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**EXPERT COMMITTEE ON
FILARIASIS**

(WUCHERERIA AND BRUGIA INFECTIONS)

Report

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EXPERT COMMITTEE ON FILARIASIS

(*Wuchereria* and *Brugia* infections)

Geneva, 25 July - 1 August 1961

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EXPERT COMMITTEE ON FILARIASIS

(*Wuchereria* and *Brugia* infections)

Report

The Expert Committee on Filariasis met in Geneva from 25 July to 1 August 1961. Dr J. F. Kessel was elected Chairman, and Dr N. G. S. Raghavan, Vice-Chairman. Mr C. B. Symes was appointed Rapporteur.

In opening the meeting on behalf of the Director-General of the World Health Organization, Dr P. Dorolle, Deputy Director-General, recalled that the programme set forth by the Organization in the field of filariases had developed along two parallel lines, one covering the infections due to *Onchocerca volvulus*, the other the infections caused by the *Wuchereria* and the *Brugia* parasites, a distinction that had proved necessary because of the differences in the epidemiology and the clinical symptomatology of these two groups of filariases. The Expert Committee on Filariasis would be concerned with the *Wuchereria* and *Brugia* infections. Its first task would be to review the recent advances made in the epidemiology of the diseases, and to discuss the experience gained in various control projects that had been carried out in endemic areas throughout the world. A critical evaluation of present therapeutic and control techniques was equally needed in order to assess their merits under differing epidemiological conditions. Dr Dorolle further invited the experts to give their advice on the implementation of the programme of activities WHO intended to undertake in accordance with the suggestions made by the Study Group on Filariasis and subsequent scientific groups.

1. EPIDEMIOLOGY

1.1 Changes in nomenclature and terminology

The discovery of animals naturally infected with various species of *Wuchereria* has given opportunities for a study of the adult worms on a larger scale than has been possible hitherto. As a result of these studies, the old genus *Wuchereria* has now been separated into two genera, *Wuchereria* and *Brugia*. The genus *Wuchereria* contains only one species,

W. bancrofti. The genus *Brugia* includes three species, *B. malayi*,¹ *B. pahangi*,² and *B. patei*.³

Various terms have been used to describe the behaviour of the embryos of these and other filariae in the peripheral blood, and, as knowledge increases, some of these terms are proving unsatisfactory. There is evidence that the term "non-periodic" does not give an adequate description of the behaviour of the microfilariae of *W. bancrofti* as found in Polynesia, and objections have been made to the term "semi-periodic", as applied to the infections with *B. malayi* found in the swamp forest areas in Malaya. The following terms are suggested as approximating more closely to the observed patterns of microfilaria occurrence in the peripheral blood:

(1) *Periodic occurrence*: Infections showing a pronounced peak in microfilaria count at some point in each 24-hour period, while for a considerable part of the 24 hours microfilariae are absent or very scanty; the peak may be either nocturnal (e.g., periodic *W. bancrofti*, periodic *B. malayi*), or diurnal (e.g., *Loa loa*).

(2) *Continuous occurrence*: Microfilariae are present in appreciable numbers throughout the 24 hours. They may, however, be *sub-periodic*, showing a consistent minor peak. This peak may again be nocturnal (e.g., in man for *B. malayi* in the swamp-forest areas of Malaya), or diurnal (e.g., *W. bancrofti* in Polynesia). Alternatively, they may be non-periodic, with no detectable rhythmic variation in numbers, e.g., *Mansonella ozzardi*, *Acanthocheilonema perstans*.

In this terminology, the *W. bancrofti* infections of Polynesia would be described as *diurnally sub-periodic*, and the *B. malayi* infections in man in swamp-forest areas of Malaya would be described as *nocturnally sub-periodic* in contrast to the nocturnally periodic infections with these filariae found elsewhere. The term non-periodic would be restricted to those infections in which no repetitive microfilaria rhythm had yet been demonstrated.

1.2 Review of recent advances in epidemiological knowledge

1.2.1 Animal reservoirs and experimental infections

Results of investigations in Malaya have stimulated a general re-assessment of the epidemiology of filariasis. *W. bancrofti* has not yet been found in animals, nor have the few attempts to transmit it experimentally to animals proved successful. Surveys of animals and transmission experi-

¹ Brug, S. L. (1929) *Trans. Far East. Ass. trop. Med.*, 3, 279

² Buckley, J. J. C. & Edeson, J. F. B. (1956) *J. Helminth.* 30, 1

³ Buckley, J. J. C., Nelson, G. S. & Heisch, R. B. (1958) *J. Helminth.* 32, 73

ments have not yet been carried out on a sufficiently large scale to exclude the possibility of an animal reservoir of *W. bancrofti*, and obviously must be continued. Attention should perhaps be concentrated on the strains of *W. bancrofti* found in remote rural areas rather than on the strains found in urban populations.

The periodic *B. malayi* appears to be the form responsible for much of the filariasis in rural areas throughout Asia. Natural infections with this form in animals are decidedly rare, but again many areas have yet to be surveyed. Periodic *B. malayi* can, however, be transmitted experimentally to cats and rhesus monkeys, and the search for natural infections should be expanded. It is important that negative as well as positive findings be reported.

The sub-periodic form of *B. malayi* occurs as a natural infection in various Malayan forest and domestic animals, and can be readily transmitted to them experimentally (see Annexes 1, 2, pages 34, 35). An animal reservoir of infection is, however, likely to be of epidemiological importance only in the swamp-forest type of environment. In such areas of Malaya, the reservoir is the leaf monkey, *Presbytis* spp., of which 75% are naturally infected, and the vectors are species of *Mansonia*, particularly *M. dives* and *M. bonneae*, which breed in enormous numbers in the swamp forest and feed readily on man, monkey, and other animals, either at ground level or in the forest canopy, thus creating a link between human and animal infection. Similar swamp forests bordering on human settlements may exist in Thailand, Borneo, Sumatra and other territories in the Malaysian zone, but they have not yet been investigated. In this type of habitat other *Brugia* infections may be present in animals and must be differentiated from *B. malayi*.

Studies of microfilaria periodicity in animals found with natural infections in the Malayan swamp forests have indicated that, while the microfilariae of *B. malayi* in cats are sub-periodic as in man, those in monkeys have a nocturnal periodicity. Transmission experiments have shown that infections in monkeys become sub-periodic when transmitted to cats, and infections in cats become periodic when transmitted to monkeys. Clearly the influence of the host on microfilaria periodicity requires much further study.

1.2.2 Human reactions to infections by animal filaria parasites

It is still not clear from recent data obtained from India, Africa, Malaya and other places to what extent tropical pulmonary eosinophilia is caused by infection with animal or human filaria parasites. It seems that this condition may be associated with a number of infections, and particularly with nematodes. The results of the few experimental infections in volunteers with *B. pahangi* from animals, and with *B. malayi*, periodic and

sub-periodic, suggest strongly that reactions to such infections depend more upon the allergic constitution of the individual concerned than upon the species of filaria. Published records, however, cover only the experimental infections of five persons, and more data are needed.

1.2.3 *Differential diagnosis of developing forms in vectors*

Inability to identify filaria larvae found in mosquitos has reduced seriously the value of many of the field studies of vectors. In recent work in Kenya, it is recorded that six different species of animal filariae have been found in *Aedes pambaensis* collected in houses. This species had previously been mistakenly recorded as a vector of *W. bancrofti*.

The urgent necessity of dealing with this inadequacy has been stressed by successive meetings of specialists. For some species such as *B. malayi* and *B. pahangi*, where morphological differences are not discernible, mature larvae have been identified by inoculation into receptive hosts and the subsequent recovery of adult worms. Serological techniques for identification should also be investigated.

Very useful diagnostic descriptions and a key have recently been produced in Kenya for a number of species of filariae found in mosquitos (see Annex 3, page 36).

The Committee recommends that :

(1) Surveys of animals for filaria infections be expanded rapidly. This work could well be done at any institute where large numbers of animals are being examined. Blood samples must be collected by night and day and examined for microfilariae, and adult worms should also be sought (see Annex 2, page 35, for description of methods). It is most important that negative as well as positive findings should be reported.

(2) Any new infections found in animals should be the subject of mosquito feeding experiments, and descriptions of the developing stages of the filariae in mosquitos should be published quickly. The existing knowledge of development stages in mosquitos should be made widely known.

(3) Studies on periodicity should be expanded, with special reference to the influence of different hosts on periodicity. All existing knowledge on periodicity in man and animals should be critically reviewed. Experiments in the transmission to man of various filariae from man and animals should be expanded, and the human reactions to infection carefully observed.

1.2.4 *Variations shown by local strains of vectors and parasites*

Evidence of variations in potential of vector strains and of variations in adaptability of strains of filariae to mosquito species is accumulating.

In the Pacific area, strains of *Culex fatigans* in New Caledonia and Australia have been readily infected by feeding upon a donor infected with a New Caledonian strain of sub-periodic *W. bancrofti*, and mature-larva rates of 80%-90% have been noted. In Fiji the local strain of this same mosquito gave an infective potential, in laboratory feedings, of about 45% and in Tahiti somewhat less than this. These differences could not be explained by differences in the microfilaria densities of the donors.

In the same region *Aedes vigilax* in Sydney gave a mature-larva rate of 98% in artificial infections when fed on a donor from New Caledonia with a microfilaria density of 57 to 154 per 20 mm³. About a year later this mosquito, having been newly recorded in Fiji, when fed on a local donor with a microfilaria density of 241 per 20 mm³, gave only a 6% mature-larva rate and when fed on densities of 5, 7 and 42 could not be infected at all. Another most important observation on infective potential of "strains" came recently from experimental work in Liverpool with *Aedes aegypti*, where, by selective breeding from a laboratory colony, experimental infection with *B. malayi* was increased from about 31% to over 90% in one generation.

The first direct evidence of the influence of strains of the filariae on infectibility of a mosquito species came from Malaya, where a Kuantan colony of *C. fatigans* was intolerant to local rural strains of *W. bancrofti* but was receptive to an urban strain from Singapore. It seems probable that physiological strains of the filariae may be as important as those of vectors in their influence on infective potentials of some species of mosquito. The importance and influence of such strains can be assessed only after much more study.

The Committee recommends that studies that may now be proceeding on the strains of the *Culex pipiens fatigans* complex and their interactions to strains of *W. bancrofti* infections be supplemented and accelerated by the following means.

- (1) Expert taxonomic studies should be initiated as soon as possible of mosquito forms associated with different infective potentials in the Pacific area, the Kuantan region of South-East Asia, East Africa, and other areas that may be selected after critical examination of recent epidemiological data.

- (2) Studies on bionomics should be undertaken on some of these mosquito strains (including selective breeding for infection potential) as an essential supplement to, and perhaps a substitute for, taxonomic differentiation.

- (3) If expert taxonomic and biologic studies should perhaps prove to be inadequate for differentiation, then genetics should be employed. Similar studies on *Aedes vigilax* and perhaps other vectors should be initiated as soon as possible.

1.2.5 Ecology of the different vectors

Studies which have been made on various aspects of the ecology of the vectors in different areas serve mainly to emphasize the necessity for more detailed knowledge. The culicine mosquitos, which constitute the majority of human filaria vectors, include day-biting bush mosquitos of the *Aedes pseudoscutellaris* group; night-biting and house-haunting *C. p. fatigans*; *Anopheles* species, whose habits vary from those of *A. gambiae* and *A. funestus*, which are largely domestic and man-biting, to those of *A. farauti* and *A. letifer*, which prefer to bite out of doors and rarely enter houses to rest; and *Mansonia* species which, in some areas, have become partially domestic because of man's activities but which are typically swamp and forest mosquitos. Though there is a considerable body of data on certain of these vectors, it is probably true to say that in no area is sufficient knowledge available for a successful control programme to be undertaken.

The *C. pipiens* complex, including *C. p. fatigans* as vector of periodic *W. bancrofti*, and *C. fatigans*, a secondary vector of sub-periodic *W. bancrofti* in the Pacific, continues to become of increasing importance. The significance of *C. fatigans* in urbanization is stressed elsewhere in this report (see page 12) and its spread to new and different habitats will undoubtedly lead to the development of new strains adapted to local conditions. The apparent influences of vector and parasite strains on the infection potential of *C. fatigans*, as already mentioned (see page 8), serve to emphasize the urgent necessity for more studies. The few attempts to determine the age structure of wild populations of *C. fatigans* have yielded unexpected results indicating that in India and in Indonesia most of these mosquitos are nulliparous.

Mansonia (Mansonioides) mosquitos have long been recognized as the main vectors of *B. malayi* in South-East Asia. In certain parts of Africa, however, they are of considerable importance as nuisance mosquitos and they have recently been shown to transmit *W. bancrofti* in New Guinea. Their laboratory colonization, both under tropical conditions and in London, provides opportunities for more detailed studies on their adult and larval biology. It has been shown that the Polovodova technique of age-grading females by the examination of the ovaries for follicular relics can be applied to *Mansonia uniformis* but difficulties were encountered in field estimations of the age structure of *M. dives* and *M. bonneae* populations. The presence of hydrachnid mites may also be of value in identifying young mosquitos in that they are rarely found in old or multiparous specimens. Studies in Africa have indicated that *M. uniformis* prefers different plants for oviposition from those which are utilized for larval attachment, while in India and New Guinea the range of plant hosts has been greatly extended. A variety of trees, palms and other plants found in swamp

forest have been identified as the hosts of *M. dives* and *M. bonneae*, and a technique has been developed for the finding of larvae in this habitat. Information on the biting cycles is confined mainly to African species, but recent findings in Malaya indicate that while *M. dives* and *M. bonneae* may bite at all times of the day at ground level in swamp forest and in shaded situations near houses, they feed in the canopy only at night when they readily attack monkeys which form a reservoir of *B. malayi*. In observations with *Mansonia* and *Aedes albopictus* mosquitos, the *Aedes* mosquitos appeared to bite indiscriminately on all parts of the body whereas *Mansonia* preferred the lower parts, particularly below the knee.

The importance of larval breeding-habits has been emphasized by recent studies in the Pacific. Elimination of all peri-domestic breeding-places of the *Aedes pseudoscutellaris* group will undoubtedly have a considerable effect on the numbers of adult mosquitos around houses, but the relative importance of these breeding-places, though apparently small compared with that of crab-holes and those occurring in bush, will vary in different areas. More study is needed. *Aedes (Finlaya) fijiensis*, which in nature breeds in the axils of a forest species of *Pandanus*, is in its absence apparently able to maintain itself by utilizing a related species of *Pandanus* which is cultivated by the Fijians. Another species of *Finlaya* in the Philippines, *Aedes poicilius*, transmits *W. bancrofti* in abaca plantations; and *Aedes togoi* is a vector of *W. bancrofti* and of *B. malayi* in Japan.

Anopheles species have particular importance as vectors of filariasis in Africa where ecological studies have been reported from Ukara Island. The effect of spraying with residual insecticides in Africa to control malaria transmitted by these mosquitos has apparently upset the ecological balance in certain areas in favour of *C. fatigans*. Very little is known of the ecology of *Anopheles barbirostris* recently identified as a major vector of *B. malayi* in Malaya. In New Guinea where *Anopheles farauti* and related species are considered to be the usual vectors of *W. bancrofti*, local ecological conditions have apparently provided unusually close contact between the human population and other mosquitos, and *Culex annulirostris* and *Culex bitaeniorhynchus* have become important vectors.

The Committee recommends that the following studies be undertaken :

(1) Evaluation of the Detinova and Polovodova techniques for the age-grading of culicine mosquitos, particularly *C. p. fatigans* and the *Aedes pseudoscutellaris* group.

(2) A critical review of information on the ecology of the *C. p. fatigans* group of mosquitos.

(3) Studies on the preferred times and places where filaria vectors bite. These studies should include observations on biting-sites on the body and comparative data on the attractions of babies, young children and older age-groups to mosquitos.

(4) A study of the relative importance of bush versus village transmission.

(5) A study of larval and adult ecology of *Mansonia* (*Mansonioides*) mosquitos with particular reference to (a) the importance of different plants as oviposition sites, and as host plants for larvae; (b) the ability of the larvae to develop on plants other than the preferred host plant; and (c) the effect of the removal of the host plant on mosquito populations.

(6) A study of the present distribution of *Aedes vigilax* in the Pacific, its bionomics and vector potential; and continued observations on distribution and vector potential in Fiji and neighbouring islands.

1.2.6 *Filariasis and C. fatigans problems in current programmes of urbanization in areas of endemicity*

Urbanization multiplies or creates man-made facilities for species of mosquitos that are able to make use of them. Numbers and distribution of malaria vectors such as *Anopheles gambiae* have been promoted by this process, but perhaps the species that have benefited most are the filariasis vectors of the *C. pipiens* group: *C. p. fatigans*, *C. p. pallens* (in the Japan region) and *C. pipiens* itself in Egypt.

Breeding facilities common to urbanization in most areas of the world include cesspits and soakage pits, ill-maintained drains (lined and unlined), septic tanks, water storage tanks, pitted roads, quarries, borrow pits, and so on. These occur both on private and public lands. In some areas new water supplies are installed without the provision of adequate drainage. The influence of these on the numbers and spread of *C. fatigans* has been recorded recently in the cities of Ceylon, notably Colombo, and in India where few towns have proper provision for water supplies and waste disposal, and where the numbers of soakage pits and cesspits accompanying the growth of villages and townships not only facilitates the breeding of *C. fatigans* but promotes its spread, and that of filariasis infection, into rural areas.

Migrations of human populations accompanying the urbanization process are also dangerous factors in the production of a possible rapid increase of *W. bancrofti* infection in the expanding towns.

Evidence of the kind already demonstrated in Ceylon and India is accumulating for several urban areas in Africa, and there is no reason to doubt than an increase of infection through urbanization is already on its way, even if it is as yet undetected, in large areas of Africa and Asia. In South America bancroftian filariasis is still confined to cities where facilities for the vector *C. fatigans*, of the type mentioned above, are abundant, particularly in coastal belts. In Belém the continuous existence of urban breeding-grounds and their multiplication with the extension of

the town is seriously hindering the progress of the filariasis control scheme based on mass treatment of the infected population.

In view of the large scale and speed of urbanization occurring in so many developing countries in Asia and Africa, many of which have recently gained independence, and of this very dangerous spread of *W. bancrofti* infection, the Committee considers that a greater effort should be made by public health services to plan measures for the prevention of the spread of *C. fatigans*.

The Committee recommends that studies should be conducted in a number of these areas of uncontrolled urbanization, with a view to designing control measures appropriate to the different local conditions.

1.2.7 Dynamics of natural transmission

The level at which infection in a population is maintained depends upon a number of factors including the frequency and degree of re-infection. In field observations in Malaya attempts have been made to obtain crude estimates of the number of re-infections, i.e., infective mosquito bites, required to maintain the degrees of infection known to occur in certain communities. As a preliminary to this activity it was determined by adequate observation that a captive goat was as attractive as a man to the species of vectors concerned. A goat was then used as a bait on which to capture biting mosquitos at standard times; catching and dissection continued for three years. In one area in which the *B. malayi* microfilaria rate in the population is 40% it was estimated that 30 infective bites of *Mansonia* species per man per year maintained this level of infection. In a population with a 10% microfilaria rate of *W. bancrofti*, carried by *Anopheles letifer*, it is estimated that one infective bite in about two years maintains this infection level, whilst in a third area a *B. malayi* infection rate of 10% is maintained by eight infective bites of *Mansonia* sp. per person per year. In many other territories in which data are available on mosquitos in houses, or their biting cycles, it may be possible to obtain similar rough estimates of the amount of mosquito transmission required to sustain levels of infections in man. It may be useful to adopt the goat-bait method, or some other animal whose attractiveness for mosquitos, relative to man, may be known or ascertained. A human bait may, of course, be used if this is possible. Estimates of degree of re-infection, based upon infective bites and quantity of mature larvae transferred per bite, might be useful in helping to define the amount of vector reduction that may be necessary to achieve cessation of transmission.

A method of this kind, employed to estimate the intensity of transmission, has been based on the biting and infection rates of mosquitos coming to bite man in timed collections. It has been utilized in Tahiti to measure progress of the control programme and the following standards were

set as objectives. The method of estimation is given in Annex 4, page 37.

Microfilariaemia rate	5.0 %
Density of microfilariae per 20 mm ³ of blood (in the population as a whole)	1.0 %
Percentage of mosquitos positive for "all-stage" larvae	5.0 %
Percentage of mosquitos positive for "infective-stage" larvae.	1.0 %
Density of "all-stage" larvae in mosquitos	0.1
Prevalence of mosquitos, i.e., average number caught per minute on a human bait who sits in the shade for ten minutes within ten metres of each dwelling	0.1

Theoretical treatment of the probabilities of transmission was considered by the Committee.¹ It may be of value to test the assumptions on which many of the deductions are based and to try to fit local experimental data into the framework provided by the authors.

The Committee recommends that workers in this field should correlate mature larvae rates and densities in vectors with microfilaria rates and densities in the human populations.

1.2.8 Incidence and severity of clinical manifestations

The Committee considered the information available on differences of clinical manifestations reported from different areas, (1) for the infections produced by *W. bancrofti* and for those produced by *B. malayi*, (2) for the infections produced by the different local strains of *W. bancrofti*, and (3) for the infections produced by the different local strains of *B. malayi*.

(1) All the information at present available indicates that the main lesions resulting from *B. malayi* infection almost always affect the limbs, most commonly the legs, whereas *W. bancrofti* infection produces a much greater range of lesions, and tends to produce greater deformity. The transmission experiments with *B. malayi* mentioned earlier indicate, for the first time, that lymphangitis and lymphadenitis may develop in human subjects within a month or two of exposure to infection, and well before microfilariae can be found in the blood.

(2) The existence of considerable clinical differences between different areas of endemicity of *W. bancrofti* is confirmed by accumulating data. These differences concern incidence and severity of some of the clinical manifestations reported from different areas. So while chyluria is rare in the Pacific and in Indian areas of endemicity, it appears to be frequent in China, Africa and South America. Elephantiasis seems to be distributed in a more uniform way throughout the different geographical areas of

¹ Beye, H. K. & Gurian, J. (1961) The epidemiology and control of filariasis (*W. bancrofti* and *B. malayi*), Geneva (Unpublished working document WHO/Fil./26)

distribution of clinical filariasis, but its incidence and severity are very different in different areas (see Annexes 5, 6, pages 39, 40). The opinion that prevalence of elephantiasis in a population is proportionate to the percentage of microfilaria carriers in the same area, is not supported by the well-known fact that clinical filariasis may be low or absent even where prevalence of microfilariaemia is high. Surveys carried out in Polynesia and Melanesia suggest that elephantiasis prevalence in a population as a whole is related more to the microfilaria density than to microfilaria rate. In two surveys carried out in West Africa, however, this explanation is not confirmed.

As the information available is scarce, additional observations are required before the relationship suggested above can be substantiated for areas other than those of the Pacific. Also, it should now be possible to approach this problem in the study of laboratory animals infected with *B. malayi* by making observations on the relationship between adult worm load and microfilaria density.

(3) There is at present no evidence of differences in the types of lesion produced by different local strains of *B. malayi*. As with *W. bancrofti*, however, there is considerable local variation in the ratio of microfilaria rate to elephantiasis rate. The greater frequency of location of elephantiasis in the lower limbs, observed in *B. malayi* infections, has been suggested as being possibly due to the biting habits of the local vectors. Recent studies (see Annexes 6, 7, pages 40, 41, for details of analyses of clinical surveys) show that 60% of bites from *M. dives* and *M. bonneae* are below the knees, as compared with 18% from *Aedes albopictus*. Such observations are suggestive but not conclusive, and the tagging of larvae with isotopes to trace the path of their migration, might be a useful procedure in the investigation of this problem.

The Committee recommends that studies should be made by infecting laboratory animals that are proved to be susceptible to human filaria infections (e.g., *B. malayi*) to determine any relationships between adult worm load, microfilaria density, and possibly the type of pathology involved. Detailed studies should also be made of the life cycle of all filaria parasites.

2. STANDARDIZATION OF METHODS AND TECHNIQUES FOR EPIDEMIOLOGICAL SURVEYS

2.1 Data to be collected from the human population

Information should be obtained on the presence and number of microfilariae in the blood, the presence of clinical signs and symptoms, and the sex and approximate age of each person examined. The size of the population to be examined will largely determine the method of sampling

to be used. Where the population is small, efforts should be made to examine everyone; in a large population, some method of random sampling must be adopted. Probably the most convenient method will be sampling on an area basis, selecting at random groups of houses or individual houses within which every person should be examined. Statistical advice should be obtained when surveys are being planned. Local habits and customs, which may have a bearing on degree of exposure to infection, should be recorded, as should details of population movement. In accordance with Articles 6 and 15 of the *World Health Organization Regulations regarding Nomenclature... with respect to Diseases and Causes of Death*,¹ information should be tabulated by sex and age group, using the age groups commonly employed in public health statistics, e.g., under 1 year, 1-4 years, 5-9, 10-14, 15-19, 20-24, and so on.

2.1.1 Presence of microfilariae in the blood

Many different methods of collecting and examining blood for microfilariae have been employed. Where a particular technique has proved reliable, a change may be undesirable, particularly if it is likely to affect comparison with previous findings. In many different areas, however, it is routine practice to take 20-mm³ samples of peripheral blood, and this amount should be the minimum. Many workers use the haemocytometer pipette, but the calibrated capillary tube and Sinton pipette technique as used in Malaya has the advantage of adaptability, and the development of a standard-bore tube would add greatly to its value. Surveys based on 20-mm³ samples will tend to underestimate the prevalence of infection to a variable extent, depending on the proportion of low microfilaria counts in the population examined. Wherever possible, a "correction factor" should be estimated by the collection of larger quantities of blood (60-80 mm³ of peripheral blood; 1 ml of venous blood) from a representative sample of the population.

Reports should give full information about the technique employed; the source of blood, whether venous or capillary, the amount collected and time of collection, the method of staining and examination, and any other details which may have a bearing on the results. In studies of periodicity, measured blood samples should be collected not less frequently than every two hours for a minimum period of 24 hours, and the times should be recorded by the international system of 00.00 to 24.00 hours local time; the hours of daylight and darkness should be specified, and working and sleeping habits of the subjects should be stated.

The identification and counting of microfilariae demands the staining of the films and the careful examination of the full film under a low-power

¹ *Manual of the International Statistical Classification of Diseases, Injuries, and Causes of Death*, 1957, Geneva, World Health Organization, vol. 1, p. 389

objective with a magnification of about 100. The choice of stain depends largely on local conditions, and on the need to differentiate easily between *Wuchereria*, *Brugia* and other species of filariae. The stains commonly used are Giemsa's, Wright's, haemalum, haematoxylin and methylene blue.

The following terms should be used in recording the results :

Microfilaria rate : The percentage of a sample of a population found to be carrying microfilariae. Separate figures should be recorded for those with clinical filariasis and those without.

Microfilaria density : The microfilaria count per unit volume of blood—e.g., mm^3 or multiple thereof. The average count should be given for all films, and separately for microfilaria carriers only. A frequency distribution of densities should be worked out where experimental work and control is involved. The levels suggested are 1-5, 6-10, 11-25, 26-50, 51-100, 101-200, 201 and over, per 20 mm^3 .

2.1.2 *Presence of clinical manifestations*

These vary from one area to another. The prevalence of the common major lesions, elephantiasis, hydrocele, chyluria, lymph scrotum, should be recorded separately for each age group, as should other conditions—for example, thickened spermatic cord, enlarged testis or epididymis, lymphatic varix, where sufficiently common. Lymphadenitis, lymphangitis, and abscess are seldom found during surveys, and their prevalence can only be estimated by questioning the persons examined. Other manifestations, such as enlarged lymph-nodes, cutaneous nodules, bronchitis, asthma, and eosinophilia, may result from filarial infection or from some other cause.

In addition to calculating a rate by sex and age group for each of the major lesions, a clinical filariasis rate should be reported. A total filariasis infection rate should also be calculated by adding the number of persons showing microfilariae in the blood to the number with negative blood but showing one or more signs of clinical filariasis, and expressing this total as a percentage of the number examined in each sex and age group.

2.1.3 *Presentation of results*

The results of surveys should be presented so that comparison with other similar surveys is possible. Tables showing prevalence rates should be divided into the standard age and sex groups, stating clearly the number examined in each group, and the prevalence of the commoner major lesions. The prevalence rates for the whole sample will be influenced by the proportion of males to females and of adults to children in the total examined, and comparison with other surveys can properly be made only

after the use of some method of standardization to correct for differences in age and sex distribution.

2.2 Data to be collected on vectors

2.2.1 Collection and identification of local species

Regular collections (by various techniques) of mosquito adults and larvae should be made in representative zones of the area concerned during all seasons of the year over an adequate period of time. The possibility of vectors other than mosquitos (e.g., bed bugs, lice, fleas or other insects that bite man) should be borne in mind. Mature larvae of *B. malayi* and *W. bancrofti* have recently been reported in bed bugs in Kerala, India, though attempts in Malaya and India to infect bugs by feeding them on infected people have failed.

Accurate identification of mosquitos is, of course, essential, and it is advisable that samples of all specimens be submitted to specialists for identification and subsequent return to serve as reference material. Keys exist for identification of mosquitos of certain regions.

It should also be borne in mind that, with progress in studies, mosquito nomenclature is subject to change. For instance, *Mansonia longipalpis* of Malaya is now known to embrace two vector species—*M. dives* and *M. bonneae*; and what was once known as *Aedes pseudoscutellaris*, a vector in many islands of the Pacific, was found to include two very similar vector species, *Aedes pseudoscutellaris* and *Aedes polynesiensis*, and new species of this group and of the *Aedes kochi* group are being described. The *Anopheles leucosphyrus* group has been revised, and a review of the *A. barbirostris* group is being produced.

A list of species now considered to be vectors of *Wuchereria* and *Brugia* filariae is included in Annex 8, page 42. For some of the species mentioned, data on which claims to vector status are based are, however, inadequate and a critical survey of all data of this kind is long overdue.

The catches are essential in any studies of mosquito population or activities, and also before, during, and after the application of control measures.

Catches in houses and other buildings are best made at well-chosen, regular and comparable times, by some form of space-spraying, though for preliminary collections of undamaged specimens and for studies of resting-sites, hand captures should be made. There is now available mechanical fogging equipment that produces rapidly large volumes of relatively dry, finely atomized pyrethrum solution. This quickly disperses to all parts of large rooms and houses and brings down the great majority of, if not all, mosquitos onto sheets that are laid on floors and furnishings. Specimens so knocked down are usually quite suitable for identification and dissection if the fog or mist is not used in excessive quantity.

Captures of mosquitos outside are usually made by two or three men, acting as bait, who capture all alighting specimens in a catching tube specially designed for the purpose or in small glass or plastic phials. It is best to design a variety of catching methods to suit the habits of the particular species of mosquito and the activities of the people concerned. Thus for a day-biting "bush" vector of the *Aedes polynesiensis* type, daytime catches have included:

- (1) half-hour or one-hour catches at 10 m from occupied houses ;
- (2) 5-minute catches at 45-m intervals along radii through bush starting in villages and ending at 450 m ;
- (3) 5-minute catches at 45-m intervals along used paths and tracks leading to gardens and plantations ;
- (4) catches over a variety of times in gardens ;
- (5) 5-minute catches at selected places under high (or low) vegetation considered to be preferred concentration areas ;
- (6) half-hour catches on platforms at selected heights in trees.

2.2.2 Determination of vectors

2.2.2.1 Natural infections

The finding of the human filariae in vectors is an essential contribution to the determination of vectors. Presence of infections of animal origin may be misleading ; it should therefore be emphasized that correct identification of developing forms of filariae found in mosquitos must be based upon adequate reference material from species of mosquitos artificially infected with as many as possible of the local filariae in man and animals.

Species to be dissected will be those which appear to be most closely in contact with man, according to the results of regular adult catches (particularly those of the bait catches) and of the precipitin tests.

It is, of course, advisable to dissect specimens within a short period of capture. In many places, however, this is not possible, and preservation of specimens can be made, alive, at low temperature (e.g., in an ice-box) or dead in a preserving fluid such as 80% alcohol glycerol solution (see Nelson¹ and Annex 9, page 45). The periods of time between capture, killing for preservation, and dissection, must be recorded for each specimen.

2.2.2.2 Experimental infections

Any species in which natural infection has been found must be submitted to artificial infection by the species of filaria concerned. (Suggested techniques are given in Annex 10, page 46). It is suggested that all third-

¹ Nelson, G. S. (1960) *Indian J. Malar.*, **14**, 588

stage worms should be termed "third" or "mature" in preference to the term "infective", which is liable to misinterpretation.

2.2.3 *Bionomics of vectors*

It is essential to obtain as much data as possible on the following:

- (1) breeding-places;
- (2) feeding preferences and biting cycles of adults;
- (3) age of wild-caught adults at different seasons by determination of ovarian development by the method of Detinova,¹ or Davidson;²
- (4) seasonal prevalence;
- (5) flight range and dispersal;
- (6) resting habits in houses and outside;
- (7) times of entry and exit for species that enter houses.

Though many of these data will be obtained from routine catches, many special studies will almost certainly be necessary, especially for feeding habits, biting cycles and resting habits. The very important host preferences can best be determined by exposing a variety of animals, including birds, and human baits, under strictly comparable conditions. Records should be kept of the numbers of mosquitos of each species entering houses, and of the times of their entry, by catches from human-baited houses with window traps; or perhaps by a comparison of catches from very early morning fogging with those obtained in nocturnal hourly captures indoors on human bait.

Information must be recorded on the habits and movements of people and their degree of contact with the vectors. This is particularly necessary with vectors that do not normally enter houses, such as the day-biting, bush-breeding *Aedes* vectors of the Pacific area.

If the use of insecticides is contemplated, the susceptibilities of the vectors concerned must be ascertained by the tests designed by WHO.³

2.3 *Filaria infections in domestic and wild animals*

Infections of animal origin may confuse the results of mosquito dissection. It is necessary, therefore, to obtain a satisfactory knowledge of the species of Filarioidea locally parasitizing animals. Representative blood samples of the local fauna, including domestic and wild animals, especially wild carnivores closely in contact with man and human dwellings,

¹ Beklemishev, W. N., Detinova, T. S. & Polovodova, V. P. (1959) *Bull. Wld Hlth Org.*, **21**, 223

² Davidson, G. (1955) *Ann. trop. Med. Parasit.* **49**, 24

³ *Wld Hlth Org. techn. Rep. Ser.*, 1960, 191

must therefore be examined for the presence of microfilariae of such parasites ; and adult worms should also be sought (see Annex 2, page 35).

3. THERAPY AND CONTROL

3.1 Review of recent advances and experience in therapy

3.1.1 *Treatment of the individual patient with diethylcarbamazine*

Diethylcarbamazine has proved highly successful in the treatment of microfilaria carriers and has frequently been shown to reduce the frequency and severity of attacks of lymphangitis. Doses usually range from 4 to 6 mg of diethylcarbamazine citrate per kg of body-weight, given daily for 10-14 days. Higher doses or longer periods of treatment are used occasionally. Direct evidence of the effect of treatment on the adult worms in man is still lacking, but it has been proved that diethylcarbamazine will kill the adults of *B. malayi* in experimentally infected cats. Indirect evidence that the adults of *W. bancrofti* and *B. malayi* in man are either frequently killed or permanently sterilized by the drug is provided by follow-up examinations of treated microfilaria carriers, many of whom have remained free from microfilariae for up to 10 years after a course of treatment.

Adequate treatment with diethylcarbamazine will reduce considerably the liability to recurrent attacks of fever, lymphadenitis and lymphangitis, and will thus arrest the further development of elephantiasis, but its value in the treatment of developed elephantiasis, of chyluria and of hydrocele remains disappointing. Although in elephantiasis a combination of drug treatment and bandaging or surgery may produce good immediate results, recurrences are common.

The reactions sometimes associated with diethylcarbamazine treatment—for example, the febrile reaction in microfilaria carriers—seem to be little affected by antihistamine drugs but may be controlled by prednisolone, prednisone, or similar compounds.

The Committee recommends that detailed investigations should be carried out (1) to confirm further the effect of diethylcarbamazine on the individual patient in filariasis ; and (2) to improve the results of treatment in patients with chyluria, hydrocele, and advanced elephantiasis.

3.1.2 *Individual therapy with drugs other than diethylcarbamazine*

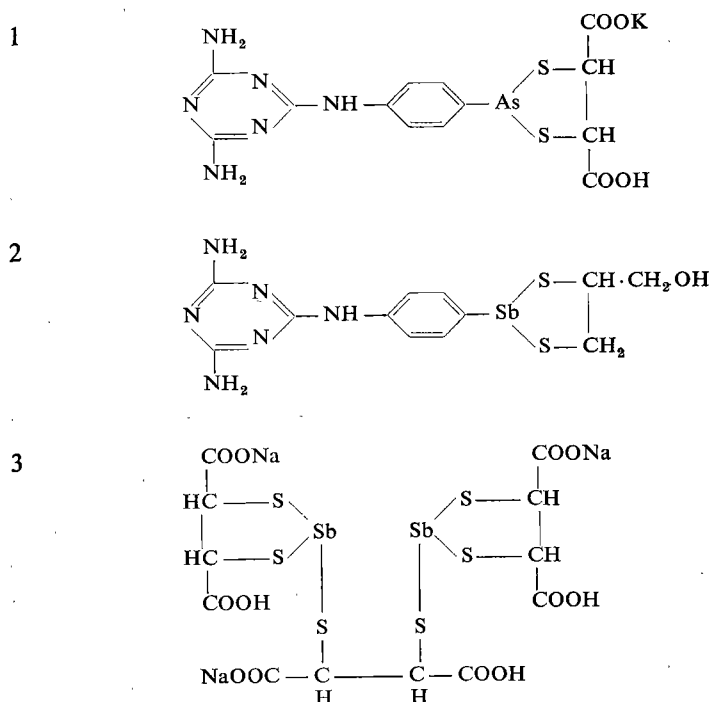
Various compounds of arsenic and antimony used for the treatment of filariasis in the past had the disadvantage of requiring administration by intravenous injection, and of having a relatively small margin between therapeutic dose and toxic dose. Recent work has produced several compounds which can be given intramuscularly, subcutaneously, or by mouth,

and which have a much greater margin of safety at dosage levels that are apparently active against microfilariae and adult worms (see following table). The compounds Mel W and MSbB are still at the clinical trial stage, but show promise of being valuable additions to the chemotherapy of filariasis.

The Committee recommends that the arsenic compound Mel W (see 1 below) so far studied in Netherlands Guinea, Cook Islands and East Africa, and the antimony compound MSbB (see 2 below) so far studied in East Africa, be submitted to larger-scale clinical trials in different parts of the world.

The antimony compound TWSb (see 3 below) that has been shown to have filaricidal properties in animal filariasis, in human onchocerciasis, and in Bancroftian filariasis, should be included in these trials.

STRUCTURAL FORMULAE OF Mel W, MSbB AND TWSb



3.2 Review of recent advances and experience in control

The long-term aim of control must be the eradication of filaria infection. The immediate aim is to reduce the transmission to a level at which

clinical manifestations do not occur. The methods used fall into two categories: (1) measures against the parasite by the administration of drugs; (2) measures against vectors.

3.2.1 *Measures against the parasite: mass treatment for parasite control*

Within the past few years a great deal of information has been collected from many countries about the treatment of whole populations with diethylcarbamazine as a method of controlling filariasis in different epidemiological situations. Dosage schedules have varied greatly from place to place, as has the proportion of the population that could be treated; nevertheless certain general conclusions may be drawn. Although reductions in microfilaria density and microfilaria rate may follow the distribution even of small amounts of the drug, the best results have been obtained where doses of 4-6 mg of diethylcarbamazine citrate have been given under supervision to a high proportion of the population at risk (80% or more) for a minimum of 5 or 6 doses in some areas, and 9 to 12 doses in others. The drug may be given either on consecutive days or at weekly or monthly intervals; those microfilaria carriers whose infections persist should be given further courses of treatment.

Where these conditions have been fulfilled, a high degree of control has been achieved against both *W. bancrofti* and *B. malayi*. Microfilaria rates have been reduced from 30% or more to 5% or less, and mean microfilaria counts from 32 per 20 mm³ film to less than 1.0. Where rates have been held at these low levels for several years, as in Tahiti, transmission of filariasis has almost ceased, and the prevalence of clinical filariasis has been greatly reduced. Recent interest in the possible effectiveness of large doses of diethylcarbamazine, given either singly or at long intervals, suggests that such methods might help to overcome the disadvantages of frequent smaller doses and merit further investigation.

The Committee recommends the following measures:

(1) That countries where filariasis has been shown to be a public health problem should consider the organization of filariasis control programmes, in which mass treatment is used. Advice should be sought from those with experience in successful schemes elsewhere, as the success of such programmes depends on careful preliminary planning, adequate drug dosage and subsequent effective follow-up operations. It is hoped that advice and support will also be forthcoming from international agencies.

(2) That additional studies be made on the effect of large single doses of diethylcarbamazine with special reference to the effect on transmission.

3.2.2 Measures against vectors

3.2.2.1 Insecticides¹

(1) *Adult control.* The house-spraying programme with wall deposits of DDT, so widely used with success against malaria vectors, has proved inadequate against filariasis carried by *C. fatigans*. As far as is known, none of the chlorinated hydrocarbon insecticides applied as residual deposits has been successful against *C. fatigans* over adequate periods because of its resistance to these compounds.

Many organo-phosphorus compounds have been tested against *fatigans* in houses, and the most promising experimentally appear to be Baytex and malathion. Baytex, however, may not be employed for general application until more data are available on its toxicity.

Other organo-phosphorus and carbamate compounds are under investigation and show promise.

Vectors of the *Mansonia* group react in different ways to house deposits of insecticides. In Malaya where there is a forest reservoir of *B. malayi*, residual spraying with dieldrin, though effective against mosquitos that entered houses, had little or no effect on infection rates in mosquitos.

In parts of India, however, where *M. annulifera* and *M. uniformis* appear to spend longer periods in houses, dieldrin and DDT deposits have had greater success. While the control of these vectors in these areas in India by the indoor application of dieldrin appears to be satisfactory, however, this measure has led to a remarkable increase in the prevalence of dieldrin-resistant strains of *C. fatigans* in the villages concerned. This increase and spread of *C. fatigans* might well facilitate the spread of *W. bancrofti* infection, which is endemic in areas situated in, or contiguous to, the *B. malayi* belt.

In the Pacific the vectors include two day-biting *Aedes* mosquitos (*A. pseudoscutellaris* and *A. polynesiensis*) that live and breed essentially in the bush and that enter houses rarely: *Aedes fijiensis*, that breeds in leaf axils and enters houses freely to feed and rest; and *C. fatigans*, that breeds in almost any type of stagnant water and enters houses in large numbers to feed. Residual spraying of houses can obviously affect only two of these vectors. Experiments in Fiji have shown that dieldrin used in this way reduces day populations of these mosquitos in houses very considerably; and though they contribute, perhaps, notably less than 50% of the total transmission, residual spraying of houses may be useful in any control campaign provided a careful watch is kept on the possible occurrence of resistance.

¹ The nomenclature and chemical nature of the insecticides referred to in this section are given in *Wld Hlth Org. techn. Rep. Ser.*, 1960, 191, 98

Anopheles are among the vectors of filariasis in many areas. In some places DDT or dieldrin applied as residual spray has eliminated or greatly reduced these (e.g., *A. gambiae* and *A. funestus* in parts of East and West Africa; *A. darlingi* in parts of Central and South America; and others). Though this reduction of vectors has, without doubt, helped to reduce transmission of filariasis in these areas, it has facilitated a considerable increase of *C. fatigans* in at least one of these places.

(2) *Larvae control*. It should be emphasized that the permanent control of *C. fatigans* and other culicines is best effected by sanitation measures aimed at source reduction, and that insecticidal treatments should be applied only when they are a necessary adjunct to such sanitation measures or where adequate sanitation cannot be implemented.

In many areas the main and urgent requirement for the control of *C. fatigans* is a reorganization of public health administration and the application of simple sanitary measures for the abolition of the numerous breeding facilities usually arising from urbanization. There are areas in which, for various reasons, this cannot be applied. In such situations insecticides may be used. It must, however, be pointed out that insecticide applications for larval control will fail unless the insecticide formulations and the planning and methods of application are made to suit the local environment. This means that sound ecological studies must be the basis of all insecticide operations.

Chlorinated hydrocarbon compounds have failed because resistance to them in *C. fatigans* develops very quickly. However, organo-phosphorus compounds show considerable promise as larvicides, and although in Douala resistance to malathion has developed in *C. fatigans*, a number of organo-phosphorus compounds have been used successfully in some parts of America for periods of up to six years against *Aedes* and *Culex* mosquitos. Baytex appears to be the most promising of organo-phosphorus insecticides, and many other compounds of this group and of the carbamate group are being investigated.

In spite of the development of resistance to many compounds, there is little doubt that *C. fatigans* can be controlled for a period of years in most places if carefully planned use is made, perhaps in succession or combination, of available insecticides in suitable formulations and dosage, and applied by suitable methods.

In some countries a return has been made with success to the use of high-spreading oil, and to Paris green applications, especially in granulated or pellet form.

It is very desirable that experiments of adequate size be set up in *C. fatigans* areas in which use is made of well-planned applications of a variety of insecticides.

Insecticides in usual formulations and applications are not suitable for *Mansonia* species that live submerged and attached to roots of aquatic

plants and trees. Insecticides incorporated in pellets or briquettes offer a possible means of larval control where breeding occurs in ponds or tanks on *Pistia* and *Salvinia*.

The three *Aedes* vectors of *W. bancrofti* in the Pacific breed in tree-holes, crab holes, rock holes, coconut husks, small discarded containers, plant axils and rat-eaten coconuts, which are not easily accessible to ordinary applications of insecticides. Nevertheless, many breeding-grounds of two of these vectors, *A. pseudoscutellaris* and *A. polynesiensis*, in containers in and around villages can be eliminated by simple cleaning-up measures if good organization and constant enthusiasm are available. In experiments, dieldrin pellets applied to tree-holes were very promising. For crab-hole breeding it was found that destruction of the crabs with BHC rice-bran bait was found to be a feasible method in experiments, and it is considered that this method could be used successfully in restricted areas. Breeding in heaps of coconut husks was checked experimentally by spraying with dieldrin. Banding of coconut trees to prevent rat attack on coconuts has proved successful in some territories.

3.2.2.2 *Susceptibility to insecticides*

Determination of the susceptibility of the vector population to the types of insecticides under consideration should be made for each species before any use of insecticides is started in the area.

For these determinations the WHO standard test kits for determining the susceptibility or resistance of adult mosquitos and/or of mosquito larvae to insecticides should be used.

Detailed instructions for carrying out the tests and the principles for (a) establishing the base line of susceptibility, and (b) subsequent routine checks, are given in the tenth report of the WHO Expert Committee on Insecticides.¹

Regular susceptibility determinations should, of course, be carried out at suitable intervals, if vector control measures are started, to detect possible development of resistance to insecticides. Test kits are available without charge upon application to WHO.

3.2.2.3 *Herbicides*

These have particular application for the destruction of *Pistia*, *Eichornia* and *Salvinia* plants to which *Mansonia* larvae are attached. Applications of pentachlorophenol and 4-chloro-2-methylphenoxyacetic acid (MCPA) have been used successfully in the special conditions of Ceylon. It is probable that other compounds may become available in the near future.

¹ *Wld Hlth Org. techn. Rep. Ser.*, 1960, 191

3.2.2.4 *Biological control*

Studies are being made on the introduction and spread of *Coelomomyces* for the control of mosquitos of the *Aedes pseudoscutellaris* group naturally breeding in small containers in some islands of the Pacific. Though the parasite has established itself, results are not yet adequate for evaluation. Biological control of disease vectors is receiving much attention. The introduction of *Toxorhynchites* mosquitos to many islands of the Pacific for the control of the same group of *Aedes* has not been successful.

3.3 **Relative merits of parasite and vector control**

In early control programmes against filariasis, a number of attempts were made to accomplish the objective by mosquito control alone. In all such campaigns that have been brought to the attention of this Committee, no significant success was achieved because of the unusual difficulties encountered in reducing filaria vectors.

The introduction of diethylcarbamazine provided a completely new method of control by reducing the reservoir of infection in the human population, a factor of the greatest importance owing to the length of life of the parasite. The effect of the drug as a filaricide is no longer in doubt in individual and mass treatment. The chief disadvantages in mass treatment are the frequency of reactions in symptomless carriers, the lack of effect against advanced elephantiasis, and the need to give repeated doses. These disadvantages, however, can be overcome by adequate preliminary health propaganda.

In control programmes against filariasis to date, it would appear that the best procedures reverse the order now accepted in malaria eradication schemes, and that chemotherapeutic control should be used first, thereby reducing microfilariaemia to a minimum as early in the programme as possible. Administration of diethylcarbamazine should be accompanied, or followed as rapidly as possible, by the most effective mosquito control programme which can be applied. If vector mosquito density could be sufficiently reduced immediately after the reduction of microfilariaemia, transmission of filariasis would cease.

In a recent questionnaire sent to 12 areas in the Pacific which either have instituted filariasis control programmes or are planning to do so in the near future, only one of the authorities replying considered that it might be possible to control filariasis by employing mosquito control methods alone. This one authority was in doubt, after three years, concerning the area results but wished from a scientific point of view to carry on the observation for a continued period. Replies from the other 11 areas advocated the use of diethylcarbamazine alone or a combination of the two methods.

The control programme in Tahiti employed a combination of both methods. In 1949 before any procedures were instituted, 14.2% of 1452 children examined in the 0-9 age-group were positive with a corresponding density of 4.1 microfilariae per 20 mm³ while after control, in 1960, 1070 of this same age-group, who had never received diethylcarbamazine, showed 0.9% to be positive with a microfilarial density of 0.03.

This low infection rate in the early age-group indicates that filariasis transmission has reached a low point of less than 1%. This finding, together with the fact that few or no new cases of elephantiasis developed during the same period indicates that in the areas under thorough control, transmission has reached a level where clinical filariasis is no longer apparent as an important public health problem.

With the great reduction in microfilariaemia and in clinical filariasis that has occurred in a number of successful filariasis control programmes, the question arises as to whether an effort should be made to proceed to the eradication stage, on the lines of the eradication programmes that have been developed for malaria. This decision will have to be determined by evaluating the existing conditions of each area in question.

The Committee considers that the most effective and rapid control programme for filariasis known at present is the combination of mass treatment with diethylcarbamazine and effective mosquito control.

4. SUGGESTIONS FOR RESEARCH

Many recommendations involving research have been made in earlier sections of this report. For convenience they are listed below, with reference to the appropriate section.

4.1 Epidemiology

- (1) Studies on filaria infections in animals (1.2.1)
- (2) Studies on developing larvae in mosquitos (1.2.3)
- (3) Experimental transmission to man (1.2.1)
- (4) Studies in microfilaria periodicity (1.2.1)
- (5) Studies on *C. fatigans* and strains of *W. bancrofti* (1.2.4)
- (6) Studies on *Aedes vigilax* and strains of *W. bancrofti* (1.2.4)
- (7) Correlation between microfilaria prevalence rates and mosquito transmission (1.2.7)
- (8) Relation between worm load and microfilaria density (1.2.8)

- (9) Vector ecology
 - (a) Review of ecology of *C. pipiens* group (1.2.5)
 - (b) Taxonomic, bionomic and genetic studies on *C. fatigans* (1.2.4, 3.2.2)
 - (c) Evaluation of techniques of mosquito age determination (1.2.5)
 - (d) Studies on ecology of *Mansonia* (1.2.5, 3.2.2)
 - (e) Studies on ecology of *Aedes vigilax* (1.2.4, 1.2.5)
 - (f) Integrated studies on the *C. fatigans* problem (3.2.2)
 - (g) Preferential biting-times, -places, and -sites on body (1.2.5)

4.2 Therapy

- (1) Studies to confirm the effect of diethylcarbamazine in individuals (3.1.1)
- (2) Studies to improve results of treatment in patients with advanced lesions (3.1.1)
- (3) Clinical trials of new drugs (3.1.2)

4.3 Control

- (1) Studies on effect of large single doses of diethylcarbamazine (3.2.1)
- (2) Control of *C. fatigans* in conditions of uncontrolled urbanization (1.2.6)
- (3) Studies on vector susceptibility to insecticides and development of resistance (3.2.2)
- (4) Studies on control of *C. fatigans* by sanitation (3.2.2)
- (5) Studies on biological control measures (3.2.2)

4.4 Other subjects

- (1) Biochemical research on the metabolic reactions supplying energy required for the motor activity of microfilariae and, wherever possible, of adult worms.
- (2) Co-ordination and support of studies on the clinical evolution of filariasis to include the effect of trauma and bacterial infection on the production of clinical manifestations.
- (3) Co-ordination and support of the use of isotopes in studying the life-cycles of the filariae, and in clinical and therapeutic problems.
- (4) The Committee endorsed the plans and development of the research programme undertaken by WHO, as proposed by a scientific group on filariasis in 1959, in the fields of: (a) immunochemistry of filaria infections; (b) filaria infections in animals; (c) clinical evolution and

therapy of filaria infections ; (d) vectors of *Wuchereria* and *Brugia* infections ; and (e) studies on the effect which malaria eradication activities may have on local epidemiology of the *Wuchereria* and *Brugia* infections under the different conditions existing in the different endemic areas. Subjects (b), (c), and (d) have already been mentioned. The results of the Committee's discussions on (a) and (e) are as follows :

(a) *Immunochemistry of filaria infections.* Serological and immunological studies in filariasis have been seriously handicapped heretofore by the lack of suitable laboratory animals. Study of infections in cats with *B. malayi* and *B. pahangi* and in rabbits with *Dirofilaria uniformis* should obviate some of the difficulties.

As specific antibodies are not frequently detected during active infections, a thorough search should be made for the presence of free antigens in the serum, urine, hydrocele or fluid from lymph-nodes of infected individuals. Furthermore, testing for antibodies could be carried out after treatment with diethylcarbamazine (Hetrazan) to remove excess antigen supposedly produced by the circulating microfilariae. Search for antibodies in old chronic human infections should also be conducted.

In the light of recent advances in the field of immunology (agar-gel diffusion techniques, immuno-electrophoresis) a comparison of the antigenic structure of microfilariae, infective larvae and adults, should be made to determine antigenic differences, and to select the stage which is the most suitable for use in the various diagnostic tests. This should be followed by attempts at chemical and physical fractionation and purification of antigens to be used in the intradermal and serological tests.

Serological techniques employing soluble antigens (flocculation, complement-fixation, haemagglutination tests) should be evaluated with the purified products. Furthermore, the possible value of techniques employing whole organisms (microfilaria immobilization test, fluorescent antibody test) should be fully evaluated.

In general, studies in immunology in filariasis should be stimulated and supported in those institutions which are interested and competent to develop programmes along similar lines to those currently applied for other helminthiases.

The Committee recommends :

(i) Search for free antigen, using available techniques, in infected humans and animals before and after treatment.

(ii) Comparison of the antigenic structure of microfilariae, mature larvae, and adults by agar-gel diffusion techniques and immuno-electrophoresis.

(iii) Chemical and physical fractionation and purification of antigens.

(iv) Search for new tests (fluorescent antibody, flocculation tests, etc.) with special emphasis on tests employing minute amounts of blood.

(v) Evaluation of intradermal tests, and serological tests, employing purified antigens, under field conditions.

(vi) Sero-immunological investigations of populations living in endemic areas.

(e) *Studies on the effect that malaria eradication activities may have on local epidemiology of the Wuchereria and Brugia infections under the different conditions existing in the different endemic areas.* The Committee considers that activities in this field should be started as soon as possible, so that general policy lines may be suggested regarding filariasis control in areas where malaria control or eradication activities are, or will be, carried out. The aim must be to take advantage of the organization and methods of malaria control operations, through adequate and timely application of supplementary filariasis control measures, suitable to the different epidemiological conditions of the different areas where the two diseases co-exist but have a different combination of vectors. The Committee suggested that different local combinations of vectors should be studied in different areas, and that the effect of the use of insecticides on the different species of vectors of filariasis be evaluated, so that the expediency of additional measures for their control could be assessed in areas where malaria control programmes have produced, or are likely to produce, insecticide resistance in the filariasis vectors. This applies particularly to *C. fatigans*, and to the expediency of suggesting that adoption of antilarval and other measures against this species should accompany the application of malaria control operations.

The effect of malaria eradication operations in terms of reduction of filariasis, in areas where these diseases have vectors either totally or partly in common, among which all or some are susceptible to insecticides, should be investigated also, in order to be able to advise on policy lines of local supplementary measures regarding (i) the expediency of starting mass drug treatment against filariasis in areas where anopheline control has resulted in great reduction of filariasis vectors; and (ii) the advisability of prolonging insecticide operations after their cessation in malaria control, to maintain the reduction of filariasis vectors over the period of drug administration. Geographical areas of common endemicity, and different combinations of malaria and filariasis vectors, suggested as types for urgent and profitable investigations are :

Areas in India and Malaya, or other Asian countries, where *Anopheles* vectors of malaria co-exist with species of *Mansonia* vectors of filariasis.

Areas of Malaya where *A. barbirostris* is a common vector of filariasis and malaria.

Areas of India, the Philippines, Indonesia, or other Asian countries, where *Anopheles* vectors of malaria co-exist with *C. fatigans*.

Areas of Africa where *A. gambiae* and *funestus* co-exist with *C. fatigans*.

Areas where *A. gambiae* and *funestus* alone are the common vectors of malaria and filariasis.

Areas of South America where *Anopheles* vectors of malaria co-exist with *C. fatigans*.

Areas of South America where *A. darlingi* or other vectors of malaria are also the vectors of filariasis, in the absence of *C. fatigans*.

Areas of New Guinea where *Anopheles* are the common vectors of malaria and filariasis.

The different areas in the Pacific where malaria and filariasis co-exist and where different combinations of vectors are locally responsible for transmission of both diseases.

5. OTHER SUBJECTS

5.1 Coding for the International Classification of Diseases

The Committee was requested to give advice on the coding of filariasis in the International Classification of Diseases; recognizing that changes are expedient, it recommends that the following coding should replace the existing one:

Diseases caused by <i>Filarioidea</i>	127
Filariasis (to include infections caused by <i>Wuchereria</i> sp. and <i>Brugia</i> sp.)	127.1
Loiasis: infection with <i>Loa loa</i>	127.2
Onchocerciasis or onchocercosis: infection with <i>Onchocerca volvulus</i>	127.3
Acanthocheilonemiasis: infection with <i>Acanthocheilonema perstans</i>	127.4

5.2 Terminology

The Committee discussed the draft of a document on terminology for malaria prepared by the Organization in order to examine the extent to which it covers needs of workers in filariasis. The Committee noted that

this was a useful document which requires supplementation by some terms particular to filariasis.

The Committee therefore recommends that consideration be given to the possibility of using this document as a basis for the issue of a supplement prepared by specialists in the field of filariasis.

5.3 Identification of different stages of Filarioidea

The Committee stresses the urgent need for a manual on the identification of all stages of the Filarioidea of man.

5.4 Training

The Committee recommends that assistance be given to members of national staffs for training in the different techniques associated with filariasis research. This could be done on an individual basis or by the organization of training courses.

5.5 Support for filariasis research

The Committee recommends that assistance be given where necessary to institutes engaged in filariasis research and to control programmes likely to advance knowledge, with the object of supporting their continuation until significant results are obtained.

ACKNOWLEDGMENT

The Committee acknowledges the special contribution made by Mr J. W. Wright, Chief, Vector Control and Pesticides, Division of Environmental Sanitation.

Annex 1

**RECORDS OF THE OCCURRENCE OF *BRUGIA* INFECTIONS
IN ANIMALS IN EAST PAHANG, MALAYA:
SURVEY RESULTS TO 30 MAY 1959 ***

Animals	Number examined	Infected with		
		<i>B. malayi</i>	<i>B. pahangi</i>	both species
Primates :				
White-handed gibbon (<i>Hylobates lar</i>)	6	—	—	—
Long-tailed macaque (<i>Macaca irus</i>)	116	4	—	—
Short-tailed macaque (<i>M. nemestrina</i>)	16	—	—	—
Dusky leaf-monkey (<i>Presbytis obscurus</i>)	25	19	—	—
Slow loris (<i>Nycticebus coucang</i>)	25	—	8	—
Carnivores :				
Domestic cat (<i>Felis domestica</i>)	88 **	5	14	4
dog (<i>Canis familiaris</i>)	25 †	—	5	—
Tiger (<i>Panthera tigris</i>)	1	—	1	—
Wild cat (<i>Felis bengalensis</i>)	7	—	—	1
" (<i>F. planiceps</i>)	1	—	1	—
Civet-cat (<i>Paradoxurus hermaphroditus</i>)	44	1	12	—
" (<i>Arctogalidia trivirgata</i>)	3	—	1	—
" (<i>Viverra zibetha</i>)	1	—	1	—
" (<i>Arctictus binturong</i>)	1	—	—	—
Otter (<i>Lutra sumatrana</i>)	2	—	—	—
Pholidota (Edentata) :				
Pangolin (<i>Manis javanica</i>)	11	1	—	2
Insectivores :				
Tree-shrew (<i>Tupaia glis</i>)	40	—	—	—
Moon-rat (<i>Echinosorex gymnurus</i>)	4	—	2	—
Rodents :				
Squirrel (<i>Callosciurus</i> spp.)	50	—	—	—
Giant squirrel (<i>Ratufa</i> spp.)	11	—	1	—
Rat (<i>Rattus</i> spp.)	107	—	—	—
Porcupine (<i>Hystrix brachyura</i>)	2	—	—	—
" (<i>Athero macrourus</i>)	2	—	—	—
Bats :				
Flying fox (<i>Pteropus vampyrus</i>)	30	—	—	—
Insect-eating bat (<i>Microchiroptera</i> sp.)	6	—	—	—
Ungulates :				
Water-buffalo (<i>Bubalus bubalos</i>)	18	—	—	—
Domestic cattle (<i>Bos taurus</i>)	3	—	—	—
Goat (<i>Capra hircus</i>)	38	—	—	—
Sheep (<i>Ovis aries</i>)	32	—	—	—
Wild pig (<i>Sus scrofa</i>)	10	—	—	—
Mouse-deer (<i>Tragulus javanicus</i>)	7	—	—	—
Birds :				
Domestic fowl (<i>Gallus gallus</i>)	32	—	—	—
Domestic duck (<i>Anas</i> sp.)	5	—	—	—
Ground dove (<i>Geopelia</i> spp.)	27	—	—	—
Owl (<i>Ketupa ketupu</i>)	3	—	—	—
Teal (<i>Dendrocygna javanica</i>)	2	—	—	—
Hornbill (<i>Buceros rhinoceros</i>)	1	—	—	—

* After Laing, A. B. G., Edeson, J. F. B. & Wharton, R. H. (1960) *Ann. trop. Med. Parasit.*, 54, 95

** In 1958 survey only; in previous surveys, 50 out of 159 had *malayi*-like microfilariae

† In 1958 survey only; in previous surveys 4 out of 5 had *malayi*-like microfilariae

Annex 2**ANIMALS AS RESERVOIR HOSTS FOR *WUCHERERIA*
AND *BRUGIA* INFECTIONS**

In the search for animal hosts of *Wuchereria* and *Brugia* infections, it should be remembered that the microfilariae of both infections in man are nocturnally periodic in most areas, and that the examination of the blood of animals by day will give results of doubtful significance.

Blood for examination for microfilariae may be obtained by pricking the finger or ear, or the skin between the toes, and the aim should be to examine a film at least 20 mm³ thick and stained in the usual way.

A technique for finding adult worms which are living in the lymph-nodes has recently been described.¹ The main feature is that the lymph-nodes are first placed in saline and are then confined between two blocks of 2-inch × 4-inch plate glass and examined under a stereoscopic microscope. Adult or developing worms which can then be seen lying in the vessels or lymphatic tissues may be dissected out and preserved in glycerol.

A useful method for the examination of preserved material is to mount the adult worm in a hanging drop of glycerol.

Experimental infections

Mature filaria larvae can be successfully inoculated into animals by the following method.²

(1) Experimentally infected mosquitos are dissected in normal saline 10-15 days after feeding on a suitable microfilaria carrier (preferably with 20-100 microfilariae in 20 mm³ of blood).

(2) Mature larvae are picked up on the point of a fine needle and transferred individually to a small drop of saline in a glass cavity-block or watch-glass.

(3) 20-30 larvae is a convenient number of larvae for a single inoculation.

(4) A small quantity of saline is drawn into a syringe fitted with a wide-bore needle, then the drop of saline containing the larvae is drawn into the syringe (the drop should be small enough for few, if any, of the larvae to be drawn into the body of the syringe).

¹ Buckley, J. J. C. & Edeson, J. F. B. (1956) *J. Helminth.*, 30, 1

² Edeson, J. F. B. & Wharton, R. H. (1957) *Trans. roy. Soc. trop. Med. Hyg.*, 49, 604

(5) The larvae are inoculated subcutaneously.

(6) The piston of the syringe is withdrawn, a small quantity of saline is pipetted into the syringe and the contents are then emptied into a glass cavity-block to see whether all the larvae were inoculated.

Annex 3

KEY TO THE DIFFERENTIATION OF IMMATURE FILARIA LARVAE IN MOSQUITOS *

- | | |
|--|--|
| (1) Caudal papillae or protuberances present | (2) |
| No caudal papillae | (9) |
| (2) Length more than 1100 μ | (3) |
| Length less than 1100 μ | (6) |
| (3) Three equal bubble-like caudal papillae | <i>W. bancrofti</i> |
| Three caudal papillae of various shapes and sizes | (4) |
| (4) Terminal or dorsal papilla prominent | (5) |
| All three papillae poorly developed; anal ratio less than
4.5 | <i>B. malayi</i> and <i>B. pahangi</i> |
| (5) Terminal dorsal papilla "dog's nose" shape; lateral papillae
poorly developed; larva narrows between anus and extremity;
anal ratio averages 4.5 | <i>B. patei</i> |
| Terminal papilla large and central; two small lateral sub-
terminal alae; anal ratio less than 3 | <i>Setaria equina</i> |
| (6) Anus more than 50 μ from extremity | (8) |
| Anus less than 50 μ from extremity | (7) |
| (7) Three small terminal papillae | <i>Dirofilaria corynodes</i> |
| One small terminal papilla with or without two very small sub-
terminal papillae | <i>Dirofilaria immitis</i> or <i>D. repens</i> |
| (8) Three prominent terminal papillae | <i>D. arbuta</i> |
| (9) Length usually less than 1100 μ ; anus less than 50 μ from
extremity | <i>Foleyella</i> spp. |
| Length 1000 μ - 1250 μ ; anus more than 50 μ from extremity
. | <i>C. flavescens</i> |

* After Nelson, G. S. (1959) *J. Helminth.*, 23, 233

Annex 4

POTENTIAL TRANSMISSION INDEX *

In the Tahitian control programme, methods were developed to procure quantitative information on the prevalence rates of mosquitos, and the occurrence in them of *W. bancrofti* infections. Mosquitos are caught on human bait for a ten-minute period within 10 m of each house in a given district. The average number of mosquitos caught per minute is the unit selected by which to record prevalence rates. Standardized proportions of each catch are dissected, and the numbers of larvae for each stage of *W. bancrofti* are recorded. From these records the percentage of mosquitos positive for each stage of larvae, and the larval density for each stage in the mosquito, are determined. The results have been used not only to designate the existing conditions of transmission before control procedures are begun, but also to measure the progress of the control campaign against filariasis.

A summary sheet of an Intensive Survey in an area before control measures were instituted is presented below.

ANALYSIS OF INTENSIVE MOSQUITO SURVEY OF A TYPICAL
DISTRICT BEFORE CONTROL MEASURES SHOWING COLLECTIONS
AND DISSECTIONS OF *AËDES POLYNESEIENSIS* FOR
WUCHERERIA BANCROFTI LARVAE

District : 1		
(1) Number of stations	112	
(2) Number of mosquitos collected	615	
(3) Number of stations with mosquitos	75 or	66.9 %
(4) Number of stations with 10 or more mosquitos	20 or	17.8 %
(5) Number of stations with mosquitos positive for larvae of <i>W. bancrofti</i>	29 or	25.8 %
(6) Number of mosquitos caught per minute	0.54	
(7) Number of mosquitos dissected	422	
(8) Mosquitos containing developmental-stage larvae	56 or	13.2 %
(9) Mosquitos containing mature larvae	32 or	7.6 %
(10) Density of developmental-stage larvae per dissected mosquito	0.74	
(11) Density of mature larvae per dissected mosquito	0.19	

* After Kessel, J. F. (1957) *Bull. Wld Hlth Org.*, 16, 633

Of special importance are points (6), (8), (9), (10) and (11). These data, from such a standardized type of survey, provide significant information concerning the prevalence of *W. bancrofti* larvae in mosquitos, and from them an impression of the amount of transmission in progress can be determined.

Each of the points (8) to (11) in itself might be used as an index for comparison with other surveys. However, a single index compiled from a combination of the most pertinent points provides a standard susceptible of general comparison.

The potential transmission index is derived by multiplying item (6)—0.54—by item (10)—0.74—which gives 0.4. A whole number, capable of easy comparison, is desirable for such an index. If 0.4 is multiplied by 1000 and divided by 4, the figure 100 is obtained.

This index was developed in order to compare transmission data before and after control measures. The density of larvae could be based either on developmental stages present in the mosquito, that is, beyond the microfilarial stage, or on mature larvae only. The Tahitian study group elected to use larval density based on all developmental stages rather than on mature larvae alone because inclusion of all larvae represented a more severe test for their control measures. In epidemiological studies on the relation between microfilaria prevalence rates and infection rates in mosquitos it might be preferable to consider only the mature larvae.

Annex 5

**COMPARISON OF PREVALENCE OF ELEPHANTIASIS,
PERCENTAGE OF POPULATION POSITIVE FOR MICROFILARIAE,
AND DENSITY OF MICROFILARIAE PER 20 mm³ OF BLOOD
IN DIFFERENT AREAS OF THE PACIFIC ***

	Percentage with elephantiasis	Percentage with microfilariaemia	Density of microfilariae in whole population	Density of microfilariae per positive case
Hitiāa	9.9	43.6	48.1	110
Society Islands	7.0	32.0	35.0	110
Maiao	5.0	27.0	33.0	127
Marquesas	3.0	33.7	19.7	58
Apia, West Samoa	1.6	22.6	7.5	46
Maupiti	1.0	26.0	12.0	46
Kandavu, Fiji	1.0	25.3	9.0	36
Mau, New Caledonia	1.0	33.0	7.0	20
Tikehau	0.5	29.2	8.0	22
Austral Islands	0.3	30.0	12.0	40
Labasa, Fiji	0.3	9.0	0.9	10

* After Kessel, J. F. (1957) *Amer. J. Trop. med. Hyg.*, 6, 402

Annex 6

CLINICAL MANIFESTATIONS OF *W. BANCROFTI* INFECTIONS IN INDIA

Area	Number of persons		Number of persons with elephantiasis					Number of persons with lymphangitis	Sources
	examined	with filaria disease	location						
			upper extremity	lower extremity	both limbs	genitalia	other		
Trivandrum, Kerala	31 005	1 025	—	547	—	96	2	380	Iyengar, M.O.T. (1938) <i>Ind. J. med. Res.</i> , Suppl. no. 30
Ernakulam, Kerala	7 672	245	24	200	3	2	—	16	Malaria Institute of India (1955) Report for the year 1955
Cuttack Suburbs (Orissa)	1 446	77	3	38	12	23	1	—	Raghavan (unpublished data)
	40 123	1 347	27	785	15	121	3	396	

Annex 7

CLINICAL MANIFESTATIONS OF *W. MALAYI* INFECTIONS IN DIFFERENT AREAS

Area	Number of persons		Number of persons with elephantiasis						Number of persons with lymphangitis	Sources	
	examined	with filaria disease	location				genitalia	other			
			upper extremity	lower extremity	both limbs						
Thailand		213		204	9						
Malaya											
Perak, Pahang	—	322	1	320	—	1	—	—	—	—	Iyengar, M.O.T. (1953) <i>Bull. Wild Hlth Org.</i> , 9, 731
Pahang	—	279	1	276	2	—	—	—	—	—	Poynton, J. P. & Hodgkin, E. P. (1938) <i>Bull. Inst. med. Res.</i> , F.M.S., No. 1
Kedah/Pahang	—	108	1	101	6	—	—	—	—	—	Wilson (personal communication to Turner, 1959)
Pahang	—	37	—	33	3	1	—	—	—	—	Wilson (personal communication to Turner, 1959)
India											
Shertallai, Kerala	6 404	1 473	9	821	76	5	1 (scro-	561			Turner, L. H. (1959) <i>Trans. roy. Soc. trop. Med. Hyg.</i> 53, 154
Ambalapuzha, Kerala	3 071	442	7	208	18	1	1 (scro-	207			Turner, L. H. (1959) <i>Ann. trop. Med. Parasit.</i> , 53, 180
Karunagapalli, Kerala	2 000	68	—	25	—	—	—	43			Iyengar, M. O. T. (1938) <i>Ind. J. med. Res.</i> , Suppl. no. 30
Sri Harikotta, Andhra Pradesh	709	102	17	76	7	2	—	—			Iyengar, M. O. T. (1938) <i>Ind. J. med. Res.</i> , Suppl. no. 30
Shertallai Taluk, Kerala	8 463	2 429	57	1 300	148	5	—	919			Raghavan, N. G. S. & Krishnan, K. S. (1949) <i>Ind. J. Malar.</i> , 3, 39
	20 647	5 473	93	3 864	269	15	2	1 730			Singh, J. et al. (1956) <i>Ind. J. Malar.</i> , 10, 317

Annex 8

**THE VECTORS OF HUMAN *WUCHERERIA* AND *BRUGIA*
INFECTIONS IN NATURE**

Many of the early records concerning the identity of human filaria vectors in nature are of doubtful value. For this reason the list which follows may not be completely reliable since all the vectors listed may not satisfy the conditions which are required. Briefly, these are the finding of mature larvae identifiable as either *Wuchereria* or *Brugia* in wild mosquitos, confirmed by observations on the development of the infection under laboratory conditions. The list is not regarded as complete and is based on the review by Raghavan¹ using mainly the mosquito terminology of Stone, Knight & Starcke.²

Vectors of periodic *Wuchereria bancrofti*

Neotropical region :

Culex pipiens fatigans Wiedemann
Anopheles (Nyssorhynchus) darlingi Root
Anopheles (Nyssorhynchus) aquasalis Curry

Mediterranean region :

Egypt :

Culex pipiens group

Ethiopian region :

Anopheles (Myzomyia) gambiae Giles
Anopheles (Myzomyia) funestus Giles
Culex pipiens fatigans Wiedemann

Oriental region :

India :

Culex pipiens fatigans Wiedemann
Anopheles (Myzomyia) philippinensis Ludlow
Anopheles (Myzomyia) sundaicus Rodenwaldt

¹ Raghavan, N. G. S. (1961) *Bull. Wld Hlth Org.*, **24**, 177.

² Stone, A., Knight, K. L. & Starcke, H. (1959) *A synoptic catalog of the mosquitoes of the world*, Washington.

Oriental region (*continued*) :

Ceylon

Culex pipiens fatigans Wiedemann
Anopheles (Myzomyia) subpictus Grassi

Maldive Islands

Culex pipiens fatigans Wiedemann
Anopheles (Myzomyia) tessellatus Theobald

Malaya

Culex pipiens fatigans Wiedemann
Anopheles (Anopheles) letifer Sandosham
Anopheles (Anopheles) sinensis Wiedemann

Borneo

Anopheles (Anopheles) barbirostris van der Wulp
Anopheles (Blanchard) leucosphyrus Dönitz

Indonesia

Culex pipiens fatigans Wiedemann

Indochinese peninsula

Culex pipiens fatigans Wiedemann
Anopheles (Anopheles) sinensis Wiedemann

China

Culex pipiens pallens Coquillett
Anopheles (Anopheles) sinensis Wiedemann

Philippines

Culex pipiens fatigans Wiedemann
Aedes (Finlaya) poicilius Theobald

Japan

Culex pipiens pallens Coquillett
Aedes (Finlaya) togoi Theobald

Australasian region :

Melanesia (New Guinea, Solomon Islands)

Anopheles (Myzomyia) punctulatus Dönitz group
Anopheles (Anopheles) bancroftii Giles
Mansonia (Mansonioides) uniformis Theobald
Culex annulirostris Taylor
Aedes (Finlaya) kochi Dönitz

Micronesia

Culex pipiens fatigans Wiedemann

Vectors of sub-periodic *Wuchereria bancrofti*

Polynesia (including Fiji)

Aedes (Stegomyia) pseudoscutellaris Theobald group*Aedes (Stegomyia) polynesiensis* Marks*Culex pipiens fatigans* Wiedemann*Aedes (Finlaya) fijiensis* Marks

New Caledonia

Aedes (Ochlerotatus) vigilax Skuse**Vectors of *Brugia malayi***

Oriental region :

India

Mansonia (Mansonioides) annulifera Theobald*Mansonia (Mansonioides) indiana* Edwards*Mansonia (Mansonioides) uniformis* Theobald*Anopheles (Anopheles) barbirostris* van der Wulp group

Ceylon

Mansonia (Mansonioides) uniformis Theobald*Mansonia (Mansonioides) annulifera* Theobald*Mansonia (Mansonioides) indiana* Edwards

Thailand

Mansonia (Mansonioides) annulifera Theobald*Mansonia (Mansonioides) uniformis* Theobald*Mansonia (Mansonioides) indiana* Edwards*Mansonia (Mansonioides) longipalpis* van der Wulp(from which the forms *M. dives* Schiner, and*M. bonneae* Edwards, have been described)*Anopheles (Anopheles) barbirostris* van der Wulp group

Indochinese peninsula

Mansonia (Mansonioides) indiana Edwards

China

Anopheles (Anopheles) sinensis Wiedemann

Japan (Hachijo-Komisha Island)

Aedes (Finlaya) togoi Theobald

Indonesia

Mansonia (Mansonioides) annulata Leicester*Mansonia (Mansonioides) indiana* Edwards

- Mansonia (Mansonioides) longipalpis* van der Wulp
(from which the forms *M. dives* Schiner, and
M. bonneae Edwards, have been described)
Mansonia (Mansonioides) uniformis Theobald
Anopheles (Anopheles) barbirostris van der Wulp
- Malaya—Periodic *B. malayi*
Anopheles (Anopheles) barbirostris van der Wulp
Mansonia (Mansonioides) uniformis Theobald
Mansonia (Mansonioides) annulifera Theobald
- Malaya—Sub-periodic *B. malayi*¹
Mansonia (Mansonioides) dives Schiner
Mansonia (Mansonioides) bonneae Edwards
Mansonia (Mansonioides) annulata Leicester
Mansonia (Mansonioides) uniformis Theobald

Annex 9

METHOD FOR THE PRESERVATION AND STAINING OF MOSQUITOS WHEN EXAMINING FOR FILARIA LARVAE *

A routine method for the examination for filaria larvae of mosquitos preserved in alcohol has been tested successfully under field conditions.

(1) Mosquitos collected alive are transferred to 80% pure ethyl alcohol where they may remain indefinitely.

(2) Alcohol is removed by taking the mosquito specimens through descending dilutions to water; they are then stained for three days in Mayer's acid haemalum, differentiated for three days in distilled water, and transferred to glycerol to await dissection. (Fresh acid haemalum should be prepared every 2-3 months as the stain gradually deteriorates.)

(3) Stained mosquitos are dissected in glycerol. All larval stages in the thorax are readily seen, and mature larvae can be picked up and transferred to a fresh slide under a coverslip for examination with high magnifications.

¹ The distribution of this parasite coincides with that of the closely related animal parasite *B. pahangi*, the mature larvae of which are indistinguishable from those of *B. malayi*.

* After Nelson, G. (1958) *Bull. Wld Hlth Org.*, 19, 204

Annex 10

SUGGESTED METHODS FOR EXPERIMENTAL INFECTIONS

(1) Select a suitable person infected with the filaria concerned; take blood samples, preferably three of 20 mm³ each from the finger and from the surface of the arm, and 1 ml from the median cubital vein. Smear carefully on slides, dry, dehaemoglobinize, fix in methyl alcohol, dry, stain in 50% Giemsa or similar stain, wash, dry; count microfilariae. Similar sampling and blood counts should be made at the end of the feeding period.

(2) Feed about 50 to 100 (or more) laboratory-bred mosquitos of the species concerned in an 18-inch-cube gauze cage by having the infected donor insert an arm or leg. This must be done at the time of normal feeding activity of the infected mosquito, previously determined by studies of biting-cycle. Usually about half-an-hour's exposure is sufficient if the mosquitos have been previously starved of blood and allowed to feed only upon apple slice or some other suitable fruit sugar.¹ After feeding, specimens may be stored in the exposure cage, and fed with apple slice or 10% sugar solution; preferably, however, they should be transferred to individual tubes fitted with blotting- or filter-paper on which they are fed with a drop of sugar solution each morning and a drop of distilled water each afternoon.

(3) Store in an identical manner a second batch of the same species, of the same age, laboratory-bred but not exposed to infection, for comparative observations on death rates.

(4) Estimate the total infection rates in a suitable sample of mosquitos each day by killing them and dissecting and examining them for all stages of developing filarial forms.

(5) Estimate mature larva rates by dissection and examination of an adequate sample of fed specimens after a period approximately equal to the total period of full development in the mosquito. This period must be ascertained by preliminary observation. It is about 10 days in some species of *Mansonia*, 12 days in some Pacific species of *Aedes*, and 14 days in some strains of *C. fatigans*.

(6) Counts of mature larvae made at examinations as described under (4) and (5) can be averaged to obtain a mature larva density for those specimens dissected at or after the period of full development. Dead

¹ Some species may not feed in small cages; feeding may be tried under full-sized mosquito bed-nets or cages of similar size.

mosquitos found each day should be dissected and recorded. The survival rate of infected mosquitos should be compared with that of the comparative batch (see (3)).

(7) Estimate a laboratory index of efficiency by the following, or some equally suitable, method.

Index of experimental infection

$$a = \text{Survival rate} = \frac{\text{Number of mosquitos surviving incubation (i.e., full development) period}}{\text{Number of mosquitos fed}}$$

$$b = \text{Mature larva rate} = \frac{\text{Number of mosquitos with mature larvae}}{\text{Number of mosquitos surviving incubation period}}$$

$$c = \text{Mature larva density} = \frac{\text{Number of mature larvae}}{\text{Number of mosquitos with mature larvae}}$$

$$a \times b \times c = \frac{\text{Number of mature larvae}}{\text{Number of mosquitos fed}}$$

(provided all mosquitos are dissected at about the same interval after the infecting blood meal)

m = Number of microfilariae found in the peripheral blood per mm^3 at the time of the infecting blood-meal

$$\text{Index of experimental infection} = \frac{a \times b \times c}{m}$$

This is an expression of the number of mature larvae that might be expected for each mosquito that fed on a donor with 1 microfilaria per mm^3 in the peripheral blood. In order to obtain a satisfactory index a series of experiments should be made using donors with various microfilaria counts, and averaging the results.

Annex 11

**CHEMOTHERAPY OF FILARIASIS AND ITS CONTROL
BY DIETHYLCARBAMAZINE ***

Diethylcarbamazine continues to be the chief compound for the therapy of bancroftian and malayan filariasis. World-wide experience, during which this compound has been administered to several million persons, has confirmed its safety; no fatal case of idiosyncrasy to it has ever been encountered. It has long been believed, on indirect evidence, that it can kill or permanently sterilize the adult worms of *W. bancrofti* and *B. malayi*. Direct evidence that diethylcarbamazine actually kills the adult worms of *B. malayi* has been provided by Edeson & Laing¹ using cats experimentally infected with the human parasite. A valuable review on diethylcarbamazine has been written by Singh & Raghavan.²

Dosage schedules used in a number of programmes follow.

Pacific Area

Tahiti : 6 mg per kg of body-weight of diethylcarbamazine citrate once monthly for 12 months.

Niue, Cook Islands : 50 mg of diethylcarbamazine monthly to all persons over 6 years.

Fiji : 400 mg of diethylcarbamazine citrate given at weekly intervals for six doses, followed by a second course six months later.

Asia

India : 4 mg of diethylcarbamazine citrate per kg (i.e., 200 mg/adult) daily for five days.

Ceylon : 100 mg of diethylcarbamazine three times a day for 7 days.

China : (1) 200 mg of diethylcarbamazine citrate three times a day for 7-10 days.

(2) 400 mg of diethylcarbamazine citrate four times a day for 1 day, or 500 mg twice daily for two days.

* From Hawking, F. (1961) *The chemotherapy of filariasis and control by diethylcarbamazine*, Geneva (Unpublished working document WHO/Fil./25)

¹ Edeson, J. F. B. & Laing, A. B. G. (1959) *Ann. trop. Med.*, **53**, 394

² Singh, J. & Raghavan, N. G. S. (1957) *Bull. nat. Soc. Ind. Malar.*, **5**, 35

Japan : 2 mg of diethylcarbamazine citrate per kg of body-weight daily for 5 days, or
2 mg of diethylcarbamazine citrate per kg of body-weight every 10 days for 10 times.

Malaya : 5 mg of diethylcarbamazine citrate per kg of body-weight either weekly or monthly for six doses.

Africa

Kenya : 2.2 g of diethylcarbamazine citrate per person daily for six days.

Tanganyika : 200 mg of diethylcarbamazine citrate monthly or 200 mg every second month for 1-2 years.

Gambia : 5 mg of diethylcarbamazine base daily for 5 successive days.

South America

Brazil : 6 mg of diethylcarbamazine per kg daily for 7 days, repeated annually.

It will be seen that four main types of dosage schedules have been employed.

(1) An interrupted dose of 5-6 mg/kg monthly or weekly for 6-12 doses. This has been employed in Tahiti, Fiji, Malaya and Tanganyika. It seems to be more effective and less toxic than the other schedules, but it is more laborious to administer.

(2) One dose (e.g., 4 mg/kg) daily for five (or seven) days. This is the standard schedule in India and it has also been employed in Japan, Kenya, Gambia and Brazil. This schedule is chosen particularly for its greater convenience of administration.

(3) A single large dose (e.g., 1.0 g). This has been recommended mainly in China (where conditions may be different from the rest of the world), but it is also recommended in Brazil. Although it is probably less effective and more toxic than the other schedules, it seems so much more convenient to administer that it probably deserves more consideration than it has received in the past.

(4) Treatment of microfilaria carriers only has been employed in Ceylon, a dose of 100 mg per person being given three times a day for seven days. Such restriction of the treatment to microfilaria-positive cases only must be evaluated by comparing the cost, labour and efficiency of detecting carriers, with the cost and acceptability of treating a whole population.

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