*Bull. Org. mond. Santé*
*Bull. Wld Hlth Org.* } 1968, 38, 29-59

# Analysis of the *Wuchereria bancrofti* Population in the People of American Samoa

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*Recent interest in mathematical descriptions of the epidemiology of helminth parasites has prompted several attempts to obtain quantitative estimates of reproduction and survival at all stages in their life-histories. The availability of microfilarial counts, repeated on many people over 4 1/2 years, suggested that these estimates could be made for* Wuchereria bancrofti. *Important values that were calculated are the duration of patency for single infections (2 1/2 years), the maximum density of microfilariae achieved by 1 female (70/60 mm³ of peripheral blood), the average output of larvae by a female during her lifetime ($1.32 \times 10^7$), the death rate of mated females (0.02–0.05 per month), the average load of reproducing female worms per blood-positive person (6.91 for men, 6.07 for women, 2.93 for children), and the average total load of worms in infected people (11.18 for men, 7.70 for women, 4.02 for children).*

*The implications of the approach taken are important from the standpoints of epidemiology and control. The construction of a complete ecological life-table for the parasite is a distinct possibility. Life-tables from several areas differing in transmission rates should make it possible to predict the critical density of hosts, below which the parasite population should die out spontaneously.*

The four parts of this paper represent an attempt to derive quantitative conclusions about the life-history of *Wuchereria bancrofti* and the epidemiology of filariasis. The reader may well conclude that for some parts of the analysis the data are less than adequate. Certainly, the data do not meet all our wishes, and we recognize that potentially the numerical values have a large and unspecifiable error. In some sense, therefore, the use of exact numbers is deliberately provocative. We hope that the papers will stimulate both further thought on improvements in the mathematical approach and further research to improve the quantity and quality of available data.

## I. FLUCTUATIONS IN MICROFILARIAL DENSITY IN INDIVIDUAL CASES

The need for quantitative data on helminth infections has been recognized for several decades, especially to assess the intensity of infections with intestinal nematodes (Stoll, 1923; Keller & Leathers, 1936; Andrews, 1942; Pesigan et al., 1958). Studies on various intestinal helminths have led to commonly accepted and widely quoted values relating the numbers of eggs in faeces with the numbers of female worms present.

For parasites, including the filarial worms, living in the tissues or vascular systems of their hosts, the quantitative data that have been amassed have not yielded such satisfactory interpretations. The relevance of such data as microfilarial counts is undetermined. The first and most obvious reason for this is that direct counts of the numbers of adult worms present in people are not available. A second reason is related to the failure to find a relationship between the severity of symptoms, particularly elephantiasis, and the number of microfilariae per unit volume of blood. This observation, however, applies to individual cases only, since the

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frequency of occurrence of symptoms seems to be related to the mean density of microfilariae, when the entire human population is considered (WHO Expert Committee on Filariasis, 1962). The distinction between the situation in individuals and that in the population has thus given rise to some confusion. Further confusion has arisen because the mean density of microfilariae in the population is not always related to prevalence. This lack of relationship is most probably due to heterogeneity of exposure, with the result that some people acquire much heavier worm burdens than would be expected from the proportion of people positive in the entire population. The *a priori* expectation would be for symptoms to be related to worm burden. Therefore, a less-than-perfect correlation between frequency of symptoms and over-all prevalence is not surprising, if some areas under study have the heterogeneity of exposure described above.

A third set of reasons for the lack of consensus in interpreting counts of microfilariae derives from the extreme variations that exist in these counts from person to person and from time to time. The latter are complicated by the phenomenon of periodicity, which brings about such great differences within a few hours that other sources of variation have sometimes appeared to be inconsequential.

Differences between individuals and variations within individuals over periods of months or years have received less attention than they deserve. The existence of a strain of *Wuchereria bancrofti* in which periodicity of microfilariae is weak or non-existent facilitates the study of these other sources of variation.

The data on which the present series of papers is based were collected in American Samoa over a period of 4½ years. During that time, the same individuals were examined repeatedly (see below), but always at the same time of day, so that even such minor diurnal variation as occurs (Mattingly, 1962) did not influence the results. The purpose of this part of the paper is to demonstrate long-term variations in microfilarial density in these individuals, and to show that a pattern exists. In parts II–IV the pattern is considered in detail, an explanation for the pattern is suggested, and differences between infected individuals are analysed. An attempt is made to explain the extreme differences in microfilarial density that exist between individuals when a survey is made of the population, and an approach is made towards using microfilarial densities to estimate worm burdens.

## METHODS

The data on which this analysis is based were collected in American Samoa between September 1948 and March 1953. Records were available for 1575 Samoans who were examined during that period. Of these, 358 were treated after 1–3 examinations, which limited the usefulness of the records. The remaining 1217 individuals were never treated and, as shown in the following tabulation, were examined 1–10 times:

| | *No. of examinations* | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *1* | *2* | *3* | *4* | *5* | *6* | *7* | *8* | *9* | *10* |
| No. of cases | 224 | 159 | 159 | 138 | 141 | 147 | 144 | 83 | 18 | 4 |

The examinations were distributed unevenly in time. For most of the villages, the first 5 surveys were carried out at 3-month intervals from September 1948 to October 1949. Additional surveys were made in March of the years 1950, 1951 and 1953. For Aoloau and the villages where treatment was carried out, the first 7 surveys were taken at 2-month intervals, and the remaining 3 were taken in the same months as the other villages.

From each person, three 20-mm³ samples of peripheral blood from finger-punctures were collected between 14.00 and 17.00 hours. Results were recorded as the total number of microfilariae in 60 mm³ of blood. The sampling error in such counts is related to the mean count, as might be expected (see below).

## SOURCES OF VARIANCE IN MICROFILARIAL COUNTS

### *Sampling error*

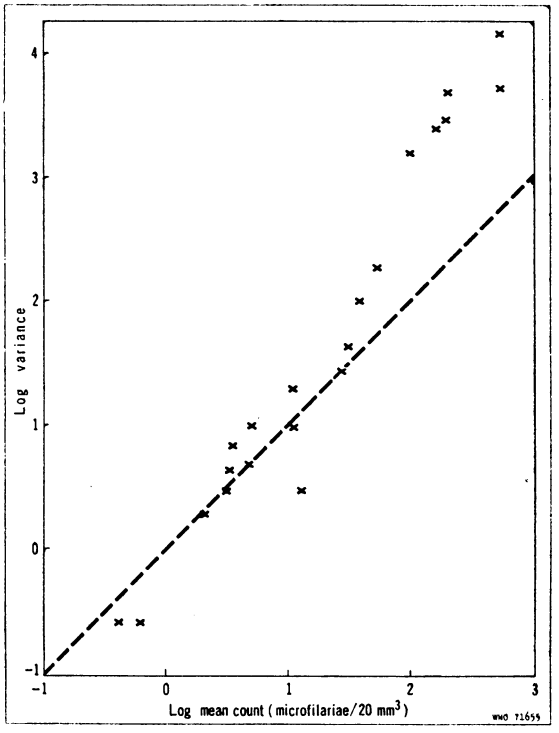
In the previous section, it was pointed out that a certain amount of sampling error exists with regard to any count of microfilariae. One approach to the evaluation of the possible causes of this error is to compare variances and mean counts over a large range of values. The first such comparison involves counts taken on blood from the same individual within a short period. For this comparison, the data of Rosen (1955) are available. Rosen made 8 separate counts on each of 21 Tahitians infected with *W. bancrofti*. The 8 specimens were taken over a period of little more than 1 hour—a time too short to be affected by the small amount of diurnal periodicity in this strain of parasite.

If the larvae had been distributed randomly in the blood stream, as current theory postulates, the variance should be equal to the mean count, since the counts from the 8 samples should con-

form to the Poisson series. The expectation is not borne out, and the variance rises more rapidly than the mean (Fig. 1). Thus, a randomizing effect can only be a part of the source of error in counts from skin-punctures. A plausible explanation for

FIG. 1

RELATION BETWEEN VARIANCE AND MEAN COUNT OF MICROFILARIAE FOR 21 PEOPLE;[a] EIGHT SMEARS TAKEN WITHIN A SHORT TIME[b]




[a] Broken line indicates Poisson distribution.
[b] Data from Rosen (1955) on subperiodic *W. bancrofti*.

the additional variance comes from a consideration of the problem faced by a microfilaria 200 $\mu$–300 $\mu$ long by 6 $\mu$–8.5 $\mu$ in diameter in negotiating capillaries 10 $\mu$ or less in diameter (McCarthy, 1956; Burton, 1964). Only a slight amount of retardation would be necessary to give a disproportionately high density of larvae on the arterial side of a capillary bed. The number of larvae in a measured volume of blood from a skin-puncture would thus be determined in part by the exact source of the blood. The source of the blood would be determined by a variety of factors involved in the technique of its collection. The exact site of the incision, its depth, the amount of blood taken, and the pressure exerted to ensure an adequate volume could all influence the exact source of blood, and hence the number of microfilariae per unit volume.

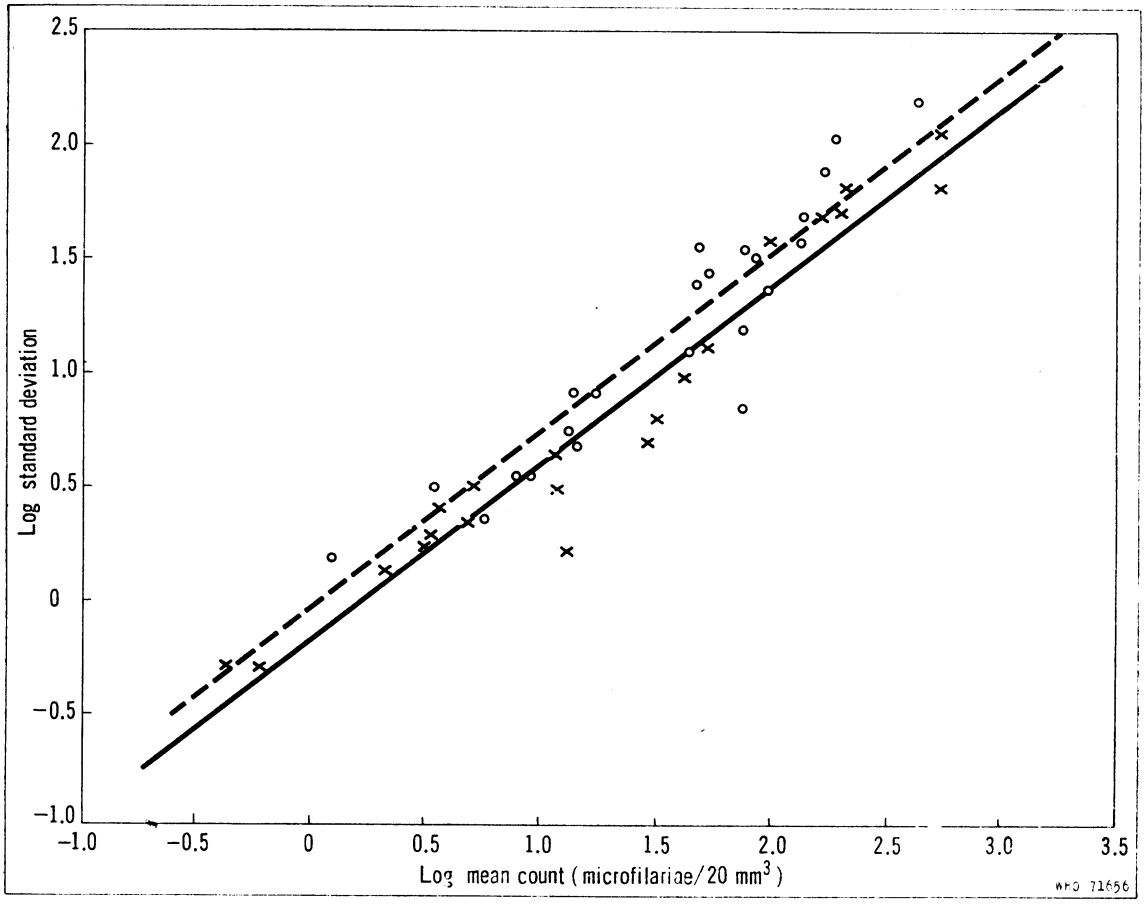
One interesting aspect of this hypothesis is that it yields at least two testable predictions. The first is that blood withdrawn by venepuncture should have a lower mean density than that taken from the skin of the same subject. Such a discrepancy has in fact been reported, but different observers have not obtained uniform ratios for the two densities. Thus, the graph published by Hawking & Thurston (1951) indicates a difference of only about 10% in a monkey infected with *Dirofilaria* sp. Yorke & Blacklock (1917) reported skin blood to contain twice as many larvae as venous blood in the case of periodic *W. bancrofti* observed by them. Amos, quoted by Kessel (1957), obtained data on subperiodic *W. bancrofti* indicating a much higher ratio, but the report is not in a form which permits an exact estimate.

*Variance caused by diurnal periodicity*

The second prediction that comes from the hypothesis requires some explanation. In Fig. 2, regression lines have been included, and the relationship shown is between the standard deviations and the means. The change is a necessary correction for the curvature that exists when variances are related to means (Fig. 1) and must be made to meet the assumption of linearity required for calculating the regressions. Curvature does not appear to be a problem when the standard deviation is used instead of variance. If the variance (above that expected from a Poisson distribution) is really due to retardation of microfilariae entering the capillary bed, deviation from randomness should depend only on the mean density, and the slope of the regression line should be constant, regardless of any additional sources of variance, such as those imposed by diurnal periodicity. Thus, for counts on the same individual, taken over 24 hours, the regression line should be higher than, but parallel to, the regression of standard deviation on mean count for counts taken at the same time. The implied prediction is borne out (Fig. 2). The data are those of Edgar et al. (1952), who took samples every 6 hours for 24 hours. The slopes of the two regression lines, with 95% confidence limits, are $0.770 \pm 0.258$ for Rosen's data, and $0.828 \pm 0.085$ for the data of Edgar et al., and are clearly not significantly different.

FIG. 2

RELATIONSHIP BETWEEN STANDARD DEVIATION AND MEAN DENSITY OF MICROFILARIAE OF SUBPERIODIC *W. BANCROFTI* IN TWO SETS OF OBSERVATIONS [a]




×——× 21 subjects with 8 samples taken over little more than 1 hour. Data from Rosen (1955).

o– – –o 24 subjects examined every 6 hours for 24 hours. Data from Edgar, Beye & Mille (1952).

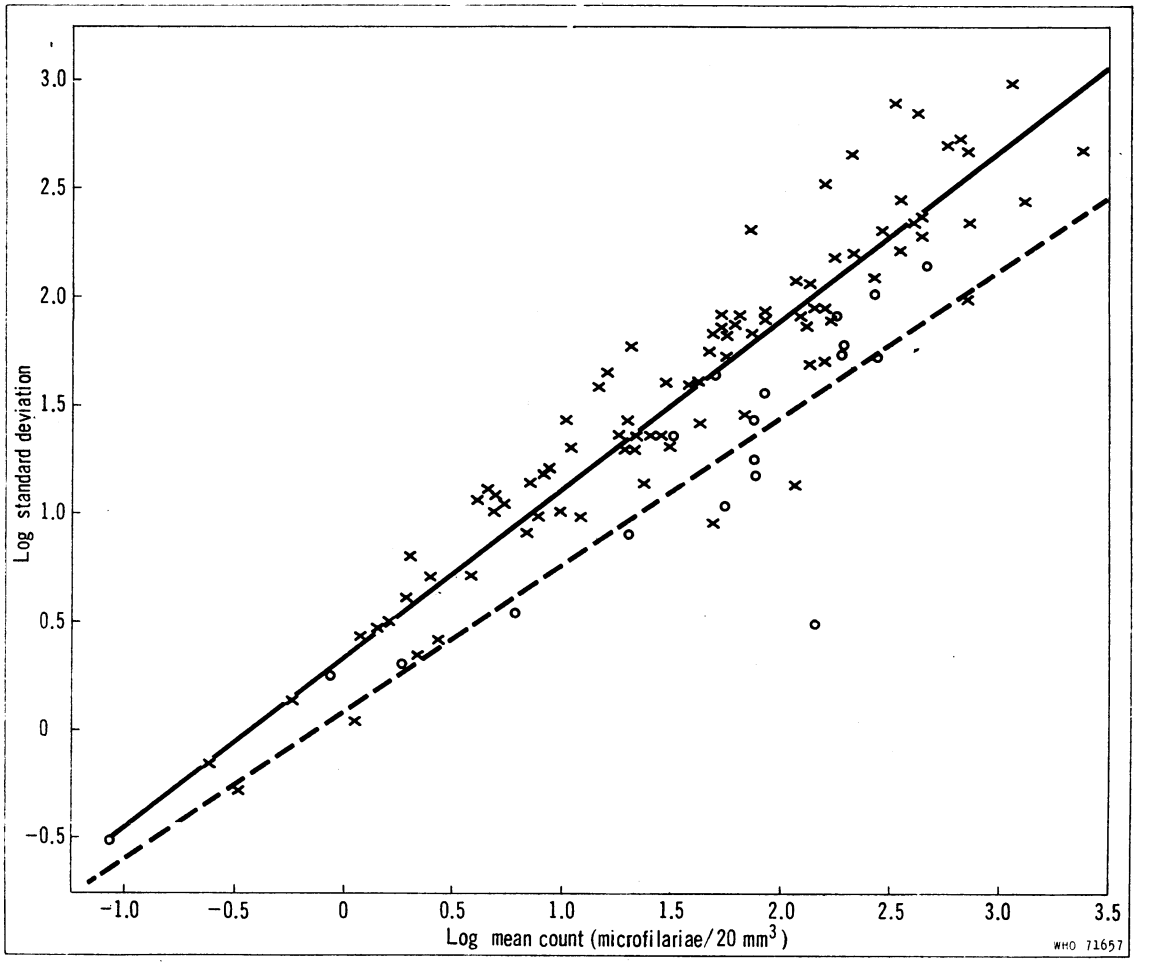
[a] Both lines are least squares regressions for their respective points.

*Variance caused by long-term fluctuations*

In the data on which the present series of papers is based, a very wide range of microfilarial counts from single individuals was observed. Detailed examination of the data shows that, as a general statement, the longer the time over which counts were obtained, the greater the range in microfilarial densities. This range is greater than that caused by the small amount of periodicity in this strain of *W. bancrofti*. If the prediction stated in the previous section is correct, a regression of log standard deviation on log mean count should show the same slope as the lines in Fig. 1 and 2, but the whole regression line would be expected to be still higher than either of the others. Comparable data are available from at least one other study on the Pacific strain of *W. bancrofti*. Edgar et al. (1952) examined a series of individuals each month for a year. A comparison of the two long-term studies is of interest (Fig. 3). Clearly, up to 4 ½ years, the longer the period over which the same individuals are examined, the greater the variance around each mean count. Of special interest here is the fact that the regression lines remain essentially

FIG. 3

RELATIONSHIP BETWEEN STANDARD DEVIATION AND MEAN DENSITY OF MICROFILARIAE OF SUBPERIODIC *W. BANCROFTI* IN TWO SETS OF LONG-TERM OBSERVATIONS[a]




×——× 90 subjects examined over a period of 4½ years. Data from present study.

o- - - -o 20 subjects examined monthly for 1 year. Data from Edgar, Beye & Mille (1952).

[a] Both lines are least squares regressions for their respective points.

parallel. The slopes, with 95% confidence limits, are 0.672 ± 0.151 for the data of Edgar et al. and 0.775 ± 0.055 for the present data.

The increase in variance with the time over which the samples were taken is best explained by the hypothesis that long-term trends exist in mean microfilarial density within any given individual. Moreover, it must be postulated that the trend covers a period longer than 12 months, since the increase in variance after that time would thus be explained. In the following section, the data will be examined for the existence of such a trend.

## LONG-TERM VARIATION IN MICROFILARIAL DENSITY

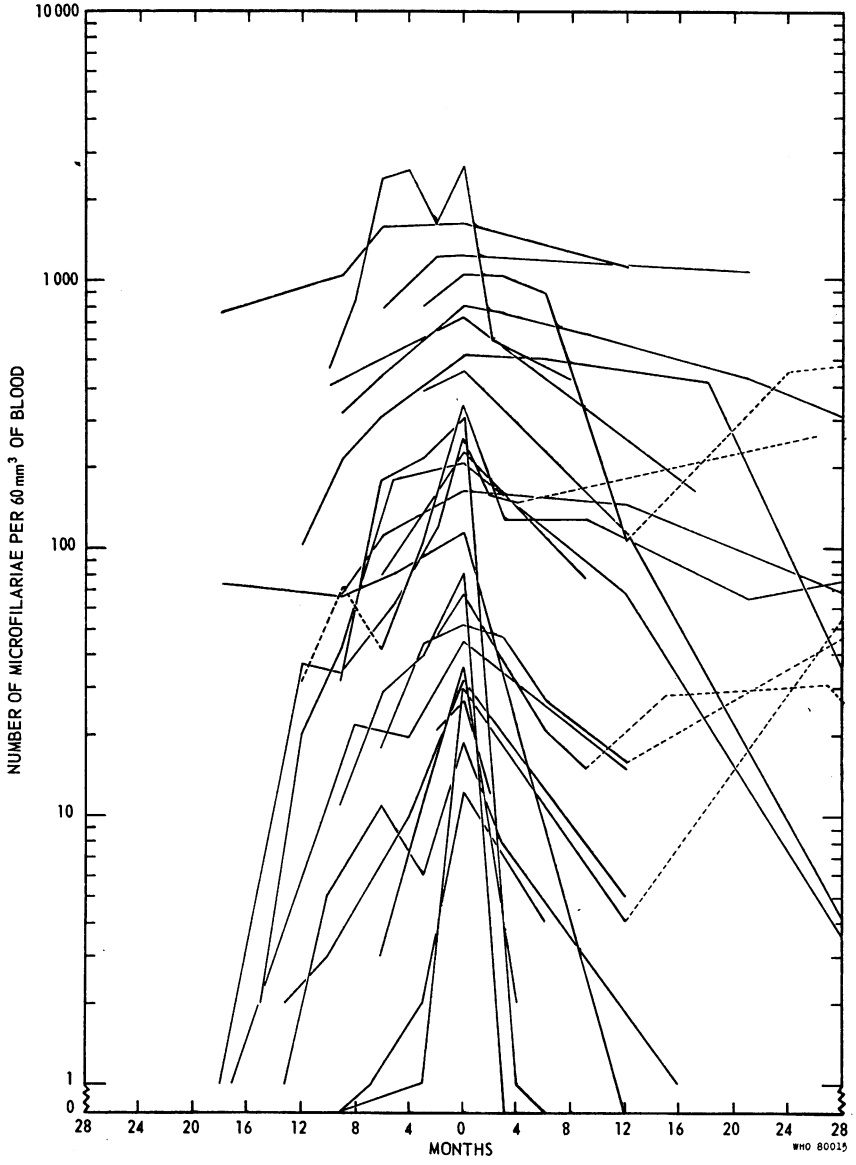
The postulation was made that significant trends must occur in microfilarial counts when these are made from the same individual over a period of from 1 to 4 ½ years. Examination of the detailed data from individual subjects shows that in many

cases the trend takes the form of a wave-like progression of counts with a definite peak. The peak is preceded by an increase in microfilarial density lasting from 3 to 24 months, and is followed by a decrease of somewhat shorter duration. No evidence was obtained to show that the peaks occurred during a particular season, and the pattern appears in persons of all ages and both sexes.

The general trend, which appears to be followed in most infected individuals, is best demontrated by placing peak counts at the same time and making linear connexions to earlier and later counts (Fig. 4).

FIG. 4

CHANGES IN MICROFILARIAL DENSITY IN 25 UNSELECTED CASES OF SUBPERIODIC *W. BANCROFTI* [a]




[a] The histories were moved in time so that the peak densities would coincide in the same month. Broken lines indicate additional peaks for the same individual.

A large number of complete patterns might be expected but the actual number is not great. Of 1217 people examined and not treated, 338 were positive on at least one examination. Of these, 76 were only examined once or twice, and could not possibly show the pattern described. Of the remaining 262, 21 had no more than 1 microfilaria per 60 $mm^3$ of blood on any examination. Thus, 241 cases are available for a realistic description of trends in microfilarial density. As might be expected, examination of many of these cases ended before the peak was demonstrable, or began after the peak had been reached. Other cases show more than one peak, as though two of the described patterns were overlapping.

Among the 241 available cases, 122 showed at least one peak in microfilarial density; 92 showed either a continuous rise or a continuous fall; 24 showed a decline followed by an increase. Only 3 failed to reveal any change to a value outside the 50% confidence limits of their mean counts.

The 25 cases shown in Fig. 4 indicate that in general the changes immediately before and after the peak are less marked than are the initial rise and final drop in microfilarial density, although there are exceptions. Making allowance for undetected overlaps, the entire pattern appears to require 2 to 4 years for its completion.

## DISCUSSION

The long-term trends in microfilarial density provide an explanation for the very great differences that are found when a survey is made of the human population. Since the peaks are not co-ordinated in time, any survey will reveal only a portion of the infected persons with microfilarial densities near their maxima. Other people will be found near the beginning or the end of the wave-like progression of microfilarial densities. Thus, the variation in counts taken at any moment will be much greater than the variation between peak counts for the same group of people. The point is important in any attempt to relate microfilarial counts to worm load, since only the peak counts would be relevant.

To account for the long-term pattern that has been described, we advance the hypothesis that the pattern reflects the reproductive activity of one or more female worms. This hypothesis requires a long average interval between mosquito bites resulting in reproductive female worms. Otherwise, there would be such a high proportion of overlapping infections that the trends described would be difficult or impossible to detect. The average time between such infective bites can be estimated from the average duration of an infection, estimated above as 2–4 years, and the proportion of cases showing evidence of overlapping infections. Among the 241 cases that could be analysed, 53, or a proportion of 0.22 showed more than a single wave of microfilarial density. The average time between bites leading to a positive condition, therefore, should be 2/0.22 to 4/0.22 or 9.1–18.2 years. The probability that any case will become positive in any one year should be the reciprocal of these values, or 0.11–0.055, which should approximate to the expected rate of cases becoming positive per year among the population. These calculations assume a random distribution of worms among people. This, however, is not true, as will be shown below. With a contiguous distribution, the above estimates of the rate of becoming positive would be too high. An independent method of estimating these rates, presented in Part II of this paper yields values of 0.037 to 0.074 per year. Thus, at least one prediction implied by the hypothesis is adequately confirmed. A similar pattern of changes in microfilarial density has been observed in at least one other species of filaroid worm, *Loa loa* (Duke, 1960). In experimentally infected monkeys, the " primary wave " of microfilarial density (Fig. 5) is remarkably like the one deduced here for subperiodic *W. bancrofti*, although in the case of *Loa*, the entire wave only occupies 9–22 weeks.

Duke's demonstration that it is the host's reaction, rather than declining reproduction of the worm, that causes the microfilariae to disappear from the circulating blood would require a major revision of our hypothesis were it not for two important facts. The first of these facts is that, in addition to the numerous cases that showed evidence of overlapping waves, a number of the cases among Samoans became positive again a few months after becoming negative. Duke's experimental transplantations show that such a phenomenon is absent from superinfections with the same strain of *Loa*. The second fact is that the time scale is 7–14 times as long in *Wuchereria* as in *Loa*. Thus, any host reactions in *Wuchereria* infections appears to have a different effect on the parasite.

In Part II of this paper, the peak counts are analysed for evidence that they can be used to estimate worm loads.


FIG. 5
MICROFILARIAL DENSITIES DURING TWO EXPERIMENTAL INFECTIONS WITH *LOA LOA* [a]

[a] Data replotted from Duke (1960). This figure should be compared with Fig. 4.

## II. ESTIMATION OF THE NUMBER OF REPRODUCTIVE FEMALE WORMS PER PERSON

If the hypothesis outlined in Part I, that fluctuations in the numbers of microfilariae in the peripheral blood of infected persons are a reflection of the reproductive history of one or more female parasites acquired simultaneously or during a short period, is correct, only those counts made at or very near the peak will be of use in relating microfilarial density to the number of worms present. The peak counts, then, should tend to occur as multiples of some number representing the maximum number of microfilariae produced by a single reproducing female worm. This will now be discussed.

### DISTRIBUTION OF PEAK COUNTS AMONG PEOPLE

#### *Adjustment for size*

Before the distribution of peak counts can be analysed for the existence of multiples of any number, the influence of the size of the host on the density of microfilariae must be considered. Assuming that the female worm does not respond to the size of her host, corrections must be made for the increased density in children. No data have been found on the growth of Polynesians, but Spector (1956) gives growth data for a number of races. Using average values for Asians, the size of each year-group of each sex was related to the size of adult males. These factors cannot be used directly because of the non-random distribution of larvae in the body (Hawking & Thurston, 1951; Rowlands, 1956) and the differential rates of growth of the various organs concerned. These factors were taken into account by using the average relative densities of microfilariae given by Hawking & Thurston for animals killed near the time of maximal circulation of the larvae and the weights of the different organs

at different ages given by Spector. The final corrections for counts from women and children, relative to adult men, are given in Table 1. It has been assumed that adult males weigh 55 kg and have 70 ml of blood per kg, giving a total volume of 3.85 litres of blood.

TABLE 1

CORRECTION FACTORS FOR MICROFILARIAL COUNTS IN WOMEN AND CHILDREN, RELATIVE TO COUNTS IN MEN [a]

| Age (years) | Correction factor | |
|---|---|---|
| | Males | Females |
| 1 | 0.208 | 0.203 |
| 2 | 0.276 | 0.263 |
| 3 | 0.329 | 0.306 |
| 4 | 0.351 | 0.341 |
| 5 | 0.391 | 0.368 |
| 6 | 0.422 | 0.397 |
| 7 | 0.447 | 0.428 |
| 8 | 0.474 | 0.455 |
| 9 | 0.499 | 0.477 |
| 10 | 0.524 | 0.521 |
| 11 | 0.549 | 0.586 |
| 12 | 0.586 | 0.638 |
| 13 | 0.633 | 0.681 |
| 14 | 0.700 | 0.723 |
| 15 | 0.767 | 0.763 |
| 16 | 0.833 | 0.802 |
| 17 | 0.896 | 0.832 |
| 18 | 0.945 | 0.853 |
| 19 | 0.976 | 0.872 |
| 20 | 0.997 | 0.895 |
| ⩾21 | 1.000 | 0.919 |

[a] Calculations based on relative distribution of larvae among organs at time of maximum circulation of larvae, and upon the relative rates of growth of the different organs.

*Systematic variations in peak count*

These corrections were applied to counts for women and children, and the data examined for maximal microfilaria counts in all series of examinations covering 9 months or more. Because of the change in abundance in microfilariae in individual people, the only maxima used were those occurring before March 1950. Cases in which two peaks occurred were considered to be overlapping infections, and both peaks had to be reduced. This was done by using the average slopes of observed changes in density (see below, Part III). An example of how overlapping data were handled will be given for a 33-year-old woman from the village of Failolo. The original counts (microfilariae per 60 mm³ of blood) and the counts corrected for body-size are given in the following tabulation:

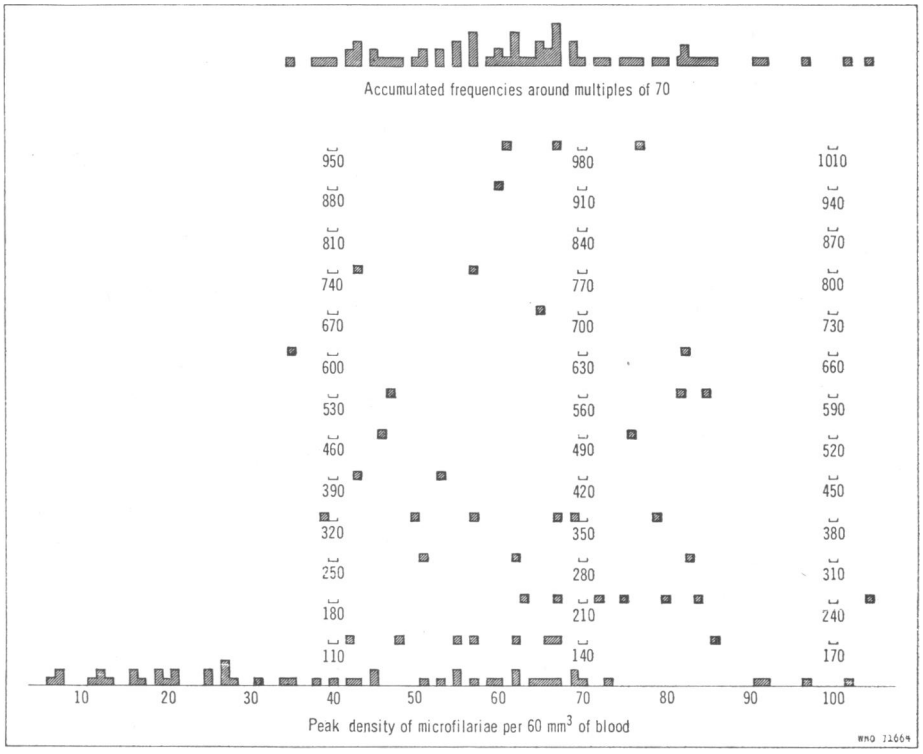
| | *Year and month of survey* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *1948* | | *1949* | | | *1950* | *1951* | *1953* |
| | *Sept.* | *Dec.* | *Mar.* | *June* | *Oct.* | *Mar.* | *Mar.* | *Mar.* |
| Original count | 83 | 189 | 209 | 96 | 167 | 122 | 121 | 204 |
| Count corrected for body size | 76 | 174 | 192 | 88 | 153 | 112 | 111 | 187 |

From the average slopes of increase it is estimated that the 153 larvae in October 1949 would have been reached from densities of 78 in March, or from 44 in December, 1948. This would leave, as a first approximation, 130 in December and 114 in March belonging to the earlier peak. Thus, December rather than March is considered to be the time of the actual peak. The 130 larvae in December would have declined to 26 by October, leaving 127 actually belonging to the second peak. These would represent an increase from 41 in December, leaving (as a second approximation) 132 as the likely magnitude of the first peak. The two peaks were recorded as 132 and 127, after making allowance for the overlapping infections.

When all of the corrections and adjustments were completed, groups of cases were found to be concentrated within the following ranges of density: 1–45, 51–73, 125–137, 203–214, etc. The group in the lowest range will be considered later; the remaining clusters suggest a series of multiples of 70.

The data are arranged so that a statistical test can be applied to this conclusion (Fig. 6). With 70 and its multiples lined up one above the other and made the midpoints of successive histograms up to the point where 68 of the 75 cases concerned are included, the accumulated distributions of cases around these midpoints can be tested for randomness of distribution. The top histogram in Fig. 6 represents the accumulated distributions around multiples of 70. If there were no tendency for peak counts to occur near multiples of 70, the histogram should show no particular shape, except perhaps a slight skewness to the lower numbers. Most of

FIG. 6
FREQUENCY DISTRIBUTION OF ALL PEAK COUNTS OF MICROFILARIAE EXCEPT 7 CASES WITH PEAK COUNTS OF MICROFILARIAE BETWEEN 1185 AND 2450 [a, b]




[a] Omitted for reasons of space.
[b] The figure is arranged to show the grouping around 70 and its multiples.

the counts do, however, occur near the middle of the distribution. This tendency is not likely to be accidental, as is shown by dividing the upper histogram arbitrarily into 5 parts of equal length and testing for randomness by the $\chi^2$ test (Table 2). Further to test the conclusion that peak counts represent a series of multiples of 70, the same method was applied to accumulated frequencies of other assumed peak counts, using multiples of counts from 30 to 100. The results are shown in Fig. 7. The large values of $\chi^2$ are clustered between 67 and 77, with the highest value at 71. One other piece of information has been added to the graph. This is an indication of those values for which the maximum number of cases fall in the centre of the histogram. Since this indicates that the cause of the non-randomness is likely to be related to a tendency for counts to accumulate around multiples of the central value, rather than to some other and

TABLE 2
DISTRIBUTION OF PEAK COUNTS AROUND MULTIPLES OF 70, FOR ALL PEAK COUNTS BETWEEN 35 AND 1015

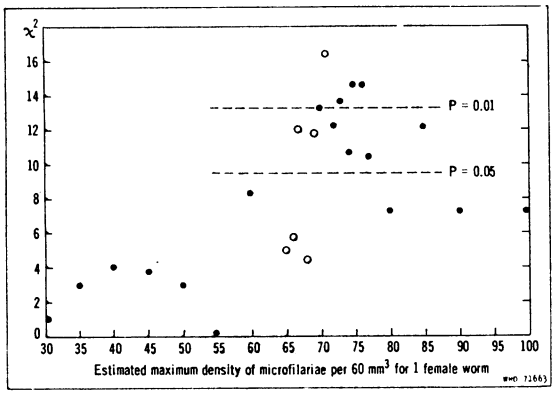
| Part of distribution | No. of cases: Observed (o) | No. of cases: Expected (e) | o−e | $\frac{(o-e)^2}{e}$ |
|---|---|---|---|---|
| Lowest 14 numbers | 15 | 13.8 | 1.2 | 0.104 |
| Next 14 numbers | 21 | 13.8 | 7.2 | 3.757 |
| Middle 14 numbers | 19 | 13.8 | 5.2 | 1.959 |
| Next 14 numbers | 9 | 13.8 | −4.8 | 1.670 |
| Highest 14 numbers | 5 | 13.8 | −8.8 | 5.612 |

$\chi^2 = 13.102$

[a] Counts from women and children corrected as described in text. Data are the same as in top histogram in Fig. 6.

FIG. 7

RELATIVE DEGREE OF NON-RANDOMNESS OF DISTRIBUTION OF PEAK COUNTS OF MICROFILARIAE AROUND MULTIPLES OF VARIOUS NUMBERS BETWEEN 30 AND 100 PER 60 mm³ OF BLOOD [a]




[a] Open circles indicate those distributions with maximum number of cases in centre of histograms in Fig. 6.

unexplained reason, more importance should be placed on values from 65 to 71 than would otherwise be the case. For this reason, and because the probable error of mean counts in this range is of the order of 10%, it seems best to use 70 as the basis for further calculations.

The method of analysis of the data does not permit a direct statement of the probability of obtaining by chance the pattern described. The following numerical experiments were therefore kindly conducted for us by the World Health Organization. Uniform random numbers were generated on the computer in the range between 0 and 1000, and by following the above procedure, apparent periodicities of the distribution were examined for possible peaks around each number between 30 and 100 and its multiples. The $\chi^2$ test was applied in the same way as shown above. Out of 200 experiments, each based on different series of random numbers, 11 yielded results that were comparable with those in our analysis. Inasmuch as this proportion of 0.055 is very close to the commonly acceptable limit of 0.05, we feel that the results are sufficiently encouraging to continue with the analysis. We emphasize that the numbers calculated should not be regarded without some caution. We maintain that this is preferable to making no estimates, as it seems that comparable data do not exist.

Thus, a single female worm normally reaches a peak output of microfilariae sufficient to produce a density of 70/60 mm³ of blood from skin-punctures in an average adult male host. Microfilarial output is not reduced as a result of any possible crowding effects up to a density of at least 14 female worms (Fig. 6). Thus for each person with microfilariae, from an adequate number of examinations a reasonably accurate estimate can be obtained of the number of actively reproducing female *W. bancrofti* present in the body.

For those cases for which reasonably accurate estimates can be made, this method gives a mean of 4.67 reproducing female *W. bancrofti* per infected person. The information becomes more interesting when the human population is divided into groups more or less homogeneous with respect to exposure.

## CHANGE IN PREVALENCE WITH AGE

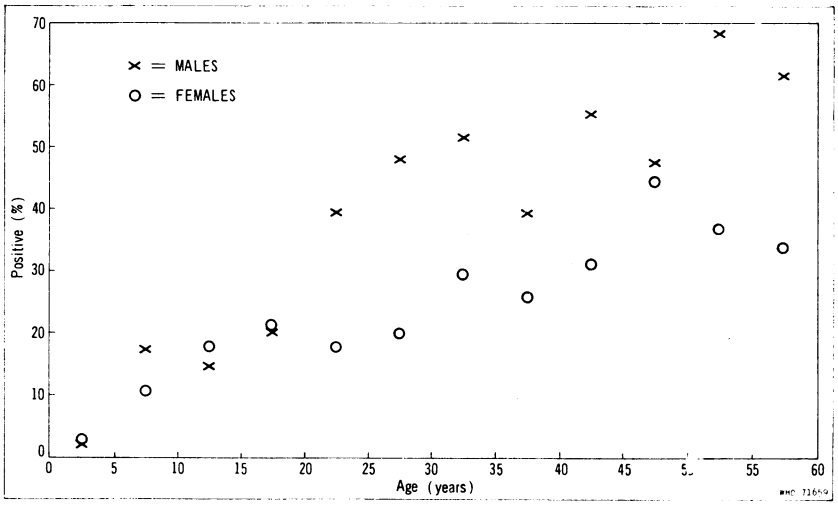
The prevalence data for endemic diseases seem most meaningful when presented separately for different age-groups. Since the age of each person examined was recorded, the present data can be presented in such a fashion.

Because of the timing of the various surveys, March is taken as the last month of the year; and all surveys made during a 12-month period were pooled. An individual found positive on any of the three or four surveys was considered positive in that year, but not necessarily in other years. This means that the years ending in March 1949 and March 1950 show slightly higher proportions positive than the single surveys of March 1951 and March 1953. This does not make an important difference in the calculations. Individual people, of course, move from one age to another in the successive samplings, and a strict check was maintained on this factor in calculating age-specific prevalence values. The details are given in Fig. 8. The 10- to 20-year-olds consistently failed to show a prevalence that would produce a monotonic curve over the whole range of ages. This phenomenon was investigated further by calculating the prevalences for each year of age up to 20, and for each year's survey. Data for males and females were so similar up to the age of 15 that they could be pooled (Table 3).

The sharp increase in prevalence after the age of 15 in men, and to a lesser extent after 20 in women, suggests a marked increase in exposure. Since transmission is high in the bush (Jachowski & Otto, 1952), the data reflect the likelihood that men spend more time in the bush or on the farms than do other

FIG. 8

RELATIONSHIP BETWEEN PREVALENCE OF *W. BANCROFTI* [a] AND AGE FOR BOTH SEXES OF SAMOANS




[a] Prevalence determined by presence of microfilariae in 60 mm³ of blood.

people, and that women spend more time there than do children. For these reasons, the population was divided into males aged 15 years and older, females 20 years and older, and a third group composed of boys up to 15 and girls and women up to 20 years. These three groups were then considered separately in estimating the numbers of reproducing female worms harboured.

TABLE 3

PREVALENCE OF *W. BANCROFTI* IN SAMOA [a]

| Age-group (years) | Children [b] | | | Age-group (years) | Adult males | | | Adult females | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | No. examined | No. positive | Percentage positive | | No. examined | No. positive | Percentage positive | No. examined | No. positive | Percentage positive |
| 0–2 | 29 | 0 | 0 | 15–19 | 163 | 34 | 20.9 | | | |
| 3–4 | 93 | 3 | 3.2 | 20–24 | 107 | 42 | 39.3 | 143 | 26 | 18.1 |
| 5–6 | 229 | 17 | 7.4 | 25–29 | 82 | 40 | 48.8 | 115 | 24 | 20.9 |
| 7–8 | 243 | 30 | 12.3 | 30–34 | 71 | 37 | 52.1 | 84 | 25 | 29.8 |
| 9–10 | 214 | 28 | 13.0 | 35–39 | 90 | 36 | 40.0 | 80 | 21 | 26.3 |
| 11–12 | 182 | 25 | 13.7 | 40–44 | 59 | 33 | 55.9 | 91 | 29 | 31.9 |
| 13–14 | 174 | 37 | 21.3 | 45–49 | 50 | 24 | 48.0 | 51 | 23 | 45.1 |
| 15–16 | 64 | 12 | 18.8 | 50–54 | 58 | 40 | 69.0 | 32 | 12 | 37.5 |
| 17–18 | 62 | 13 | 21.0 | 55–59 | 8 | 5 | 62.5 | 26 | 9 | 34.6 |
| 19 | 26 | 6 | 23.1 | 60–64 | 27 | 17 | 63.0 | | | |

[a] Values are given separately for the three subsets of the population, as described in the text. Data from all surveys have been combined.

[b] Including males under 15 years and females under 20.

## ESTIMATION OF THE NUMBER OF REPRODUCING FEMALE WORMS PRESENT

### *Different age and sex groups*

From a previous section, it appears that a single female worm is capable of producing enough microfilariae to raise the density to a maximum of 70/60 mm$^3$ of peripheral blood. The number of larvae produced per female seems unaffected by increases in the number of females present. In order to estimate the number of actively reproducing female worms in a person, it is necessary only to make the corrections for body-size and for any overlapping, and divide the peak counts by 70. A clearly discernible peak must be preceded and followed by lower counts within 6 months at most. These restrictions reduce the number of cases that can be analysed to 51 males 15 years old and older, 38 females 20 years old and older, and 22 of the remainder of the population.

Among blood-positive adult men, the average parasite load estimated in this way is 5.65 female worms; among blood-positive women the load is 4.74, and among children it is 2.27. Account was taken of cases where repeated infections were apparently overlapping; these were recorded as separate infections.

In making these estimates, it became apparent that the sequence of dates on which the population was examined had imposed a bias on the estimates of average worm load. Under the conditions stated above for establishing the height of a peak density of microfilariae, four of the examinations were made at times that precluded the possibility of their use as peak counts. This left only counts taken during a 10-month period, and meant that the lighter infections of short duration would be more likely to reach a peak during that time than would the heavy infections. Therefore, the estimates are likely to be biased downwards. This bias can be partly, but not completely, overcome by considering all peaks, regardless of the interval between observations. This procedure yields estimates of 6.10 actively reproducing female worms per blood-positive adult male Samoan, 4.47 per positive adult female, and 2.36 per positive child. These estimates can legitimately be raised by taking into account those blood-positive people for whom the exact height of the peak cannot be demonstrated because the maximum count found was either not preceded or not followed by another count. When estimates of worm load based on these maxima are included, the average calculated worm loads increase to 6.91 per man, 6.07 per woman and 2.93 per child. The distributions of worms among positive people are given in Table 4. These estimates may be considered minimal for the reasons given above.

### *Villages with different rates of transmission*

The distributions shown in Table 4 cover all available data for Samoa. As Jachowski & Otto

TABLE 4

DISTRIBUTION OF ESTIMATED NUMBERS OF ACTIVELY REPRODUCING FEMALE *W. BANCROFTI* AMONG BLOOD-POSITIVE PEOPLE IN THREE SUBSETS OF THE POPULATION

| No. of worms per person | Number of cases among: | | |
|---|---|---|---|
| | Adult males (≥15 years) | Adult females (≥20 years) | Children (both sexes) |
| 1 | 35 | 21 | 33 |
| 2 | 23 | 10 | 13 |
| 3 | 26 | 8 | 6 |
| 4 | 7 | 4 | 0 |
| 5 | 10 | 1 | 4 |
| 6 | 6 | 5 | 2 |
| 7 | 8 | 3 | 0 |
| 8 | 8 | 2 | 4 |
| 9 | 3 | 5 | 1 |
| 10 | 3 | 1 | 1 |
| 11 | 3 | 2 | 1 |
| 12 | 2 | 1 | 1 |
| 13 | 2 | 0 | 1 |
| 14 | 2 | 4 | 0 |
| 15 | 1 | 0 | 0 |
| 16 | 0 | 0 | 0 |
| 17 | 3 | 0 | 0 |
| 18 | 0 | 1 | 0 |
| 19 | 1 | 0 | 0 |
| 20 | 1 | 0 | 0 |
| 21 | 2 | 0 | 0 |
| 22 | 1 | 0 | 0 |
| 23 | 1 | 1 | 0 |
| 24 | 3 | 0 | 0 |
| >24 | 6 | 3 | 0 |
| Total | 157 | 72 | 67 |

(1952) have pointed out, prevalence and mean microfilarial density were higher in certain of the villages than in others. Among villages large enough to yield statistically valid data, Vaitogi had the lowest prevalence (45.2% for men, 30% for women and 15.8% for children). Tula and Masausi had the highest rates (50%–58% for men, 39%–51% for women, and 24% each for children, respectively). It is therefore of interest to examine the estimates of worm loads and their distributions for the two situations. The results are given in Table 5. Because of the smaller populations analysed, only males 15 years old and older are shown; blood-positive women and children were not numerous enough to give meaningful data. In Vaitogi, the average positive male had 5.93 female worms, while in Tula and Masausi combined, the average was 8.32.

TABLE 5

DISTRIBUTION OF ESTIMATED NUMBERS OF ACTIVELY REPRODUCING FEMALE *W. BANCROFTI* AMONG ADULT MALES IN TWO SITUATIONS DIFFERING IN INTENSITY OF TRANSMISSION

| No. of worms per person | No. of cases among adult males in: | |
|---|---|---|
| | Vaitogi (lower transmission) | Tula + Masausi (higher transmission) |
| 1 | 8 | 6 |
| 2 | 6 | 4 |
| 3 | 6 | 6 |
| 4 | 1 | 0 |
| 5 | 2 | 4 |
| 6 | 4 | 0 |
| 7 | 3 | 3 |
| 8 | 5 | 1 |
| 9 | 2 | 1 |
| 10 | 1 | 1 |
| 11 | 1 | 0 |
| 12 | 1 | 0 |
| 13 | 1 | 0 |
| 14 | 0 | 1 |
| 15 | 0 | 0 |
| 16 | 0 | 0 |
| 17 | 0 | 2 |
| 18 | 0 | 0 |
| 19 | 0 | 1 |
| 20 | 0 | 0 |
| >20 | 2 | 4 |
| Total | 43 | 34 |

## DISCUSSION

If it were possible to confirm the conclusion that a single female *W. bancrofti* is capable of producing enough microfilariae to raise their density to 70 per 60 mm³ of blood, the conclusion would have important implications for studies of the dynamics of transmission, as well as for estimates of worm burden such as those that we have made. Therefore, confirmatory evidence from any source is worth examining.

Although direct evidence from independent sources is not available, it is nevertheless possible to consider whether the estimate is realistic. At least two approaches are available. The first of these, which will be considered in detail below, is to consider the reproductive capabilities of other parasitic nematodes of comparable size. It will be shown that *W. bancrofti*, according to the conclusions drawn, produces larvae at about the same rate as hookworms produce eggs, when the reproductive products are put in terms of percentage of the parent's volume per day.

The second approach is to consider the effects of natural selection in arriving at an optimal larval output per female worm. It may be considered axiomatic that, within physiological limits, natural selection will have operated to bring about the maximal average reproductive success per female worm. This average reproductive success is dependent upon survival through the mosquito, and it has been shown repeatedly in mosquito feeding experiments that both high and low densities of microfilariae are deleterious to success in producing third-stage larvae. From among the numerous studies, we have selected those of Rosen (1955) as most pertinent, since the same vector (*Aedes polynesiensis*) and the same strain of *W. bancrofti* were used as are concerned in the present study. The factors reflecting the average success of larvae may be listed as follows:

(1) the proportion of mosquitos infected after a blood meal;

(2) the survival of mosquitos after feeding;

(3) the survival of larvae in infected mosquitos;

TABLE 6

INFLUENCE OF MICROFILARIAL DENSITY UPON EFFECTIVENESS OF TRANSMISSION OF SUBPERIODIC *W. BANCROFTI* BY *AEDES POLYNESIENSIS*

| No. of microfilariae per 60 mm³ of blood | Proportion of mosquitos infected (*a*) | Proportion of mosquitos surviving to development of 3rd-stage larvae (*b*) | No. of 3rd-stage larvae per mosquito/No. of larvae in 3 mm³ of blood (*c*) | Relative contribution (i.e. $a \times b \times c$) |
|---|---|---|---|---|
| 1-3 | 0.08 | 0.65 | 1.25 | 0.065 |
| 4-15 | 0.33 | 0.65 | 2.31 | 0.495 |
| 16-30 | 0.65 | 0.65 | 1.62 | 0.684 |
| 31-60 | 0.80 | 0.59 | 1.53 | 0.722 |
| 61-150 | 0.88 | 0.54 | 1.27 | 0.604 |
| 151-300 | 0.90 | 0.47 | 0.82 | 0.347 |
| 301-600 | 0.95 | 0.41 | 0.65 | 0.253 |
| ⩾601 | 1.00 | 0.22 | 0.35 | 0.077 |

[a] Calculations made from data in Rosen (1955).

(4) the ratio between the density of larvae in the blood and the average number carried by a mosquito which has fed on that blood.

Rosen showed that all these factors were influenced by the density of larvae in the blood. At low densities of larvae (1–15/60 mm³), few mosquitos became infected; at high densities (more than 150/60 mm³), both survival of mosquitos and the ratio of larvae reaching the third stage of development to the number presumably ingested were low. The details are given in Table 6. The average success of larvae through the mosquito phase of the life-cycle is 10 times as great for intermediate densities as for either high or low densities. The densities of microfilariae at which survival through the mosquito is greatest are those which are estimated to be achieved by 1 or 2 female worms. From these considerations based on natural selection, it would be expected that reproduction would be adjusted to produce the frequency of densities at which transmission would be optimal. From Table 4, it can be seen that 30% of all positive cases are estimated to be carrying single mated females, and that nearly half carry either 1 or 2 mated females. Thus, the rate of reproduction leading to the most efficient mosquito transmission is such that the maximum number of effective human carriers is maintained, and expectations based on natural selection also indicate that the estimates are realistic.

It must be emphasized that neither source of evidence demonstrates the *accuracy* of the estimates that we have made. They could only indicate serious discrepancies, probably of the order of a factor of 2–5. More accurate checks must be obtained from other sources.

## III. REPRODUCTIVE HISTORY OF FEMALE WORMS

This part of the paper is concerned with the details of changes in microfilarial density which have been interpreted as reflecting the reproductive history of single infections. The analysis leads to a prediction of the patent period of *W. bancrofti* infections, and is important in making estimates of reproduction by female worms.

Improvements can be made in the understanding of the epidemiology of helminth parasites by applying the principles and methods of population ecology to the parasite population (Beye & Gurian, 1960; Hairston, 1962, 1965a; Macdonald, 1965). Such an approach requires a knowledge of the age-specific reproduction and mortality rates. It is these rates

which can be deducted from analysing the changes in microfilarial density.

## DURATION OF THE PATENT PERIOD

Two methods are available for estimating the average time during which a person remains blood-positive as a result of a single infection: direct observation, and the rates of increase and decrease in microfilarial density.

Direct observation requires the selection of those individual records that show a complete or nearly complete history of a rise and decline in microfilarial density. Records in which the peak counts remained below 10 microfilariae per 60 mm³ were disregarded, as were those showing more than a single peak density, unless the peaks were separated by counts lower than 10. These criteria permitted the use of 34 cases in which it was possible to state the minimum duration, and 5 cases in which the maximum duration could be stated with confidence. The distributions are shown in the following tabulation:

| | *Months* | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *6* | *8* | *9* | *12* | *14* | *15* | *18* | *21* | *24* | *27* | *30* | *54* |
| No. of cases lasting longer than indicated months | 2 | 1 | 1 | 7 | 1 | 2 | 6 | 3 | 1 | 3 | 7 | |
| No. of cases lasting less than indicated months | | | | 1 | | | | | | 1 | 2 | 1 |

The average of those cases in which the minimum duration is known is 18.9 months, and the longest minimum is 30 months. The average of the cases where the maximum is known is 30.6 months. Except for one case of 12 months' duration, these all lasted 27 months or more. It can be estimated from this approach that the duration of the patent period is of the order of 2–2 ½ years. This estimate is believed to be somewhat low as an over-all average. In the first place, the duration of the records would cause more prolonged cases to be rejected as not being complete enough to use; in the second place, most of the cases that could be used were light infections, which would be expected to remain positive for a shorter time than heavy infections. The error, however, is not likely to be very great, especially from the second cause. The rate at which people became negative after being removed from endemic areas indicates an average annual loss of 0.30–0.33 of the positive cases, if the assumption is permitted that the rate of loss is constant. The average duration of a positive case under these conditions can be calculated as 2.5–2.9 years.

Estimation of the patent period from changes in microfilarial density has the advantage of using a larger number of cases, but is somewhat more involved than the direct method, and requires several steps.

*Rates of increase in microfilarial density*

In order to discover if there were changes in the rate of increase in density of microfilariae, the data were analysed by taking the peak counts as starting-points and working backwards for successive 2- or 3-month periods. The ratio of increase from 2 or 3 months before the peak up to the peak was calculated for all available cases; then the 2- or 3-month period beginning 4 or 6 months before the peak was analysed in the same way, and finally the ratio of increase was calculated for any period known to have ended 6 months or more before the peak count. Cases in which either count was less than 10 microfilariae per 60 mm³ were rejected because of the large proportional error of such counts. The slopes of these increases were calculated from the equation:

$$\log N_t = \log N_0 + xt \qquad (1)$$

where $N_t$ is the number present after $t$ months, $N_0$ is the number at the beginning of the period, and $x$ is the slope. The monthly ratio of increase, or the factor by which the average count increased each month, is calculated as the antilog of $x$. The results of the calculations are given in Table 7. It can be seen that the density increases faster early in the patent period than it does in the 6 months immediately before the peak is reached. It is concluded that the worm starts reproduction by increasing its microfilarial output at a rate which gives

TABLE 7

AVERAGE RATES OF INCREASE IN MICROFILARIAL DENSITY AT VARIOUS PERIODS BEFORE THE TIME OF THE PEAK COUNT

| Number of months before peak | No. of cases | Slope | Ratio of increase per month |
|---|---|---|---|
| 2-3 | 54 | 0.088 | 1.23 |
| 4-6 | 31 | 0.086 | 1.22 |
| >6 | 16 | 0.124 | 1.404 |
| Uncertain, probably >6 | 75 | 0.152 | 1.404 |
| | | 0.147 | |

a slope of 0.147 per month, and then slows down to a rate that gives a slope of 0.087 per month for 6 months.

In order to reach the peak density of 70 microfilariae per 60 mm³ at a rate of increase which gives a slope of 0.087 per month, the density 6 months before the peak would be expected to be 20.6/60 mm³. Prior to reaching this density, the slope of the increase is 0.147 per month. To reach a density of 20.6 from the lowest detectable density of 1/60 mm³ would require 8.95 months. These calculations were made using equation (1).

*Rates of decrease in microfilarial density*

The decline in microfilarial density after the peak count was analysed in the same way as the increase up to the peak. These is no trend in the data at all, and the average rate of decrease for 42 cases was 0.161 per month. However, these calculations could not make use of the numerous cases that declined to zero or to very low counts, since the equation used to obtain the rate of decrease cannot be used for zero values. The equation is

$$l_t = e^{-at}, \qquad (2)$$

where $l_t$ is the proportion remaining at $t$ months and $a$ is the rate of decrease per month. There are 31 cases in the records in which this decline to zero occurred, with the time element relative to the peak count reasonably certain. The very rapid decline started at least 9.8 months and not more than 17.2 months after the peak count.

In addition to the 31 cases that dropped to zero, there are 15 in which an equally sudden decline took place, but to counts of various magnitudes. Therefore, many of the negative persons had some microfilariae left, but at densities below the threshold of discovery. The average exponential rate of decrease among the 15 cases was 0.757 per month.

This increased rate of loss of microfilariae from the blood is interpreted as occurring after the female has stopped producing larvae. If this is really the case, the mean length of life of microfilariae in the blood can be calculated as

$$1/(1-e^{-0.757}),$$

or 1.88 months, or 56.4 days. This estimate is much closer to the observation of 70 days made by Rao (1933) than to the estimate of 14 days made by Knott (1935).

A rapid decline in microfilarial density took place between 9.8 and 17.2 months after the occurrence of the peak count. Because of the timing of the surveys, the maximum estimate is more subject to error than is the minimum. For purposes of further calculation, therefore, 12 months represents a good estimate of the duration of the post-peak reproduction.

Further use of equation (2) can be made to obtain the density ($X$) of larvae expected from an infection with a single reproducing female worm at the end of its reproductive life, 12 months after the peak density of microfilariae is reached.

$$(X/70) = e^{-(0.161\times 12)},$$
$$X = 10.15 \text{ microfilariae/60 mm}^3.$$

The time required for this to be reduced to the threshold of detectability, 1/60 mm³, can be calculated as

$$1/10.15 = e^{-0.757t};\ t = 3.06 \text{ months}.$$

The duration of the blood-positive period for an infection with 1 mated female worm can now be estimated from all of the average rates of increase and decrease:

| *Period* | *Duration (months)* |
|---|---|
| First detectability to 6 months before peak density | 8.95 |
| Remainder of time to peak | 6.0 |
| Survival of female after peak | 12.0 |
| Decline of microfilariae to limit of detectability | 3.06 |
| | 30.01 |

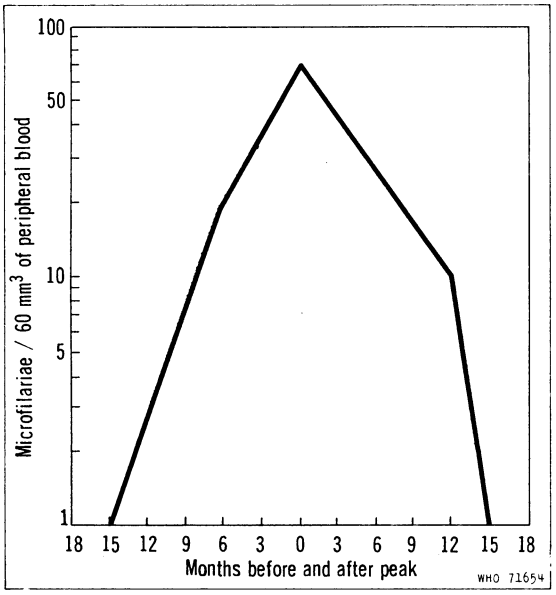
This, of course, would be the minimum normal patent period, since infections with more than 1 worm would be detected earlier and would also require longer for the microfilariae to disappear. Men over 30 years of age have the highest average number of reproducing female worms of any part of the population, averaging 10.61 per blood-positive man. Such an infection would be detectable on blood examination for 39.91 months. Thus, the patent period, estimated from changes in density of microfilariae, is 2.5–3.3 years, depending on the number of female worms present. These estimates are in excellent agreement with both of those given above. The history of blood examinations from such a hypothetical average case, infected with 1 female worm, is shown in Fig. 9.

## DISCUSSION OF THE REPRODUCTIVE HISTORY

These estimates are fundamental to all that follows, and they must be compared with the conclusions of other workers, and an attempt be made to explain any contradictions with the literature bearing on the subject.

FIG. 9

REPRODUCTIVE HISTORY OF SUBPERIODIC *W. BANCROFTI*, SHOWING DENSITY OF MICROFILARIAE IN THE PERIPHERAL BLOOD DURING THE PATENT PERIOD [a]




[a] An average case carrying 1 mated female worm is shown.

Microfilarial periodicity and the reproductive history of filarial worms have been the subjects of a large amount of research over the past 15 years (Hawking & Thurston, 1951; Edgar et al., 1952; Rosen, 1955; Jachowski & Otto, 1955; Hawking, 1956; McFadzean & Hawking, 1956; Rowlands, 1956; McCarthy, 1956; McCarthy & Fitzgerald, 1956; Edeson & Buckley, 1959; Edeson, 1959a, 1959b; Jordan, 1960; Duke, 1960; Hawking et al., 1964; Wong, 1964).

*Relevance of periodicity*

With regard to microfilarial periodicity, very nearly all of the available data support the findings of Hawking that the larvae accumulate in the lungs when they are rare in the peripheral blood, and that the daily cycle is produced by the liberation of this reservoir into the general circulation. The only really contradictory data are those showing that the examination of 1 ml of venous blood reveals about as many positive cases as the examination of 20 mm³ of blood obtained by skin-puncture, and fewer cases than the examination of 60 mm³ of blood obtained by skin-puncture (Amos, quoted by Kessel, 1957). As already indicated, a somewhat contradictory consideration comes from the problem faced by the microfilariae in passing through the capillaries. Although Hawking & Thurston were unable to observe any disturbance of red cells in arterioles and capillaries until circulation slowed before the death of the host, the easy passage of larvae through the capillaries is difficult to visualize, to say nothing of the simultaneous passage of microfilariae and red blood cells. As a matter of fact, the data of Hawking & Thurston suggest that even after the reservoir in the lungs is released into the general circulation, the capillaries retard the flow of larvae, leaving a disproportionatally small fraction in the larger blood lessels.

Thus, they estimated that 10.6%–38% (average 23.9%) of the larvae were in the lungs at night, and that 11.2%–38.5% (average 27.1%) were in the rest of the tissues. Both these percentages are excessive for an assumption of randomness of distribution of larvae over the total blood volume, although the estimates are almost certainly minimal, at least for the lungs, since there was evidence of the release of a large number of microfilariae from the lungs immediately before death.

Estimates of the average distribution of blood in different parts of the circulatory system indicate 15% for the entire pulmonary circulation, and 6% for the capillaries and arterioles in the rest of the body (Guyton, 1959). Hawking & Thurston quote estimates of 7.8%–9.1% for the fraction in the capillaries of the lungs of dogs, and 5%–6% for monkeys. Assuming a large value within this range, at most 15% of the total blood volume would be in all capillaries taken together. Hawking & Thurston estimate that of the larvae found in tissues, an average of 51% were seen in capillaries and other vessels less than 25 $\mu$ in diameter. Since an average of 49.1% of the total estimated larvae were in the lungs and other tissues, a minimum of 51% of 49.1%, or 25%, of the larvae were in a maximum of 15% of the blood at a time when they were supposedly distributed randomly through the blood-stream. From their ratios of microfilarial densities in right and left ventricles, minimum estimates of the larvae escaping the lungs immediately before death can be calculated, and the 25% should be raised to 30%. At least twice as many larvae seem to be in the arterioles and capillaries as there should be for an assumption of randomness of distribution. For the lungs alone, using the data of Hawking & Thurston, at least 16.5%

of the larvae are in 7.8%–9.1% of the blood; this leads to an identical conclusion—that, even during the nightly increase in larval density in peripheral blood, larvae are held up in arterioles and capillaries throughout the body. The minimum ratio of densities (approximately 2 : 1) is that found by Yorke & Blacklock (1917) for night blood in periodic *W. bancrofti*, is more than the 1.1 : 1 shown in Hawking & Thurston's graph for *Dirofilaria* sp. in a monkey, and is much less than the 50 : 1 deduced from Amos' statements.

### ESTIMATION OF THE NUMBER OF LARVAE PRESENT

Although the hypothetical synthesis of data from various sources seems plausible, there remains a serious problem in attempting to estimate the number of microfilariae in the body from samples of either venous blood or that obtained from skin-punctures. The problem is complicated by the conclusion mentioned earlier that it makes a great deal of difference whether the blood comes from the arterial or the venous side of the capillary bed. If half comes from each side, and the density of larvae on the arterial side is twice as great as that on the venous side, then finger-punctures will give a density 1.5 times that found in venous blood. This value is a fair compromise between the observations cited above, with the sole exception of that of Amos. This point is discussed again below.

In order to calculate the number of larvae in the body, it is necessary to translate the ratios deduced above. If blood from finger-puncture contains 1 microfilaria per 60 $mm^3$, the density would be two-thirds of this in the 85.5% of the blood that is not in arterioles and capillaries. For a man weighing 55 kg, with 70 ml of blood per kg, there would be

$$0.66 \times (0.855 \times 3.85 \times 10^6)/60 = 36\,000$$

microfilariae in the heart, veins and arteries. In the 14.5% of the blood in arterioles and capillaries there would be

$$1.33 \times (0.145 \times 3.85 \times 10^6)/60 = 12\,400$$

microfilariae.

The total microfilariae in the body would thus be on the order of 49 000. The discrepancy between the proportional distribution calculated above and the estimate of approximately equal numbers in organs and peripheral blood made by Hawking & Thurston can be accounted for by the vessels in the organs with a diameter larger than 25 $\mu$, which contain about half of the larvae in the organs. It should be noted that higher densities in the tissues would raise the ratio between finger-puncture and venous blood above the 1.5 estimated above. This could only be counteracted by assuming that a larger fraction of the finger-puncture blood came from the venules.

As a very crude check on these calculations, it can be estimated from the data of Hawking & Thurston that among their dogs and monkeys the total larvae per living female *Dirofilaria* recovered were 3.73 million (dog 1), 0.59 million (dog 9), 8.97 million (dog. 4) and 4.31 million (monkey 185). Both extremes are questionable, as dog 9 was killed early in the infection while microfilarial counts were still rising, and dog 4 was autopsied 44 hours after death, and the number of adult worms recovered may be less accurate than for the other animals. We have estimated that a single female *W. bancrofti* produces sufficient larvae to raise the density in skin-puncture blood to 70/60 $mm^3$. This would make an estimated 70 × 49 000, or 3.43 million larvae as the maximum standing crop per female. This value agrees surprisingly well with estimates for *Dirofilaria*, made from the data of Hawking & Thurston.

In connexion with the proposed relationship between worm loads and microfilarial density, the work of Wong (1964) is important, and must be considered here. Her results show a rapid response to artificial alterations in microfilarial density, and this is interpreted as indicating that the female worms adjust their rate of reproduction to the density of microfilariae. Such a phenomenon would upset the present analysis were it true at all levels of density of females, and were it true of *W. bancrofti*. The rapidity of response can be accounted for easily from Hawking's studies. With a large reservoir of larvae in the arterioles and capillaries, considerably less than one-fourth of the microfilaria population would have been removed at each transfusion. Thus, a relatively small shift from reservoir to general circulation would restore the microfilarial density in the latter. The number of adult worms present in the experimental animals is not stated, but the microfilarial counts in the peripheral blood are extraordinarily high, barely overlapping the highest counts in the animals studied by Hawking & Thurston. Thus, the number of adult females must have been great—almost certainly enough to make up the loss of 5.6–27 million larvae weekly without undue assumptions about reproductive

capacity. For example, the animal infected with *Brugia pahangi* had a density of 31 microfilariae per mm³, and had received 458 infective larvae. If only 13% survived (the average recovery for *B. malayi* in cats, according to Edeson & Buckley, 1959) and half were females, there would have been 30 females; with the highest recovery rates of Edeson & Buckely (49%), there would have been more than 100 females in the animal. These values indicate that an increase in production of as little as 8000 larvae per female per day would have been required to make up the weekly loss of 5.6 million. In a succeeding section of this paper, we estimate the daily production of 75 000 microfilariae by each female *W. bancrofti*. Thus, an increase in reproduction of as little as 10% could account for Wong's results. Even so, the unnatural loss of larvae was made up, implying that before the experiments the females were reproducing at less than capacity. It is our interpretation that this phenomenon occurs only at high densities of female worms and of microfilariae, and does not reflect the situation at lower and more usual densities. There can be little selective advantage to the parasite in only reducing microfilarial densities to the levels shown in Wong's data, since the survival of mosquitos is low to nil when they have fed on hosts carrying densities of larvae such as those used in her experiments; moreover, the proportion of available larvae surviving to the infective stage in the remaining mosquitos is also very low under these conditions (Rosen, 1955; Wharton, 1957). Hence, regulation of microfilarial output by the adult females would appear to be unusual under natural conditions.

## NUMBER OF MICROFILARIAE PRODUCED

The number of microfilariae in the blood at any moment represents the opposing factors of rate of output by the female worm or worms and the death rate of microfilariae in the body. The latter has been estimated above as amounting to an exponential rate of 0.757 per month. The finite loss rate would be $(1-e^{-0.757})$, or 0.531 per month. The number of microfilariae produced in any one month ($M_t$) can be calculated from the difference between successive counts of larvue ($N_t$) and from the finite rate of loss of larvae ($a$) by the following expression:

$$N_{(t+1)} - N_t + a \times (N_{(t+1)} + N_t)/2 = M_t. \quad (3)$$

Algebraically, this simplifies to the following expression:

$$N_{(t+1)}(1 + \tfrac{1}{2}a) - N_t(1 - \tfrac{1}{2}a) = M_t \quad (4)$$

or

$$1.266\, N_{(t+1)} - 0.735\, N_t = M_t.$$

The result must be multiplied by 49 000, the number of larvae in the body of a mature man when the density is 1/60 mm³ of peripheral blood. The results of these calculations are given in Table 8.

TABLE 8

CALCULATED MICROFILARIAL OUTPUT BY 1 FEMALE *W. BANCROFTI* DURING THE PERIOD WHEN THE INFECTION IS DETECTABLE BY EXAMINATION OF 60 mm³ OF PERIPHERAL BLOOD

| Microfilarial output before peak density | | Microfilarial output after peak density | |
|---|---|---|---|
| Month before peak | Microfilarial output | Month after peak | Microfilarial output |
| 15 | 50 814 | 1 | 1 138 951 |
| 14 | 73 301 | 2 | 1 007 834 |
| 13 | 98 586 | 3 | 867 297 |
| 12 | 142 880 | 4 | 710 990 |
| 11 | 197 615 | 5 | 617 299 |
| 10 | 284 494 | 6 | 520 734 |
| 9 | 403 271 | 7 | 441 339 |
| 8 | 534 341 | 8 | 380 680 |
| 7 | 751 949 | 9 | 325 850 |
| 6 | 862 695 | 10 | 277 833 |
| 5 | 1 017 688 | 11 | 227 803 |
| 4 | 1 266 203 | 12 | 204 673 |
| 3 | 1 521 794 | | |
| 2 | 1 905 262 | | |
| 1 | 2 253 505 | | |

| | |
|---|---|
| Total detectable microfilariae | 18 085 181 |
| Estimated No. before detectability | 94 438 |
| Total microfilariae produced | 18 179 619 |

A female worm is thus estimated to be capable of producing $1.82 \times 10^7$ microfilariae during its life. At the maximum rate of production, it releases 75 128 microfilariae per day, a number that is consistent with other parasitic nematodes of comparable size. Thus, at a size of 90 mm by 0.25 mm, the volume of a female, calculated as a cylinder, would be 4.42 mm³. If the larvae are $270 \times 8.5$ $\mu$, and 75 128 are produced daily, this would amount to 1.15 mm³, or 26% of the volume of the adult

female. The range for different quoted sizes of females and larvae is from 16% to 39%. Hookworms have a volume of about 1.32 mm³ (*Necator*) to 3.39 mm³ (*Ancylostoma*). Regarding eggs as prolate spheroids, and assigning median estimates of the numbers laid per female, the volume produced daily would be 0.529 mm³ (*Necator*) or 0.908 mm³ (*Ancylostoma*). These are 40.1% and 26.8%, respectively, of the volumes of the adult females.

The calculations are based upon what is interpreted to be the normal reproductive history of female *W. bancrofti*. It also represents a maximum value. As noted previously, there are several complete records from man which never showed a peak count as high as 70 microfilariae per 60 mm³. Counts greater than 45/60 mm³ can reasonably be assumed to be due to failure to catch the exact peak, or to sampling error, or both. Those in which the highest count was 5 or less per 60 mm³ can be attributed to accidental overrepresentation when the true abundance should have been represented by less than 1. There remain 30 cases in which the peak count was 6–45 microfilariae per 60 mm³. This represents 30.9% of the cases showing distinct peaks greater than 5. Of the 30 cases, 20 are adults and 10 are children 5–17 years old. The average age of those older than 15 (34.29 years) is the same as the average age of positive adults generally, and it is not possible to attribute the reduced count to built-up host response. The most likely explanation for the low peak counts is that the worms died before the normal life-span was completed.

These cases represent losses to the parasite population. The extent of the loss is better assessed by comparison with the median " normal " counts than with the mean, because of the greatly skewed distribution of the normal counts. The median peak count for those greater than 45/60 mm³ is 224. The median of counts between 6 and 45 is 25. Hence, 30.9% of the cases have counts only 11.2% as high as normal. The loss to the worm population can thus be estimated as 88.8% of 30.9%, or 27.4%. Subtracting this from the calculated average output per normal female worm, it is estimated that the average mated female worm produces $1.32 \times 10^7$ microfilariae, or $6.6 \times 10^6$ female microfilariae, if an equal sex ratio is assumed. The reciprocal of this last number must approximate to the probability that one of them will be transmitted and find a mate.

The estimated reproduction is less than 40% of the $3.5 \times 10^7$ larvae estimated by Beye & Gurian (1960), and the difference would help account for their 10-fold error in attempting to balance births and deaths of female worms.

## DISCUSSION

A proper understanding of animal populations is the concern of the rapidly developing field of population ecology (Leslie & Ranson, 1940; Birch, 1948; Evans & Smith, 1952; Andrewartha & Birch, 1954; Slobodkin, 1962). The principles and methods have been applied to a variety of species, most recently to populations of helminths (Beye & Gurian, 1960; Hairston, 1962, 1965a; Macdonald, 1965). The information that is required for an analysis of population phenomena consists of age-specific data on both reproduction and survival. The various sources of mortality on filarial worms have been considered in detail by Beye & Gurian (1960). They attempted to obtain from the literature sufficient data to make quantitative estimates of the probability of survival through most of the steps in the life-history of the parasite. They did not, however, have adequate data on reproduction, and assumed reproduction to be constant over an assumed 10 years of life in the human host. Data from the present study indicate that reproduction is not constant for very long, and that the mean length of life is considerably less than 10 years. Time-specific reproductive rates are given in Table 8. These could be made age-specific if the time from infective bite to the appearance of microfilariae were known. Various observations indicate a maximum of 14 months for the duration of the immature period, but it may be considerably shorter.

A beginning can be made at obtaining age-specific survival from the cases that showed peak densities of microfilariae substantially lower than 70/60 mm³ blood. If these are correctly interpreted as representing deaths before the end of the normal reproductive life, their survival can be estimated from the height of the peak microfilarial density. From these survivals, the mean exponential death-rate can be estimated as 0.02 per month during the first 7 months after detectability, and 0.05 per month thereafter until the end of the normal reproductive period. These estimates are admittedly based on a tenuous series of logical steps, but they do show that it is possible in principle to make a start at constructing an ecological life-table for the parasite (Hairston, 1965a).

## IV. EPIDEMIOLOGICAL RATES AND THE ESTIMATION OF THE TRUE WORM BURDEN

In areas where filariasis is endemic many people suffering from symptoms of the disease are negative on blood examination. For the strain of parasite under study, an outstanding example of such findings is the work of Beye et al. (1952), who found the proportion of people with symptoms of filariasis to be higher than the proportion with demonstrable microfilaraemia in all age-groups. The commonly accepted explanation for this state of affairs is that such people have been blood-positive in the past, and that the worms have died. However, another possible state of parasitization which has been generally overlooked is the condition where an infected individual harbours living worms of only one sex (Hairston, 1965b). It can be shown that the annual rate of becoming positive is low; Hayashi (1962) has calculated values ranging from 0.0005 to 0.045 per year for different endemic areas in Japan. Since single-larva infections are common in mosquitos, it follows that at any given time an appreciable part of the human population is carrying single parasites. A smaller fraction will have unisexual infections consisting of 2 or more worms. Therefore, the possibility that unmated worms may be responsible for some of the symptoms of filariasis seems to be important. Furthermore, if such worms appear to be important in this respect, it is desirable to estimate their numbers in order to arrive at a realistic assessment of the average worm load in the human population.

### RATES OF BECOMING POSITIVE AND NEGATIVE

Age-prevalence data may be analysed so as to estimate the rates of acquiring and losing the infection, and the model selected for this analysis depends upon the underlying assumptions that can be permitted (Muench, 1959). Hayashi (1962) has analysed filariasis data from several locations in Japan, using the two-stage catalytic model of Muench. This model assumes that an individual who has lost his infection never becomes positive again. Ample evidence shows that this model is not appropriate to the Samoan data, as there are 14 cases which certainly went from positive to negative and back to positive, and a larger number of apparently overlapping infections. More appropriate is the reversible catalytic model which assumes a constant rate of infection and a constant rate of loss of infection. The rate of becoming positive is the same for those persons who have lost the infection as for those who never had it. Although the assumptions may not be met completely by the facts, the approximation appears to be close enough for the model to be very useful, as it describes the changes in prevalence quite accurately.

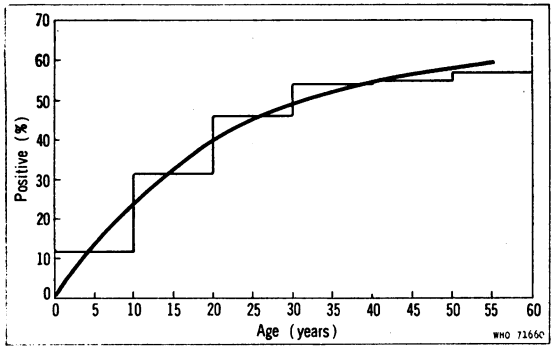
The equation given by Muench for the situation that apparently holds in subperiodic *W. bancrofti* is

$$y = a/(a + b)(1 - e^{-(a + b)t}), \quad (5)$$

where $y$ is the expected proportion positive after $t$ years, $a$ is the instantaneous rate of becoming positive per year and $b$ is the instantaneous rate of becoming negative per year. Muench gives a nomogram which permits the easy calculation of $a$ and $b$ from age-prevalence data. When applied to the data of Beye et al. (1952), an excellent fit is obtained (Fig. 10); this is sufficiently encouraging to pursue

FIG. 10

OBSERVED PREVALENCE AT SUCCESSIVE AGES [a] AND AGE-PREVALENCE [b] CALCULATED FROM THE REVERSIBLE CATALYTIC MODEL OF MUENCH [c]




[a] Histogram.

[b] Curve; parameters of curve, *a* (annual rate of becoming positive) = 0.0306; *b* (annual rate of becoming negative) = 0.0174.

[c] Data from Beye et al. (1952).

the analysis further. The question of people carrying dead worms can be resolved by assuming that death of worms is the reason for counts becoming negative. The proportion of the population that has ever been positive can be calculated by ignoring the loss-rate, and is equal to:

$$1 - e^{-at}. \quad (6)$$

The curve describing this relationship gradually rises above that for the proportion positive, as is shown in Fig. 11.

The parameters in expressions (5) and (6) also permit us to estimate the proportion of people carrying unmated worms. The proportion positive obviously represents the combined chances of obtaining male and female worms. If it is assumed that they are acquired independently of each other, then the proportion positive should equal the product of the proportion with males and the proportion with females. If we further assume an equal sex ratio, the proportion positive should equal the square of the proportion with either sex (Hairston, 1962). Similarly, the proportion of persons who have ever been infected with females can be calculated as the square root of the proportion of those who were ever positive. A final estimate that is important is the proportion who have ever been infected with worms of either sex. Under the assumptions already made, if $x$ is the proportion of persons ever to have been infected with females, $(1 - x)$ is the proportion who were never infected with females, and $(1 - x)^2$ would be the proportion having escaped all infections. The proportion of persons ever infected with worms of either sex, then, would be

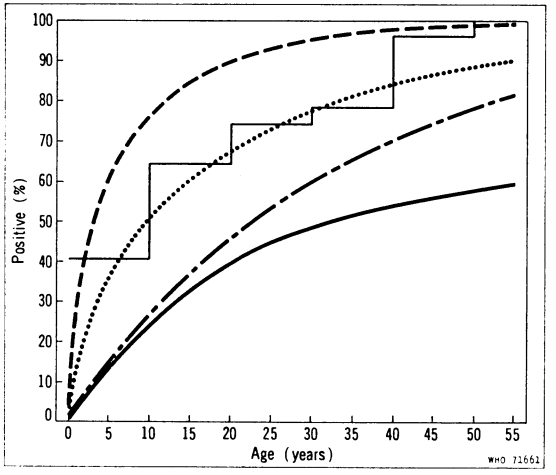
$$1 - (1 - x)^2 \text{ or } x(2 - x). \qquad (7)$$

The calculated proportions of those who have ever been infected with female worms and the proportion ever infected with worms of either sex, mated or not, have been added to Fig. 11. There is a striking similarity between the proportion with symptoms of filariasis and the proportion calculated ever to have been infected with female worms, mated or not. This similarity may be fortuitous because the assumption of complete independence of acquiring the two sexes is oversimplified. The obvious nonrandom distribution of mated female worms indicates that the independence is less than complete, and the calculated proportions which include unmated worms are probably too high. Thus, the proportion with symptoms may correctly reflect the proportion ever infected with worms of either sex.

The important point, here, however, is that it is not possible to account for symptomatic cases which are microfilaria-negative on the basis of dead worms alone, and unmated worms are shown to be an important factor. In this connexion, unmated worms provide the most reasonable explanation for the high frequency of symptoms without microfilaraemia among servicemen infected in the South Pacific area during the Second World War (Wartman, 1947; Trent, 1963). In these cases, the hypothesis of dead worms as the cause of symptoms seems generally untenable.

FIG. 11

OBSERVED RELATIONSHIP BETWEEN AGE AND THE PREVALENCE OF SYMPTOMS OF FILARIASIS[a] AND FOUR RELATIONSHIPS CALCULATED FROM MICROFILARAEMIA RATES




——— Calculated proportion of blood-positive persons.

— — — Calculated proportion of those ever blood-positive.

..... Calculated proportion of those ever infected with female worms.

– – – – Calculated proportion of those ever infected with worms of either sex.

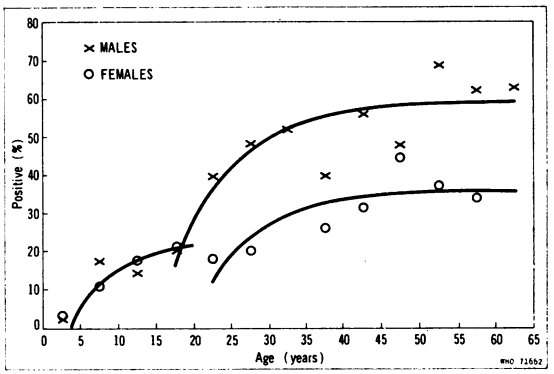
[a] Histogram.

## ANALYSIS OF AGE-PREVALENCE DATA FROM SAMOA

The results of the surveys taken for the present study indicate that the population is less homogeneous with regard to exposure than the population studied by Beye et al. (1952). The 10- to 20-year-olds consistently failed to show a prevalence that would produce a monotonic curve over the whole range of ages. There is a sharp increase in prevalence after 15 years in men, and to a lesser extent after 20 in women. For these reasons, the population was divided into the three groups already described. The three separate models agree quite well with the data, as is shown in Fig. 12 and Tables 9–12.

FIG. 12

AGE-PREVALENCE DATA FOR CHILDREN, ADULT MALES AND ADULT FEMALES [a]




[a] Continuous lines calculated from Muench's reversible catalytic model (see text).

TABLE 10

PREVALENCE RATES FOR FEMALES 20 YEARS OLD AND OLDER, AND EXPECTED RATES BASED UPON THE REVERSIBLE CATALYTIC MODEL OF MUENCH [a]

| Age-group (years) | No. examined | No. positive | Proportion observed | Positive expected |
|---|---|---|---|---|
| 20–24 | 143 | 26 | 0.182 | 0.111 |
| 25–29 | 115 | 24 | 0.209 | 0.239 |
| 30–34 | 84 | 25 | 0.298 | 0.300 |
| 35–39 | 80 | 21 | 0.263 | 0.328 |
| 40–44 | 91 | 29 | 0.319 | 0.342 |
| 45–49 | 51 | 23 | 0.451 | 0.352 |
| 50–54 | 32 | 12 | 0.375 | 0.353 |
| 55–59 | 26 | 9 | 0.346 | 0.354 |

[a] Expected rates calculated from equation (5) as given on p. 50 and Table 9. Values of terms: $a = 0.053$, $b = 0.097$.

TABLE 9

PREVALENCE RATES FOR MALES 15 YEARS OLD AND OLDER, AND EXPECTED RATES BASED UPON THE REVERSIBLE CATALYTIC MODEL OF MUENCH [a]

| Age-group (years) | No. examined | No. positive | Proportion observed | Positive expected |
|---|---|---|---|---|
| 15–19 | 163 | 34 | 0.209 | 0.158 |
| 20–24 | 107 | 42 | 0.393 | 0.359 |
| 25–29 | 82 | 40 | 0.488 | 0.468 |
| 30–34 | 71 | 37 | 0.521 | 0.526 |
| 35–39 | 90 | 36 | 0.400 | 0.558 |
| 40–44 | 59 | 33 | 0.559 | 0.574 |
| 45–49 | 50 | 24 | 0.480 | 0.583 |
| 50–54 | 58 | 40 | 0.690 | 0.588 |
| 55–59 | 8 | 5 | 0.625 | 0.591 |
| 60–64 | 27 | 17 | 0.630 | 0.592 |

[a] Expected rates calculated from equation (5) as given on p. 50. Values of terms: $a = 0.074$, $b = 0.050$.

TABLE 11

PREVALENCE RATES [a] FOR CHILDREN OF BOTH SEXES UNDER 15 YEARS OF AGE, AND FOR FEMALES 15–19 YEARS OLD

| Age-group (years) | No. examined | No. positive | Proportion observed | Positive expected |
|---|---|---|---|---|
| 0–2 | 29 | 0 | 0 | 0 |
| 3–4 | 93 | 3 | 0.032 | 0.034 |
| 5–6 | 229 | 17 | 0.074 | 0.090 |
| 7–8 | 243 | 30 | 0.123 | 0.128 |
| 9–10 | 214 | 28 | 0.131 | 0.156 |
| 11–12 | 182 | 25 | 0.137 | 0.177 |
| 13–14 | 174 | 37 | 0.213 | 0.192 |
| 15–16 | 64 | 12 | 0.188 | 0.204 |
| 17–18 | 62 | 13 | 0.210 | 0.212 |
| 19 | 26 | 6 | 0.231 | 0.216 |

[a] Expected rates calculated from equation (5) as given on p. 50. Values of terms: $a = 0.037$, $b = 0.122$.

## ESTIMATION OF THE TRUE WORM LOAD

If the assumptions and calculations already presented are correct, it should be possible to reconcile the independent estimates of the distributions of female worms among people and the rates at which the infection is acquired. Furthermore, the total worm burden, including unmated worms in apparently worm-negative people could be calculated. In calculating the proportion of people carrying unmated female worms, the method already given above was applied to the values for the Samoan data. Thus the proportion of men carrying female worms, mated or not, should be equal to the square-

TABLE 12

METHOD OF APPORTIONMENT OF CASES INFECTED WITH UNMATED FEMALE WORMS INTO PERSONS HAVING 1, 2, 3, 4, 5, AND 6 WORMS EACH [a]

| No. of females per person | No. of cases | Most probable No. of worms of both sexes in infected persons | Most probable ratio of cases with mated worms to those with unmated females (from binomial) | Expected No. of cases with unmated females | No. of cases interpolated for odd Nos. of female worms |
|---|---|---|---|---|---|
| 1 | 35 | 2 | 2 : 1 | 17.50 | 9.57 (3) |
| 2 | 23 | 4 | 14 : 1 | 1.64 | 1.03 (5) |
| 3 | 26 | 6 | 62 : 1 | 0.42 | |
| >3 | | | | negligible | |
| Total No. of cases with more than 1 unmated female | | | | 30.16 | |
| Total No. of cases with unmated females | | | | 57.19 | |
| Difference: total No. of cases with 1 unmated female | | | | 27.03 | |

[a] Data are for males 15 years old and older.

root of the proportion blood-positive at the average age of men 15 years old and older (34.15 years). From the reversible catalytic model, the expected proportion positive is 0.539; its square-root is 0.733. According to this method of estimation, 0.733 of Samoan men are infected with living female worms, 0.733 with living male worms, and only $(1-0.733)^2$, or 0.071, are really uninfected. As noted above, this method of calculation requires an unrealistic assumption. This is shown by the obvious non-random distribution of worms among people.

The tendency for some people to acquire more worms than would be expected on the basis of a random distribution means that the acquisition of male and female worms is not completely independent. This being the case, the proportion of people with unmated worms is overestimated, but the error appears to be unavoidable if any estimate at all is to be made and, as will be shown below, the error does not appear to be excessive.

Accepting this approach as a feasible method for estimating single-sex infections, the 157 infections among men shown in Table 9 represent 157/0.733, or 214.19 individuals who are infected with female worms. Thus, 57.19 cases have female worms only. Although a large proportion of these may be carrying single females, it is unlikely that all of them do so, and the problem of the apportionment of the 57.19 unrecognized infections among those who have 1, 2, 3, etc., unmated worms needs to be considered. As with the problem of the number of cases involved, a solution to this problem can only be approximated. The rationale of the solution requires the assumption that the acquisition of microfilariae by a mosquito is random with respect to the sex of the microfilariae. Unless an unequal sex ratio exists, this assumption is difficult to challenge. The distribution of the sexes among larvae in a mosquito, then, should follow a binomial form. Thus, among mosquitos infected with 2 larvae, one-fourth should have 2 females, one-fourth should have 2 males, and one-half should have 1 larva of each sex. The infections resulting from the bite of such a mosquito should follow the same distribution, and similar considerations apply to any infection of people with 2 worms, whether acquired at the same infective bite or not. From this reasoning, the patent infections with single females would represent one-half of all 2-worm infections, and two-thirds of those 2-worm infections that contain females. Hence, the undetected single-sex infections with 2 female worms would be one-half as abundant as the patent infections observed to have 1 female, provided that a single mated female were always accompanied by one male. This is certainly not exclusively so, but it is the most probable arrangement under the binomial. Moreover, errors from this source should tend to cancel each other, rather than to accumulate. Following the same reasoning as was used for cases with 2 unmated female worms, it can be shown that the number of cases with 4 unmated females is most

likely to be one-fourteenth of the number with 2 mated females, and the number of cases with 6 unmated females is most likely one sixty-second of the number with 3 mated females. Higher densities of unmated females would be so rare as to be negligible.

The approach has now given a method by which estimates can be obtained of the numbers of cases with 2, 4, or 6 unmated female worms. Direct calculation of the number of cases with 3 or 5 unmated females is not possible, but they can be approximated by linear interpolation between the numbers of cases with even numbers of unmated females. The number of cases with 1 unmated female must be the difference between the total with 2–6 unmated females and the total estimated to be carrying unmated females. The method is illustrated for the adult male part of the population in Table 12. The same method was applied to data from women and children. When the distribution of unmated females has been calculated, the numbers are added to the respective numbers of cases with mated females to give the distribution of all female worms in the human population.

In order to complete the distribution, the number of people with no female worms, mated or unmated, must be estimated. The 214.19 adult males represent the proportion 0.733, as noted above. This means that they represent a total population of 292.21 adult males, including 78.02 men who have no female worms at all. The entire distribution has now been estimated. The results are given in Table 13, along with the distributions of female

TABLE 13

ESTIMATED DISTRIBUTIONS OF ALL FEMALE WORMS, MATED OR NOT, AMONG MEN, WOMEN, AND CHILDREN

| No. of female worms per person | No. of cases among: | | | | | |
|---|---|---|---|---|---|---|
| | Males 15 years old and older | | Females 20 years old and older | | Children and adolescents | |
| | Estimated from data | Expected (negative binomial) | Estimated from data | Expected (negative binomial) | Estimated from data | Expected (negative binomial) |
| 0 | 78.02 | 78.02 | 94.05 | 94.05 | 247.93 | 247.93 |
| 1 | 62.03 | 44.94 | 58.01 | 38.25 | 105.90 | 85.46 |
| 2 | 40.50 | 32.27 | 20.50 | 23.33 | 29.50 | 39.28 |
| 3 | 35.57 | 24.69 | 13.54 | 15.82 | 14.72 | 19.56 |
| 4 | 8.64 | 19.48 | 4.57 | 11.26 | 0.93 | 10.11 |
| 5 | 11.03 | 15.64 | 1.32 | 8.24 | 4.52 | 5.34 |
| 6 | 6.42 | 12.70 | 5.06 | 6.15 | 2.10 | 2.86 |
| 7 | 8.00 | 10.40 | 3.00 | 4.65 | 0 | 1.55 |
| 8 | 8.00 | 8.57 | 2.00 | 3.55 | 4.00 | 0.85 |
| 9 | 3.00 | 7.09 | 5.00 | 2.73 | (>8) 5.00 | 1.66 |
| 10 | 3.00 | 5.88 | 1.00 | 2.11 | | |
| 11 | 3.00 | 4.89 | (>10) 9.00 | 6.91 | | |
| 12 | 2.00 | 4.08 | | | | |
| 13 | 2.00 | 3.41 | | | | |
| 14 | 2.00 | 2.48 | | | | |
| 15 | 1.00 | 2.07 | | | | |
| >15 | 18.00 | 15.60 | | | | |
| Mean No. per person | | 4.10 | | 2.18 | | 0.81 |
| Mean No. per infected person | | 5.59 | | 3.85 | | 2.01 |
| Mean No. per positive person | | 6.91 | | 6.07 | | 2.93 |

[a] These distributions are compared with negative binomial distributions having the same mean numbers and variances.

worms among women and children. The columns giving the expected values will be discussed below.

We have already pointed out that different villages were not exposed to the same transmission rates. Vaitogi, for example, had a lower prevalence and the people had lighter estimated loads of reproductive female worms than they had in Tula and Masausi. A comparison between the calculated distributions of all female worms in the two situations is presented in Table 14.

Thus far, no estimate has been made for male worms. If an equal sex ratio exists the estimated densities in the two tables should be doubled for " mean number per person " and for " mean number per infected person ", in order to obtain the estimates of total worm burden.

These distributions reflect a combination of three factors: (1) the distribution of infective bites among people, (2) the distribution of infective larvae among mosquitos, and (3) the probability that a given infective larva will be transmitted at any particular bite. The distributions in Tables 13 and 14 are thus potentially important in understanding the transmission of the parasite since they are strongly " clumped ". (" Clumping " is the tendency for some individuals in the same population to acquire more worms, and others fewer, than would be expected on a chance basis. This phenomenon is very common in the distributions of all animals in nature, and is virtually universal among parasites. Thus, the smaller the constant, the greater is the tendency for parasites to be clumped in certain individuals.)

TABLE 14

ESTIMATED DISTRIBUTIONS OF FEMALE *W. BANCROFTI* AMONG MALE SAMOANS IN TWO SEPARATE SITUATIONS DIFFERING IN TRANSMISSION RATE [a]

| No. of female worms per person | No. of cases in: | | | |
|---|---|---|---|---|
| | Vaitogi (less transmission) | | Tula + Masausi (more transmission) | |
| | Estimated from data | Expected (negative binomial) | Estimated from data | Expected (negative binomial) |
| 0 | 21.37 | 21.37 | 14.22 | 14.22 |
| 1 | 16.45 | 13.81 | 11.55 | 8.20 |
| 2 | 10.00 | 10.04 | 7.00 | 6.00 |
| 3 | 8.22 | 7.57 | 7.65 | 4.70 |
| 4 | 1.43 | 5.81 | 0.29 | 3.80 |
| 5 | 2.27 | 4.51 | 4.20 | 3.13 |
| 6 | 4.10 | 3.52 | 0.10 | 2.62 |
| 7 | 3.00 | 2.76 | 3.00 | 2.21 |
| 8 | 5.00 | 2.17 | 1.00 | 1.87 |
| 9 | 2.00 | 1.71 | 1.00 | 1.59 |
| 10 | 1.00 | 1.35 | 1.00 | 1.36 |
| >10 | 5.00 | 5.22 | 8.00 | 9.31 |
| $\chi^2$ | 8.94 | | 10.48 | |
| | $9 > DF > 5$ | | $9 > DF > 5$ | |
| | $0.10 < P < 0.50$ | | $0.10 < P < 0.50$ | |
| Mean No. per person | 3.52 | | 5.12 | |
| Mean No. per infected person | 4.82 | | 6.75 | |

[a] These distributions are compared with negative binomial distributions having the same means and variances.

A measure of the clumping can be obtained if the form of the distribution is known.

Three mathematical distributions were tried and compared with the data: Neyman's type A contagious distribution, Thomas' double Poisson series, and the negative binomial distribution. The first two were calculated according to the methods given by Thomson (1952), and the third by the method of Bliss & Fisher (1953). The negative binomial gave much the best fit, and the expected distributions based upon it are compared with the estimated data in Tables 13 and 14.

The fit of the expected negative binomial is materially better for the separate villages than for Samoa as a whole. This reflects the fact that the data are more homogeneous where exposure is more nearly uniform. In fact, for these data, the observed and expected distributions do not appear to be significantly different, although the method of obtaining the " observed " values makes it difficult to know the exact number of degrees of freedom for the comparison. The distributions are described in a satisfactory manner from the mathematical standpoint. One of the parameters of the negative binomial is a constant that is inversely related to the amount of clumping in the data.

Knowledge of the frequency of uninfected individuals and of the mean number of female worms per person makes it possible to calculate the constant ($k$ in the following equation):

$$\text{proportion of negative counts} = 1/[1 + (\text{mean}/k)]^k. \quad (8)$$

Direct solution of this equation is not possible, but trial and error produced values of 0.67 for males aged 15 and over, 0.50 for females aged 20 and over, and 0.60 for children. Inasmuch as $k$ can vary from 0 at maximal possible clumping to infinity in a random distribution, clumping is strong in all groups and is most pronounced among women and least among adult males.

## DISCUSSION

Most areas where filariasis is endemic the parasite populations have long ago reached equilibrium conditions, with reproduction and mortality equal, at least over any reasonably long period of time, such as 1 year or more. If this were not the case, the parasite population would have to be either increasing or decreasing, with cases among humans increasing in frequency or else tending to die out altogether. Such an equilibrium may be reached over a wide range of rates of transmission. At some point, however, a further reduction in the probability of successful transmission cannot be compensated for by the fecundity of the average female worm. At this point, in population terms, the net reproductive rate is less than unity, and the parasite population will decline to zero unless the probability of transmission is increased. This is the " critical transmission level " discussed by Beye & Gurian (1960), and is straightforward and easy to visualize. A point that is less obvious is that the net reproductive rate is equal to unity for any parasite population that is at equilibrium, *regardless of how much the rate of transmission exceeds the critical level.*

The relevance of this point can be seen when it is realized that transmission is more than the minimum necessary to maintain the population; thus, some of the reproduction must be either curtailed through crowding effects in the human host or wasted through crowding effects in the mosquito. In the case of subperiodic *W. bancrofti*, the data at hand indicate no evidence for any crowding effect in the human host, at least up to the analysable densities reached in this study. Thus, any wastage of reproductive potential appears to occur elsewhere. The most likely place appears to be in the mosquito. Infection experiments with mosquitos are most successful when the human host has an intermediate density of microfilariae in the peripheral blood as shown by Rosen (1955) for *Aedes polynesiensis* and subperiodic *W. bancrofti*. Mosquitos receiving large numbers of larvae have a low survival, and the survivors have fewer than the expected number of larvae, either because of a differential mortality or because of loss of some larvae from heavily infected mosquitos. A somewhat paradoxical situation arises in that the most heavily infected people represent a large amount of loss to the parasite population, since the chance of successful transmission from such people is much reduced. Obviously, the distribution of worms among people is an important piece of information in this connexion.

If, in this situation, the transmission rate were reduced, there would be fewer heavily infected people; the loss to the parasite population would be proportionally less, and the system would again come into equilibrium with a net reproductive rate of 1. A considerable range of transmission rates over which these compensating mechanisms work probably exists. At the lower levels of transmission, another factor becomes important—the increased probability that worms successfully transmitted to

people will fail to become mated (Hairston, 1962, 1965a; Macdonald, 1965). At the point where this effect becomes self-accelerating, the critical transmission level has been reached, and the parasite population will decline automatically to zero.

An ideal and complete analysis, using the approach indicated in this paper, and by Hairston (1962, 1965a), would include numerical estimates of all factors in the life-cycle of the parasite, especially the biting rate and the survival of larvae in naturally infected mosquitos. Equally important would be a repetition of the analysis from several areas where the parasite and hosts are the same, but among which the rate of acquisition of infection varied as widely as possible. Were such data available, a predictive model might be produced in which the critical level could be stated in terms of the number of mosquito bites per person per day, or of the permissible densities of microfilariae in people. This seems possible to obtain, at least in principle. The amount and kinds of data required could be stated with confidence, and with the proper personnel in a favourable location, such data could be obtained.

From both the standpoint of a scientific understanding of the population dynamics of the parasite and from the practical advantage of having a concrete goal in control efforts, a study of this type would be highly desirable.

## RÉSUMÉ

Cette étude représente une tentative d'exprimer quantitativement certains aspects de l'évolution naturelle de *Wuchereria bancrofti* et de l'épidémiologie de la filariose. Les données de base en ont été recueillies dans une population de 1575 habitants des Samoa orientales de septembre 1948 à mars 1953.

L'analyse des densités microfilariennes, à courts intervalles, chez les mêmes personnes montre que contrairement à la théorie généralement admise, les larves ne sont pas réparties uniformément dans l'ensemble du système circulatoire lorsque la densité dans le sang périphérique est élevée. Une explication plausible de ce phénomène est que les microfilaires, en raison de leur taille, voient leurs déplacements retardés lors du franchissement des réseaux capillaires. Des numérations périodiques pratiquées chez des filariens pendant 4 ans et demi indiquent que la microfilarémie évolue sous la forme d'une onde, caractérisée par une augmentation progressive de la densité pendant 3 à 24 mois, aboutissant à un clocher, suivie par une phase de déclin plus brève. La durée totale de ce cycle est de 2 à 4 ans, le clocher s'observant indépendamment de la saison ou de l'année. Cela peut expliquer les fortes variations de la densité microfilarienne constatées au cours des enquêtes. Les auteurs avancent l'hypothèse selon laquelle ces particularités reflètent l'activité reproductrice d'un ou de plusieurs parasites femelles.

L'analyse statistique des données recueillies chez les mêmes personnes pendant une période pouvant atteindre 4 ans et demi indique que les densités microfilariennes maximales sont de l'ordre de 70 microfilaires par 60 $cm^3$ de sang ou d'un multiple de ce nombre. Si l'on prend comme base des chiffres inférieurs ou supérieurs (de 30 à 100), on n'obtient pas des résultats semblables. On en conclut qu'un seul parasite femelle produit une quantité de microfilaires suffisante pour déterminer une densité de 70 microfilaires par 60 $mm^3$ de sang périphérique.

A partir de cette conclusion, on a calculé le nombre de filaires femelles reproductrices présentes chez des Samoans porteurs de microfilaires. On a obtenu des valeurs moyennes de 6,91 ; 6,07 et 2,93 parasites respectivement chez les hommes adultes, les femmes adultes et les enfants. Ces chiffres sont considérés comme des minimums. Dans trois villages différant fortement sous le rapport de l'intensité de la transmission, le nombre de parasites femelles chez les filariens adultes de sexe masculin a été estimé en moyenne à 5,93 (village à prévalence faible) et à 8,32 (villages à prévalence forte).

Une estimation numérique de la capacité reproductrice des filaires femelles a été faite sur la base des taux moyens de variation de la microfilarémie pendant plusieurs mois ou années. On considère qu'un sujet infecté par une seule femelle fécondée reste positif (examen portant sur un échantillon de 60 $mm^3$ de sang) pendant 2,5-2,9 années, ce qui correspond à la période patente de l'infection. La durée de vie d'une microfilaire est en moyenne de 56 jours.

Des observations faites au cours de cette enquête et des données recueillies par d'autres auteurs, il ressort que les microfilaires sont retenues dans les capillaires, même lorsqu'elles ont quitté les poumons de l'hôte pour gagner la circulation périphérique. On a calculé que la densité microfilarienne dans les capillaires et les artérioles est deux fois plus élevée que dans les vaisseaux périphériques. On estime que lorsque le sang prélevé au doigt contient 1 microfilaire par 60 $mm^3$, le nombre total de larves présentes dans l'organisme du porteur est de $4{,}9 \times 10^4$. La production totale maximale de larves par parasite femelle serait de $3{,}43 \times 10^6$ microfilaires.

L'étude des variations mensuelles de la densité microfilarienne et du taux supposé de mortalité des filaires adultes permet de préciser certains aspects de la reproduction chez *W. bancrofti* femelle. Le taux de reproduction

atteint un maximum de 75 000 larves par jour 15 mois après le moment où l'infection peut être décelée, chiffre correspondant à celui avancé pour d'autres nématodes de taille équivalente. Chaque femelle produit au total $1{,}82 \times 10^7$ microfilaires, mais par suite de la mort précoce de certains vers adultes ce nombre est ramené à $1{,}32 \times 10^7$ microfilaires en moyenne. Le taux de mortalité des filaires femelles est évalué à 0,02-0,05 par mois.

Grâce à l'utilisation du modèle catalytique de Muench, on a pu calculer les taux annuels d'acquisition et de perte de l'infection filarienne et dresser une courbe âge-prévalence. Ces deux paramètres ont permis d'établir la proportion de la population hébergeant des vers morts et le pourcentage d'habitants porteurs de parasites non fécondés. La fréquence élevée des cas d'infections symptomatiques sans microfilarémie apparente ne peut s'expliquer que si l'on tient compte des porteurs de vers non fécondés.

Appliquée aux habitants de Samoa, la méthode montre que les taux annuels d'acquisition et de perte de l'infection varient dans trois groupes de population: sujets de sexe masculin âgés de 15 ans et plus, où les taux sont respectivement de 0,074 et de 0,05; sujets de sexe féminin âgés de 20 ans et plus (taux: 0,053 et 0,097); reste de la population (taux: 0,037 et 0,122).

On a pu de la sorte évaluer la proportion de sujets porteurs de femelles non fécondées et, en tenant compte de la distribution des femelles reproductrices, en déduire la distribution des femelles non fécondées chez les sujets où la recherche des microfilaires reste négative. En combinant les données relatives à la distribution des femelles reproductrices et les données concernant les femelles non fécondées, on peut calculer la charge parasitaire dans chaque groupe de population. Cette charge est en moyenne de 8,20 parasites chez les hommes, de 4,36 chez les femmes et de 1,62 chez les enfants. Si l'on ne considère que les sujets infectés, les chiffres sont respectivement de 11,18, 7,70 et 4,02. Chez certains sujets, la charge parasitaire peut atteindre 75 à 100 vers des deux sexes.

Les implications de ce genre de recherches en ce qui concerne l'épidémiologie et la lutte contre la filariose sont examinées. Si les populations du parasite sont de toute évidence capables de persister dans des régions où la transmission est d'intensité très variable, il n'en va plus de même lorsque la transmission est tellement faible qu'un grand nombre de parasites, après la pénétration chez l'hôte, ne peuvent trouver un partenaire pour s'accoupler. Il serait important d'obtenir des données permettant de prévoir ce niveau critique.

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