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Tanzania Filariasis Project: a provocative day test with diethylcarbamazine for the detection of microfilariae of nocturnally periodic *Wuchereria bancrofti* in the blood

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In coastal Tanzania, an area where the microfilariae (mf) of Wuchereria bancrofti *exhibit nocturnal periodicity, the administration of 2 mg diethylcarbamazine (DEC) per kg body weight in the daytime provoked mf to enter the peripheral blood. In persons on normal daily activities the daytime DEC provocative method proved to be as sensitive in detecting microfilaraemia as was the examination of night blood. Its use in routine surveys is therefore justified. Although mf densities by day and night were highly correlated (r = 0.83) they tended to be lower after provocative daytime DEC than in the corresponding night blood, except in very light infections. This method was also useful in assessing the parasitological response to mass chemotherapy with DEC, but, in comparison with the results of the night blood examinations, the sensitivity and magnitude of the counts in persons remaining positive progressively decreased as the period of DEC administration increased. A correction factor has to be calculated to take account of this, and/or additional night blood samples must be taken.*

The dose of 2 mg of DEC per kg body weight used was readily acceptable to the people in coastal East Africa, whose cooperation is difficult to obtain for night blood surveys. Apart from W. bancrofti, *the only human filarial infection occasionally encountered in this area was* Dipetalonema perstans. *Because of the risk of a severe Mazzotti reaction the test is contraindicated in onchocerciasis endemic regions. Severe reactions may also occur in subjects with loaiasis.*

A filariasis project commenced in the United Republic of Tanzania in 1973 (*13*) and during Phase I the distribution, prevalence, and density of microfilariae in the Tanga region were investigated.

Katamine et al. (*7*) in Japan have shown that, in areas where microfilariae (mf) of *Wuchereria bancrofti* are nocturnally periodic, they can be stimulated to appear in the blood during the day by administration of diethylcarbamazine (DEC). This finding has since been adopted as a survey method and Manson Bahr & Wijers (*9*) in East Africa have shown that 100 mg of DEC is sufficient in adults to induce a peak of mf in the peripheral blood 45–60 min after drug administration.

In the United Republic of Tanzania, where the mf of *Wuchereria bancrofti* are nocturnally periodic, it was necessary for surveillance purposes to use this daytime DEC provocative method because the villagers sometimes objected to the taking of blood at night. It was also desirable to standardize methods with those used in the neighbouring Kenyan project (*14*).

The dose of DEC administered and its rate of absorption are important factors governing the rate of increase of circulating mf during the day (*5, 10, 7*). After making preliminary experiments (McMahon et al., unpublished data, 1974) using doses of 2–6 mg/kg body weight and examining subjects 5–60 min after administration, we decided to adopt the 2 mg/kg dose recommended by Manson Bahr & Wijers (*9*).

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METHODS, MATERIALS, AND RESULTS

Samples of 0.1 ml of finger-prick blood were examined for mf. A modification of the counting chamber method of Denham et al. (*1*) was used.

Replicate counts

Counts of replicate samples of finger-prick blood were analysed statistically, (i) to establish the pattern of variability and choose a suitable transformation for further statistical analysis, and (ii) to investigate any tendency for drift in counting, shown by an upward or downward trend in the transformed counts.

Replicate counts considered were:

(*a*) counts on 2 or more samples (up to 10) from the same finger prick;

(*b*) counts on 2 samples, 1 from the left and 1 from the right hands of the same person—the left was taken first and counted first;

(*c*) counts of samples taken on each of 2 or 3 consecutive nights, each group of 2 or 3 from the same person; and

(*d*) counts of 2 samples from finger-pricks 2 months apart, each pair of samples from the same person.

The blood was taken either by day 50 min after provocation by 100 mg of DEC (in adults) or by night without prior administration of DEC.

The pattern of variation is considered by reference to the relationship between the mean and the variance of each group of counts on a finger prick for (*a*) above, and on a person for (*b*), (*c*), and (*d*) above, plotted on double logarithmic scales. The plots omit groups with all counts zero, and those with zero variance. These plots are shown for (*a*) in Fig. 1, and for (*c*) in Fig. 2. The plots for (*b*) and (*d*) resemble Fig. 2. The gradient of a straight line through the points, allowing for random scatter about the line, indicates the appropriate statistical transformation (*12*). Gradients of 1 (Fig. 2) and 2 (Fig. 1) are shown here. These indicate logarithmic and square-root transformations, respectively, and they are found to be reasonable transformations with these data. A logarithmic transformation is indicated for procedures (*b*), (*c*), and (*d*). The logarithm of the count plus one, referred to hereafter as log $(c+1)$ is used. The square-root transformation is indicated for procedure (*a*) and is thus useful for comparison of counts in the same specimen of finger-prick blood.

An upward or downward trend is assessed by the mean value of the difference between the transformed values of the first and second counts in each group. Groups with both these counts at zero are omitted. The mean value is compared with its standard error to test whether there is any statistically significant evidence of the long-run average being different from zero.

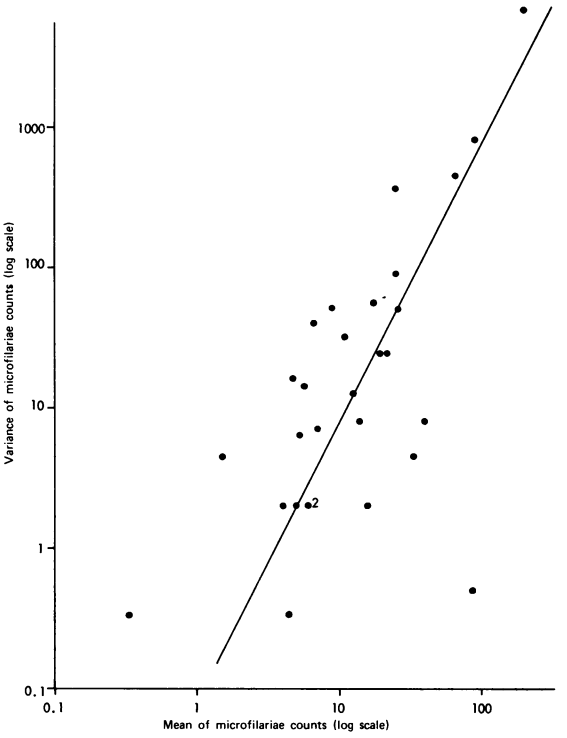

Fig. 1. Microfilariae counts on 2–10 samples from the same finger.

The numbers in each group and the results are shown in Table 1. The mean difference is calculated for groups (i) to (iv). Statistically significant evidence of a fall in average counts is found only for samples taken 2 months apart, using procedure (*d*) ($P<0.001$), but there is no evidence of drift when procedure (*a*), (*b*), or (*c*) is used.

Comparison between microfilariae counts immediately before and 50 min after administration of 100 mg of DEC in hospitalized and nonhospitalized subjects

The results are shown in Table 2. Series A covered a cross-section of the community and consisted of subjects who had not been examined previously. Series B and C consisted of persons known to be positive for microfilariae.

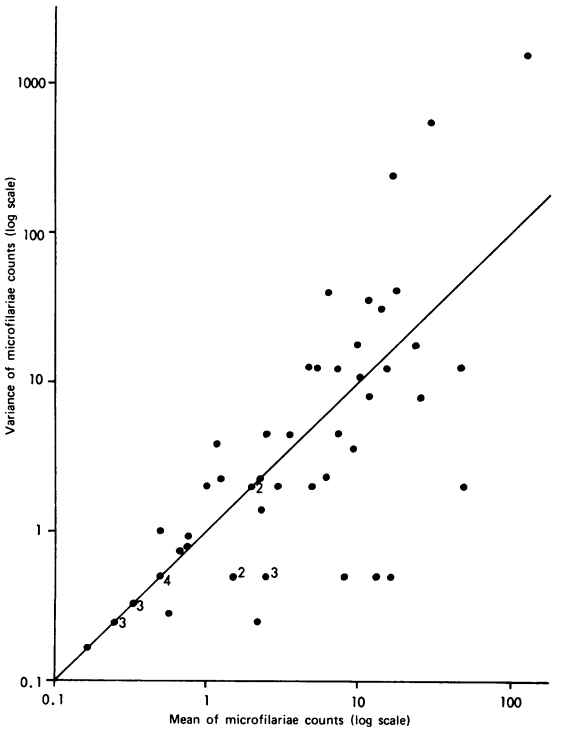

Fig. 2. Microfilariae counts repeated on same subjects for 2 or 3 consecutive nights. Numbers beside a point indicate the number of cases with the same value.

The statistical significance of the changes following DEC administration was determined by the mean and standard error of the differences between the 2 counts for each person, transformed by log $(c+1)$ (as was done for replicate counts, see above), and also by McNemar's test (*2*) for the change in percentage positive between the two counts.

Among patients on normal daily activity at 10 h 00, the rise in mean transformed count and in percentage positive were both statistically significant ($P<0.001$ and $P<0.01$, respectively). At night (21 h 00), the fall in both measures was statistically significant ($P<0.001$ and $P<0.01$, respectively). Among hospitalized patients tested by day, there was no statistically significant evidence of any effect of DEC provocation.

In fact, more detailed information on 4 hospital patients suggests a fall in microfilarial count following administration of DEC. The 4 were tested at 15-min intervals after administration (Table 3).

Comparison of night counts and daytime DEC provocative counts before and after mass chemotherapy with DEC

Before mass DEC chemotherapy. The provocative test performed at 10 h 00 was compared with the usual night test done at 22 h 00 the night before. Village volunteers known to be positive from previous investigations gave a sample of blood on each occasion. The results are shown in Fig. 3. Five volunteers were negative by night and positive by day, while 2 were negative by day and positive by night. This does not provide statistically significant evidence of different sensitivities. However, exclusion of those previously found negative will have limited the inclusion of persons with light infections in the group. The continuous line is fitted to the data mathematically and is the first principal component

Table 1. Results of analysis for statistical transformations and trend in mean counts

	Study group[a]			
	(*a*)	(*b*)	(*c*)	(*d*)
Number of groups of counts of which:	115	52	31	71
no. with more than two counts	32	–	13	–
no. with all counts zero	51	30	0	0
no. with first two counts zero	53		0	
no. with all counts the same (and not zero)	7	2	1	5
Transformation indicated	square root	logarithmic	logarithmic	logarithmic
Mean difference between the first two transformed counts	0.067	0.057	0.024	–0.222
Standard error of mean difference		0.055	0.049	0.038

[a] For definitions of the groups, see text under the heading "Replicate counts" (page 760).

Table 2. Comparison between microfilariae counts before and 50 min after administration of 100 mg of DEC

	No. examined	Before DEC		After DEC	
		No. positive	Geometric mean count among positives	No. positive	Geometric mean count among positives
Subjects on normal daily activity, at:					
A. 10 h 00–10 h 50	50	7 (14%)	2.4	14 (28%)	7.0
B. 21 h 00–21 h 50	31	30 (97%)	14.1	20 (65%)	6.7
Hospitalized patients, at					
C. 10 h 00–10 h 50	50	41 (82%)	5.9	35 (70%)	6.7

Table 3. Microfilariae counts prior to DEC administration and every 15 min after administration (at 10 h 00) to 4 hospital patients

	10 h 00[a]	10 h 15	10 h 30	10 h 45	11 h 00	11 h 15	11 h 30
Case No. 1	7	2	1	0	1	2	0
Case No. 2	69	52	35	15	21	9	12
Case No. 3	22	13	11	6	not done	9	16
Case No. 4	14	5	5	2	2	7	3

[a] Prior to DEC administration.

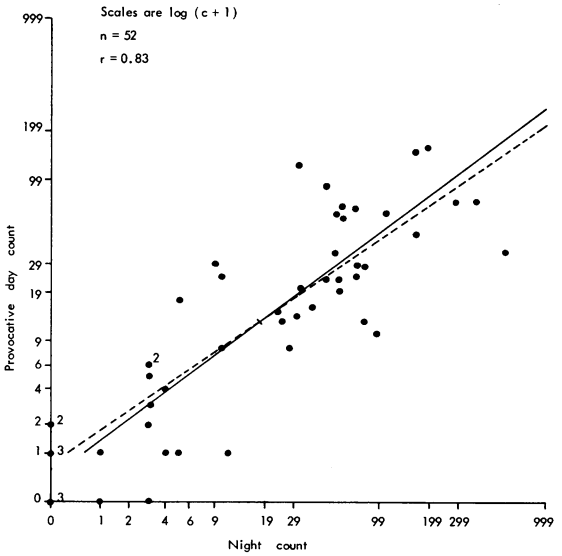

Fig. 3. Comparison of night and provocative day microfilariae counts prior to chemotherapy with DEC. The continuous line was fitted to the data mathematically by the principal component technique. The dashed line was calculated by the same technique to have the same slope in Fig. 3, 4, and 5. Negative results by the provocative test were excluded when fitting the line.

(*11*). The fitting of a line to the data by this technique can be more appropriate than ordinary linear regression if the error variances of each method, on the logarithmic scales, are assumed to be the same. The 5 negatives by the provocative test were excluded when fitting the line.

After mass DEC chemotherapy. DEC in daily doses of 6 mg/kg was administered on 3 consecutive days followed by 6 mg/kg once monthly for 6 months. In collecting these data, those found previously negative were not excluded. The results at 3 and 6 months after commencement of DEC administration (Fig. 4 and 5) are analogous to those prior to chemotherapy (Fig. 3) but mass chemotherapy is associated with a depression of the provocative count when compared with a given night count. The dashed lines in Fig. 3, 4, and 5 are calculated to have the same slope in each figure, again by the principal component technique. In none of these figures do the dashed lines show a large deviation from the continuous lines, bearing in mind the scatter of points. The vertical displacements of the dashed lines from each other are taken as a measure of the depression in provocative count following mass chemotherapy. They suggest that the "count plus one" $(c+1)$ associated with 3 months' mass chemotherapy was depressed by a factor of 1.8 (i.e., $(c+1)$ after chemotherapy = $(c+1)$ before

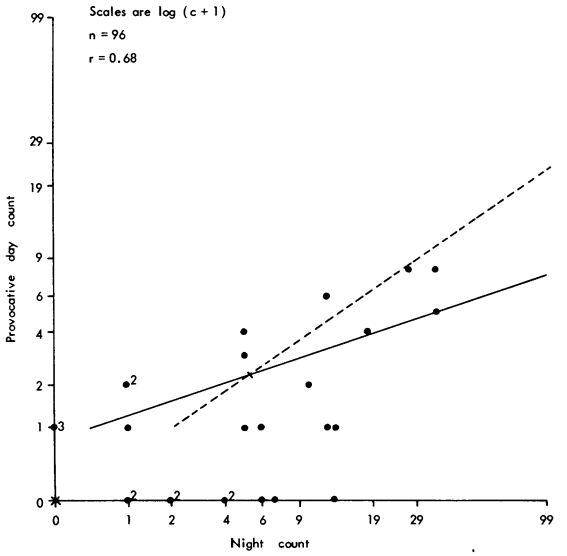

Fig. 4. Comparison of night and provocative day microfilariae counts 3 months after commencement of mass DEC chemotherapy. 67 subjects were negative on both counts. (See also the explanations to Fig. 2 and 3.)

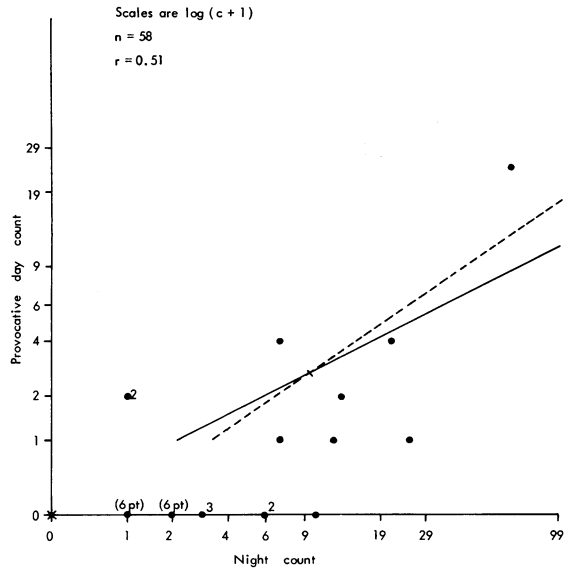

Fig. 5. Comparison of night and provocative day microfilariae counts 6 months after commencement of mass DEC chemotherapy. 30 subjects were negative on both counts. (See also the explanations to Fig. 2 and 3.)

chemotherapy divided by 1.8); and that after 6 months' mass chemotherapy ($c+1$) was reduced by a factor of 2.4. The sensitivity of the provocative test (i.e., its ability to detect positives) with reference to different levels of microfilaraemia found by the night counts is shown in Table 4. The data in the three columns of this table are the same as those used in Fig. 3, 4 and 5. The first two proportions in the first column (before mass chemotherapy) are bracketed to indicate that they are not strictly comparable with the proportions in other columns. This is because of the implicit selection bias in taking only volunteers previously found positive. The selection bias can be neglected except in the case of volunteers who were lightly infected or not infected. With due attention to this and to the small numbers, it is clear that there is a fall-off in sensitivity following mass chemotherapy but that this is likely to be important only with light infections.

Table 4. Proportion of subjects found positive by the provocative test in comparison with their original night count before and following 3 and 6 months' mass chemotherapy

Night count	Proportion of subjects positive by day provocative test		
	Before chemotherapy	After 3 months' chemotherapy	After 6 months' chemotherapy
0	[5/8][a]	3/64	0/30
1	[2/3][a]	3/7	2/8
2		0/2	0/6
3-4	7/8	0/2	0/3
5-8	2/3	4/6	2/5
9-16	4/4	4/5	2/3
17-32	6/6	2/2	2/2
>32	20/20	2/2	1/1

[a] Not strictly comparable with the proportions in the other columns.

DISCUSSION

The mechanism by which DEC induces an increase in circulating mf is not understood. Iwamato (*6*) administered 0.1 or 0.2 mg of DEC per kg body weight 4 times a day for several days to patients with nocturnally periodic *W. bancrofti*. The number of mf in the peripheral blood increased gradually day by day but no microfilaricidal effect was demonstrated. Fujimaki (*3*) noted that the minimum concentration of DEC necessary to kill *W. bancrofti* mf in the blood was approximately 0.8 μg/ml.

In our experience (Table 2), which is similar to that of Katiyar et al. (*8*), DEC administration at night decreases circulating mf. On the other hand, administration during the day, at the low point of the cycle, to persons on normal activities results in increased microfilaraemia. This may be due to release of mf from the reservoir in the lung capillaries (*4*). However, hospitalized patients are often mf positive during the day and tend to show a decrease in count following DEC administration. These patients frequently receive several drugs, some of which may be capable of provoking mf to enter the peripheral circulation. This, plus altered sleep rhythms, may provide an explanation for the alteration in the circadian rhythm of their microfilariae.

In the present studies, the analysis of replicate counts indicates that there is a need to carry out statistical analysis on transformed data, and that logarithmic transformations are appropriate except when comparing samples from the same blood specimen. The demonstration that there was no detectable drift in counting on the same day or on consecutive days is important as a verification of the accuracy of counting. The drift detected over 2 months could be explained by seasonal change resulting in a lower level of microfilaraemia in the population.

The counting of mf after a provocative daytime dose of DEC appears to be as sensitive a method for detecting positives as is the examination of night bloods (Fig. 3). The use of this method in surveys is therefore justified. Day and night densities are highly correlated ($r = 0.83$), but the densities tend to be lower in the day blood following provocation than in the corresponding night blood, except in very light infections.

The DEC provocative method is also useful in assessing the parasitological response to mass chemotherapy with DEC, although here the sensitivity and magnitude of the counts in persons remaining positive are less than those obtained from night counts (Fig. 4 and 5).

During the early stages of a mass DEC campaign to control nocturnal bancroftian filariasis the overall mf prevalence rate should decrease but the number of light infections would be expected to increase. If, in such situations, the provocative day test is used to assess the parasitological response, it might be necessary to calculate correction factors for these low grade microfilaraemias and/or to examine a sample of the population at night.

The dose of 2 mg of DEC per kg used for provocation in the present studies was readily acceptable to the population. A small number (less than 2%) complained of scrotal pain or pruritus of the skin. Because of the risk of a severe Mazzotti reaction, the test is contraindicated in onchocerciasis and it is likely that febrile reactions will arise in *Brugia* infections (*15*). Fortunately, none of these other filarial infections was encountered on the coastal belt around Tanga. In these circumstances, the use of this method effectively circumvented the otherwise almost unsurmountable difficulty of persuading the inhabitants of the region to allow blood samples to be taken at night.

RÉSUMÉ

PROJET DE RECHERCHE SUR LA FILARIOSE EN TANZANIE: TEST DE PROVOCATION PAR LA DIÉTHYLCARBAMAZINE

Dans les régions côtières de Tanzanie, les microfilaires de *Wuchereria bancrofti* présentent une périodicité nocturne; cependant, l'administration de diéthylcarbamazine (DEC) durant le jour provoque l'apparition de microfilaires dans le sang périphérique.

Pour procéder à la détection des microfilaires, des échantillons de sang de 0,1 ml sont prélevés par piqûre au doigt, puis placés dans une cellule de numération et examinés, cinquante minutes après l'administration orale de 100 mg de DEC aux sujets adultes, 150 mg aux enfants de 5 à 9 ans et 75 mg à ceux de 10 à 14 ans.

Chez les personnes ayant des activités journalières normales, la méthode de provocation par la DEC s'est révélée aussi sensible pour la détection de la microfilarémie que l'examen de sang prélevé la nuit, ce qui justifie son emploi pour les enquêtes de routine. Bien que les densités diurne et nocturne des microfilaires soient en étroite corrélation ($r = 0,83$), la microfilarémie tend à être plus faible après administration d'une dose DEC de provocation que dans le cas de sang prélevé la nuit chez le même individu, sauf si l'infection est très légère.

La méthode de provocation diurne par DEC est également utile pour l'évaluation de la réponse parasitologique à la chimiothérapie de masse par la DEC, mais la sensibilité de l'épreuve et les résultats numériques du comptage par rapport aux résultats obtenus par l'examen de sang nocturne décroissent progressivement, chez les personnes demeurant positives, pendant la période d'administration

de DEC. On peut remédier à cet inconvénient en appliquant un facteur de correction et/ou en prélevant la nuit des échantillons de sang supplémentaires.

Le test de provocation diurne par administration d'une dose de 2mg/kg de DEC a été bien accepté par les populations côtières d'Afrique de l'Est, qui sont moins enclines à coopérer à des enquêtes par prélèvement nocturne de sang. D'autre part, il s'agit d'une région où, à part *Dipetalonema perstans,* occasionnellement présent, *W. bancrofti* est le seul parasite à l'origine de l'infection humaine. Dans les régions d'endémie onchocerquienne, le test est contre-indiqué en raison du risque de réaction grave de Mazzotti. Des réactions graves peuvent également se produire chez les sujets atteints de loase.

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