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WORLD HEALTH ORGANIZATION  
TECHNICAL REPORT SERIES

No. 359

# WHO EXPERT COMMITTEE ON FILARIASIS

(*WUCHERERIA* AND *BRUGIA* INFECTIONS)

**Second Report**

WORLD HEALTH ORGANIZATION

GENEVA

1967

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PRINTED IN FRANCE

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WHO EXPERT COMMITTEE ON FILARIASIS

(*WUCHERERIA* AND *BRUGIA* INFECTIONS)

Geneva, 19-24 September 1966

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## WHO EXPERT COMMITTEE ON FILARIASIS

(*WUCHERERIA* AND *BRUGIA* INFECTIONS)

### Second Report

A WHO Expert Committee on Filariasis met in Geneva from 19 to 24 September 1966. Dr F. Hawking was elected Chairman, Professor M. Sasa Vice-Chairman, and Professor L. A. Jachowski, jr, Rapporteur.

Opening the meeting on behalf of the Director-General, Dr A. M.-M. Payne, Assistant Director-General, said that filariasis is of great concern to many governments, particularly in developing countries, where rapid urbanization and population movements have greatly aggravated the situation. Greater and more sustained efforts will be required if any progress is to be made in its control. The present state of filariasis and of the control schemes undertaken in several countries has been assessed at a WHO Inter-regional Seminar on Filariasis held at Manila in 1965, and it has become apparent that, in spite of advances in knowledge of the epidemiology of filariasis, large-scale efforts to control the disease by mass drug treatment and by anti-mosquito measures have not everywhere yielded the expected results. Even in areas with the best results, complete interruption of transmission of the infection has not been achieved. It is recognized that, where results have not been satisfactory, this has often been due to non-technical causes such as shortage of funds and of trained staff, which have compelled the use of less expensive but also less effective control measures. Nevertheless, there is still no general agreement on the most effective and most economical ways of applying the control measures now available. Comprehensive assessments of past and present control projects are therefore needed, but require generally acceptable assessment methods to achieve comparability of results.

Because of the dynamics of the transmission of filariasis and the long life-span of the adult worm, control of the infection depends greatly on chemotherapy. Although opinions conflict on the value of diethyl-carbamazine for mass treatment, it is doubtful whether this drug alone will ultimately achieve cessation of transmission. A better filaricide is needed.

Much the same situation exists in regard to vector control measures. Opinions differ widely, which is not surprising considering the great variety of vectors involved. Recognizing the importance and urgency of

collecting basic information on *Culex pipiens fatigans*, the chief vector of *Wuchereria bancrofti*, towards the end of 1962 WHO established a Filariasis Research Unit in Rangoon, Burma. This unit has made significant contributions to knowledge of the bionomics of *C.p. fatigans* and of the epidemiology of filariasis caused by periodic *bancrofti*, and its work is continuing. However, to make vector control measures against filariasis as efficient as possible, many gaps must be filled in knowledge of the ecology of other vector species and of specific control methods, both ecological and engineering.

## 1. SOME RECENT ADVANCES IN KNOWLEDGE OF FILARIASIS

### 1.1 The parasites

The separation of what was formerly the genus *Wuchereria* into *Wuchereria* and *Brugia* is now generally accepted. The genus *Wuchereria* still contains only one species, *W. bancrofti*, a parasite found only in man. The taxonomic position of *W. bancrofti* var. *vauceli*, a microfilaria reported from man in Madagascar, is still uncertain; the adult worms have not yet been described. The genus *Brugia* now contains eight species, *B. malayi*, *B. pahangi*, *B. patei*, *B. beaveri*, *B. buckleyi*, *B. ceylonensis*, *B. guyanensis*, and *B. tupaiae*. Only *B. malayi* is known to occur as a natural infection in man. The other seven species have been discovered in animals in various countries, the last five having been described since 1961.

A new microfilaria has recently been found in man in the island of Timor at the eastern end of the Indonesian archipelago. In stained blood films it has some resemblance to the microfilaria of *B. malayi*, but shows constant morphological differences; the clinical manifestations associated with this parasite are similar to those caused by *B. malayi*. The adult worms have not yet been discovered, and the specific status of the parasite remains uncertain. Until more is known about it, the name "Timor microfilaria" must be retained.

The recognition of species is based on morphology. Perhaps equally important is the determination of forms and strains within each species. A difference in the periodicity of the microfilariae in the peripheral blood is the primary basis for the separation of *W. bancrofti* into two distinct forms, and two forms of *B. malayi* are distinguished by this criterion. Objections having been made to the previous classification of microfilarial periodicities,<sup>1</sup> this subject was reconsidered. The terminology used in this report is based on the following considerations.

<sup>1</sup> *Wld Hlth Org. techn. Rep. Ser.*, 1962, 233, 6.

The filarial infections of man and animals in which microfilariae appear in the peripheral blood can be divided into two broad groups. The first group would contain the infections—periodic and subperiodic—in which the variations in the numbers of microfilariae occur in a rhythmic pattern, repeated during each 24-hour period. The second group consists of infections in which, although variations in the microfilaria count may be observed, no rhythmic pattern of variation can be detected—examples are non-periodic infections such as *Dipetalonema perstans*. *Periodic infections* show a pronounced peak in the microfilaria count at some point in each 24-hour period, while for a considerable part of the 24 hours microfilariae are either absent or very scarce. The peak may be either nocturnal (as in periodic *W. bancrofti* or periodic *B. malayi* infection) or diurnal (*Loa loa*). In *subperiodic infections*, a consistent but much less pronounced peak in the count occurs at some point in each 24-hour period, and there is a tendency for microfilariae to be present at all times. Again, the peak may be either nocturnal (subperiodic *B. malayi* infection in man in Malaya) or diurnal (subperiodic *W. bancrofti* infection in Polynesia).

The differences in the pattern of periodicity described above are associated with other biological differences suggesting the existence of biologically distinct forms of the parasites concerned. In all the filarial infections a further breakdown into strains is possible on the basis of infectivity to the various species of insect vectors, and this probably applies to periodic, subperiodic, and non-periodic infections alike. Examples of the influence of strains on the infectivity of microfilaria carriers to mosquitos have come from Malaya and elsewhere. Strains of periodic *W. bancrofti* from a rural area developed readily in the anopheline mosquitos that were the natural vectors, but only to a limited extent in *Culex fatigans*, in which however an urban strain of *W. bancrofti* developed readily. The determination of the form and strain, as well as the species, of parasite may be an essential step towards an understanding of the epidemiology of filariasis in a locality, but insufficient work has been done on this subject and considerable difficulties are involved.

The geographical distribution of *Wuchereria* and *Brugia* can be described only in general terms. The periodic form of *W. bancrofti* has a very wide but focal distribution in the humid tropical zone and is transmitted by night-biting mosquitos. It has disappeared from the mainland of Australia, from North America, and from some of the islands in the Caribbean, but is invading new areas in the growing towns of Asia, Africa, and South America. The diurnally subperiodic form of *W. bancrofti* is restricted to the South Pacific area, where it is transmitted mainly by day-biting mosquitos; the periodic form is absent from this area. The periodic form of *W. bancrofti* may occur either as a rural or an urban infection; the subperiodic form is predominantly rural.

Human infection with *B. malayi* has not been recognized outside Asia. The periodic form has a focal distribution in Asia, and is transmitted by the *Mansonia* of open swamps, by some anophelines, and by *Aedes togoi*. The subperiodic form, originally described in Malaya, and transmitted by the *Mansonia* of swamp forests, has recently been found in Palawan Island in the Philippines. Both forms of *B. malayi* are predominantly infections of rural populations. The only well-substantiated reports of a decrease in *B. malayi* have come from Ceylon, where various control measures have been in use for a number of years. Reported increases in *B. malayi* infection are probably due to better detection of endemic areas rather than to true increases in the incidence of infection.

## 1.2 Clinical manifestations

### 1.2.1 Relation to developmental stages of worms

Microfilariae produce remarkably few lesions either in the lungs or in other tissues. The lesions observed in the lungs of hypersensitive subjects are discussed under eosinophilic lung (1.2.2). The adult worms often produce lesions in the lymphatic vessels (causing the clinical signs of lymphangitis, elephantiasis, etc.). The acute lesions consist of patches of local inflammation, mild or intense, round the adult worms. According to recent work<sup>1</sup> on the lesions produced by *B. pahangi* in cats and dogs, these inflammatory reactions mostly occur at the times when the worm liberates secretions, i.e., at the third or fourth moult, before the production of microfilariae, or when it begins to die, either spontaneously or following chemotherapy. The reactions result in the formation of lymphocyte thrombi near or round the worms, thickening of the vessel walls, dilatation of the lymphatic vessels, etc. The worm may survive apparently unharmed, or it may be killed.

### *Elephantiasis*

The presence of adult worms in the lymphatics apparently leads to incompetence of the valves, either directly as a result of the excretions of the worms or indirectly as a result of the acute inflammation just described. As a consequence there is a back-flow of lymph, which is accentuated by the high hydrostatic pressure due to man's erect posture. The lymphatics become dilated and tortuous with extensive varicosities, as can be demonstrated by radiological techniques and intralymphatic injection of opaque substances. Lymph stasis and lymphoedema in dependent parts of the body lead to the development of elephantiasis. Lymphangiography has proved useful in differentiating filarial from non-

<sup>1</sup> Schacher, J. F. & Sahyoun, P. F. (1966) *Trans. roy. Soc. trop. Med. Hyg.*, **61**, 234.

filarial elephantiasis in areas like the highlands of East Africa where non-filarial elephantiasis occurs.

*Relation of lesions to number of worms present*

Although the individual reaction of the host is important and the various strains and species of filarial worms differ in their pathogenicity, there is evidence that the frequency and severity of lesions tend to be proportional to the number of worms present. This evidence is indirect, and is provided by (1) animal experiments and (2) analysis of epidemiological information about the human population in endemic areas. In experiments with *Litomosoides carinii* infection in cotton rats, the extent of the late pathological changes is usually dependent on the number of adult worms per host, the mortality being higher among animals with high parasite burdens, as, for example, more than 50 worms per rat. Epidemiological surveys of different endemic areas of human filariasis have shown that the prevalence of clinical lesions is usually correlated with the microfilaria rate and density, especially the latter.

In animals some correlation can be observed between the peak microfilaria count and the number of adult worms as subsequently determined at autopsy. In man it is seldom possible to ascertain the relation between the microfilaria count as measured at some particular time and the hypothetical peak value that would be found if the count were measured repeatedly during an untreated infection; it is similarly rarely, if ever, possible to count the number of adult worms at autopsy. Nevertheless, some indication of the number of adult female worms in a population (even if not in an individual) might possibly be provided by the microfilaria density in the blood of such a population. Attempts should be made to obtain further information along these lines.

1.2.2. *Eosinophilic lung*

The finding of microfilariae in lung biopsies from patients with eosinophilic lung (tropical eosinophilia) suggests that filariasis causes this syndrome. That microfilariae are concerned is further suggested by the rapid disappearance of symptoms after treatment with antifilarial drugs.

The filariae involved have not been identified. Since most cases of eosinophilic lung have been reported from areas in which human and animal filariasis is endemic, the origin of the condition is difficult to determine. The syndrome has, however, been produced experimentally in a human volunteer by injection of infective third-stage larvae of both *B. malayi* and *B. pahangi*.

Most cases of this allergic phenomenon have been reported from south-east Asia, Brazil, and Africa. In some areas routine chest X-rays

have revealed pulmonary fibrosis suggestive of eosinophilic lung. Additional studies are needed to determine the geographical distribution of the syndrome and to establish the role of the filarial parasites in its causation.

### 1.2.3 Geographical variations

The pattern of clinical manifestations in filariasis depends not only on the intensity of transmission but also on the species and form of the parasite. *Brugia* infections generally differ from those due to *W. bancrofti* in that hydrocele is very rare and elephantiasis often less marked. Both species of parasite give rise to elephantiasis mainly affecting the legs. Observations of the distribution of nodular swellings in the lymphatics after treatment with high doses of diethylcarbamazine<sup>1</sup> suggest, however, that the anatomical distribution of the adults of *W. bancrofti* and *B. malayi* differs.

Geographical differences in the pattern of lesions are often quite marked, even with infections of the same species of parasite. For example, hydrocele is the most obvious complication of *W. bancrofti* in both Africa and Japan; chyluria is rarely seen in Africa, but occurs in over 1% of the population examined in some endemic areas of Japan. In the Pacific, where both periodic and subperiodic forms of *W. bancrofti* occur, differences in parasite physiology and behaviour may be important in the clinical variations observed. Geographical variations in the clinical manifestations of filariasis must be considered in comparative studies of the effect of control measures in different regions.

## 2. METHODOLOGY IN EPIDEMIOLOGICAL ASSESSMENT

### 2.1 Standardization of techniques and methods

#### 2.1.1 Parasitological

##### *The presence of microfilariae in the blood*

Preliminary investigations are necessary to establish the identity of the species of microfilariae and its periodicity. This will indicate the optimum time for taking blood samples for assessing the prevalence rate and for determining the microfilaria density. Samples of peripheral blood, preferably in measured quantities of not less than 20 mm<sup>3</sup>, can be taken either from the finger or from the ear for the preparation of thick smears. The technique developed in Japan<sup>2</sup> of making several

<sup>1</sup> Ch'en Tzu-Ta (1964) *China med. J.*, **83**, 625.

<sup>2</sup> Sasa, M. (1963) *Bull. Wld Hlth Org.*, **28**, 437.

linear smears per slide from each person presents advantages for specific epidemiological purposes. Densities should be expressed as the number of microfilariae per unit volume of blood, which in practice is usually per 20 mm<sup>3</sup>. The differentiation and counting of microfilariae demand staining of the films and careful examination of the entire film. The Romanovský stains, such as Giemsa's, give a good differentiation of the species and can be used for mass staining. Haematoxylin stains may be necessary in special cases. Counting can be done with a magnification of about 100 ×, but higher magnifications may be required for species identification. No absolute standardization in staining techniques is possible because the quality of stains varies and the microfilariae from various geographical areas stain differently. In blood films, if the nuclei of the white cells are well stained the microfilariae present will also be well stained.

#### *Presence of filaria larvae in mosquitos*

At least 30 species of filaria worms are known to complete their development in mosquitos. Of these only two, *B. malayi* and *W. bancrofti*, are infective to man. The problem of differentiating filaria larvae in mosquitos depends on the parasites prevalent in domestic and wild animals in any particular area. Before any species of mosquito can be incriminated as a vector of either *W. bancrofti* or *B. malayi*, the stage of development of the larvae in the mosquito must be identified.

Four larval stages are found in mosquitos:

(1) The microfilaria, which may be found in the stomach or in other parts of the insect soon after a blood meal. Under certain circumstances, it is useful to examine blood-fed mosquitos for microfilariae because at this stage they can still be identified with certainty. However, it is only when the larvae have started to develop in the thoracic muscles that the mosquito should be recorded as infected.

(2) The first-stage larva : a short, sluggish, sausage stage which in *Wuchereria* and *Brugia* is confined to the thoracic muscles, but which in other species occurs in the malpighian tubules or fat body.

(3) The second-stage larva : a long sausage stage with a developing intestinal canal. In *Brugia* and *Wuchereria* it is confined to the thorax and is relatively inactive.

(4) The third-stage larva : the final stage of development in the mosquito. The larva at this stage is very active and can be found in any part of the insect, not just in the head or proboscis. Only this stage should be referred to as infective.

In studies on transmission it is the third-stage infective forms that require special attention. Simple morphological characteristics, such

as length, breadth, position of the anus, and shape of the caudal extremity, can be used to separate *Wuchereria* and *Brugia* from almost all infective forms in animals that have been described, but it is not possible to separate the different species of *Brugia* one from another except by injecting them into susceptible animals and waiting for their full development.

Entomologists engaged on filariasis work should be able to recognize the characteristic features of *Wuchereria* and *Brugia* infective larvae, and they should know the techniques for the preservation of the larvae. A practical procedure that has proved of value in the field is to mount the larvae in a minute drop of glycerol on a cover slip and to invert this over a cavity slide ringed with a sealing agent such as Euparal. This permanent hanging-drop preparation can be kept for future study or sent elsewhere for identification.<sup>1</sup>

There is an urgent need for reference centres as depositories for infective larvae of known species and for the identification of all stages of the parasites.

### 2.1.2 Entomological

The large number of different species of vectors known to be involved in the transmission of filariasis makes it impossible to give standardized techniques for all situations.

The purpose for which the entomological data are to be collected will naturally vary, but the Committee's main interest was focused on indices that will help to define the parameters involved in transmission. As far as these are concerned, the following were thought to be important and susceptible of some degree of standardization. The examination of the mosquito fauna and the identification of the vector or vectors were discussed by a previous Expert Committee<sup>2</sup> and need no further elaboration here.

#### 1. *The human blood index*

This index reveals the proportion of vectors that feed on man and gives an indication of vector contact with both human and animal hosts. The method consists of collecting blood-fed mosquitos at all their resting places and subjecting their stomach contents to a precipitin test. Sampling is very important indeed and great care must be taken to ensure that it does not lead to bias in interpreting the results. Under the same sampling conditions, changes in the human blood index following control by

<sup>1</sup> Full details of the technique for preserving and identifying infective larvae in insects can be found in Annex 2 to the Second Report of the WHO Expert Committee on Onchocerciasis (*Wld Hlth Org. techn. Rep. Ser.*, 1966, 355, 79).

<sup>2</sup> *Wld Hlth Org. techn. Rep. Ser.*, 1962, 233, 18.

insecticides may indicate changes in vector behaviour (which is of epidemiological interest) or reflect selective control of that part of the vector population associated with man. Where the vector is known to feed mainly on man and birds, direct examination of its stomach contents as soon as possible after a blood meal provides a ready means of identification, since human and avian erythrocytes are morphologically distinct.

### 2. *Vector density relative to man*

The index referred to as the "vector density relative to man" is an important parameter. It can be determined at all seasons of the year and in all situations where man-vector contact is possible. By its seasonal changes in vector density will be revealed and may have a bearing on the design and timing of control campaigns.

The technique consists of catching vectors coming to feed on man over a defined period. The time of catching will depend on the 24-hour biting cycle of the vector, and the duration of the catch on the vector density. For *Culex fatigans* the peak of biting occurs after midnight and, since this is an awkward hour, sampling has been done over the two hours immediately preceding midnight. At this time the density is high enough to yield a significant number of females for subsequent analysis. In the Pacific the day biter *Aedes polynesiensis* bites in such numbers that catching has to be restricted to a very short period, e.g., 10 minutes during the peak feeding activity. In Africa all-night catches are required for *Anopheles gambiae* and *A. funestus*.

Since the object is to be able to compare catches at different seasons and at different times during a campaign, it is essential that the technique and timing be standardized for each situation and be kept constant throughout.

### 3. *Receptivity of the vector to infection*

A species of mosquito, for a variety of reasons, may be a vector in one locality and not in another. To obtain information on its receptivity to infection, it is necessary to feed laboratory-reared females on a microfilaria carrier and determine the development period and the numbers of parasites that reach the infective third stage.

The technique for establishing an index of experimental infection was dealt with in the first report of the Expert Committee,<sup>1</sup> and the only further comment concerns the factor *a* in the formula proposed for the survival rate.<sup>2</sup> This was derived from the number of mosquitos surviv-

<sup>1</sup> *Wld Hlth Org. techn. Rep. Ser.*, 1962, 233, 46.

<sup>2</sup>  $a = \text{survival rate} = \frac{\text{Number of mosquitos surviving incubation period}}{\text{Number of mosquitos fed}}$

ing extrinsic incubation of the parasite divided by the number which fed. The Committee felt that great care has to be taken in assessing this factor because of the difficulty of keeping some species of mosquitos alive in laboratories. The mortality could be due to causes other than incompatibility between the vector and the parasite, and the possibility of excluding it from the calculation of the index of experimental infection should be considered.

The relative importance of locally occurring vectors will be determined in time by the results of dissection of wild-caught biting mosquitos.

#### 4. *Survival of the vector*

This parameter, which is very valuable in campaigns based on vector control, is determined from data on the proportion parous, i.e., the proportion of adult females that have laid eggs at least once, and on the length of the gonotrophic cycle. Parity is readily determined from an examination of the ovarian tracheoles by the method described by Detinova,<sup>1</sup> which can be adapted to field projects. The mosquitos caught for determining the density relative to man can be used for this purpose and special collections need not be made.

The gonotrophic cycle can be determined in the field and should, if possible, be confirmed in the laboratory. Due regard should be paid to certain complicating factors, such as the possibility of delay in oviposition due to feeding on sugar or the periodicity of the cyclical rhythms of the species.

The survival of female mosquitos for one day and the expectation of their infective life can then be obtained from charts such as those proposed by Garrett-Jones & Grab (Fig. 1 and 2) for malaria mosquitos.

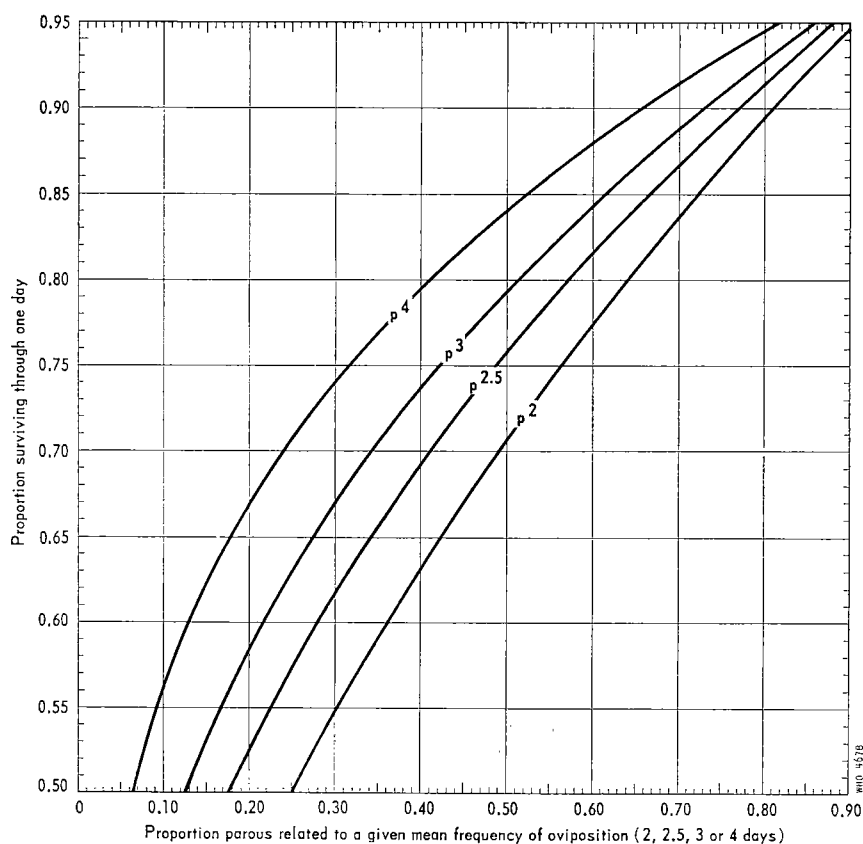
#### 5. *Use of the proportion parous among adults in control schemes*

As a guide to control programmes the proportion parous among adult mosquitos serves as a useful measure of the efficacy of the methods applied. With the prevention of the emergence of newly hatched adults through larval control the proportion parous will naturally rise. As this older section dies off from natural causes the proportion will tend to become constant again. Thereafter, any sudden change in parity serves as a useful indicator that control has broken down somewhere. On the other hand, adult control reducing the expectation of life will lead to an increase in the proportion nulliparous.

A ready means of recognizing such young nulliparous populations is provided by the genetically controlled green coloration that occurs in young adult *C. fatigans* in some places.

<sup>1</sup> Detinova, T.S. (1962) *Age-grouping methods in Diptera of medical importance*, World Health Organization, Geneva (*Wld Hlth Org. Monogr. Ser.*, No. 47).

FIG. 1.\* CURVES FOR DERIVING THE PROPORTION SURVIVING THROUGH ONE DAY ( $p$ ) FROM OBSERVED PROPORTIONS PAROUS REPRESENTING  $p^2$ ,  $p^{2.5}$ ,  $p^3$  OR  $p^4$



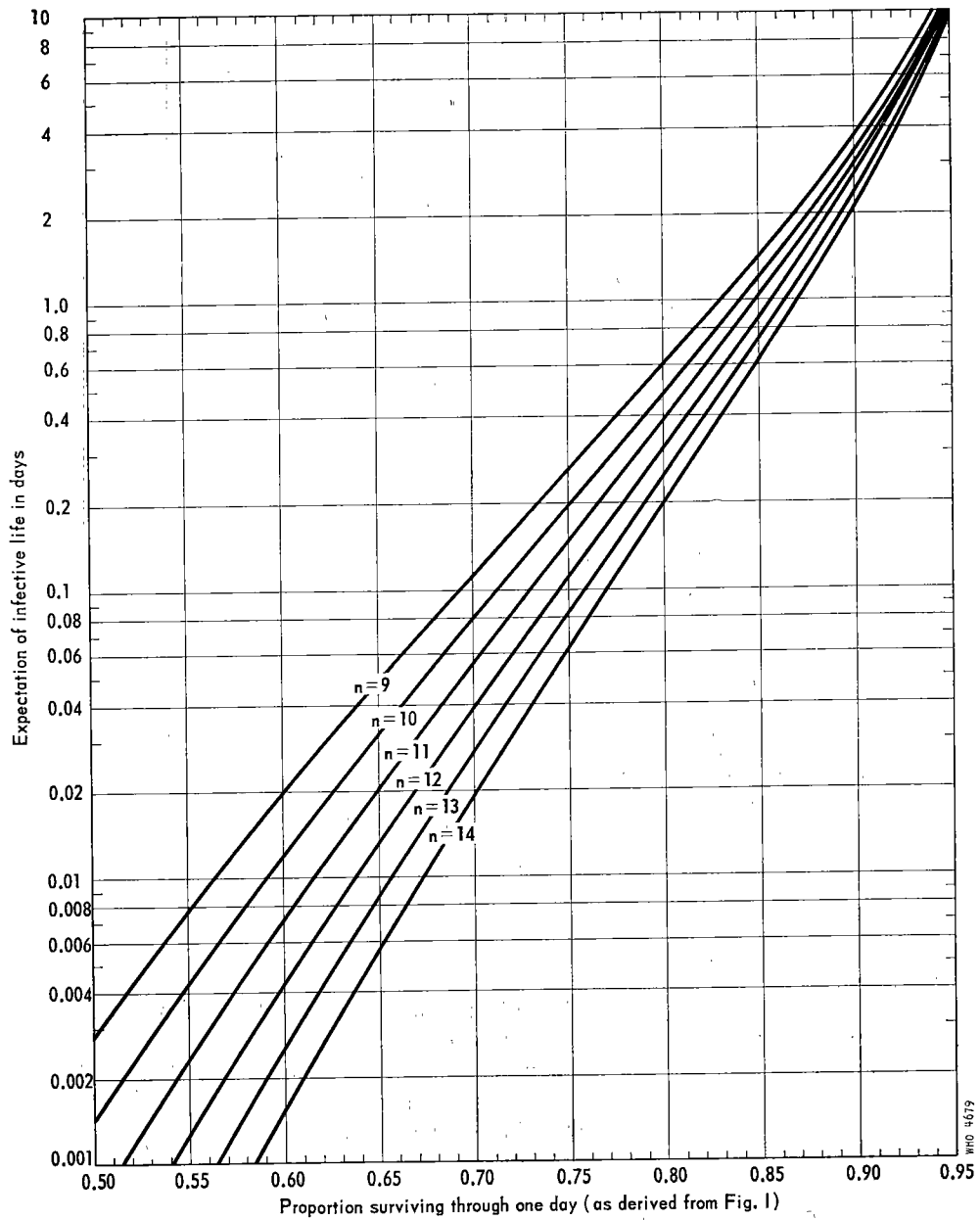
\* From Garrett-Jones, C. & Grab, B. (1964) *Bull. Wld Hlth Org.*, 31, 71.

## 6. Natural infections in the vector

It is important to identify with certainty the larvae of human filariae and to record all larvae found according to their stage of development. Data collected in this manner may be most useful for determining other indices, as for example the rate of survival,<sup>1</sup> and when combined with the density relative to man they furnish a good assessment of the vector threat.

<sup>1</sup> Laurence, B.R. (1963) *Bull. Wld Hlth Org.*, 28, 229.

FIG. 2. \* CURVES FOR DERIVING THE EXPECTATION OF INFECTIVE LIFE OF A VECTOR FROM KNOWN VALUES OF  $p$ , FOR SPOROGENIC PERIODS OF 9 TO 14 DAYS IN THE PARASITE.



\* From Garrett-Jones, C. & Grab, B. (1964) *Bull Wild Hlth Org.*, **31**, 71.

### 2.1.3 *Clinical*

For assessment purposes, the principal acute and chronic clinical manifestations of filariasis, e.g., lymphangitis, adenopathy, elephantiasis, and hydrocele, should be recorded, if possible, although it is recognized that it is often difficult to do so. Clinical records will be utilized mainly in determining the public health importance of the disease and in the long-term evaluation of progress in its control. Unless the assessment is made by a physician, the results (apart from records of gross abnormalities such as hydrocele and elephantiasis) are of doubtful value. For practical reasons it is seldom possible to examine all the persons from whom blood samples are taken. On the other hand, the group examined must be a representative sample of the population. This is not the case with patients at a filariasis clinic, who may consist mostly of persons who have lesions.

The following lesions may be considered to be manifestations of filarial infection :

— Acute lymphangitis of the legs or genitals. This can seldom be observed directly, and all evidence is obtained by questioning the patients. It is probably desirable to ask all persons examined whether they have experienced such attacks (acute pain in the legs or genitals with swelling, redness, and fever) during the past 12 months, but the replies must be assessed critically and with caution.

— Chronic enlargement of lymph nodes in the inguinal and femoral regions (and, in Pacific countries, of the epitrochlear glands). This has often been mentioned in older surveys. It is difficult, however, to distinguish enlargement due to filariasis from that due to non-specific causes, and it is considered that records are usually of little value.

— Hydrocele. This is a valuable objective sign.

— Elephantiasis of the genitals, legs, or arms. Mild forms may offer some difficulty in diagnosis. Elephantiasis of the legs and arms can usually be detected even during mass surveys. This, therefore, is an obvious sign to be recorded. It must be remembered, however, that both hydrocele and elephantiasis may sometimes be due to non-filarial causes.

— Chyluria.

In all cases the lesions observed should be classified according to year of onset and age and sex of the persons examined. It will also be value to record length of residence in the area under survey and to add notes specifying the criteria observed in judging whether borderline cases have been regarded as positive or negative.

The occurrence of asthma and chronic bronchial symptoms in a filarial area is worth recording as a potential indication of eosinophilic lung. The response to treatment with diethylcarbamazine and the presence or absence of eosinophilia should be determined whenever possible, to permit assessment of whether or not the asthma can be classified as probable eosinophilic lung.

#### 2.1.4 *Immunological*

The usefulness of immunological tests as tools in the epidemiological assessment of filariasis has been considered by various investigators. The tests—most frequently the intradermal test—have been used as a complement to, and sometimes as a substitute for, parasitological examination. Since the results obtained are not always easy to interpret, there are conflicting opinions as to the reliability of these methods, especially when they are used for diagnostic purposes. This is understandable, since most of the antigens have been obtained from different sources, are variable in their composition, and have been prepared by various methods. Crude preparations may contain certain antigenic components shared by unrelated parasites, or the antigens may lack specificity. Furthermore, a specific antigen is not necessarily the “functional antigen” that possesses the strongest immunogenic potential and intervenes during the course of the infection. Other possible explanations of failure to detect all cases of filariasis by any of present immunological techniques may be neutralization of circulating antibody by large numbers of microfilariae and insufficient cross-reaction to a heterogeneous species.

It is doubtful whether the standardization of immunological methods could help at present towards a better interpretation of the results. Standardization is only possible with isolated and characterized antigens but, before that stage is reached, the reactivity and specificity of the antigens must be determined. Once an antigen is isolated and characterized, standardization becomes an easy undertaking, and interpretation of the results of a test using the antigen (provided that the test is uniformly applied) can then be meaningful. A detailed protocol must, therefore, be generally agreed upon for the application of most immunological tests.

The present situation in regard to filarial antigens can be summarized as follows. An antigen was isolated from *Dirofilaria immitis* by Professor Sawada, Gunma University, Maebashi, Japan. The material was isolated by an elaborate chromatographic technique, characterized as a protein, and analysed for its amino acid composition. Its reactivity has been demonstrated in intradermal tests carried out in filariasis endemic areas—Ceylon, New Guinea, and Rangoon. Although the source of material is an animal filaria, the possible common antigenicity between

this species and human filariae could be put to use for epidemiological assessments, but the diagnostic value of the reagent requires further investigation.

In Ceylon, Japan, New Guinea, and Rangoon, where the results of intradermal tests using Sawada's antigen have been recorded by measuring the diameter of the wheal and considering a diameter of more than 7 mm as positive, a high percentage of positives were reported. Conflicting results were, however, obtained, from other areas such as Brazil and India. Extensive experience of the evaluation and interpretation of skin tests applied in other parasitic diseases indicates that greater precision is achieved if the total wheal area is calculated.

For evaluation purposes, it is necessary to undertake parallel parasitological and serological tests, including skin tests, using a defined antigen on the same individuals under uniform conditions. Skin reactions should be read with a device for measuring the total wheal area. In this way, valid comparisons may be made. These investigations would have to be carried out in endemic areas as well as in areas where filariasis is non-existent, in order to exclude non-specific reactions due to other parasitic diseases or to unrelated hypersensitivity conditions.

It has been suggested that skin tests repeated at regular intervals (every three months) on the same individuals might provide useful information for the assessment of the degree of transmission occurring in a given area, and eventually of the effects of control measures. The rate of conversion from negative to positive reactions or the proportion of persons in whom the intensity of reaction has increased would be considered as an indicator. Further investigation is essential, however, to determine whether repeated injections of the same antigen would sensitize the individuals tested. Studies should also be carried out in areas where filariasis is non-existent.

The filarial skin test antigen labelled FST-Sawada is now available for testing and further evaluation by investigators interested in filariasis.<sup>1</sup> Although there is insufficient information concerning the specificity of this material, preliminary tests in proven cases of *W. bancrofti*, *Loa loa*, and *Onchocerca volvulus* infections gave positive results, suggesting that there is group specificity.

Further information concerning the application of immunological tests is provided in the report of a WHO Expert Committee on Immunology and Parasitic Diseases.<sup>2</sup> A document entitled "Instructions for the performance and interpretation of the intradermal test" is also available.<sup>3</sup>

<sup>1</sup> This antigen is provided free of charge on request to Parasitic Diseases, Division of Communicable Diseases, World Health Organization, Geneva, Switzerland.

<sup>2</sup> *Wld Hlth Org. techn. Rep. Ser.*, 1965, 315.

<sup>3</sup> On request from Parasitic Diseases, Division of Communicable Diseases, World Health Organization, Geneva, Switzerland.

## 2.2 Selection of the methods to be used in surveys

The scope of any epidemiological survey of filariasis will be determined by the purpose of the investigation. Once the presence of filariasis in an area has become known, a special survey is essential in order to define the extent of the problem. The collection of blood films from representative samples of the population in different localities may be sufficient to indicate the distribution of infection, and the public health importance of the disease may be assessed by clinical examination of the people living within the areas thus detected.

Much more detailed investigation is necessary before any extensive control programme can be started. The methods suggested in earlier sections of this report must be employed to obtain the fullest possible information on microfilaria rates and densities and on the prevalence of clinical lesions in all age and sex groups of the population, as well as on the vector mosquitos and their bionomics and on possible animal reservoirs of infection. Several years may have to be spent in assembling the data on these subjects; unless this is done, no proper planning of extensive control campaigns will be possible and baseline information essential for any later assessment of the effects of control measures will be lacking.

Once control measures have started, the methods of assessing the results will vary to some extent with the type of control measures utilized. A rapid reduction in microfilaria rates and densities in the human population is to be expected after mass chemotherapy with diethylcarbamazine; mosquito control measures should produce a change in vector density and in the infection rate in the vector. Every control programme aims at interrupting transmission, or at reducing it to a level at which new infections cease to develop, but years may elapse before this degree of control is reached. The methods of assessment used throughout the control campaign should be the same as those originally used to obtain the baseline information, otherwise the assessment of progress becomes extremely difficult. Whatever control measures are adopted, observations in a comparable untreated area are desirable in order to estimate the extent to which natural fluctuations in transmission levels may have occurred.

## 2.3 Presentation and analysis of parasitological survey data

The information collected in field surveys is of little value until it has been analysed. The data must be assembled, preferably in some standardized manner, in order to make their meaning clear. Far too often an assessment of the results of an investigation is rendered difficult or impossible because the data are so poorly presented that they

defy analysis. Guidance for field workers on the handling and understanding of their data is thus an essential part of the methodology in epidemiological assessment.

The results of microfilaria surveys of a population may be evaluated from various standpoints, such as the microfilaria rate, the frequency distribution of microfilaria counts, and the distribution of the microfilaria positive grade. All these present different aspects of the prevalence of the parasite among the population, and the values obtained from different populations are all useful in comparing the prevalence in various areas or in evaluating the effectiveness of control measures in the same population. The results should be presented in a form that allows statistical treatment, so as to permit evaluation of the significance of differences among the populations surveyed (see annex).

#### *Microfilaria rate*

The percentage obtained by dividing the number of microfilaria carriers by the total number of persons examined is frequently used to indicate the prevalence of microfilaraemia in an endemic area. This percentage is the microfilaria rate. It is not the infection rate, since microfilaria carriers are usually only a part of the population infected with the parasite. Patients in the prepatent stage of the disease as well as many in the chronic stage show no microfilaraemia.

The microfilaria rate of a population is subject to variation according to the volume of blood examined, the time of day at which the blood is taken, and the sex and age of the group studied.

In a filariasis endemic area there are always some microfilaria carriers, usually people with low microfilaria densities, who are recorded as negative either by technical error or because microfilariae happen to be absent in the particular blood sample examined. Errors due to the first cause may be minimized by the training of microscopists. Errors due to the second depend largely on the amount of blood examined. Another factor causing discrepancy in successive surveys of the same population is variations of microfilaria density in the same individuals due to uneven distribution in the blood stream, periodicity, and the tendency of the microfilariae to increase or decrease over several months or years. In mass blood surveys of a population it is usually difficult to determine the causes of errors, but it is useful to make successive surveys on the same population.

#### *Microfilaria density*

Microfilaria counts per unit volume of blood in samples from individual persons and the distribution of these counts in a population have been found useful in various aspects of the epidemiology and control

of filariasis. Although the counts are subject to variation, they are considered to be an indication of the intensity of infection in individual cases. In the treatment of microfilaria carriers with diethylcarbamazine, the grade of the fever reaction was found to have a high correlation with the microfilaria density.

The microfilaria density may be utilized for comparison of the degree of endemicity in different areas. The efficiency of a control measure may be evaluated by the change of density in the same population before and after it is applied. For such purposes, it has been the usual procedure to take the difference between simple arithmetic means as a basis of comparison. Use of the arithmetic mean has been questioned, however, because the microfilaria density in a population is usually skewed in its distribution.

In studying the microfilaria density in a population, it is desirable to work from the start on its frequency distribution, such as listing the numbers of cases with all the respective numbers of microfilaria counts, starting from 0, 1, 2, 3, and proceeding to the highest counts observed. A form on which this can be recorded is given in the table. Recent information accumulated from a number of endemic areas of *W. bancrofti* has shown that the frequency distribution of the logarithms of microfilaria density is approximately normal; thus the median microfilaria count may be used as the most reliable criterion for comparison of the densities of different populations (see annex).

#### *Determination of correction factor*

The microfilaria rates and densities estimated by examination of small amounts of peripheral blood are likely to under-represent the true infection rate. The degree of under-representation will depend upon the technique employed, the experience of the technician, the volume of blood examined, and the distribution of microfilaria counts in the population. If a standard technique is used, a correction factor can be estimated covering the volume of blood examined and the distribution of microfilaria counts. A direct method for determining a correction factor is described in the annex.

### 3. CHEMOTHERAPY

#### 3.1 Present status of diethylcarbamazine in filariasis control

The chief importance of diethylcarbamazine lies in its use as a possible public health measure to remove most of the microfilariae from the population in a given endemic area and so to reduce the human reservoir of infection. Much experience has shown that if people can be persuaded to take an adequate amount of diethylcarbamazine their

**FREQUENCY DISTRIBUTION OF MICROFILARIA COUNTS  
AND OTHER BLOOD SURVEY DATA <sup>1</sup>**

(Blood sample : 30 mm<sup>3</sup>)

Microfilaria counts	No. of filariasis cases according to microfilaria count <sup>2</sup>											Cumulative No. of cases	
	Units											Total <sup>3</sup>	No.
Tens	0	1	2	3	4	5	6	7	8	9	Total <sup>3</sup>		
0	9 644	57	48	30	25	16	14	11	13	11	225	225	66.6
10	11	9	9	9	4	1	2	6	1	3	55	280	82.8
20	2	2	3	2	5	2	3				19	299	88.5
30	1	1	1		1	1	1	1	1	1	9	308	91.1
40	1			1				1			3	311	92.0
50	2		1							1	4	315	93.2
60			1					1			2	317	93.8
70	1							1			2	319	94.4
80			1	1							2	321	95.0
90			1					2			3	324	95.9
100-199	100, 104, 105, 105, 117, 117, 126, 133, 151, 154, 181, 195										12	336	99.4
200-299	258										1	337	99.7
300-399	344										1	338	100
400-499													
500-599													
600-699													
700-799													
800-899													
900-999													
1000 over													

<sup>1</sup> Total population 22 698. Number examined : 9,982. % examined : 44 %. % positive : 3.39 %. Total microfilaria count : 5 513. Average microfilaria count : per positive 16.31; per total 0.552. Mf positive grade : N<sub>3</sub> : 144, N<sub>2</sub> : 85, N<sub>1</sub> : 109; total : 338

<sup>2</sup> Counts of 100 and more microfilariae per slide are recorded individually.

<sup>3</sup> Not including the negative (00).

microfilariae (and probably some of their adult worms)<sup>1</sup> will be destroyed. For practical purposes, an adequate amount seems to be a total dose of about 72 mg of diethylcarbamazine citrate per kg body weight.<sup>2</sup> The period over which this amount has been administered has varied from area to area; spaced doses of 6 mg/kg once a week or once a month give as good a microfilaricidal effect as and are less likely to cause adverse reactions than daily dosage. *B. malayi* is more susceptible to treatment with diethylcarbamazine than is *W. bancrofti*, but febrile reactions are frequent in microfilaria carriers. The main obstacle to the effective use of diethylcarbamazine in mass treatment is the difficulty in persuading people to take enough of the drug. This difficulty arises partly from the nature of the infection—many infected persons are free from symptoms for long periods—and partly from the onset of side effects, particularly after the first dose. Persuasion and health education may be fairly easy with a small population, but are much more difficult with large populations.

Two methods have recently been suggested whereby people might be persuaded to accept the compound. In the first, in Japan, low doses of the drug have been incorporated in popular foods such as children's orangeade or *miso* soup, and have been administered either to microfilaria carriers only or to all the population of small communities (e.g., dormitories of industrial workers). In both groups the compound was readily accepted when thus presented and produced its usual microfilaricidal effect.

For the second method, it has been proposed that the compound should be incorporated into some article of universal consumption such as cooking salt. This method has already been widely used for the administration of iodine, and also for the administration of chloroquine to control malaria. In connexion with chloroquine, great experience has been gained in the many technical and administrative problems that arise during the preparation and distribution of medicated salt. Many difficulties have been encountered in practice, and very careful planning and supervision are necessary to make the administration a success. This would also be certainly true of any antifilarial campaigns. There are reasons, however, for hoping that the use of diethylcarbamazine against filariasis might prove more successful than that of chloroquine against malaria. They are: (a) the different course of filariasis infection means that elimination of the parasite may not be necessary for control; a reduc-

<sup>1</sup> Ch'en Tzu-Ta (1964) *China med. J.*, **83**, 625; Hawking, F. (1966) *Fortschr. Arzneimittelforsch.*, **9**, 192.

<sup>2</sup> The products of different drug firms vary widely in the amount of diethylcarbamazine that they contain. For example, a Notezine tablet may contain 100 mg diethylcarbamazine base compared with 25 mg in Banocide and Hetrazan tablets (50 mg of the citrate salt). The importance of checking the base content of the preparation used will be obvious.

tion of the worm load and of the incidence of complications may be achieved before this level is reached; (b) treatment repeated at intervals may be sufficient to obtain adequate control; (c) diethylcarbamazine is practically tasteless; and (d) according to present knowledge, there is no likelihood of drug resistance of the filariae to diethylcarbamazine being produced. Nevertheless, the real value of this procedure can be judged only by a series of pilot experiments on a gradually increasing scale in different parts of the world.

To date, experience is limited. Laboratory experiments have shown that diethylcarbamazine mixed with the diet is stable on boiling and autoclaving, and does not lose its therapeutic activity or develop toxicity. (Investigators from India, however, report that, if the compound is cooked with tamarind containing tartrates, most of it may be changed so that it can no longer be recognized by chemical means.) In pilot trials in Brazil, the compound thus mixed has been administered to two institutionalized groups of 1000 and 1300 adults respectively, at concentrations up to 0.3% or 0.4%, corresponding to a daily intake of 50 to 100 mg of diethylcarbamazine citrate per head. This procedure was found to be convenient to carry out and acceptable to the consumers. No reactions were observed among the microfilaria-positive members of the groups (or among any others). Satisfactory reductions of the microfilaria counts were observed in small groups of microfilaria carriers during observations which were still continuing at the time this Committee met.

Further pilot trials with various dosages and under various conditions should be undertaken so that the results of this procedure can be evaluated. When the results are known, consideration can then be given to the other aspects that must be borne in mind before such a procedure can be put into wide use.

A third method for the administration of diethylcarbamazine that should be investigated consists of utilizing the schools in filarial districts. The children are under partial discipline, and courses of treatment might be administered under supervision by the teaching staffs at suitable ages (e.g., at school entry and before leaving) to determine whether incipient filarial infections in juveniles could be destroyed thereby. Administration of diethylcarbamazine to schoolchildren should merely *supplement*, not *replace*, proper antifilarial measures for the whole community.

### 3.2 Present status of Mel W

The Expert Committee that met in 1961 recommended that clinical trials with the arsenic compound Mel W should be made on a larger scale in different places.<sup>1</sup> The information now available indicates that Mel W

<sup>1</sup> *Wld Hlth Org. techn. Rep. Ser.*, 1962, 233, 22.

is active against either periodic or subperiodic *W. bancrofti*, its main effect being produced on the adult worms. There have, however, been reports of several deaths following its use, apparently due to encephalopathy, and such risks should not be incurred during the treatment of filarial infections, which are normally non-fatal. Any physician contemplating the use of Mel W in filarial infections should be fully aware of the possible risks involved, and all possible precautions should be taken to reduce these risks, including strict limitation of the size of dose.

### 3.3 The development of drug screening

Whenever possible in drug screening it is preferable to test compounds in experimental animals against the target organisms, in this case *W. bancrofti* and *B. malayi*. However, methods have not yet been developed to the point that any small animals suitable for large-scale work can be infected satisfactorily for drug studies. A number of experimental models are available in the laboratory for screening drugs, of which *Litomosoides carinii* in the cotton rat is the most widely used.

Reliance upon *L. carinii* is encouraged by its use in the discovery of the antifilarial activity of diethylcarbamazine. On the other hand, complete reliance upon it is discouraged by some false leads from it, notably in the case of the cyanine dyes during the Second World War. Nevertheless, practical considerations favour primary screening with *L. carinii* infections, which readily permit evaluation of the effects obtained against adult worms, microfilariae, and even immature worms. Primary screening should be designed to detect even slight activity and reproduce the results, in the hope that further chemical work may lead to more effective compounds. However, like that of all animal models its usefulness is limited, and the test should be supplemented by other experimental systems using other parasites such as *Brugia*, *Dipetalonema*, or *Dirofilaria*. WHO can assist by stimulating research on other filarial life cycles and by the dissemination of material and information. Recent developments in the preservation of a variety of microfilariae, including *W. bancrofti*, in the deep freeze and in the maintenance of some of them in laboratory hosts could open up new fields for drug screening.

It is desirable, but not essential, to examine new drugs secondarily in some of the other available experimental infections, e.g., *B. pahangi* or *B. malayi* in cats or dogs, *Dipetalonema witei* in gerbils and hamsters, *Dirofilaria immitis* in dogs, or *Dirofilaria uniformis* in rabbits. Such additional testing has the dual advantage of utilizing several types of filariae and several types of animals. The fact that a drug is widely active in safe doses against several types of filariae in animals would clearly justify preclinical toxicity studies.

### 3.4 Testing of new compounds in man

Principles for the preclinical testing of drug safety have been laid down by a WHO Scientific Group,<sup>1</sup> and the Committee recommended that these principles be followed in all preclinical studies of new filaricidal drugs.

The Committee wished to refer also to a tentative protocol for the conduct of clinical trials of antischistosomal drugs<sup>2</sup> by stages, which may serve as a guide in the clinical evaluation of filaricidal drugs. It includes initial trials in properly equipped hospitals and centres where the necessary clinical, biochemical, pharmacological, and parasitological observations can best be made with a view to determining the efficacy and safety of the drugs in carefully selected patients. In this respect, WHO can render valuable assistance in the selection of suitable centres for clinical drug trials and may contribute to the establishment of and operation of such centres.

When adapting the above protocol to the clinical testing of filaricidal drugs, attention must be paid to the need for observations relating to different geographical regions according to the species and forms—both periodic and subperiodic—of the parasite. The reduction in microfilaria counts observed over long periods of time will serve as the main criterion for assessing the efficacy of a drug. While histopathological studies of the adult worm may theoretically be desirable, it should be remembered that they are not warranted in most cases since they involve trauma to lymph vessels already susceptible to pathological change. An exception might be the recovery of adult worms from the spermatic cord during hydrocele repair.

Adverse effects must be carefully recorded. They fall into two groups: (a) those resulting from the administration of the drug itself, including local and systemic reactions; and (b) those resulting from the interaction between the drug and the parasite, such as febrile and allergic disturbances due to the death of microfilariae and inflammatory reactions around dying adult worms.

The clinical trial should be extended to include more severely affected patients only when the earlier studies produce encouraging results.

The same careful medical supervision is necessary in phase II trials, which will include larger numbers of patients, some of whom may be out-patients. The number of patients admitted to the trial must be sufficient to enable a proper statistical analysis of the results to be made, and at this stage a properly controlled comparison with a reference drug is desirable.

<sup>1</sup> *Wld Hlth Org. techn. Rep. Ser.*, 1966, **341**.

<sup>2</sup> *Wld Hlth Org. techn. Rep. Ser.*, 1966, **317**, 38.

The first field trials should be conducted under the guidance of the staff concerned in the earlier trials. The aim of these trials will be to determine the suitability of the drug for general treatment and its later use as a control measure. Adequate provision must be made to detect side effects that may occur in special groups such as young children, pregnant women, or persons with concurrent infections or complications of low incidence not detected in the smaller groups at the initial trials. Standardized forms should be provided to record this information.

As field trials are extended to different geographical areas, particular care will be needed to determine variations in the effect of the drug.

#### 4. VECTOR CONTROL

##### 4.1 Assessment of vector control methods

Vectors of filariasis can be classed into four general groups :

*Anopheles* species

*Aedes* species

*Mansonia* species

*Culex pipiens* complex.

The diversity in the bionomics of these vector species calls for careful consideration of control measures.

##### 4.1.1 Control of *Anopheles* species

The principal anopheline vector species are : in Africa the *A. gambiae* complex, *A. funestus*; in South America *A. darlingi*, *A. aquasalis*; in South East Asia and the Western Pacific *Anopheles* of the *barbirostris* group, the *A. punctulatus* group, *A. minimus flavirostris*.

Their control has been undertaken as part of antimalaria campaigns. Unfortunately, the results of house-spraying with DDT or dieldrin on filariasis transmission have been evaluated only in Indonesia (West Irian), where the transmission of *W. bancrofti* by the *A. punctulatus* group was said to have been interrupted. Elsewhere it is known that DDT house-spraying has brought about the rapid elimination of *A. campestris* in parts of Malaya, and of *A. darlingi* in some coastal regions of South America; however, for *A. funestus* and *A. gambiae* results have varied, these species disappearing or losing contact with man in some areas, yet in others persisting and continuing to transmit malaria.

Among filariasis vectors, resistance to dieldrin has been observed in *A. gambiae*, *A. funestus*, *A. minimus*, *A. flavirostris sundaicus*, *A. aquasa-*

lis, as well as double resistance to both DDT and dieldrin in *A. subpictus* in Indonesia.

#### 4.1.2 Control of *Aedes* species

*Ae. togoi*, *Ae. kochi* and *Ae. poecilus* are the only *Aedes* vectors of periodic *W. bancrofti*. The latter species, which is relatively endophilic, was controlled in the Philippines by house-spraying with DDT.

*Ae. polynesiensis*, *Ae. tongae*, *Ae. upolensis* and *Ae. samoanus* are vectors of diurnally subperiodic filariasis in the Pacific Islands. Diurnal or nocturnal, they bite and rest mainly outdoors. They were apparently eliminated on a few islands by aerial DDT spraying more than 15 years ago, but three years later they reappeared.

To protect villages from the above *Aedes* species it is necessary only to control the breeding places within a perimeter of 100 m, because of the very short flight range of these mosquitos. The mechanical destruction of some of the breeding places is technically feasible, but an obstacle is the indifference of the local population. The treatment of small breeding places with dieldrin briquettes has been successful. An experiment involving integrated control of *Ae. polynesiensis* is in progress in the Tokelau Islands.

*Ae. vigilax* is a salt-marsh mosquito, the larvae of which could be reached by either aerial or ground spraying.

Resistance to DDT has developed in *Ae. poecilus* in the Philippines, in *Ae. fijiensis* in Fiji. Through laboratory selection DDT resistance has been obtained in *Ae. pseudoscutellaris*.

#### 4.1.3 Control of *Mansonia* species

*Mansonia* species (*M. annulifera*, *M. indiana*, *M. annulata*, *M. bonnae*, *M. dives*, *M. uniformis*) are vectors of *Brugia*; the last-named species transmits *W. bancrofti* as well in Indonesia (West Irian). Their control has been attempted in both the adult and the larval stages.

DDT house-spraying in the past has yielded results which were good against *M. uniformis* in Ceylon but of uncertain efficacy in India. In Thailand, house-spraying in a limited area with DDT at 2 g/m<sup>2</sup> in conjunction with chemoprophylaxis resulted in the elimination of infection in the mosquito.

In Malaya, on the contrary, the vectors of subperiodic *B. malayi* in bush villages were only slightly affected by residual insecticide spraying campaigns. So far, no insecticide resistance has been recorded in *Mansonia* species.

Antilarval control has been based essentially on the destruction of the plants that serve as supports for the *Mansonia* larvae: *Eichhornia*,

*Pistia*, *Salvinia*, *Scirpus*, etc. The mechanical destruction of these plants has proved ineffective and most costly. However, the use of herbicides such as 2,4-D and Phenoxylene 30 has been highly satisfactory in Ceylon. New herbicides (Reglone, Weedazol, Dowpon, and derivatives of 2,4-D) have been used successfully in Egypt to control the same plants, which there serve as supports for aquatic snails.

#### 4.1.4 *Current control practices against the C. pipiens complex*

As regards current methods for the control of *C.p. fatigans*, the main urban vector of filariasis, most large control campaigns have not so far been successful. In many cases the information available on the bionomics of the vector was inadequate to base a scientific campaign on, or insufficient advantage was taken of the biological information already available.

Adult control campaigns utilizing chlorinated hydrocarbons as residual sprays have failed because this species is little susceptible to DDT and develops resistance to this group of insecticides. A large segment of the adult mosquito population both feeds and rests outdoors, and adults entering houses prefer resting on clothes, curtains, and other surfaces that are often not sprayed. Campaigns utilizing insecticidal fogs have not succeeded in obtaining more than a very temporary measure of control and mosquito numbers have rapidly returned to normal.

Most larval control campaigns have also been only of limited effectiveness; again the chlorinated hydrocarbons have failed to provide adequate control, as a high degree of larval resistance to them quickly develops. Larvicidal oils give only partial control and their effectiveness rapidly disappears.

In most cases, the organization, administration, and trained personnel of control campaigns appear to have been inadequate for the complicated task of control. The Committee noted with satisfaction the work being undertaken at the WHO Filariasis Research Unit in Rangoon, Burma, on non-chemical and integrated methods of control; on insecticide resistance; and on the screening of organophosphorus and other new larvicides and larvicide formulations emanating from the WHO scheme for the evaluation and testing of new insecticides. It particularly remarked upon the valuable analyses being made of the organization, administration, and costing of the experimental field trial now under way. It hoped that this experimental control trial, coupled with accurate assessment of vector and parasite densities, will lead to the development of an effective and economically acceptable method for the interruption of disease transmission through control of the vector. The Committee also noted the valuable control studies now under way in Ceylon, India, and Thailand.

## 4.2 Review of current research and its trends

### *Ecology and biology*

Increased attention is being given to the possibilities of vector control, which may be defined as "Measures of any kind directed against a vector of disease and intended to limit its ability to transmit the disease".

Following the recommendations of the WHO Expert Committee that met in 1961, the overall emphasis in current research in vector control is on vector biology and ecology. Determined efforts are being made to encourage studies in this direction, as it is now realized that some failures in the past can be directly attributed to incomplete or mistaken knowledge of this subject.

### *Studies on resistance and genetics*

The Expert Committee was informed of the investigations in progress on insecticide resistance and the genetic control of insect vectors of disease. It is clear that the extensive use of insecticides is likely to select out resistant strains. Careful studies are therefore being made on the resistance-inducing properties of new insecticides. Global surveys of the spread of resistance in insect vectors of disease are being undertaken, and a computer programme for the handling of resistance data on a global basis has been developed by WHO.

Recent research has shown that exposure of insect populations to an insecticide will lead to cross-resistance, not only to chemically related compounds but also to other chemical groups. Study of the resistance spectrum is considered very useful as, apart from its practical value in the choice of alternative insecticides, it also provides independent information on the identity of the resistance mechanisms involved. A number of WHO collaborating laboratories are carrying out investigations to determine the resistance spectrum of various species to a series of insecticides. At the same time, selection experiments are being carried out to determine the speed of development of resistance to different groups of insecticides when these compounds are used in a certain sequence and in combination with other insecticides. The view is gaining ground that for satisfactory control of a particular species a series of insecticides should be available which can be used in a predetermined manner to delay the development of resistance.

Genetical studies include investigation of the identity of the genes responsible for resistance to new compounds, of the frequency of these genes in natural populations, and of the gene action involving biochemical mechanisms. Laboratory studies are also in progress on the discriminating dosages leading to characterization of natural populations into genotypes.

*Genetic control*

The Committee took note of the WHO programme on vector genetics and manipulation of vector populations by genetic control. A recent WHO Scientific Group<sup>1</sup> discussed the possibilities at present of using genetic mechanisms for the control of the *Culex pipiens*, *Aedes*, and *Anopheles gambiae* complexes and of *Aedes* species.

Genetic control has so far been limited to the release of insects sterilized by ionizing radiations or chemosterilants. However, a great many other possibilities exist for the manipulation of genetic factors already present in natural populations, such as cytoplasmic incompatibility, hybrid sterility, and sex distortion factors. Among these, the following appear most promising :

(1) The induction of dominant lethal mutations—the so-called sterile male technique. This involves the rearing, sterilization, and release of male insects into the environment in sufficient numbers to have a significant impact on the reproductive potential of the natural population.

(2) The use of chemosterilants for inducing sterility in mosquitos, a technique in which considerable progress has been made. The chemicals might be used in two ways : (a) in the sterilization of mosquitos for release, or (b) in the application of chemicals to natural habitats to induce sterility in large portions of the natural populations. *Culex fatigans* has been found to be a most suitable species for this type of approach.

(3) Cytoplasmic incompatibility. Within certain complexes of mosquitos, such as *Culex pipiens* and *Aedes scutellaris*, the cytoplasm of the egg causes incompatibility between isolated populations, and crosses between certain strains of mosquitos produce no offspring. Control can be effected by mass rearing of the males of one crossing type and their release into an area populated by an incompatible strain. A number of strains of the *Culex* complex have been discovered which are incompatible with each other. A pilot experiment on the control of *C. fatigans* by this method is under way in Rangoon.

(4) Other approaches, such as hybrid sterility in *A. gambiae* and sex distortion in *Aedes aegypti*, are also under investigation.

It was stressed that, though promising, many aspects of genetic control require further study before the value of this approach can be assessed in the field.

*Biotic factors*

The Committee reviewed present knowledge of the possible use of predators and parasites against mosquito vectors of filariasis. Some

<sup>1</sup> *Wld Hlth Org. techn. Rep. Ser.*, 1964, 268.

of these, such as predacious fish, may be used as ancillary means of control under limited circumstances. Some predatory mosquito species of the genera *Lutzia* and *Toxorhynchites* may limit other larval populations, but so far they have not provided effective control.

More promise is shown by an integrated approach using both a larvicide and a *Coelomomyces* fungus, and the possibility of extending studies and field trials of such combinations should be further explored.

## 5. ORIENTATION OF FILARIASIS CONTROL

### 5.1 Advantages and disadvantages of chemotherapy and vector control

The orientation of an antifilarial campaign requires in the first place a careful study of the local conditions, since the optimum methods to be adopted vary greatly in different places. However, the main advantages and disadvantages of the two chief methods, chemotherapy and vector control, may be summarized as follows :

#### *Chemotherapy* (with diethylcarbamazine)

##### *Advantages :*

- (1) It produces an immediate reduction of the microfilarial level in the population, and so of the sources of further infection. (Apart from certain regions in Malaya, there is no known animal reservoir of infection.)
- (2) Experience during the past ten years has afforded many examples of its having been successful on a small scale.
- (3) It is applicable to rural as well as to urban areas.
- (4) In addition to its value as a control measure, it has a beneficial effect on the already infected population by curing acute symptoms, reducing the worm burden, and thus diminishing the danger of pathological lesions developing.

##### *Disadvantages :*

Success depends upon obtaining good co-operation from the population in accepting the compound. In the past, this has usually been difficult to obtain when *large* populations are concerned, as in India. It is possible that this disadvantage might be diminished by the development of more acceptable methods of administration and a dynamic health education programme.

### *Vector Control*

#### *Advantages:*

(1) Mosquito control in towns is desirable on the general grounds of public hygiene and amenity, and it should be possible to pay special attention to the vectors of filariasis inside the general organization for this purpose.

(2) Larviciding programmes do not depend to any great extent upon obtaining the co-operation of the local population. They are particularly applicable to urban areas in many countries where filariasis is an urban problem.

#### *Disadvantages:*

Vector control is slow to affect the prevalence of filarial infection in the population. Thus, the number of available vectors or their longevity must be reduced to a very low level and must be maintained at it for several years before the existing worms in the population die out and transmission is interrupted. Prolonged control may give rise to insecticide resistance.

### *Combined control methods*

Combined control involves the use of both drug control and mosquito control methods, either simultaneously or successively. Each of these methods, when used alone, has come near to achieving interruption of transmission—for example, in one part of Japan the microfilaria rate dropped from 5% to less than 1% within five years with diethylcarbamazine, and in Mauritius, where *A. funestus* was eradicated and *A. gambiae* was reduced by residual spraying, the microfilaria rate of *W. bancrofti* fell from 28% to 1.2% over a period of 12 years.

Although an exact quantitative evaluation of each method is difficult, the combined approach, aimed on the one hand at the destruction of adult worms and their microfilariae in man and on the other hand at the destruction of the vector, appears to be the most logical. A programme using combined methods will certainly reach its goal more rapidly than a programme using a single method. Integration of the various approaches of control depends, however, upon the epidemiology of the disease in each area, the resources of the country, and the personnel available.

## **5.2 Relation of filariasis control to malaria control**

In areas where filariasis and malaria are transmitted by the same species of mosquitos, as in much of Africa and in West Irian, vector control measures will affect the transmission of both diseases. The same

may be true of areas where, although different species of vectors may be involved, these species have very similar habits. Before malaria control measures are started in either type of area, it is important that the filariasis surveys should have been carried out so that an assessment of the results of malaria control on the transmission of filariasis can later be made.

In many places, however, the vectors of filariasis are completely different from the vectors of malaria. Even so, greater co-ordination is needed in the application of control measures against both diseases. For example, the field testing of a new insecticide for malaria control, and the selection of the area for such trials, might well take into consideration the presence of filariasis and other mosquito-borne diseases in the locality.

Where malaria control in urban areas is based mainly on antilarval measures, it should be possible to extend these activities to attack the breeding places of the vectors of filariasis as well.

To anyone organizing a filariasis control scheme experience gained from malaria eradication campaigns will be of considerable assistance. In particular, the training facilities for malaria eradication may be made available to the technical staff required in filariasis control. A minimum of training would be necessary if it were possible to take over already experienced staff no longer needed for malaria programmes.

### **5.3 Clinical filariasis and the control programme**

Unless the clinical manifestations of the disease can be treated with some success, the local population is unlikely to give full support to control projects based on either mass chemotherapy or vector control.

A scheme for the treatment of the more obvious lesions must be prepared at the outset of any campaign, and it should be designed with the expectation that the scheme will last for at least 15 years. The parasitological and entomological work should run concurrently with a carefully organized medical and surgical programme.

It should be made clear to the people that individuals with advanced elephantiasis are unlikely to benefit from the control project, and that no surgical procedures are available for mass treatment of this condition. Elephantiasis may not be common in some areas, and more can be done to help persons still liable to recurrent attacks of fever and lymphangitis, or those with hydroceles. People with lymphangitis will benefit from effective mass chemotherapy, but advance warning should be given about possible adverse reactions; without this warning the whole campaign may be discredited.

In areas of bancroftian filariasis, the hydrocele rate in adult males is often more than 20%. Of all the complications of filariasis this is the

one that can be most readily treated by simple surgical procedures. Itinerant surgical teams should be organized to deal with the worst cases. The successful treatment of only a few cases can greatly help in getting the goodwill and co-operation of the people in the campaign.

In Eastern Asia special consideration has to be given to the treatment of chyluria.

Although many patients with advanced pathological lesions have no microfilariae in their blood, it is essential to treat them with filaricidal drugs because live adult worms are often present in the tissues.

## 6. TERMINOLOGY

The need is felt in filariasis work for a standard terminology in order to avoid confusion and misunderstanding arising from lack of uniform and precise definitions for certain concepts which tend to acquire different meanings or shades of meaning.

Without this uniformity a comparison of studies carried out in various parts of the world is made unnecessarily difficult. The Committee therefore welcomed the initiative taken by WHO in drawing up a terminology of filarial infections as it recently did for the terminology of malaria and malaria eradication. After examination of the glossary in its draft form, the Committee recommended that the work be continued to completion. The present report already incorporates some of the terms and definitions agreed upon by the Committee.

## 7. RESEARCH NEEDS

In attempting to eliminate the many gaps in our knowledge of filariasis, it is difficult to suggest an order of priority that would have universal application. This list of research needs is therefore grouped under the following subject headings: parasitology, entomology, epidemiology, chemotherapy, pathology and immunology, and other needs.

### 7.1 Parasitology

#### *Parasitological reference centres*

Reference centres are needed where living and preserved specimens of all stages of filarial worms can be maintained and field workers can send material for identification. The need for help in identification is most urgent for the larval stages of the vectors. The centres could also assist in the training of staff.

### *Maintenance in laboratory animals*

Attempts should be made to establish the complete transmission cycle of *Brugia* species in small laboratory animals. Efforts to transmit *W. bancrofti* to animals should be continued. Filarial infections of animals, especially rodents, should be studied further. Any of these investigations might provide a much needed model for biological studies and drug testing.

### *Biological studies*

Basic studies on the physiology, biochemistry, histology (including fine structure) and histochemistry of filarial worms should be expanded.

### *Reservoir hosts*

A search for possible reservoir hosts of *W. bancrofti* should be made in rural foci of this infection, especially in Asia. More information is needed about the importance of animal reservoirs of *B. malayi* outside Malaya.

### *Parasites of uncertain status*

Efforts should be made to clarify the taxonomic status of the Filarioidea, especially that of *W. bancrofti* var. *vauceli* and of the Timor microfilaria, and to determine their hosts and vectors.

### *Other filarial infections*

In view of conflicting reports on the clinical significance of infections caused by *Dipetalonema perstans* and *Mansonella ozzardi*, more information is needed on the biology and pathogenicity of these parasites.

More attention should be given to infections caused by *Loa loa* and *Dipetalonema streptocerca*.

## **7.2 Entomology**

### *Biology of vectors*

Research on the taxonomy, genetics, bionomics, and vectorial capacity of known vectors should be continued and expanded in different endemic areas. There is also a need for investigation into the adaptability of human parasites to new species or strains of vectors. These studies could also include investigations of the preferential biting habits of the vectors in relation to infection rates in different age and sex groups and differences in the distribution of clinical lesions.

### *Vector control*

The WHO programme on vector control should be continued and intensified. Special attention should be directed towards :

(1) research on insecticides with special reference to new formulations to achieve better persistence in larvicides; on insecticides that will not cause cross-resistance to develop; on the speed of development of resistance to different groups of insecticides when used in a certain sequence and in combination with other insecticides; and on new application techniques, e.g., for use in area control; and

(2) research on genetic and non-chemical control methods and methods of integrated control utilizing chemical, genetic, biological, and environmental approaches.

## **7.3 Epidemiology**

### *Transmission dynamics*

Careful studies in different endemic areas are needed to correlate infection rates and microfilaria densities in the human population with exposure to infective mosquito bites. An assessment should be made of the effects of single or combined methods of control on transmission dynamics, which should provide valuable evidence for determining the transmission threshold and the level at which transmission is interrupted.

### *Natural fluctuations in the prevalence of filariasis*

Very little is known about the extent to which natural fluctuations in the prevalence rates may occur. Such fluctuations may assist or detract from the effects of control measures. Longitudinal studies are needed.

### *Effects of malaria control*

More information is needed on the effects of malaria control activities on the prevalence of filariasis. Methods of co-ordinating control activities against these two diseases are desirable.

### *Morbidity*

There is a great need for an evaluation of the morbidity caused by filariasis in various endemic areas and also in infected persons who have moved to non-endemic areas.

## **7.4 Chemotherapy**

### *Diethylcarbamazine*

Improved methods should be sought of administering diethylcarbamazine on a mass basis, especially by incorporation in common articles

of diet such as cooking salt. A study is also needed of pharmaceutical formulations for depot action, prophylactic action, and the suppression of adverse reactions.

#### *Mel W*

The antifilarial action, pharmacology, and toxicology of Mel W. require study. Special attention should be paid to the mechanisms producing encephalopathy and to ways and means of avoiding it.

#### *Other compounds*

Investigation is needed of the possible antifilarial activity of suitable compounds known to be active against other nematodes.

#### *Search for new compounds*

The search for new antifilarial compounds should be encouraged, especially by impressing upon commercial firms the desirability of such a search. WHO might assist by dissemination of information.

#### *Clinical trials*

Clinical trials of new compounds shown to have suitable antifilarial activity and low toxicity in laboratory experiments should be organized. Support should be given by WHO to centres prepared to carry out such trials under an agreed protocol.

### **7.5 Pathology and immunology**

Further studies are needed on the pathogenesis of elephantiasis, hydrocele, and chyluria, and the relationship of these lesions to microfilaria densities and worm loads. Attempts should be made to produce these lesions in experimental animals, so that methods of treating acute and chronic lesions, especially elephantiasis and hydrocele, can be developed. The occurrence of renal changes in filarial infections should be studied.

#### *Tropical eosinophilia*

More information is required about the role in the causation of tropical eosinophilia of filarial worms parasitic in both man and animals.

#### *Immunological diagnosis*

There is a need to evaluate a variety of skin and serological tests, using purified antigens as an aid to diagnosis. It is particularly important to assess the effect of repeated skin tests in non-endemic areas for possible sensitization with filarial antigens.

*Serological reference centre*

When suitable antigens become available, it will be desirable to set up a serological reference centre for standardization of both sera and antigens.

*Immunology of filarial infections*

Basic studies are needed on the immunology of filariasis in man and in experimental animals.

**7.6 Other needs***Administration and costing in control schemes*

There is a need for the careful evaluation of the administration, organization, and costing of vector control campaigns involving the use of chemical, non-chemical, and environmental sanitation methods, and also of campaigns based on mass chemotherapy of the whole population or of microfilaria carriers only.

*Assessment of control programmes*

Follow-up studies of past and current control programmes are essential in providing orientation for future programmes.

*Training and health education*

Training of scientific and technical personnel for control campaigns should be undertaken with WHO assistance prior to the final formulation of control programmes. Further studies are needed on improving methods of health education.

*Terminology*

It is recommended that WHO should prepare and distribute a glossary of terms used in filariasis.

**8. RECOMMENDATIONS**

In relation to the numerous problems of filariasis control recognized and discussed by the Committee, the following recommendations merit the highest priority :

1. Pilot control projects should be established in different geographical areas to assess the effectiveness of chemotherapy and vector control, singly or combined under different epidemiological conditions.

2. Because existing filarial drugs have certain limitations for mass treatment projects, the fullest support should be given by WHO to the development of new formulations (e.g., medicated salt) of the drugs at present available and of new antifilarial compounds by fostering and assisting in suitable clinical trials.

3. Present research on vector biology and control, especially in the field of vector ecology, the development of new insecticides, resistance problems, and genetic control methods, should be continued and encouraged.

4. The usefulness of filarial antigens for immunological tests in endemic as well as in non-endemic areas should be evaluated.

5. Field workers require reference centres at which parasitological material can be identified. The need for help is most urgent in the identification of the larval stages of the filarial worms in the vectors. WHO should assist in the establishment of such centres.

6. Adaptation of *Brugia* species or *Wuchereria bancrofti* to small laboratory animals should be attempted in order to satisfy the need for additional information on the biology of the human filariae and to facilitate screening of new chemotherapeutic agents.

#### ACKNOWLEDGEMENTS

The Committee acknowledged the special contributions made to its deliberations by the following WHO staff members : Dr L. J. Bruce-Chwatt, Chief, Research and Technical Intelligence, Division of Malaria Eradication; Dr N. Gratz, Vector Control; Dr N. H. Kent, Parasitic Diseases; Dr H. Mercker, Pharmacology and Toxicology; Dr R. Pal, Vector Control; Mr K. Uemura, Chief, Health Statistical Methodology.

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## Annex

### MATHEMATICAL ANALYSIS OF PARASITOLOGICAL SURVEY DATA

The results of a microfilaria survey can be used in various ways according to the purposes of the survey and the methods adopted. When the survey is made by measured blood samples, the microfilaria count of individual cases gives valuable information, as discussed below. It is desirable to take multiple blood samples from each individual. Three measured blood films of equal size provide the data necessary to determine the microfilaria positive grade of a population and correction factors. The following methods of analysing data have had limited application, but appear to be useful methods of comparing data and trends. Further evaluation of these procedures is needed.

#### 1. Method of drawing log-probit regression line of frequency distribution of microfilaria count

Analysis of the results of microfilaria surveys in a number of endemic areas with various levels of prevalence has shown that the probit values of cumulative percentages of microfilaria positive cases are distributed almost on straight lines when plotted against logarithms of the corresponding microfilaria counts. Such a relation implies that the frequency distribution is logarithmically normal.

In order to draw a regression line, it is convenient to make a table such as Table 1. Column (A) indicates class intervals of microfilaria counts adapted for a logarithmic scale on section paper. The number of cases with the respective microfilaria counts is entered in column (B), and their cumulative numbers are shown in column (C). The cumulative percentages obtained by dividing the numbers in column (C) by the total number of positive cases are calculated as in (D), and the probit values corresponding to the respective percentages in column (E) are obtained by using a conversion table of Bliss which is available in many biostatistical textbooks. When logarithmic probability papers are available (such as are commonly used in obtaining log-dosage probit-mortality regression lines in insecticidal studies), the cumulative percentage values may be plotted directly without conversion into the probits. Fig 1 as an example of a regression line obtained from results shown in Table 1.

TABLE I. EXAMPLE OF A FREQUENCY DISTRIBUTION OF MICROFILARIA POSITIVE CASES CLASSIFIED BY DENSITY PER 30 mm<sup>3</sup> BLOOD SAMPLE

Microfilaria count (A)	Frequency (B)	Cumulative frequency (C)	Cumulative % (D)	Probit (E)
1	57	57	16.9	4.04
2	48	105	31.1	4.51
3	30	135	39.9	4.74
4	25	160	47.3	4.93
5	16	176	52.1	5.05
6	14	190	56.2	5.16
7	11	201	59.5	5.24
8	13	214	63.3	5.34
9	11	225	66.6	5.43
10	11	236	69.8	5.52
11-20	46	282	83.4	5.97
21-30	18	300	88.8	6.22
31-40	9	309	91.4	6.37
41-50	4	313	92.6	6.45
51-60	2	315	93.2	6.49
61-70	3	318	94.1	6.56
71-80	1	319	94.4	6.59
81-90	2	321	95.0	6.64
91-100	4	325	96.2	6.77
101-200	11	336	99.4	7.51
201-300	1	337	99.7	7.75
301-400	1	338	100	—

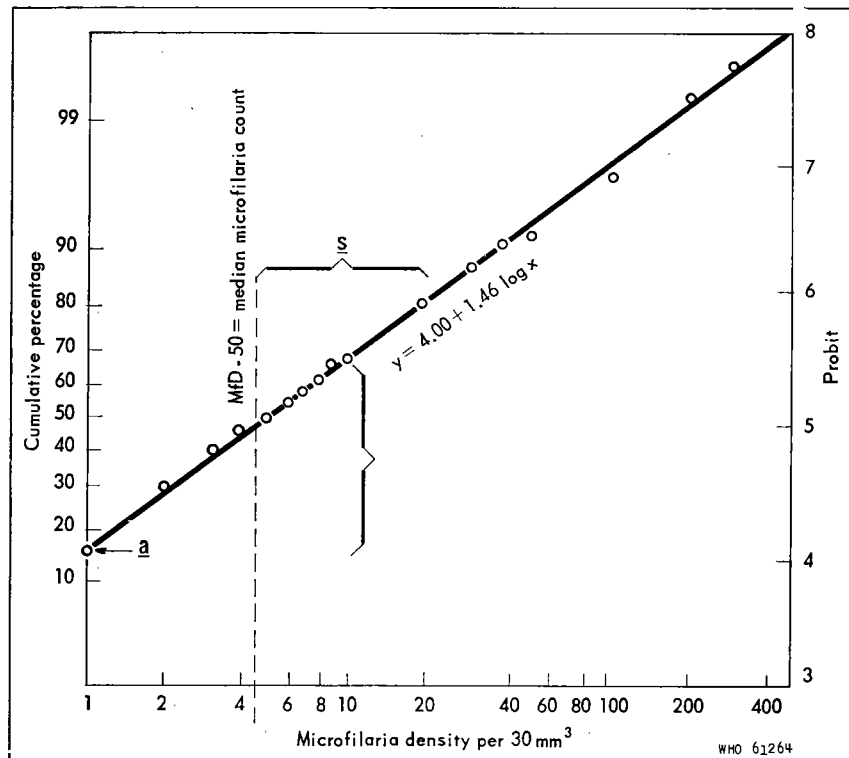
Where the frequency distribution of microfilaria positive cases according to the microfilaria density is logarithmically normal, the regression line may be represented by a simple equation :

$$y = a + b \log x,$$

where  $y$  is the probit of cumulative frequency at a density of  $x$  microfilariae, and  $a$  and  $b$  are the constants that determine the position and angle of the regression line. Although there are generally applicable mathematical methods for obtaining the most reasonable estimates of these values from the observed data, it is usually sufficient to draw the line by a simple visual fitting, as shown in Fig. 1. The value of  $a$  corresponds to the percentage of cases with microfilaria count one (or zero in logarithm), and  $b$  is the regression coefficient that determines the angle of the line to the  $x$  axis. The 50% level of microfilaria density (MfD<sub>50</sub> or median microfilaria count) is obtained by reading the value of  $x$  at the point where the regression line crosses probit five (50%) level, or by solving the equation :  $\log x = (5 - a)/b$ . The standard deviation  $s$  corresponds to the distance on the horizontal axis of the points at which the regression line crosses with probit 5 (50%) and probit 6 (84.1%).

When filariasis is highly prevalent and the microfilaria density of the population is high, the regression line stays in a lower position with a smaller angle to the  $x$  axis than in areas with lower endemic levels. The values of  $a$  and  $b$  obtained from such highly endemic areas are usually small, and both are considered as good indications of endemicity. In

FIG. 1. EXAMPLE OF A REGRESSION LINE OF THE CUMULATIVE PERCENTAGE OF MICROFILARIA POSITIVE CASES AGAINST THE MICROFILARIA DENSITY IN THE LOG-PROBIT SCALE



such a relationship the median value ( $MfD_{50}$ ) of the microfilaria density is comparable to  $LD_{50}$  in the toxicity test, and is considered more reliable than the arithmetic mean, or the average microfilaria density.

The effectiveness of a drug treatment scheme may be evaluated not only by the percentage of previously positive cases which become negative at post-treatment blood surveys, but also from the reduction in the microfilaria among those who still remain positive. In an area in which an extensive mass drug treatment programme has been in progress and the microfilaria positive rate dropped from 30.9% to 31% after administration of monthly doses of 6 mg/kg of diethylcarbamazine, a remarkable reduction in the microfilaria density was observed after treatment (Table 2). Examination of the regression lines shows that the values of  $a$  and  $b$  increased from 3.13 to 3.56 and from 1.11 to 1.39 respectively, while the  $MfD_{50}$  decreased from 47.4 to 10.7 (Fig. 2). Similar changes in values of the regression lines have been seen in other areas after the drug is adminis-

**TABLE 2. EXAMPLE OF COMPARISON OF FREQUENCY DISTRIBUTION OF MICROFILARIA DENSITY BEFORE AND AFTER ADMINISTRATION OF DIETHYLCARBAMAZINE**

Microfilaria density (20 mm <sup>3</sup> )	Before treatment		After treatment	
	Frequency	Cumulative %	Frequency	Cumulative %
1	14	5.5	11	14.7
2	10	9.4	6	22.7
3	8	12.5	6	30.7
4	8	15.7	4	36.0
5	7	18.4	2	38.7
6	4	20.0	5	45.3
7	15	25.9	1	46.7
8	3	27.1	3	50.7
9	3	28.2	1	52.0
10	4	29.8	1	53.3
11-20	36	43.9	10	66.7
21-30	10	47.8	7	76.0
31-40	12	52.5	3	80.0
41-50	6	54.9	5	86.7
51-60	12	59.6	5	93.3
61-70	6	62.0	1	94.7
71-80	10	65.9	1	96.0
81-90	6	68.2	0	96.0
91-100	6	70.6	0	96.0
101-200	23	79.6	3	100.0
201-300	20	87.5		
301-400	9	91.0		
401-500	7	93.7		
501-600	6	96.1		
601-900	7	98.8		
901 & +	3	100.0		
Total	255		75	

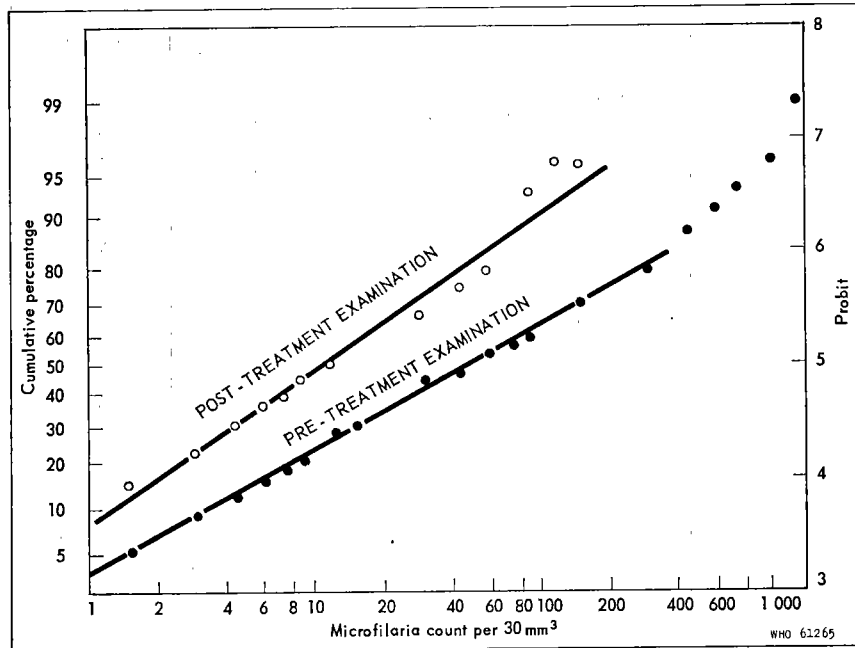
tered efficiently. The size of the differences in these values is considered to be a reflection of the effectiveness and adequacy of the respective control procedures.

## 2. Microfilaria positive grade and determination of correction factor

Where a survey is made by taking multiple blood samples from each person, the result may be used to obtain the microfilaria positive grade and further to estimate correction factors for positive rates to be obtained for surveys by different quantities of blood samples. The term "microfilaria positive grade" indicates the number of positive samples among the total samples taken at one time from the same person. When the total is 3 the possibilities are : 3:3 (all three films are positive); 2:3 (two films positive, one negative); 1:3 (only one film positive), or 0:3 (all three films negative).

Theoretically, the probability of obtaining partly positive results (i.e., 1:3 and 2:3) increases as the microfilaria density in the individuals falls, and the relative composition of the three grade (1:3, 2:3, and 3:3) is dependent on the frequency distribution of microfilaria densities in the population but not on the positive rate, though the two factors are usually

FIG 2. EXAMPLES OF REGRESSION LINES OF THE CUMULATIVE PERCENTAGE DISTRIBUTION OF MICROFILARIAL DENSITY BEFORE AND AFTER DIETHYLCARBAMAZINE ADMINISTRATION



correlated. Therefore there are no uniform correction factors applicable to certain positive rates. The correction factors for positive rates to be expected at examinations of different volumes of blood samples can be estimated directly from the observed data for each population. If three 10 mm<sup>3</sup> blood samples are examined at a survey and the numbers of cases with the positive grade of 3:3, 2:3, and 1:3 are found to be N<sub>3</sub>, N<sub>2</sub>, and N<sub>1</sub> respectively, the number of cases diagnosed as positive by examination of 30 mm<sup>3</sup>, 20 mm<sup>3</sup>, and 10 mm<sup>3</sup> is estimated by the following formulae :

$$\text{For } 30 \text{ mm}^3 : P_{30} = N_1 + N_2 + N_3$$

$$\text{For } 20 \text{ mm}^3 : P_{20} = \frac{2}{3} N_1 + N_2 + N_3$$

$$\text{For } 10 \text{ mm}^3 : P_{10} = \frac{1}{3} N_1 + \frac{2}{3} N_2 + N_3$$

The above formulae are derived from the simple assumption that one third of N<sub>1</sub> cases at 20 mm<sup>3</sup> examination and two thirds of N<sub>1</sub> cases plus one third of N<sub>2</sub> cases at 10 mm<sup>3</sup> examination are expected to be diagnosed as negative, while all others should turn out positive at the respective examinations.

An example of the application of this method to survey data from an endemic area of *W. bancrofti* before and after application of a drug control scheme is shown in Table 3.

The above direct method is applicable when three blood samples of 10 mm<sup>3</sup> each are taken from each person.

**TABLE 3. EXAMPLE OF COMPOSITION OF MICROFILARIA POSITIVE GRADES AND CORRECTION FACTORS OBTAINED BEFORE AND AFTER TREATMENT WITH A DRUG**

	Number with positive grade				% positive for 30 mm <sup>3</sup>	Correction factor for		MfD <sub>50</sub>
	N <sub>3</sub>	N <sub>2</sub>	N <sub>1</sub>	N		20 mm <sup>3</sup>	10 mm <sup>3</sup>	
Before	840	191	189	10 357	11.78	0.949	0.845	14.1
After *	68	59	103	1 988	11.57	0.851	0.616	3.3

\* Only previously positive cases were examined.

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