TRANSACTIONS OF THE ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE, VOL. 70. NO. 1. 1976.

Some observations on filariasis in Western Samoa after mass administration of diethylcarbamazine

JOAN H. BRYAN AND B. A. SOUTHGATE
Ross Institute of Tropical Hygiene, London School of Hygiene and Tropical Medicine

Summary

An extremely efficient diethylcarbamazine administration campaign to eradicate *Wuchereria bancrofti* has been carried out in Western Samoa. The use of the membrane-filtration technique has shown that a large number of people exhibit extremely low microfilarial densities, often with less than 10 in 1 ml of venous blood. It was found that one of these low level microfilaria carriers readily infected the local vector *Aedes polynesiensis* and that development took place to the infective stage. It was estimated that 497 infective larvae of *W. bancrofti* will enter the human population of Western Samoa daily from these vectors. Resumption of filariasis transmission is possible and surveillance of the human and mosquito populations should be continued for a number of years and control measures taken quickly if further transmission occurs.

Introduction

Large scale administration of diethylcarbamazine (DEC) for the control of *Wuchereria bancrofti* infections has been carried out in a number of parts of the world during the past 25 years, with varying degrees of success. It is extremely difficult to assess the effectiveness of such campaigns in interrupting the transmission of *W. bancrofti;* this is partly because there has been no standardization of either the dosages or the timing of drug administration, and partly because there has been no standardization of the techniques used to measure reductions of parasite loads either in human or mosquito hosts. In addition, transmission of *W. bancrofti* has been affected by other factors such as population migrations and the use of insecticides in some areas where control by DEC has been attempted.

It is recognized that DEC is safe when used in mass drug administration (MDA) campaigns (SASA et al, 1970) and that it is effective in lowering microfilarial rates and densities in the human population (KESSEL et al, 1970; KESSEL, 1971; SASA, 1963; SASA et al, 1963). However, DEC does not always clear microfilaraemia completely with any of the dosage schedules that have been used (HAWKING, 1962; MAHONEY and KESSEL, 1971) although in at least one instance it has achieved complete interruption of transmission (FUKUSHIMA, 1967).

Although DEC treatment has little or no effect on the established clinical lesions of late bancroftian filariasis (VAN DIJK, 1964), long term MDA has been effective in preventing the development of new cases of advanced pathology (MARCH et al, 1960; LAIGRET et al, 1966; MARSHALL and YASUKAWA, 1966). Even in the early stages of a campaign, there is a dramatic reduction in the incidence of early clinical manifestations of filariasis such as filarial fever, lymph gland and muscle sheath abscesses, lymphangitis and lymphadenitis (BURNETT and MATAIKA, 1964; LAIGRET et al, 1965; DANDO, 1967).

In areas where mass DEC administration has failed to interrupt transmission of *W. bancrofti*, the failure has been readily explicable by several factors:

(a) relatively poor coverage of the population in the MDA;
(b) lack of co-operation by the population in the campaign due to the incidence of undesirable side effects of the drug in treated individuals;
(c) relapse, recurrence or reinfection in substantial proportions of the treated population, possibly due to inadequate drug dosage schedules. Full reviews of these problems are given by VAN DIJK (1964), FUKUSHIMA (1967) and MAHONEY and KESSEL (1971).

In all studies carried out so far, the persistence of microfilaria carriers in human populations has been determined by the relatively insensitive technique of examining stained blood films of 20 mm^3 or 60 mm^3 of capillary blood obtained by finger or ear lobe puncture. It is easy to understand how levels of microfilaraemia detectable by these methods can sustain transmission of the infection. Recently much more sensitive methods for the detection and quantification of microfilaraemia, even at very low densities, have been introduced (BELL, 1967; CHULARERK and DESOWITZ, 1970; DESOWITZ et al, 1970; DENHAM et al, 1971). Use of these techniques in several areas of the South Pacific (SOUTHGATE and DESOWITZ, 1971; SOUTHGATE, 1973; DESOWITZ, 1973; DESOWITZ and SOUTHGATE, 1973; DESOWITZ et al, 1973; SOUTHGATE, 1974; DESOWITZ and HITCHCOCK, 1974) has shown that both in treated and untreated populations, large numbers of people are exhibiting extremely low microfilarial densities, many with less than 10 microfilariae in 1 ml of venous blood. These findings clearly introduce a new dimension into planning eradication programmes for bancroftian filariasis, in that these lightly infected people may act as a parasite reservoir if vector mosquitoes are not also controlled.

Therefore, in Western Samoa, where an exceptionally efficient MDA of DEC has been carried out (THIEME and PENAIA, 1972), between February and April 1973 we attempted to determine:

(a) what proportion of mosquitoes could be infected by feeding on an ultra-low density microfilaria carrier and
(b) whether mosquito infections derived from low level carriers who had been treated with a full course of DEC were capable of full development to the infective stage in the mosquito mouthparts.

This work was carried out whilst the authors were consultants to the World Health Organization Programme of Investigation on Filariasis. We are grateful to Dr. N. Ansari, Dr. L. Kartman and Dr. B. B. Waddy for their help and advice. Dr. L. Penaia, Mr. Sua Pitone Fanola, Mr. Pa S. A. Fola Sone and the members of the Western Samoan Filariasis Control Project gave invaluable assistance, as did our volunteer.

Materials and methods

The experimental work was carried out at Apia on Upolo Island but the preliminary blood surveys to find an ultra-low density microfilaria carrier were made in nine villages on Savai'i and Upolo Islands. Both coastal and inland villages were included.

Blood examination techniques

(a) The membrane-filtration technique was performed as described by DESOWITZ and SOUTHGATE (1973).

(b) The counting-chamber technique was performed as described by SOUTHGATE (1973).

Entomological techniques

(a) Collection of mosquitoes. All work was carried out on wild-caught *Aedes polynesiensis*, the main vector of bancroftian filariasis in Western Samoa (RAMALINGAM, 1968), collected by day-biting catches. Mosquitoes were captured in sucking-tubes from the head, arms, chest and back of human baits, and transferred to paper or plastic cups. Mosquitoes which had engorged before capture were rejected. Collections were made in an uninhabited area near Apia. No attempt was made to rear mosquitoes from eggs for these experiments because the insectary had become contaminated with insecticide and because the length of time for conducting the experiments was limited.

(b) Care of the adult mosquitoes. As the cages available in Apia seemed to have been contaminated with insecticide (mosquitoes died within 24 hours of being placed in them) the mosquitoes were kept in ½ gallon paper ice cream containers or in plastic or paper cups covered with mosquito netting. Up to 140 female mosquitoes were kept in the ice cream buckets and 50 in the cups. After the initial blood-meal, the females were supplied with cotton wool pads soaked with glucose solution and sultanas which had been soaked overnight in water. Initially, the glucose pads were not changed daily, but in some cases fermentation occurred, killing all the mosquitoes in the container. The fermentation was reduced if new pads were supplied daily. At the beginning of the experiments the soaked sultanas were placed in small petri dishes in the ice cream containers; however, this was discontinued as the mosquitoes drowned in the water surrounding the sultanas. Subsequently, both the glucose-soaked cotton wool and the sultanas were placed on top of the containers.

To maintain a high humidity and to protect the adults from possible exposure to insecticide, the containers were stored in plastic bags which were fastened with rubber bands, and damp pads of cotton wool were placed inside each bag.

The temperature in the insectary fluctuated from 80°F. (27°C.) to 93°F. (34°C.) and as the temperature dropped, condensation occurred on the sides of the containers. To prevent the mosquitoes getting wet in this condensed water, all the containers were lined with filter paper. Unfortunately, many of the mosquitoes became trapped between the filter paper lining and the sides of the containers. This problem could not be overcome, using the equipment available.

In some cases, filter paper cones were placed in 2 × 1 in. (5 × 2·5 cm) tubes or small petri dishes inside the containers. These were half-filled with water to provide oviposition sites. Egg laying began three days after a blood-meal. Survival of the mosquitoes seemed to be better if there was an oviposition site. When no site was provided, eggs were laid on the glucose pads on the top of the containers; the gymnastic feats involved in these ovipositions seemed to be detrimental to survival.

Feeding experiments on the human volunteer

For feeding on the volunteer, up to 80 unfed female mosquitoes were placed in a cup, covered with mosquito netting. Three or four cups were placed along the forearm of the volunteer, with the netting tops next to the skin, for periods of 20 minutes. After this time, partially and fully engorged females were removed with a sucking tube and placed into the containers described above. These containers were examined daily, and dead insects in a suitable condition for dissection were removed and dissected; the dead mosquitoes rapidly became covered with fungus which made dissection difficult in some cases.

Dissection of the mosquitoes

After removal of the legs and wings, each mosquito was divided into head, thorax and abdomen, and each part dissected separately in isotonic saline. The head was split open and the mouthparts carefully separated. The thoracic flight muscles were thoroughly teased apart. The abdomen was dissected to reveal the gut and the Malpighian tubules. The dissected mosquitoes were examined under a compound microscope at a magnification of × 50. All the filarial larvae detected were separated and identified. When a mosquito died before the blood meal was digested, the gut contents were smeared on to a glass slide, dehaemoglobinized, fixed with methyl alcohol, stained for 20 minutes with 10% Giesma and examined for parasites.

When it became apparent that infective larvae were appearing in the mouthparts on the 12th day after feeding at the ambient temperatures involved, any surviving mosquitoes were killed and dissected on the 12th or 13th day after feeding.

Control mosquito dissections

As wild-caught females were used for the feeding experiments, it was important to ensure that the mosquitoes used were not infected with *W. bancrofti* larvae before the start of these experiments. Over 300 wild-caught mosquitoes were dissected on the day of capture. A further 50 were fed on the investigators and dissected 7–10 days after feeding. No filarial larvae were detected in these mosquitoes.

Determination of the blood-meal size of Ae. polynesiensis

To determine how many mosquitoes would feed on 1 ml of blood, 100 unfed mosquitoes were weighed on a chemical balance and also 100 fed ones, after being killed with ether. The difference between the weights of the two groups was the weight of blood retained after ingestion by 100 mosquitoes. As already stated, fully and partially engorged mosquitoes were used in the feeding experiments on the volunteer. For the weighing of the fed mosquitoes, the first 100 mosquitoes, partially or wholly engorged, that would have been acceptable in the volunteer feeding experiment were chosen. This was thought to give a sample that would represent adequately the mosquitoes used in the feeding experiments.

The human volunteer

A suitable volunteer—a male of 23 years, healthy, weighing 127 lb (57·6 kg), with a low level of microfilariae (6 per ml venous blood)—was chosen for this study. His home was in Salelologa village, Savai'i Islands,

Table I – Results of 1st day feeding

Data on volunteer

Pre-feed count – 60 mm³ finger-prick blood in counting chamber: neg.

Mid-feed count – 1 ml venous blood by membrane-filtration: 5 microfilariae seen.

Post-feed count – 60 mm³ finger-prick blood in counting chamber: neg.

Data on mosquitoes

Number fed – 333

Days after blood meal	Mosquitoes dissected	Mosquitoes pos.	Filarial larvae
1	7	0	0
2	40	0	0
3	9	1	1
4	6	0	0
5	6	1	2
6	20	0	0
7	20	5	6
8	21	1	1
9	9	1	2
10	12	6	9
11	4	1	2 (infective)
12	7	0	0
13	6	0	0
Totals	167	16	23

Table II – Results of 2nd day feeding

Data on volunteer

Pre-feed count – 60 mm³ fingerprick blood in counting chamber: neg.

Mid-feed count – 1 ml venous blood by membrane-filtration: 4 microfilariae seen.

Post-feed count – 60 mm³ fingerprick blood in counting chamber: neg.

Data on mosquitoes

Number fed – 292

Days after blood meal	Mosquitoes dissected	Mosquitoes pos.	Filarial larvae
1	1	1	1
2	5	0	0
3	4	1	1
4	8	0	0
5	10	1	1
6	28	0	0
7	19	0	0
8	11	3	3
9	13	3	3
10	9	2	2
11	27	3	3 (infective)
12	8	0	0
13	7	0	0
Totals	150	14	14

Table III – Results of 3rd day feeding

Data on volunteer

Pre-feed count – 60 mm³ fingerprick blood in counting chamber: neg.

Mid-feed count – 1 ml venous blood by membrane-filtration: 8 microfilariae seen.

Post-feed count – 60 mm³ fingerprick blood in counting chamber: neg.

Data on mosquitoes

Number fed – 396

Days after blood meal	Mosquitoes dissected	Mosquitoes pos.	Filarial larvae
1	1	0	0
2	11	2	2
3	13	3	7
4	8	3	7
5	23	1	3
6	6	0	0
7	4	0	0
8	9	0	0
9	10	1	1
10	22	2	2
11	20	0	0
12	16	4	4 (infective)
Totals	143	16	26

N.B. – One mosquito dissected on day 2 contained an infective larva of *Dirofilaria immitis* in the Malpighian tubules.

Table IV – Results of 4th day of feeding

Data on volunteer

Pre-feed count – 60 mm³ fingerprick blood in counting chamber: 1 microfilaria seen.

Mid-feed count – 1 ml venous blood by membrane-filtration: 6 microfilariae seen.

Post-feed count – 60 mm³ fingerprick blood in counting chamber: 1 microfilaria seen.

Data on mosquitoes

Number fed – 383

Days after blood meal	Mosquitoes dissected	Mosquitoes pos.	Filarial larvae
1	2	0	0
2	0	0	0
3	0	0	0
4	3	0	0
5	27	0	0
6	52	3	3
7	25	3	3
8	8	2	2
9	4	0	0
10	27	2	2
11	3	1	2 (1 infective)
12	2	0	0
Totals	153	11	12

Table V – Results of 5th day feeding

Data on volunteer

Pre-feed count – 60 mm³ fingerprick blood in counting chamber: 1 microfilaria seen.

Mid-feed count – 1 ml venous blood by membrane-filtration: 2 microfilariae seen

Post-feed count – 60 mm³ fingerprick blood in counting chamber: neg.

Data on mosquitoes

Number fed – 398

Days after blood meal	Mosquitoes dissected	Mosquitoes pos.	Filarial larvae
1	0	0	0
2	0	0	0
3	0	0	0
4	11	0	0
5	2	0	0
6	0	0	0
7	44	4	5
Totals	57	4	5

and he had received the following dosage schedule of DEC:

1966–67: Six doses at weekly intervals of 5 mg DEC per kg body weight, followed immediately by 12 doses at monthly intervals of 5 mg per kg body weight.

1971: Twelve doses at monthly intervals of 6 mg per kg body weight.

Feeding was carried out on five afternoons between 1400 hours and 1630 hours; mosquitoes collected the same morning between 0800 hours and 1100 hours were used. On the afternoon of feeding, the volunteer underwent three blood examinations for microfilariae. Immediately before feeding started, 60 mm³ of finger-prick blood was examined in a counting chamber; halfway through feeding, 1 ml of venous blood was examined by the membrane-filtration technique, and at the end of feeding a 60 mm³ counting-chamber test was repeated.

Results

Human blood examination

The blood of 93 people, 92 of whom were known to have been infected before MDA, was examined by the membrane-filtration technique during the search for a suitable volunteer for the mosquito feeding experiments. Of these 93 persons, 23 were positive for *W. bancrofti* microfilariae, 17 of them having counts of 10 or less microfilariae per ml of blood. It is of interest to note that a 14-months-old girl, born in January 1972, was infected with a count of 34 microfilariae in 1 ml of blood; the last round of mass DEC administration was completed in December 1971, so at least one case of natural transmission since that time has been proved.

Feeding experiments on the human volunteer

Results of the feeding experiments on the volunteer and the results of the blood examinations conducted on the days of feeding are set out in Tables I–V. All mosquitoes from the fifth day of feeding were killed seven days after feeding as the period available for the study was at an end; it was already apparent that mosquitoes could become infected with *W. bancrofti* larvae after feeding on a person with ultra-low density microfilaraemia, and that larvae could develop to the infective stage.

It will be seen from Tables I to V that a number of mosquitoes developed multiple infections of *W. bancrofti* larvae. Detailed results are set out in Table VI and a summary in Table VII.

In 670 *Ae. polynesiensis* dissected after feeding on the volunteer, 80 *W. bancrofti* larvae were found. From the first four days of feeding, 64 larvae were found in 513 mosquitoes which died before the larvae could have matured (i.e., in the first 11 days after ingestion) and 11 larvae were found in 100 mosquitoes which survived till the 12th day after feeding. Infection rates in the two groups are not significantly different ($0{\cdot}3 > p > 0{\cdot}2$), so that mortality of the mosquitoes was independent of infection with microfilariae.

Blood meal size in Ae. polynesiensis

Results of blood meal size determination showed that the weight of blood ingested and retained by 100 mosqui-

Table VI – Distribution of multiple infections of *W. bancrofti* larvae in mosquitoes

Feed 1	
Day 5	One with two sausage forms
Day 7	One with two early 2nd stage forms
Day 9	One with two 2nd stage forms
Day 10	One with two 3rd stage forms and one 2nd stage form
	One with two 3rd stage forms
Day 11	One with two infective forms
Feed 2	
No multiple infections seen	
Feed 3	
Day 3	One with five sausage forms
Day 4	One with five sausage forms
Day 5	One with three sausage forms
Feed 4	
Day 11	One with one infective form and one 3rd stage form
Feed 5	
Day 7	One with two early 2nd stage forms

A summary of all the mosquito feeding experiments on the volunteer is set out in Table VII.

Table VII – Overall summary of mosquito feeding experiments on volunteer

Number of mosquitoes fed	1802
Number of mosquitoes dissected	670
Number of mosquitoes positive	61
Number of *W. bancrofti* larvae seen	80
Number of *W. bancrofti* infective forms seen	10

toes was 140 mg (i.e., 1·4 mg per mosquito). The mean specific gravity of human blood is 1·055, so 1·4 mg of blood has a volume of 1·327 mm³. Therefore, 1 ml of blood will be retained by 754 mosquitoes. It is realized that this calculation does not allow for the loss of fluid from the anus of mosquitoes during feeding, but our observation confirmed those of ROSEN (1955) that *Ae. polynesiensis* does not expel blood during feeding, although occasional drops of clear serum-like fluid were expelled. It is unlikely that the amount of blood ingested and retained, as shown by our weighings, differed markedly from the amount ingested.

Discussion

Our results show clearly that:

(a) *Ae. polynesiensis* readily took up microfilariae from an ultra-low density carrier;
(b) more than the expected number of larvae were ingested;
(c) the microfilariae developed to the infective stage in spite of the previous DEC treatment of the host; and
(d) the parasite loads picked up by the vectors did not cause increased mortality of the vector during the extrinsic cycle of parasite development.

Each of these factors will be discussed in detail, together with previous work which is relevant.

The threshold density of infective microfilaraemia

A number of valuable studies have been conducted in the past to determine the minimum densities of microfilaraemia compatible with infection of invertebrate hosts in various filarial-arthropod host-parasite combinations. The results of these studies vary widely, but some at least have shown significant infection rates in arthropods after feeding on hosts with microfilaria densities that were extremely difficult or impossible to detect before the introduction of the membrane-filtration technique.

For example, HIGHBY (1946) recommended xenodiagnosis as a possible technique for detecting microfilaraemia of *W. bancrofti;* his results showed infection which would have been most unlikely to have been detected by any technique in use then. It is of interest that he achieved these results in spite of using what were almost certainly two unnatural host-parasite combinations, a Samoan strain of *W. bancrofti* with *Culex fatigans* and *Ae. aegypti.* ROSEN (1955) has shown that the Polynesian strain of *W. bancrofti* is poorly adapted to these mosquitoes.

WHARTON (1957a and b) working with *Brugia malayi* and *Mansonia longipalpis* and JORDAN (1959) with *W. bancrofti* and *C. fatigans* showed that microfilarial densities as low as 25 per ml produced infection rates in mosquitoes with *infective* larvae of 7·8% and 2·6% respectively. ROSEN (1955), using *W. bancrofti* and *Ae. polynesiensis,* obtained an *infective* larva infection rate of 5·3% after feeding on a donor with 20 microfilariae per ml of blood. SYMES (1960b) studied Fijian *W. bancrofti* in *Ae. pseudoscutellaris,* a close relative of *Ae. polynesiensis,* and obtained an *infective* larva infection rate of 7·04%, with a mean load of 2·3 infective larvae per infected mosquito after feeding on volunteers whose microfilarial densities varied from 2 to 50 per ml of blood. Symes comments that "it seems that *Ae. pseudoscutellaris* at least may become infected after feeding upon people whose microfilariae are too few to be detected by ordinary survey techniques". Symes further discovered infection rates with first-stage larvae in 1·81% of *Ae. pseudoscutellaris* fed on a man whose microfilarial count was zero, using the concentration technique of KNOTT (1939).

The present study shows that ultra-low density microfilaraemia readily infects mosquitoes, at least for the Pacific host-parasite combination of *W. bancrofti* and *Ae. polynesiensis.*

The "concentrating" capacity of Ae. polynesiensis

There have also been a number of interesting studies on the problem of the "concentrating" power of arthropods when taking infected blood meals. Results have been contradictory, some showing a distinct "concentrating" effect, some a microfilarial intake by the arthropod host roughly equal to the expected number in the blood-meal volume from the vertebrate, whilst others show a "dilution" effect. Thorough reviews of the literature and valuable reference lists are given by WHARTON (1957a and b), JORDAN and GOATLY (1962), BURTON (1964), NELSON (1964) and GUBLER er al (1973), all of whom add their own experimental data.

ROSEN (1955), working in Tahiti, was unable to demonstrate a "concentrating" effect when he fed *Ae. polynesiensis* on subjects having *W. bancrofti* microfilariae, but SYMES (1960b), in his tables X(a) and X(b) showed a very marked "concentrating" effect when feeding *Ae. pseudoscutellaris* and *Ae. fijiensis* on Fijians with sub-periodic *W. bancrofti;* the "concentrating" effect is seen both in the percentage of mosquitoes infected and in the average number of infective larvae per infected mosquito.

Our results show that *Ae. polynesiensis* takes up and sustains development of many more filarial larvae than would be expected from their concentration in the human host. As 754 mosquitoes ingest 1 ml of blood, if the mosquitoes were not "concentrating" the microfilariae, an infection rate of about 1% would be expected. In fact, a total of 80 *W. bancrofti* larvae were found in 670 mosquitoes, approximately 12 times the expected number.

Effects of DEC on the infectivity and subsequent development of microfilariae

MANSON-BAHR (1952), studying the development of the Fijian strain of *W. bancrofti* in *Ae. pseudoscutellaris,* found that microfilariae persisting in the blood after administration of DEC were incapable of undergoing development in the mosquito. Other workers have shown that microfilariae persisting in the blood after DEC treatment readily develop to the infective stage in their mosquito vectors; ROSEN (1955) using the Tahitian strain of *W. bancrofti* and *Ae. polynesiensis* and JORDAN (1959) in Mwanza, Tanzania, with nocturnally periodic *W. bancrofti* and *C. fatigans.* Three of Rosen's patients had received 42 mg/kg body weight of DEC, three received 21 mg/kg body weight and two received unspecified dosages. Jordan used a more complicated series of regimens, but in one experiment a volunteer was given 500 mg of DEC five hours and again one hour before mosquito feeding, at which time "the serum level of drug would be high".

Our present study also shows that microfilariae from a DEC-treated volunteer infect *Ae. polynesiensis* readily and development to the infective stage is not affected by previous exposure of the parent worms to DEC; the volunteer had received a total dosage of 162 mg per kg of body weight over a five year period.

Host and parasite mortality in arthropod-filarial infections

FÜLLEBORN (1908) and BAHR (1912) seem to have been the first workers to suggest that the ingestion of large numbers of microfilariae might produce excessive

mortality in arthropod hosts during parasite maturation. Since this time, numerous studies of the problem and valuable reviews of the literature have been published, and the possible reasons for the early death of the hosts are given by WHARTON (1957b), LAVOIPIERRE (1958) and JORDAN and GOATLY (1962).

It is of interest that excessive mortality does not seem to occur when *Anopheles* mosquitoes are fed on high-density microfilaria carriers (HICKS, 1932), possibly because of the protective effect of the cibarial and pharyngeal armatures of these mosquitoes (COLUZZI and TRABUCCHI, 1968; BRYAN et al, 1974).

Studies on the host-parasite combinations most similar to the one studied by us have been carried out by ROSEN (1955) in Tahiti and SYMES (1960b) in Fiji. Rosen only observed significantly increased mortalities in his infected as compared with control mosquitoes when microfilarial densities in donor blood reached 9,780 per ml or higher. He did, however, record rather higher mortalities in the period 13 to 16 days after feeding on a donor with a density of 8,350 microfilaria per ml of blood. He suggested two possible explanations: either that the mosquitoes died of their heavy infection or those which survived better had lost larvae without taking a blood meal, as demonstrated by PRATT and NEWTON (1946) and GWADZ and CHERNIN (1973).

Symes suggested that excess mortality only became apparent when large numbers of microfilariae (average 57) were ingested and found that donor blood counts exceeding 5,500 microfilariae per ml were needed to produce this level of intake. Certainly, the parasite loads studied by us did not increase the daily mortality of the vectors.

As well as filarial larvae killing the vector, the vector may cause the death of the larvae. Death may be caused by genetic resistance of the mosquito (MACDONALD, 1962; MCGREEVY et al, 1974), or, particularly in the case of *Anopheles* species, by mechanical damage inflicted by the cibarial and pharyngeal armatures (COLUZZI and TRABUCCHI, 1968; BRYAN et al, 1974); death may also occur in the thoracic muscles due to a host reaction to the developing larvae. The latter type of reaction usually occurs in abnormal host-parasite systems (LAURENCE, 1970; OOTHUMAN et al, 1974). Loss of filarial larvae from the vector may occur in the discharge of fluid from the hind-gut (REID, 1953; KARTMAN, 1953b; JORDAN, 1954; JORDAN and GOATLY, 1962), but has not been observed in *Ae. polynesiensis*.

Strains of filaria from one geographical area are often poorly adapted to vectors of the same species of filaria from other areas—e.g., ROSEN (1955) showed that the Polynesian strain of *W. bancrofti* was poorly adapted to *C. fatigans* and a Martinique strain of *W. bancrofti* developed abnormally in *Ae. polynesiensis*. The same general rule applies to a number of other filarial-arthropod host-parasite relationships (KARTMAN, 1953a, b and c).

However, Pacific strains of *W. bancrofti* are well adapted to *Ae. polynesiensis*, and therefore it seems unlikely that a significant loss of developing larvae of the Samoan strain of *W. bancrofti* would occur in *Ae. polynesiensis* with the post-DEC levels of microfilaraemia. ROSEN (1955) showed that failure of *W. bancrofti* microfilariae to develop occurred when mosquitoes had fed on blood with high densities of microfilariae, but not at lower densities. Similar results occurred with *W. bancrofti* and *C. fatigans*.

Two interesting studies on the relationship between the number of ingested microfilariae and subsequent numbers of infective larvae, for a number of arthropod-filaria combinations, have recently been published by PACHECO et al (1972) and PICHON (1974). In both these studies, using different methods, the same conclusions have been reached; regardless of the presence of a "concentrating" mechanism, at low microfilaria densities, most ingested larvae develop to the infective stage. This is true until a horizontal asymptote is reached by the number of infective larvae present, regardless of subsequent increases in the number of microfilariae ingested. The numerical value of this asymptote varies with each arthropod-filaria relationship studied. PICHON (1974), using the figures of ROSEN (1955) and those from his own experiments in Tahiti, calculates the asymptotic value for the relationship Pacific *W. bancrofti* and *Ae. polynesiensis* as about 19. This level is not likely to be reached in Samoa after mass administration of DEC.

It would appear that all the factors mentioned by KARTMAN (1954) as contributing to his "Index of Experimental Infection" in filarial vectors, namely the survival rate of infected hosts, the infection rate and the efficiency ratio, are likely to achieve the maximum values possible for the local vector-parasite combination in the present circumstances in Western Samoa.

General implications of this study for filariasis eradication in Western Samoa

We have shown that:

(a) *Ae. polynesiensis* is able to ingest and "concentrate" microfilariae from an ultra-low density microfilaria carrier;
(b) the maturation of these microfilariae in the mosquitoes is not affected by the DEC treatment of the carrier;
(c) the parasite load the mosquitoes pick up from a DEC-treated carrier is insufficient to cause a significant mortality of the mosquitoes or the filariae,

and it is known that *Ae. polynesiensis* bites man in considerable numbers and that numerous microfilaria carriers still live in Western Samoa. Therefore, resumption of filariasis transmission is still possible.

To gain a clearer idea of how great this possibility is, we need to estimate (a) the number of microfilaria carriers alive at present in the country, and (b) how many infective larvae are likely to be produced daily in *Ae. polynesiensis* after feeding on these carriers.

Number of microfilariae carriers in Western Samoa

The presence of microfilaraemia in Western Samoa before the start of MDA of DEC has been variously estimated at about 19% (THIEME and PENAIA, 1972), and about 30% (MCCARTHY and FITZGERALD, 1955; MCCARTHY and CARTER, 1967); these surveys were carried out using stained 20 mm^3 thick films of finger-prick blood. Recent studies (SOUTHGATE, 1974; DESOWITZ and HITCHCOCK, 1974) have shown that prevalence rates of microfilaraemia would have been in the range of 50% to 70% if 1 ml of venous blood had been examined by the membrane-filtration method. Membrane-filtration examinations of subjects known to have been microfilaraemic before MDA have been performed twice in Western Samoa; in 1971 (DESOWITZ and SOUTHGATE, 1973) and during the present study. In each case about 23% of the patients were still microfilaraemic, the majority at ultra-low densities. It seems reasonable, therefore, to estimate the *minimum* number of microfilaria

carriers alive at present in Western Samoa as:

$$M = P\,x\,y\,z \quad \ldots\ldots (1)$$

where P is the present population of Western Samoa
= 145,000 (this is the result of estimation, not census).

x is the proportion of the present population which was alive at the start of MDA
= 0·75, from unpublished estimates of crude birth and death rates in the past six years and not from reliable figures of birth and death registrations.

y is the minimum estimate of the proportion of the population microfilaraemic at the start of MDA
= 0·19 (THIEME and PENAIA, 1972). This figure is almost certainly far too low; other published papers which we have quoted, using the same technique, give estimates as high as 0·30. In addition, the blood film technique seriously underestimates the true prevalence of microfilaraemia, and there is no evidence to suggest that DEC is more efficient at clearing low-density microfilaraemia undetectable by the 20 mm³ technique than it is at clearing high-density microfilaraemia.

z is the proportion of the previous microfilaria carriers known to be microfilaraemic at present, using the membrane-filtration technique
= 0·23 (DESOWITZ and SOUTHGATE, 1973; the present study). This figure is probably too low, as examination of 1 ml of venous blood from each individual by the membrane-filtration method in a single point-prevalence study certainly underestimates the true prevalence of microfilaraemia in a population (SOUTHGATE, 1974).

Thus equation (1) becomes:

$$M = 145{,}000 \times 0{\cdot}75 \times 0{\cdot}19 \times 0{\cdot}23$$
$$= 4{,}753.$$

It is realized that there are numerous possible sources of error in making this estimation, some of which have already been discussed. Other sources of error are:

(a) the majority of persons dying since 1967, and allowed for in the estimation of x will have been from elderly age-groups, either negative for microfilaraemia or having low-density microfilaraemia undetectable by 20 mm.³ blood films. This means that our value of 0·19 for y is too low, as is our value of 0·23 for z, since the 1971 and 1973 estimates of z were based solely on examinations of surviving microfilaria carriers who initially had fairly high densities of microfilariae;

(b) equation (1) assumes that no new cases of microfilaraemia have arisen since the start of MDA, although we detected one such case during the present study;

(c) no allowance has been made for the natural disappearance of microfilaraemia due to the death or ageing of sexually mature female worms. Although studies of the natural decline and disappearance have been published (JACHOWSKI et al, 1951; LEEUWIN, 1962; HAIRSTON and JACHOWSKI, 1968; MAHONEY and AIU, 1970), it is impossible to apply these results to Western Samoa as the situations and techniques are completely different.

An alternative method of calculating the number of microfilaria carriers in Western Samoa would be to take the proportion of carriers detected by stained blood film examination of 60 mm.³ of finger prick blood, and apply a correction factor derived from the prevalence rate studies carried out using the blood film and membrane-filtration techniques simultaneously, in other Pacific islands (DESOWITZ and SOUTHGATE, 1973; DESOWITZ et al, 1973; DESOWITZ and HITCHCOCK, 1974). These studies have been reviewed by DESOWITZ (1974), who estimated the correction factor at about two times for adults living in hypo- or hyperendemic areas, about six times for children in hyperendemic areas and between three and 6·5 times for *treated* adults. Since the conclusion of the 2nd round of MDA in Western Samoa at the end of 1971, the Samoan Filariasis Control Section has examined a sample (11,506) of the whole population. Using a stained 60 mm³ finger prick blood film, the prevalence of microfilaraemia was 0·19% (PENAIA, personal communication). Using the lowest correction factor of DESOWITZ (1974), this would give a true prevalence of microfilaraemia of 0·38‰, whereas the use of the highest factor would give a figure of 1·235‰. These figures applied to the whole population of Western Samoa give estimates of the number of microfilaria carriers of 551 for the lowest conversion factor and 1,791 for the highest.

However, we feel that this second method of estimation is invalid in the present instance, since there have been no studies carried out of whole populations using both techniques simultaneously in areas where DEC has been administrated in such high dosage or to such a high proportion of the population as in Western Samoa. We feel that with all its inadequacies, which we have discussed at length, our estimate of 4,753 as the minimum number of microfilaria carriers in Western Samoa is the best that can be made in the present circumstances.

The number of infective larvae produced daily in Ae. polynesiensis

A very rough estimate can also be made of the number of infective larvae produced daily in *Ae. polynesiensis* in Western Samoa. In making this estimation, we are assuming that all the microfilaria carriers will produce infection rates in the vector that are similar to that found in our experimental work on the DEC-treated volunteer. The number of infective larvae can be determined from the equation:

$$M\,m\,a\,p^n\,i\,b \quad \ldots\ldots (2)$$

where M is as defined in equation (1)
= 4,753

m is the mean density of *Ae. polynesiensis* in relation to man.

a is the average number of humans bitten by one *Ae. polynesiensis* in one whole day.

ma = 200 (data on the individual parameters m and a are not available for Western Samoa, but estimates of the daily man-biting rate, which is the product of these two figures, can be made from the results of a number of workers in the Pacific area—e.g., JACHOWSKI and OTTO (1952), JACHOWSKI (1954), JACHOWSKI et al (1951), BURNETT (1960), SYMES (1960a), RAMALINGAM (1968) and RAKAI et al (1974). The figures vary widely with the proximity to the breeding sites and the habitat; seasonal variations in Samoa are small (JACHOWSKI, 1954). For making this estimation, we have arbitrarily chosen the value of 200 which falls within

the ranges observed by the authors quoted above).

p is the probability of *Ae. polynesiensis* surviving through one whole day
= 0·80 (BURNETT, 1960).

n is the time elapsing between ingestion of microfilariae and the time when the mosquito is ready to feed with infective larvae in the mouthparts
= 14. This parameter varies with temperature, but we have allowed 11–12 days for the maturation of larvae after ingestion of microfilariae and 2–3 days before the mosquito takes her next blood meal.

i is the proportion of *Ae. polynesiensis* surviving the extrinsic cycle which actually contained infective larvae in the present study
= 0·09 (see Tables I to IV).

b is the mean number of infective larvae per infected mosquito
= 1·0 (from the present study; the actual value is 1·11, but the number of mosquitoes studied was small and to make a minimum estimate it is reasonable to take the value as 1).

Thus, equation (2) becomes:

$4{,}753 \times 200 \times (0{\cdot}80)^{14} \times 0{\cdot}09 \times 1$
= 3,764 infective larvae daily or 1,374,000 infective larvae annually will fall on the human population of Western Samoa. In arriving at this figure, no allowance has been made for *Ae. polynesiensis* biting hosts other than man. This mosquito will bite other animals, but man is the preferred host (JACHOWSKI, 1954).

Not all these infective larvae will enter the human population. It has been shown that in a number of mosquito-filaria systems not all the infective larvae escape from the mosquito mouthparts at a single blood meal, and of those which do escape not all succeed in entering the vertebrate host. HAIRSTON and DE MEILLON (1968), studying *W. bancrofti* and *C. fatigans* in Rangoon with much larger numbers of infective larvae per mosquito, found that 0·414 of the infective larvae present escaped at a single blood meal. It seems very unlikely that with an average of one infective larva per mosquito as in the present study, such a small proportion of the larvae would escape. HO and EWERT (1967) showed that the proportion of infective larvae of *B. pahangi* which, having escaped from the mosquito, succeeded in penetrating the vertebrate host was 0·32. If we multiply our estimate by 0·132 (the product of these two sources of loss), we obtain the figure of 497 infective larvae daily entering the human population as a minimum estimate.

Not all of the population will be equally at risk from these larvae. Young adult males who spend more time than the rest of the population in the bush areas, the preferred habitat of *Ae. polynesiensis*, will be in most danger, as will persons living and working in proximity to microfilaria carriers.

This estimate is only concerned with the infective larvae transmitted by *Ae. polynesiensis*. However, in some areas of Western Samoa, particularly in the bush villages, *Ae. samoanus* is a more important vector than *Ae. polynesiensis* (RAMALINGAM, 1968).

Conclusions

In view of our experimental findings and their implications, it seems to be absolutely essential that in Western Samoa surveillance of the human and mosquito populations be continued for several years to come. If resumption of transmission occurs, it should be dealt with promptly, either by the resumption of mass drug administration (in view of the findings of DEC-refractory microfilaraemia reported by DESOWITZ and SOUTHGATE (1973), an alternative drug to DEC is urgently needed) or by some method of vector control.

References

Bahr, P. H. (1912). Filariasis and elephantiasis in Fiji. *Research Memoirs of the London School of Tropical Medicine*, **1**, 1–192.

Bell, D. (1967). Membrane filters and microfilariae: A new diagnostic technique. *Annals of Tropical Medicine and Parasitology*, **61**, 220–223.

Burnett, G. F. (1960). Filariasis research in Fiji, 1957–1959. Part 1. Epidemiology. *The Journal of Tropical Medicine and Hygiene*, **63**, 153–162.

Burnett, G. F. & Mataika, J. U. (1964). Mass administration of diethylcarbamazine citrate in preventing transmission of aperiodic human filariasis. II. Results of a blood survey made four years after drug administration. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **58**, 545–551.

Burton, G. J. (1964). The intake of microfilariae of *Wuchereria bancrofti* by *Culex pipiens fatigans* in British Guiana. *Annals of Tropical Medicine and Parasitology*, **58**, 333–338.

Bryan, J. H., Oothman, P., Andrews, B. J. & McGreevy, P. B. (1974). Effects of pharyngeal armatures of mosquitoes on microfilariae of *Brugia pahangi*. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **68**, 14.

Chularerk, P. & Desowitz, R. S. (1970). A simplified membrane filtration technique for the diagnosis of microfilaremia. *The Journal of Parasitology*, **56**, 623–624.

Coluzzi, M. & Trabucchi, R. (1968). Importanza dell'armatura bucco-faringea in *Anopheles* e *Culex* in relazione alle infezioni con *Dirofilaria*. *Parassitologia*, **10**, 47–59.

Denham, D. A., Dennis, D. T., Ponnudurai, T., Nelson, G. S. & Guy, F. (1971). Comparison of a counting chamber and thick smear methods of counting microfilariae. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **65**, 521–526.

Desowitz, R. S., Chularerk, P. & Palumbo, N. E. (1970). Evaluation of a simplified membrane filtration technique for the diagnosis of canine filariasis. *The Southeast Asian Journal of Tropical Medicine and Public Health*, **1**, 231–232.

Desowitz, R. S. & Hitchcock, J. C. (1974). Hyperendemic bancroftian filariasis in the Kingdom of Tonga: The application of the membrane filter concentration technique to an age-stratified blood survey. *The American Journal of Tropical Medicine and Hygiene*, **23**, 877–879.

Desowitz, R. S. & Southgate, B. A. (1973). Studies on filariasis in the Pacific: 2. The persistence of microfilaraemia in diethylcarbamazine treated populations of Fiji and Western Samoa: Diagnostic application of the membrane-filtration technique. *The Southeast Asian Journal of Tropical Medicine and Public Health*, **4**, 179–183.

Desowitz, R. S., Southgate, B. A. & Mataika, J. U. (1973). Studies on filiarasis in the Pacific: 3. Comparative efficacy of the stained blood-film, counting-chamber and membrane-filtration techniques for the diagnosis of *Wuchereria bancrofti* microfilaraemia in untreated patients in areas of low endemicity. *The Southeast Asian Journal of Tropical Medicine and Public Health*, **4**, 329–335.

Fukushima, H. (1967). Selective treatment of bancroftian filariasis. *Acta Medica Universitatis Kagoshimaensis*, **9**, 25–32.

Fülleborn, F. (1908). Untersuchungen an menschlichen Filarien und deren Übertragung auf Stechmücken. *Archiv für Schiffs-und Tropenhygiene*, **12**, 353–388.

Gubler, D. J., Inui, T. S., Black, H. R. & Bhattacharya, N. C. (1973). Comparison of microfilaria density in blood sampled by finger prick, venipuncture and ingestion by mosquitoes. *The American Journal of Tropical Medicine and Hygiene*, **22**, 174–178.

Gwadz, R. W., & Chernin, E. (1973). Escape of infective larvae of *Brugia pahangi* from *Aedes aegypti*. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **67**, 814–818.

Hairston, N. G., & De Meillon, B. (1968). On the inefficiency of transmission of *Wuchereria bancrofti* from mosquito to human host. *Bulletin of the World Health Organization*, **38**, 935–941.

Hairston, N. G. & Jachowski, L. A. (1968). Analysis of the *Wuchereria bancrofti* population in the people of American Samoa. *Bulletin of the World Health Organization*, **38**, 29–59.

Hawking, F. (1962). A review of progress in the chemotherapy and control of filariasis since 1955. *Bulletin of the World Health Organization*, **27**, 555–568.

Hicks, E. P., (1932). The transmission of *Wuchereria bancrofti* in Sierra Leone. *Annals of Tropical Medicine and Parasitology*, **26**, 407–422.

Highby, P. R. (1946). A technique for xenodiagnosis of filariasis. *The Journal of Parasitology*, **32**, 433–434.

Ho, B. C., & Ewert, A. (1967). Experimental transmission of filarial larvae in relation to feeding behaviour of the mosquito vectors. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **61**, 663–666.

Jachowski, L. A. (1954). Filariasis in American Samoa. V. Bionomics of the principal vector, *Aedes polynesiensis* Marks. *American Journal of Hygiene*, **60**, 186–203.

Jachowski, L. A. & Otto, G. F. (1952). Filariasis in American Samoa. II. Evidence of transmission outside of villages. *The American Journal of Tropical Medicine and Hygiene*, **1**, 662–670.

Jackowski, L. A., Otto, G. F. & Wharton, J. D. (1951). Filariasis in American Samoa. I. Loss of microfilaria in the absence of continued reinfection. *Proceedings of the Helminthological Society of Washington*, **18**, 25–28.

Jordan, P. (1954). Microfilariae in dejecta of mosquitoes. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **48**, 537.

Jordan, P. (1959). The possible role of low-density microfilaraemia in the spread of *Wuchereria bancrofti* by *Culex fatigans* in East Africa. *Annals of Tropical Medicine and Parasitology*, **53**, 42–46.

Jordan, P. & Goatly, K. D. (1962). Bancroftian filariasis in Tanganyika: A quantitative study of the uptake, fate and development of microfilariae of *Wuchereria bancrofti* in *Culex fatigans*. *Annals of Tropical Medicine and Parasitology*, **56**, 173–187.

Kartman, L. (1953a). Factors influencing infection of the mosquito with *Dirofilaria immitis* (Leidy, 1856). *Experimental Parasitology*, **2**, 27–78.

Kartman, L. (1953b). An observation on the loss of microfilariae from the mosquito host during its infective meal. *The Journal of Parasitology*, **39**, 571–572.

Kartman, L. (1953c). Effects of feeding mosquitoes upon dogs with differential microfilaraemias. *The Journal of Parasitology*, **39**, 572.

Kartman, L. (1954). Suggestions concerning an index of experimental filaria infection in mosquitoes. *The American Journal of Tropical Medicine and Hygiene*, **3**, 329–336.

Kessel, J. F. (1971). A review of the filariasis control programme in Tahiti from November 1967 to January 1968. *Bulletin of the World Health Organization*, **44**, 783–794.

Kessel, J. F., Siliga, N., Tompkins, H. & Jones, K. (1970). Periodic mass treatment with diethylcarbamazine for the control of filariasis in American Samoa. *Bulletin of the World Health Organization*, **43**, 817–825.

Knott, J. (1939). A method for making microfilarial surveys on day blood. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **33**, 191–196.

Laigret, J., Kessel, J. F., Malarde, L., Bambridge, B. & Adams, H. (1965). La lutte contre la filariose lymphatique apériodique en Polynésie française. *Bulletin de la Société de Pathologie Exotique et de ses filiales*, **58**, 895–916.

Laigret, J., Kessel, J. F., Bambridge, B. & Adams, H. (1966). Onze ans de chimioprophylaxie par la diethylcarbamazine de la filariose lymphatique aperiodique a Tahiti. *Bulletin of the World Health Organization*, **34**, 925–938.

Laurence, B. R. (1970). Problems of the adaptation of filarial worms to their mosquito hosts. In *H. H. Srivastava Commemoration Volume*, ed. Singh, K. S., & Tandan, B. K., pp. 9–17. Izatnagar: Indian Veterinary Research Institute.

Lavoipierre, M. M. J. (1958). Studies on the host-parasite relationships of filarial nematodes and their arthropod hosts. II. The arthropod as a host to the nematode: A brief appraisal of our present knowledge, based on a study of the more important literature from 1878 to 1957. *Annals of Tropical Medicine and Parasitology*, **52**, 326–345.

Leeuwin, R. S. (1962). Microfilaraemia in Surinamese living in Amsterdam. *Tropical and Geographical Medicine*, **14**, 355–360.

McCarthy, D. D. & Fitzgerald, D. N. (1955). Researches in Western Samoa. III. Observations on filariasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **49**, 82–89.

McCarthy, D. D. & Carter, D. G. (1967). The control of filariasis in Western Samoa. *Report of the Medical Research Council of New Zealand*, **16**.

Macdonald, W. W. (1962). The selection of a strain of *Aedes aegypti* susceptible to infection with semiperiodic *Brugia malayi*. *Annals of Tropical Medicine and Parasitology*, **56**, 368–372.

McGreevy, P. B., McClelland, G. A. H. & Lavoipierre, M. M. J. (1974). Inheritance of susceptibility to *Dirofilaria immitis* infection in *Aedes aegypti*. *Annals of Tropical Medicine and Parasitology*, **68**, 97–109.

Mahoney, L. E., & Kessel, J. F. (1971). Treatment failure in filariasis mass treatment programmes. *Bulletin of the World Health Organization*, **45**, 35–42.

Mahoney, L. E. & Aiu, P. (1979). Filariasis in Samoan immigrants to the United States. *The American Journal of Tropical Medicine and Hygiene*, **19**, 629 631.

Manson-Bahr, P. (1952). The action of Hetrazan in Pacific filariasis. *The Journal of Tropical Medicine and Hygiene*, **55**, 169–173.

March, H. N., Laigret, J., Kessel, J. F. & Bambridge, B. (1960). Reduction in the prevalence of clinical filariasis in Tahiti following adoption of a control program. *The American Journal of Tropical Medicine and Hygiene*, **9**, 180–184.

Marshall, C. L. & Yasukawa, K. (1966). Control of bancroftian filariasis in the Ryukyu Islands: Preliminary results of mass administration of diethylcarbamazine. *The American Journal of Tropical Medicine and Hygiene*, **15**, 934–942.

Nelson, G. S. (1964). Factors influencing the development and behaviour of filarial nematodes in their arthropodan hosts. In *Host-parasite relationships in invertebrate hosts*, ed. Taylor, A. E. R., pp. 75–119. Oxford: Blackwell Scientific Publications.

Oothuman, P., Simpson, M. G. & Laurence, B. R. (1974). Abnormal development of a filarial worm *Brugia patei* (Buckley, Nelson and Heisch), in a mosquito host, *Anopheles labranchiae atroparvus* van Thiel. *Journal of Helminthology*, **48**, 161–165.

Pacheco, G., Atkins, M. J. & Gurian, J. (1972). Quantification of infection of ticks with *Dipetalonema viteae*. *The Journal of Parasitology*, **58**, 275–278.

Pichon, G. (1974). Relations mathématiques entre le nombre des microfilaires ingérées et le nombre des parasites chez différents vecteurs naturels ou expérimentaux de filarioses. *Cahiers ORSTOM série Entomologie médicale et Parasitologie*, **12**, 199–214.

Pratt, I. & Newton, W. L. (1946). The migration of infective larvae of *Wuchereria bancrofti* within the mosquito host and their rate of escape under laboratory conditions. *The Journal of Parasitology*, **32**, 272–280.

Rakai, I. M., Naserua, J. D., Macnamara, F. N. & Pillai, J. S. (1974). Mosquito-borne infections in Fiji. IV. Biting times for village mosquitoes and human filaria transmission potential of *Aedes polynesiensis* and *Aedes pseudoscutellaris*. *Journal of Medical Entomology*, **11**, 588–594.

Ramalingam, S. (1968). The epidemiology of filarial transmission in Samoa and Tonga. *Annals of Tropical Medicine and Parasitology*, **62**, 305–334.

Reid, J. A. (1953). Transmission of filariasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **47**, 84.

Rosen, L. (1955). Observations on the epidemiology of human filariasis in French Oceania. *American Journal of Hygiene*, **61**, 219–248.

Sasa, M. (1963). Pilot experiments in the control of bancroftian filariasis in Japan and Ryukyu. *Bulletin of the World Health Organization*, **28**, 437–454.

Sasa, M., Kanda, T., Mitsui, G., Shirasaka, A., Ishii, A. & Chinzei, H. (1970). The filariasis control programmes in Japan and their evaluation by means of epidemiological analysis of the microfilaria survey data. In *Recent advances in researches on filariasis and schistosomiasis in Japan*, ed. Sasa, M., pp. 3–72. Tokyo: University of Tokyo Press.

Sasa, M., Oshima, T., Sato, K., Mitsui, G., Sugata, F., Nishi, S., Yamamoto, H., Tada, I. & Motoi, E. (1963). Studies on epidemiology and control of filariasis: Observations on the carriers of *Wuchereria bancrofti* in the Amami Islands with special reference to the effects and side-reactions of diethylcarbamazine. *Japanese Journal of Experimental Medicine*, **33**, 213–243.

Southgate, B. A. (1973). Studies on filariasis in the Pacific: 1. A field trial of a counting-chamber technique for the determination of microfilarial rates and densities. *The Southeast Asian Journal of Tropical Medicine and Public Health*, **4**, 172–178.

Southgate, B. A. (1974). A quantitative approach to parasitological techniques in bancroftian filariasis and its effect on epidemiological understanding. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **68**, 177–186.

Symes, C. B. (1960a). Observations on the epidemiology of filariasis in Fiji. Part I. Field studies. *The Journal of Tropical Medicine and Hygiene*, **63**, 1–14.

Symes, C. B. (1960b). Observations on the epidemiology of filariasis in Fiji, Part II. Laboratory studies and human infections. *The Journal of Tropical Medicine and Hygiene*, **63**, 31–44.

Thieme, J. C. & Penaia, L. (1972). Filariasis control project in Western Samoa. *South Pacific Bulletin*, **22**, 17–18.

Van Dijk, W. J. O. M. (1964). Control of *Wuchereria bancrofti* filariasis in West New Guinea. *Tropical and Geographical Medicine*, **16**, 54–60.

Wharton, R. H. (1957a). Studies on filariasis in Malaya: Observations on the development of *Wuchereria malayi* in *Mansonia* (*Mansonioides*) *longipalpis*. *Annals of Tropical Medicine and Parasitology*, **51**, 278–296.

Wharton, R. H. (1957b). Studies on filariasis in Malaya: The efficiency of *Mansonia longipalpis* as an experimental vector of *Wuchereria malayi*. *Annals of Tropical Medicine and Parasitology*, **51**, 422–439.

Dando, B. C. (1967). *Filariasis in the Fiji Islands*. University of London: D.T.P.H. dissertation.

Desowitz, R. S. (1973). *Epidemiological investigations on filariasis in Fiji and Tonga, including a small additional survey in Western Samoa*. WHO/FIL/73.113 (mimeographed document).

Desowitz, R. S. (1974). *The application of a membrane filter concentration method in filariasis surveys in the South Pacific area*. WHO/FIL/74.131 (mimeographed document).

Southgate, B. A. & Desowitz, R. S. (1971). *Comparative efficacy of the stained blood film, counting chamber and membrane filtration techniques in the determination of microfilarial rates and microfilarial densities*. WHO/FIL/71.91 (mimeographed document).