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The Effect of Salt Medicated with Diethylcarbamazine in Bancroftian Filariasis *

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The paper describes a trial conducted in Tanzania of the effect on bancroftian microfilaraemia of common salt medicated with diethylcarbamazine at a 0.1 % (w/w) concentration, when given to a closed population of 600–700 with a known salt intake.

After good observer agreement in microfilarial counting had been demonstrated, quantitative blood surveys were performed monthly on known carriers. Paired pretreatment microfilarial counts from the same patients at an interval of 4 months suggested a natural fluctuation in microfilarial densities and emphasized the need for parallel concurrent controls during treatment.

During the trial the mean microfilarial densities fell steadily, being reduced by 73 % at 3 months and 90 % after 6 months. Tolerance to the drug–salt mixture was extremely good.

Problems of planning, salt preparation and assessment of results are discussed and the importance of correct experimental design in future trials is stressed. The authors suggest that a trial of salt containing 0.2 % (w/w) diethylcarbamazine may achieve optimum results. They point out that, while the use of diethylcarbamazine-medicated salt may prove useful in reducing transmission in closed communities or in endemic areas with no alternative source of salt, its extension to national populations as a sole means of bancroftian filariasis control would be of problematical value.

Success in the chemotherapeutic control of endemic filariasis due to *Wuchereria bancrofti* has been limited. Diethylcarbamazine, the only effective filaricide in widespread use, gives rise to minor side-effects, and since many microfilarial carriers are symptom-free over long periods, it has been difficult to persuade carriers to undergo treatment (WHO Expert Committee on Filariasis, 1967).

It is known that relatively small doses of diethylcarbamazine, administered over long periods, have a microfilaricidal effect equivalent to the normal therapeutic regime with fewer adverse reactions (WHO Expert Committee on Filariasis, 1967). Experience with iodized salt in goitrogenic areas and with salt medicated with chloroquine for malarial suppression suggested the use of salt medicated with diethylcarbamazine in the control of microfilarial reservoirs.

Hawking & Marques (1967) reviewed the theoretical aspects of the procedure, showed that diethylcarbamazine remained stable during cooking and presented the results of a pilot trial on 22 microfilarial carriers given salt medicated with diethylcarbamazine at varying concentrations up to 0.4% (w/w). Raghavan, Basu and Putatunda [1] discussed the results of a short-term trial in which 18 microfilarial carriers were given diethylcarbamazine-medicated salt at a 0.1 % (w/w) concentration of drug. Preliminary conclusions from these two trials, both conducted on nocturnally periodic *W. bancrofti*, were that the medicated salt was acceptable up to a concentration of drug of 0.4% (w/w) and that, even at a 0.1 % concentration, it diminished the microfilarial reservoir markedly. In 1968 this unit was requested to undertake a similar investigation.

MATERIAL AND METHODS

The microfilarial carriers were male prisoners at a large regional prison near Tanga, Tanzania, nor-

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[1] Unpublished working document WHO/FIL/68.82. A limited number of copies of this document is available to persons officially or professionally interested on request to Distribution and Sales, World Health Organization, 1211 Geneva, Switzerland.

mally holding between 600 and 700 inmates. Detainees were drawn from a wide area of East Africa and did not necessarily reflect either the incidence or the density of infection in the local population of Tanga. Meetings were held with the prisoners, the plan of the trial explained and their voluntary co-operation was obtained.

Microfilarial surveys

Microfilarial surveys were performed following the method of Sasa (1963) in which, between 21.00 and 23.00 hours, blood samples were withdrawn from a finger-tip and 3 linear smears each of 10 mm^3 were prepared, representing a sampling unit of 30 mm^3 of peripheral blood. Individual disposable presterilized lancets were used and the pipettes were cleansed, sterilized and dried with 0.1% benzalkonium chloride and acetone after each examination.

Slides were air-dried, dehaemoglobinized in tap-water, air-dried, fixed in methyl alcohol for one minute, and stained by warming with Mayer's acid haemalum for 5–8 minutes, after which excess stain was washed off.

Two pretreatment microfilarial surveys at an interval of 4 months were performed on the prison population in order to familiarize personnel with methodology, to assess observer variation in microfilarial counting, and to provide within-patient controls for the investigation of the stability of microfilarial densities. After the second survey the total population of prisoners was given diethylcarbamazine-medicated salt at a concentration of 0.1% (w/w) for 6 months. Microfilarial surveys were conducted at monthly intervals on known microfilaria-positive cases during this time. In the seventh month a microfilarial survey was performed on all those prisoners who had been admitted since the start of the trial and thus had received medicated salt for varying periods but whose blood had not previously been examined.

Finally a microfilarial survey was conducted on the prison warders who fed outside the prison and therefore had not received medicated salt. The microfilaria carriers in this group were treated with a therapeutic course of diethylcarbamazine at a total dose of 4.45 g given over 15 days, equivalent to 74 mg per kg for a 60-kg man.

Salt supplies

The bulk monthly supplies of salt for the prison (600 lb or about 272 kg) were delivered every 4 weeks to a central laboratory where all preparation of the drug–salt mixture took place. Diethylcarbamazine citrate powder was mixed by hand with the salt in a 0.1% concentration (w/w), 10 g of drug in 10 kg of salt, by sieving the drug in layers on to the salt in plastic buckets and by repeatedly transferring this drug–salt mixture from bucket to bucket. Finally the medicated salt was packed as units of 10 kg into plastic bags which were sealed to prevent leaching, which was known to occur with chloroquine–salt mixtures subjected to unsuitable storage conditions.

Deliveries to the prison were made once weekly. The prison store received only sealed supplies of medicated salt and no other type of salt was held in stock. Fortnightly stores checks, both in the laboratory and in the prison, ensured that ledger and actual physical stocks agreed. As a result of the strict indenting and stock-taking system adopted, it was extremely improbable that alternative supplies of salt could have been used.

Medicated salt in the prison store was issued daily to the central kitchen, where all cooking was done, and each prisoner received his daily ration of salt from the kitchen at a dining parade. Before the trial the mean daily issue of salt from the prison store was 20 lb and hence a 10-kg bag (ca 22 lb) of medicated salt was adopted as a standard daily unit for a population varying between 600 and 700 detainees whose daily ration of salt was ½ oz (ca 14 g) per head plus an indeterminate amount used in central cooking procedures. In practice a 10-kg bag was almost completely used up each day. Any remaining salt was carried forward for use on the next day.

CHECKS ON MEDICATED SALT

Replicated random samples of the drug–salt mixture were sent at intervals to the United Kingdom where the diethylcarbamazine content was analysed. The range of the mean diethylcarbamazine content of each batch during the first 5 months was 0.09%–0.15% and the over-all mean drug content of 0.117% indicated the efficiency of the mixing procedure. Assay failed to reveal the drug in samples taken in the last week of the trial, yet this only diminished the over-all mean diethylcarbamazine content to 0.098%.

SALT CONSUMPTION AND DRUG INTAKE

Diethylcarbamazine intake per head per day at a 0.1% concentration of medicated salt and a ration of 14 g of salt would theoretically be 14 mg, which, over the 6-month period of the trial, gave a total intake of about 2.52 g or about 42 mg per kg of body-weight

for a 60-kg individual. There must, however, be wide variation around this theoretical intake as many prisoners were engaged in hard physical labour such as sisal-cutting and presumably took as much salt as could be obtained, while for others engaged in less strenuous pursuits the theoretical intake figure could be regarded as a maximum.

SOME DIFFICULTIES

The population

It was not originally appreciated that in the closed population of a prison there would still be considerable population fluctuation due to short-term prisoners. For example, the first microfilarial survey of 672 prisoners yielded 148 positive cases (22%), of whom only 33 remained in prison 4 months later at the time of the second pretreatment survey. Meanwhile there had been 203 new admissions among whom the second survey showed 71 microfilaria carriers (35%), but the majority were short-term prisoners and only 31 of this group and the 33 from the first survey remained in prison at the start of the trial. This factor disappointingly reduced the numbers of continuous total observations throughout the trial but provided a sample of paired control observations for the assessment of the stability of microfilarial densities.

The salt

The bulk salt supplies, as delivered to the central laboratory, consisted of large crystal aggregates, up to 1 cm in diameter, with an appreciable admixture of moisture and foreign particles, totally unsuitable for mixing with the drug.

Four random samples, each of 50 g, from the first bulk consignment were weighed, and then reweighed at various times during drying in an oven. Water evaporation increased steadily up to 2 hours at a temperature of 60°C when the mean water content of the replicated samples was 2.295 g$\pm$SE 0.038. Expressed as a percentage, the mean water content of the crude salt was 4.59% with 95% confidence limits of 4.35% and 4.83%. In view of this, all bulk salt supplies were dried, on trays in ovens, for 2 hours at a minimum temperature of 120°C, after which the grosser foreign particles were removed and the salt ground down to finely particulate size in a large hotel-type coffee-grinder. Diethylcarbamazine was then mixed by hand as described.

Drying, grinding, mixing, bagging and sealing the drug–salt mixture were arduous and time-consuming. When 4 men were available no more than 6 bags per day could be completed. For larger trials some mechanical procedure would be essential were the locally available salt not in a form suitable for direct mixing with the drug.

MICROFILARIAL SURVEYS

Tests of experimental technique

Variation in microfilarial recovery in each line of a 3-line blood smear. To be certain that microfilarial recoveries in each line of the 3 linear smears were comparable, the microfilarial yield in each line of the 148 positive slides from the first blood survey were ranked horizontally and subjected to a distribution-free analysis of variance (Friedman, 1937, 1940). The computed statistic χ_r^2 for rank totals was 1.47 on 2 degrees of freedom, $0.5 > P > 0.3$, indicating the sampling variation associated with homogeneous material. The null hypothesis that the microfilarial yield in each line of the 3 linear smears was similar was thus accepted.

Observer variation in microfilarial counting. It is essential, before undertaking experiments in which replicated or repeated observations or both are made, to determine whether results are reproducible; in other words, whether adequate control is exercised over the experimental conditions.

In experiments involving repetitive counting we have found from previous experience in schistosomiasis that the first essential step is to determine whether any counter differs significantly in his assessments from others. If such variation exists it must be rectified before commencing the experiment.

Four observers were responsible for counting microfilariae and their performance was assessed as follows. From the first blood survey 78 microfilaria-positive slides were randomly selected plus 2 known negative slides. The 80 slides were further randomized into 4 groups of 20. The 4 groups were randomly allotted to the 4 observers, who counted and recorded the number of microfilariae on each slide. Over 4 days each observer counted each of the 20 slides in each group. The exercise was blind as the slides were known by a reference number only and there was no communication between counters. All results were tabulated separately by each observer and handed to the experiment controller for analysis.

As the true microfilarial count in any slide was unknown, the best estimate was the mean microfilarial count of 4 experienced observers. Any significant deviation from this mean would indicate significant observer variation. The null hypothesis was that

significant deviation from the mean did not exist for any of the 4 observers, and the alternative hypothesis was that significant deviation indicated significant observer variation in microfilarial counts.

As the χ^2 distribution is inappropriate for small expected numbers, 19 of the 80 slides, including the 2 negative slides, were analysed separately because their mean expectation, i.e., the mean expected number of microfilariae, was less than 5. These counts were subjected to an analysis of variance by ranks (Friedman, 1937, 1940), and the computed test statistic χ_r^2 for this subgroup was 4.453 on 3 degrees of freedom, $0.4 > P > 0.3$, indicating acceptance of the null hypothesis and insignificant variation between observers' assessments of low microfilarial counts. As a point of interest, no observer found a microfilaria in either of the negative slides.

For the remaining 61 slides where the mean expectation was greater than 5, χ_r^2 was 5.316, on 3 degrees of freedom, $0.2 > P > 0.1$, indicating again non-significance of the variation between observers. Combining the groups yielded a χ_r^2 of 5.606 on 3 degrees of freedom, $0.2 > P > 0.1$, an over-all non-significant finding.

Excellent agreement between observers' assessments of a wide range of microfilarial counts was further confirmed by a more penetrating partitioned χ^2 analysis of slides in which the mean expectation was greater than 5.[1] It was concluded that the methods and observers used in the trial would carry no risk of significant variation.

Nomenclature of microfilarial surveys

Survey A was the first pretreatment survey, performed in February 1968 on 672 prisoners, yielding 148 microfilarial carriers.

Survey B was the second pretreatment survey, performed 4 months later in June 1968 on the new admissions to the prison since Survey A. Of 203 new prisoners, 71 were microfilaria-positive but only 31 of these remained in prison at the start of the trial.

Survey C (C = controls) referred to the 33 microfilaria-positive cases of the original 148 in Survey A who were still detained 4 months later at the time of the second pretreatment survey, who were re-examined at that time, and who remained in prison at the start of the trial. (Thus 64 microfilaria-positive cases entered the trial, 33 from Surveys A and C, and 31 from Survey B.)

[1] The details of the individual observers' counts and the analysis are available to interested readers from the authors.

Survey D was the survey of those microfilaria-positive cases remaining in prison after 1 month of medicated-salt administration (62 patients).

Surveys E, F, G, H, and I were surveys performed on the microfilaria-positive cases remaining in prison after 2, 3, 4, 5 and 6 months of medicated-salt administration and represented 52, 47, 32, 28 and 22 patients respectively.

Survey J was a terminal survey of 193 prisoners admitted to prison after the commencement of medicated-salt feeding to the prison population.

Survey W was a survey of 123 prison warders who did not receive medicated salt.

Controls

As this trial was to be conducted over a 6-month period, it was necessary to determine whether any variability occurred in microfilarial densities in the same subjects over a similar time period in the absence of treatment, i.e., the natural history of microfilaraemia.

The two pretreatment blood surveys afforded 33 patients who had remained in prison in the intervening 4 months. These subjects were subclassified as Survey C (C = controls) and were re-examined at the same time as Survey B. Thus, 33 paired microfilarial counts, performed by the same method at the same time of night, by technicians in whom there was evidence of excellent agreement in microfilarial assessments, were available for analysis. It was known that no treatment for filariasis had been given to any prisoner in this interim period.

For each pair of microfilarial counts, the second count was subtracted from the first, the null hypothesis being that the mean of the sample of differences did not differ from zero. Analysed by Wilcoxon's signed ranks test $z = 2.5991$, giving a 2-tail probability of 0.0096.

Mean microfilarial count, Survey A = 50.76 per 30 mm³ blood	51 (rounded)
Mean microfilarial count, Survey C = 37.21 per 30 mm³ blood	37 (rounded)
Difference A−C = 13.55 per 30 mm³ blood	14 (rounded)

This difference of 14 microfilariae per 30 mm³ blood between the means of the 2 surveys is statistically significant at a 2-tail probability level of 9 in 1000 and it represents a 27.5% reduction in mean microfilarial count over a 4-month period without treatment, suggesting a natural fluctuation in the stability of microfilarial densities.

Hawking & Marques (1967) stated that " the relative stability of microfilarial parasitaemias is well known ", but the literature is scanty on this aspect of bancroftian microfilaraemia. Edgar, Beye & Mille (1952) in Tahiti demonstrated a significant seasonal variation in microfilarial counts repeated on the same subjects during one year. Friedheim (1962), in the course of a clinical trial in southern Tanzania, followed microfilarial densities over 6 weeks in 5 patients with *W. bancrofti* infection who were given an inactive placebo, and noted that in 4 patients the microfilarial count after 6 weeks exceeded the initial count. Analysis of his data by a χ^2 dispersion test indicates a highly significant departure from homogeneity. Hairston & Jachowski (1968) showed a wave-like progression of microfilarial counts when repeated on the same subjects in American Samoa with a peak " preceded by an increase in microfilarial density lasting from 3 to 24 months . . . followed by a decrease of somewhat shorter duration ".

These observations have an important bearing upon the assessment of the results of medicated-salt trials which are, of necessity, conducted over long periods. There will always be some variability associated with microfilarial counts. Hairston & Jachowski (1968) discussed the sampling error associated with such counts and drew attention to the many variables which may influence the number of microfilariae per unit volume of blood, e.g., site and depth of skin incision, amount of blood taken, pressure exerted, exact source of blood in the capillary bed, etc. To these must be added variation in observer's assessment of a count and natural fluctuations in microfilarial densities.

Precise assessment of the effect of medicated salt on microfilaraemia is possible only if non-treated concurrent controls are incorporated in the design of a trial in order to demonstrate any natural fluctuations in microfilarial densities which may exist.

RESULTS

Results of 2 pretreatment microfilarial surveys

Tables 1 and 2 indicate the frequency distribution of microfilarial densities in the prison population at the 2 pretreatment Surveys A and B. Point-prevalence estimates of microfilaraemia were 148/672 (22%) and 71/203 (35%). Microfilariae of *Acanthocheilonema perstans* were seen on only 2 occasions in 1224 pretreatment blood films (Surveys A, B, C, J, W), one being a lone infection and the other mixed with *W. bancrofti*.

TABLE 1

CUMULATIVE FREQUENCIES OF MICROFILARIA-POSITIVE CASES CLASSIFIED BY MICROFILARIAL DENSITY PER 30-mm³ BLOOD SAMPLE AT PRETREATMENT SURVEY A

Microfilarial count	Frequency	Cumulative frequency	
		No.	%
1	12	12	8.1
2	8	20	13.5
3	4	24	16.2
4	3	27	18.2
5	6	33	22.3
6	1	34	23.0
7	3	37	25.0
8	2	39	26.4
9	2	41	27.7
10	1	42	28.4
11–20	12	54	36.5
21–30	19	73	39.5
31–40	11	84	56.8
41–50	6	90	60.8
51–60	6	96	64.9
61–70	5	101	68.2
71–80	5	106	71.6
81–90	1	107	72.3
91–100	3	110	74.3
101–200	28	138	93.2
201–300	7	145	97.9
>300	3	148	100.0

Total screened = 672. Total microfilaria-positive = 148 (22.0 %)

Positive 1 line only = 16 (10.8 %)

Positive 2 lines only = 12 (8.1 %)

Positive 3 lines = 120 (81.1 %)

No. with positive grade [a]			% positive for 30 mm³	Correction factor		Median microfilarial density
N_3	N_2	N_1		20 mm³	10 mm³	
120	12	16	22	0.964	0.901	32.5

[a] See WHO Expert Committee on Filariasis (1967), pp. 45-46.

TABLE 2

CUMULATIVE FREQUENCIES OF MICROFILARIA-POSITIVE CASES CLASSIFIED BY MICROFILARIAL DENSITY PER 30-mm³ BLOOD SAMPLE AT PRETREATMENT SURVEY B

Microfilarial count	Frequency	Cumulative frequency	
		No.	%
1	2	2	2.8
2	2	4	5.6
3	2	6	8.4
4	1	7	9.8
5	3	10	14.1
6	1	11	15.5
7	0	11	15.5
8	2	13	18.3
9	0	13	18.3
10	1	14	19.7
11–20	15	29	40.8
21–30	5	34	47.9
31–40	5	39	54.9
41–50	2	41	57.7
51–60	3	44	62.0
61–70	5	49	69.0
71–80	3	52	73.2
81–90	2	54	76.0
91–100	2	56	78.9
101–200	8	64	90.1
201–300	4	68	95.8
>300	3	71	100.0

Total screened = 203. Total microfilaria-positive = 71 (35 %)

Positive 1 line only = 4 (5.6 %)

Positive 2 lines only = 7 (9.8 %)

Positive 3 lines = 60 (84.5 %)

No. with positive grade [a]			% positive for 30 mm³	Correction factor		Median microfilarial density
N_3	N_2	N_1		20 mm³	10 mm³	
60	7	4	35	0.982	0.93	32.5

[a] See WHO Expert Committee on Filariasis (1967), pp. 45-46.

Results of microfilarial survey on new admissions during the trial

Table 3 shows the frequency distribution of microfilarial densities in those prisoners admitted after the start of the trial who had received medicated salt for varying lengths of time, i.e., Survey J. In

TABLE 3

CUMULATIVE FREQUENCIES OF MICROFILARIA-POSITIVE CASES CLASSIFIED BY MICROFILARIAL DENSITY PER 30-mm³ BLOOD SAMPLE AT SURVEY J

Microfilarial count	Frequency	Cumulative frequency	
		No.	%
1	2	2	7.4
2	4	6	22.2
3	2	8	29.6
4	1	9	33.3
5	1	10	37.0
6	2	12	44.4
7	0	12	44.4
8	0	12	44.4
9	0	12	44.4
10	0	12	44.4
11–20	5	17	63.0
21–30	2	19	70.4
31–40	2	21	77.8
41–50	2	23	85.2
51–60	1	24	88.9
61–70	1	25	92.6
71–80	0	25	92.6
81–90	1	26	96.3
91–100	0	26	96.3
101–200	1	27	100.0

Total screened = 193. Total microfilaria-positive = 27 (14 %)

Positive 1 line only = 5 (18.5 %)

Positive 2 lines only = 3 (11.1 %)

Positive 3 lines = 19 (70.4 %)

No. with positive grade			% positive for 30mm³	Correction factor		Median microfilarial density
N_3	N_2	N_1		20 mm³	10 mm³	
19	3	5	14	0.937	0.841	12

[a] See WHO Expert Committee on Filariasis (1967), pp. 45-46.

TABLE 4

ARITHMETIC MEANS OF MONTHLY MICROFILARIAL COUNTS FOLLOWING ADMINISTRATION OF SALT MEDICATED WITH DIETHYLCARBAMAZINE AT 0.1% (w/w) CONCENTRATION (COMBINED FIGURES FOR SERIES 1 AND 2 OF TABLE 5)

	Pre-treatment (Surveys B & C)	Medicated-salt administration					
		After 1 month (Survey D)	After 2 months (Survey E)	After 3 months (Survey F)	After 4 months (Survey G)	After 5 months (Survey H)	After 6 months (Survey I)
No. of microfilarial counts (*N*)	64	62	52	47	32	28	22
Mean microfilarial count of total *N* per 30 mm³ blood ($\bar{x}$)	44.3	26.18	22.5	11.96	5.5	6.96	4.64
Percentage reduction [a] of mean microfilarial count (total units in *N* used)		40.9	49.2	73	87.6	84.3	89.5
Mean microfilarial count of paired observations in succeeding column ($\bar{x}_1$)[b] i.e., 62 D at B & C 52 E at D 47 F at E, etc.	44.18 (62 D)	28.69 (52 E)	20.89 (47 F)	9.16 (32 G)	5.68 (28 H)	5.96 (22 I)	—
Percentage reduction [c] of mean microfilarial count on paired observations only [b] i.e., 52 E at D 47 F at E, etc.		35.1 (52 E)	52.7 (47 F)	79.3 (32 G)	87.2 (28 H)	86.5 (22 I)	—
No. (and proportion) of negative microfilarial counts	6/64 [d] (0.094)	13/62 (0.21)	12/52 (0.231)	21/47 (0.447)	10/32 (0.312)	12/28 (0.428)	9/22 (0.409)

[a] Calculated as $100 - (\frac{Z}{Y} \times 100$ where Z = post-treatment mean microfilarial count at Surveys D, E, F, G, H and I, and Y = pretreatment mean microfilarial count at Survey B and C. Total units in *N* used.

[b] Figures in parentheses in rows 4 and 5 denote the number of paired observations available at the succeeding month's survey.

[c] As in *a* but calculated on paired observations in succeeding column.

[d] Six microfilarial counts in Survey C had become negative immediately before treatment but had been positive 4 months previously at Survey A.

comparison with Tables 1 and 2, both the point-prevalence of microfilaraemia of 27/193 (14%) and the median microfilarial density of 12 were reduced. Analysis of the incidence of microfilaraemia in Survey J in relation to the length of time spent in prison and hence the duration of medicated-salt administration revealed no linear trend of reduction although some sample sizes were small, thus giving a high degree of variability.

Results of repeated microfilarial surveys during medicated salt administration

The results of monthly microfilarial counts for 6 months during the administration of 0.1% medicated salt are shown in Tables 4 and 5. Discharge of prisoners led to diminishing numbers throughout. Table 4 shows the arithmetic means of the combined Series 1 and 2 of Table 5. Series 1 and 2 were tabulated separately to note whether there were any striking differences in the findings at each month. The only difference between the series was that the 33 subjects in Series 1 had 2 microfilarial counts before commencing medicated salt, at Surveys A and C, while those in Series 2 had only 1 pretreatment count, at Survey B. As can be seen from Table 5 the results for each series were very similar, giving added confidence to the validity of the combined figures.

Results of microfilarial survey and diethylcarbamazine-treatment of prison warders

Of 123 warders surveyed, 14 were microfilaria-positive (9%). The mean density was 53.7 micro-

TABLE 5

ARITHMETIC MEANS OF MONTHLY MICROFILARIAL COUNTS FOLLOWING ADMINISTRATION OF SALT MEDICATED WITH DIETHYLCARBAMAZINE AT 0.1 % (w/w) CONCENTRATION

	Series 1 Pretreatment and control surveys		Series 2 Pretreatment survey B	Medicated-salt administration					
	A	C		After 1 month (Survey D)	After 2 months (Survey E)	After 3 months (Survey F)	After 4 months (Survey G)	After 5 months (Survey H)	After 6 months (Survey I)
Series 1									
No. of microfilarial counts (*N*)	33	33		31	26	25	20	18	14
Mean microfilarial count of total *N* per 30 mm³ blood ($\bar{x}$)	50.76	37.21		21.42	20.15	11.72	7.15	8.83	6.07
Percentage reduction [a] of mean microfilarial count (Total units *N* in used)		26.7		42.4	45.8	68.5	80.8	76.3	83.7
Mean microfilarial count of paired observations in succeeding column ($\bar{x}_1$)[b] .e., 31 D at C 26 E at D, etc.		36.52 (31 D)		22.77 (26 E)	20.68 (25 F)	12.95 (20 G)	7.28 (18 H)	8.07 (14 I)	—
Percentage reduction [c] of mean microfilarial count on paired observations only [b] i.e., 26 E at D 25 F at E, etc.				37.7 (26 E)	43.3 (25 F)	65.5 (20 G)	80.8 (18 H)	77.9 (14 I)	—
No. (and proportion) of negative microfilarial counts	0/33 (0)	6/33 [d] (0.18)		6/31 (0.19)	5/26 (0.19)	12/25 (0.48)	5/20 (0.25)	6/18 (0.33)	5/14 (0.36)
Series 2									
No. of microfilarial counts (*N*)			31	31	26	22	12	10	8
Mean microfilarial count of total *N* (per 30 mm³ of blood ($\bar{x}$)			51.84	30.94	24.85	12.23	2.75	3.6	2.12
Percentage reduction [a] of mean microfilarial count (Total units in *N* used)				40.3	52.1	76.4	94.7	93.1	95.9
Mean microfilarial count of paired observations in succeeding column ($\bar{x}_1$)			51.84 (31 D)	34.62 (26 E)	21.14 (22 F)	2.83 (12 G)	2.8 (10 H)	2.25 (8 I)	—
Percentage reduction [c] of mean microfilarial count on paired observations only [b]				33.2 (26 E)	59.2 (22 F)	94.5 (12 G)	94.6 (10 H)	95.7 (8 I)	—
No. (and proportion) of negative microfilarial counts			0 (0)	7/31 (0.13)	7/26 (0.27)	9/22 (0.41)	5/12 (0.42)	6/10 (0.60)	4/8 (0.50)

[a] Calculated as $100 - (\frac{Z}{Y} \times 100)$ where Z = post-treatment mean microfilarial count at Surveys D, E, F, G, H and I, and Y = pretreatment mean microfilarial count at Survey B or C. Total units in *N* used.

[b] Figures in parentheses in rows 4 and 5 in each series denote the number of paired observations available at the succeeding month's survey.

[c] As in *a* but calculated on paired observations in succeeding column.

[d] Six microfilarial counts in Survey C had become negative immediately before treatment but had been positive 4 months previously at Survey A.

filariae per 30 mm³ of blood and the median density was 14.5. After treatment with a standard course of diethylcarbamazine, 9 of the 14 warders were microfilaria-negative on follow-up and the mean count for the series dropped from 53.7 microfilariae to 4.4 per 30 mm³, a reduction of 91.8%. This reduction was artificially low as 1 patient, in whom a pretreatment count of 97 per 30 mm³ was reduced to 53 after treatment, was strongly suspected of defaulting on dosage. The 4 remaining microfilaria-positive patients had pretreatment counts of 322, 102, 96 and 53 microfilariae per 30 mm³ reduced to 3, 4, 1 and 3 microfilariae per 30 mm³ of blood respectively.

Clinical

Clinical manifestations of filariasis in the prison were known to be uncommon. It was not possible to screen physically all the microfilaria-positive cases revealed in the 2 pretreatment surveys, but of 47 microfilaria carriers who were randomly selected for a parallel investigation of a filarial skin antigen and clinically examined, 41 (87%) had no signs or suggestive history. In 6/47 (13%) there were 4 hydrocoeles and 3 gave a reasonably definite history of chyluria. Elephantiasis was not seen.

Tolerance in the prison population of 600–700 persons to the medicated salt given at a 0.1% (w/w) concentration for 6 months was good. No complaint of any side-effects was received. This aspect was investigated particularly closely during the first 2 weeks of medicated-salt administration with the co-operation of the prison medical officer. In contrast, among 14 prison warders treated with a total dose of 4.45 g of diethylcarbamazine in 15 days, equivalent to 74 mg per kg for a 60-kg man, plus an antihistamine compound for the first 4 days, there were 7 complaints in the first week. Five of these patients developed tender thickened spermatic cords, testicular pain or both, and headache and joint pains were recorded. Although the intensity of side-effects was only moderate, they caused grumbles in a highly disciplined body of men.

Urine samples

After medicated salt had been given for 5 months (about 150 days), 10 urine samples were taken 2 hours after the main meal of the day from a random selection of those prisoners known to be microfilaria-positive at some time during the trial. The samples were flown to the United Kingdom, where the diethylcarbamazine content was estimated at the National Institute for Medical Research.

TABLE 6

DIETHYLCARBAMAZINE EXCRETION IN URINE AND CURRENT MICROFILARIAL COUNTS IN 10 SUBJECTS AFTER 150 DAYS' ADMINISTRATION OF 0.1% DIETHYLCARBAMAZINE-MEDICATED SALT [a]

Subject	Mg diethylcarbamazine per 100 ml urine	Microfilarial count per 30 mm³ blood
1	10.2	7
2	4.0	0
3	7.1	0
4	1.9	12
5	1.4	40
6	2.9	15
7	0.8	18
8	2.9	0
9	8.0	25
10	2.1	0
	Mean = 4.13	Mean = 11.7

[a] Theoretical intake = 14 mg diethylcarbamazine per day = total theoretical intake of 2.1 g drug.

The results of these estimations and the current microfilarial count of each of the prisoners from whom urine was collected are shown in Table 6. No correlation between the 2 variables was demonstrable, Spearman's r_s giving a probability of over 30%.

DISCUSSION

Diethylcarbamazine-medicated salt at a concentration of 0.1% (w/w) of drug was acceptable and nontoxic when given daily to between 600 and 700 inmates of a closed population over a 6-month period. Urinary estimations of drug excretion showed that in fact the prisoners were receiving medicated salt at that time and assays of the drug content of random samples of medicated salt confirmed that the mixing procedure was accurate in achieving the desired concentration.

Repeated microfilarial surveys demonstrated that the compound was undoubtedly effective in reducing the total microfilarial reservoir of this sample although relatively ineffective in complete eradication of parasitaemia.

There was a steady fall in mean microfilarial counts during administration of the drug–salt compound which levelled at 4–6 months. Expressed in another way, the percentage reduction of the mean microfilarial count of each survey rose from 40%–50% at 1–2 months to around 80%–90% after 4 months. From 20% to 45% of counts became negative at different times. With the exception of Series 2 at 3 months (Table 5), the agreement of mean counts and percentage reduction of the mean count (Tables 4 and 5), whether calculated on the total number of subjects available or on paired observations of those present in the succeeding month, was striking at all times. This was interpreted as indicating that the losses at each month were a true representative fraction of the total frequency distribution of the available microfilarial counts and that a disproportionate number of outlying high or low values had not been lost, which might have distorted the general pattern of response.

Perhaps the low proportion of negative counts was not surprising. One of the peculiarities of diethylcarbamazine administered in various types of filariasis both in animals and in man is that although 90% of microfilariae in the blood disappear even after small doses, a few often persist, for reasons which are obscure (Hawking, 1963). Furthermore, mass treatment campaigns (e.g., Sasa, 1967), although highly successful in reducing the mean or median count of a microfilarial population by lowering the total frequency distribution of microfilariae, left a small residue of microfilaria carriers with low counts. It might be unrealistic to expect a higher proportion of microfilaria-negative cases in this trial after a total dose of approximately 2.52 g diethylcarbamazine given over 180 days (= 42 mg per kg for a 60-kg individual) when the standard total dose of 72 mg per kg given in 12 separate doses produced only 80%–90% negativity (Sasa, 1967). It can also be surmised that the proportion of microfilaria-negative cases will vary among different populations given the standard treatment, depending on varying microfilarial densities in those populations.

The important finding was that a reduction of mean microfilarial density of some 50% had occurred in the sample after 2 months of a very low concentration of drug and this reduction increased over the duration of the trial. The results of treatment of the warders with a standard therapeutic course of the same drug suggested a normal response to diethylcarbamazine.

A basic postulate in attempting the control of endemic filariasis is that the reduction of human microfilarial reservoirs will lead to diminished transmission. It has been theorized mathematically that in principle the critical densities of host and vector below which a parasitic population cannot maintain itself are most important for those diseases in which the parasite sexes are separate, as in the filariases (Hairston, 1962, 1965; Macdonald, 1965). There has recently been a mathematical demonstration that the transmission of *W. bancrofti* from mosquito to human host is, in Burma at least, surprisingly functionally inefficient (Hairston & de Meillon, 1968). In comparing differences in the transmission of malaria and bancroftian filariasis in West Africa, Brengues et al. (1968) noted that man was the chief agent of spread of bancroftian filariasis in areas where villages were scattered, that the human carriers of *W. bancrofti* must remain in any new area for some considerable time to create a focus of infection, and that an important factor in restricting spread of filariasis was the low infection rate among vectors. Added to these mutually compatible observations the production of low microfilarial reservoirs by non-toxic means thus assumes some importance in the mechanisms of control. Hence further trials on medicated-salt administration in bancroftian filariasis to give a more precise definition of advantages and limitations are desirable.

A broad comparison between the results of this trial and the pilot trials of Hawking & Marques (1967) and Raghavan, Basu & Putatunda (*op. cit.*) reveal some discrepancies, and the comparative data are shown in Table 7. Calculations have been made on paired observations only, i.e., on data from those subjects presenting at the succeeding microfilarial survey.

The point of similarity in all trials is the marked reduction of the mean microfilarial count in the samples, commencing soon after diethylcarbamazine-medicated salt administration. The dissimilarities, which involve the degree of reduction, are probably due to 3 factors: the small size of sample, with a consequent inherently greater variation; the different pretreatment mean microfilarial counts in the samples, even correcting for the different sampling volumes of blood; and the varying amounts of compound given and taken. For example, although the series from India and Tanzania showed respectively a 94.1% and 52.7% reduction in mean microfilarial count at 2 months after comparable amounts of compound, the Indian series is composed of a smaller sample with a lower mean pretreatment

TABLE 7

COMPARISON OF RESULTS OF MICROFILARIAL SURVEYS DURING 3 MEDICATED-SALT TRIALS IN BANCROFTIAN FILARIASIS (CALCULATIONS ON PAIRED OBSERVATIONS ONLY)

	Pretreatment survey	Times of survey after medicated-salt administration										
		2 weeks	1 month	6 weeks	2 months	11 weeks	3 months	15 weeks	4 months	19 weeks	5 months	23 weeks
Trial in Tanzania [a]												
No. of microfilarial counts	62		52		47		32		28		22	
Mean microfilarial count/30 mm³	44.2		28.7		20.9		9.2		5.7		6	
Percentage reduction [b] of mean microfilarial count			35.1		52.7		79.3		87.2		86.5	
Total drug intake (mg) at time of survey	0		420		840		1 260		1 680		2 100	
Trial in India [c]												
No. of microfilarial counts	18				18							
Mean microfilarial count/20 mm³	25.3				1.5							
Percentage reduction [b] of mean microfilarial count					94.1							
Total drug intake (mg) at time of survey	0				870							
Trial in Brazil [d]												
No. of microfilarial counts	15	15		13 (25.1)[e]		10 (27.4)[e]		6 (33.2)[e]		4 (16)[e]		4 (16)[e]
Mean microfilarial count/40 mm³	24.4	3		0.6		0.9		0.3		1.0		0.25
Percentage reduction [b] of mean microfilarial count		87.7		97.6		96.7		99.1		93.8		98.4
Total drug intake (mg) at time of survey	0	950		3 750		4 450		4 450		4 975		5 675
Trial in Brazil: 2 [f]												
No. of microfilarial counts	7	6 (35.4) [e,g]		7 (50) [e,g]		6 (57.2) [e,g]		6 (57.2) [e,g]		4 (41.25) [e]		4 (41.25) [e]
Mean microfilarial count/40 mm³	50 [g]	5.8		10.6		6		7.2		9.8		6.25
Percentage reduction [b] of mean microfilarial count		83.6		78.8		89.5		87.4		76.2		84.8
Total drug intake (mg) at time of survey	0	630		810		810		810		810		810

[a] Calculated from paired observations of Table 4.

[b] Calculated as $100 - (\frac{Z}{Y} \times 100)$ where Z = post-treatment mean microfilarial count, and Y = pretreatment mean microfilarial count. Paired observations only used.

[c] Calculated from data of Raghavan, Basu & Putatunda (*op. cit.*).

[d] Calculated from data of Hawking & Marques (1967), Table 5, Group A.

[e] Figures in parentheses indicate mean pretreatment microfilarial count for that set of paired observations. Calculations in *b* relate to this pretreatment figure.

[f] Calculated from data of Hawking & Marques (1967), Table 5, Group B.

[g] Approximate. Original data incomplete.

count. Again the high percentage reduction of mean count in the Brazilian Group A is in a small sample with a low pretreatment count and given a much higher dose of active compound. Allowing for variables, it may be concluded tentatively that the dissimilarities in results are not so great as would appear at first sight.

In future trials of this form of control, the experimental design will prove of great importance. Many variables are involved: time, concentration of drug, frequency distributions of microfilariae and possibly different patterns of the stability of microfilarial densities. In addition, there will always be sampling variations in the physical techniques of blood collections and observer variation in the assessment of numbers of microfilariae present on blood slides. The former can be minimized by constant attention to detail, and a system of incorporating check counts on slides showing high or low outlying values or on those distorting the general pattern of response will guard against undesirable degrees of observer variation. In this trial all monthly microfilarial counts were reviewed and any unusual readings were checked blind by different observers. The mean of these counts was taken as the most accurate estimate of the microfilarial density.

Control observations will be essential to allow for variations in microfilarial density in time. There was a reduction of the mean microfilarial count of 27.5% in 4 months in pretreatment counts on paired observations and similar fluctuations have been noted by other investigators in various areas. The influence of this natural fall in mean count on the therapeutic estimates of the efficiency of drug–salt after 1 and 2 months cannot be assessed, but after 3 months there was little doubt of the efficiency of the compound (Table 4). Of course, it may be argued that the control sample itself was small and may have given a biased estimate of variation in stability of microfilarial density. This only indicates the need for testing of further larger samples.

In conclusion, 3 trials have shown, in small samples, that salt medicated with diethylcarbamazine in concentrations ranging from 0.1% to 0.4% (w/w) was acceptable, non-toxic and effective in reducing a microfilarial reservoir. Further trials on populations with varying distributions of microfilarial densities would more precisely determine effects and limitations, provided strict experimental design, control and technique were incorporated. A trial of diethylcarbamazine-medicated salt at 0.2% (w/w) concentration might achieve optimal microfilarial reduction combined with acceptability.

The over-all place of diethylcarbamazine-medicated salt as a sole or predominant method of chemotherapeutic control in filariasis is more problematical. It can be criticized on grounds of wide variation in individual salt consumption, the lack of control or knowledge of actual drug intake, the technical and practical difficulties of large-scale mixing and distribution of the drug–salt compound, and, in the absence of national legislation, the availability of alternative non-medicated salt, an important point in human populations not attuned to preventive medicine.

There may be doubts about the effects of an active drug, given in less than optimal dosage over relatively prolonged periods, on helminth resistance, although this is as yet undescribed. The long-continued administration of a number of therapeutically effective drugs which are completely safe for short-term use may occasionally have undesirable side-effects in a few individuals. Since the chemotherapeutic control of endemic filariasis using medicated salt would involve large populations, it would be desirable to seek further information on long-term toxicity by continuous administration of diethylcarbamazine in salt to laboratory animals. Similarly, although experiments have shown that diethylcarbamazine-medicated salt remained stable during certain cooking procedures this is no guarantee that the variety of cooking methods or the variety of foods, additives or preservatives that would be employed in mass use would not affect the stability of the drug.

Especial care would be needed in areas where *Wuchereria bancrofti* and *Onchocerca volvulus* coexisted in the human population because of the unpleasant side-effects which may occur in patients with onchocerciasis who are given diethylcarbamazine.

It may be that diethylcarbamazine-medicated salt would play a useful role as an adjunct to other, more orthodox means of control and its use can be envisaged in closed communities in endemic areas or in populations with no alternative sources of salt. Extrapolation to national populations may pose formidable difficulties.

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RÉSUMÉ

ACTION DU SEL MÉDICAMENTÉ PAR LA DIÉTHYLCARBAMAZINE DANS LA FILARIOSE DE BANCROFT

L'essai décrit dans le présent article, mené pendant 6 mois dans une prison de Tanzanie, visait à évaluer l'efficacité de la diéthylcarbamazine, administrée sous forme de sel médicamenté à la concentration de 0,1% (p/p), dans la filariose à *Wuchereria bancrofti* périodique. Deux enquêtes préliminaires avaient révélé dans des groupes de 672 et 203 détenus des taux de prévalence de la microfilarémie de 22% et 35% respectivement. En raison des fluctuations de la population de la prison, seuls 64 porteurs de microfilaires étaient présents au moment où l'essai a débuté. Au cours des examens de sang pratiqués avant et pendant ce dernier, on s'est assuré que les variations enregistrées lors de l'examen d'un même échantillon de sang par plusieurs observateurs restaient dans des limites très acceptables. Des numérations couplées effectuées chez 33 détenus à 4 mois d'intervalle, en dehors de tout traitement, ont par ailleurs mis en évidence une réduction spontanée de 27,5% de la densité microfilarienne moyenne et indiqué la nécessité de constituer des groupes témoins pour des enquêtes de ce genre.

Le sel médicamenté contenant 0,1% de citrate de diéthylcarbamazine a été préparé dans un laboratoire central. Chaque détenu a reçu quotidiennement 14 g de sel, correspondant à la prise théorique de 14 mg par jour et d'une dose totale de 2,52 g de diéthylcarbamazine en 6 mois, soit 42 mg/kg pour un homme de 60 kg. La tolérance a été très bonne et aucune réaction secondaire n'a été observée. Par contre, des plaintes ont été formulées par 7 gardiens (sur 14) traités classiquement par la diéthylcarbamazine à la dose totale de 4,45 g en 15 jours, soit 74 mg/kg pour un homme de 60 kg.

Les numérations de microfilaires pratiquées mensuellement sur les 64 porteurs présents pendant la durée de l'essai ont montré une réduction des densités microfilariennes moyennes de 40%, 50%, 73%, 88%, 84% et 90% respectivement après 1, 2, 3, 4, 5 et 6 mois d'administration du sel médicamenté. Les examens de sang sont devenus négatifs chez 9 des 14 gardiens traités par la méthode classique.

Après 5 mois de traitement par le sel médicamenté, on a déterminé la teneur en diéthylcarbamazine de 10 échantillons d'urine prélevés au hasard chez des prisonniers. Dans chaque cas, l'examen a été positif, mais on n'a noté aucune corrélation entre les taux d'excrétion et les densités microfilariennes.

La recherche des microfilaires effectuée à la fin de l'essai sur des détenus soumis au régime du sel médicamenté pendant une durée variable a montré une réduction de la prévalence des porteurs (27 sur 193, soit 14%) et une chute des densités microfilariennes moyennes par rapport aux résultats fournis par les deux enquêtes préliminaires.

Les auteurs insistent sur la nécessité de définir avec précision les conditions expérimentales de semblables essais et de disposer de groupes témoins. Il semble que l'administration de sel médicamenté renfermant 0,2% de diéthylcarbamazine permettrait d'obtenir une réduction optimale des densités microfilariennes sans apparition de réactions secondaires notables. L'emploi du sel médicamenté semble une mesure d'appoint pleine de promesses, surtout pour le traitement de collectivités fermées, mais sa généralisation à l'échelle d'un pays ne peut être envisagée qu'avec une extrême circonspection.

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