and vomiting, may have been due to the direct toxic action of the compound, but most of them were probably allergic reactions caused by the destruction of microfilariae. None of the reactions were at all dangerous to life. If they had occurred among patients inside a hospital, who expect to experience discomfort in order to be cured, most of them would have been passed over without comment. But when they occurred among the ordinary people of a village, they made a very unfavourable mental impression, and the treatment became unpopular. In this particular village, Dr. McGREGOR was known and trusted, and he was able by much effort to persuade the people to continue taking the tablets; but in a larger area, where the doctor was not known personally the treatment would probably be inacceptable. Perhaps it might be easier to give hetrazan to people in Fiji or in parts of India, where filariasis is known and dreaded. But in the Gambia, and probably most other parts of Africa, people are not conscious of it and they are not afraid of getting

elephantiasis. And therefore they will not submit to some trouble and discomfort in order that the community shall become free of this infections.

So that is the present position about the chemotherapy of bancroftian filariasis. The objective is to treat all the persons in a community who carry microfilariae. If this were done effectively, there would be no microfilariae left to infect mosquitoes and so transmit the infection to new persons. And since there is no animal reservoir for bancroftian filariasis, the infection would soon die out completely. In order to be able to treat all the carriers in this way, one must have a drug which is not too expensive and which will be readily acceptable to primitive communities. Hetrazan is a remarkable substance, but it does not in its present from completely meet these exacting requirements. Perhaps further research will reveal a more satisfactory compound. I hope that this account of our attempts to study filariasis has been of interest to you, and I thank you for the honour of being permitted to speak in your distinguished Institute.

RESUMO

O conferente depois de enunciar alguns dos problemas das filariases, que em colaboração com os seus colegas do National Institute for Medical Research de Londres, tem tentado esclarecer, apresenta alguns dos resultados obtidos nas experiências originais feitas em animais de laboratório com o fim de elucidar vários aspectos da doença. Em seguida relata alguns dos dados obtidos nos ensaios de terapêutica da doença experimental e termina a sua Conferência descrevendo uma interessante experiência de luta contra a doença entre habitantes de uma aldeia indígena na Gâmbia onde obtiveram resultados satisfatórios utiizando o Hetrazan.

RÉSUMÉ

Le conférencier après avoir énoncé quelques uns des problèmes des filariases que, en collaboration avec ses collègues du National Institute for Medical Research de Londres, il a essayé d'éclaircir, il présente quelques uns des résultats obtenus aux essais originaux faits sur les animaux de laboratoire dans le but d'éclaircir plusieurs aspects de la maladie. Ensuite, il mentionne quelques uns des résultats termine sa conférence en décrivant

une intéressante expérience de lutte contre la maladie chez les habitants d'un village indigène en Gambie où ils ont obtenu des résultats satisfaisants en employant le Hetrazan.

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