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RELATION OF AGE, SEX, AND MICROFILARIA DENSITY TO TREATMENT OF SUB-PERIODIC FILARIASIS WITH DIETHYLCARBAMAZINE*

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INTRODUCTION

The objective of a chemotherapeutic control program against Bancroft's filariasis is the interruption of its transmission by the reduction or, ideally, the eradication, of the parasite in man.

Field trials, by the oral administration of diethylcarbamazine (DEC) either to carriers of microfilariae (mf) or to the entire population in mass control programs, have been widely reported from numerous endemic areas[1-6] since the introduction of this drug by Hewitt *et al.*[7] in St. Croix.

Follow-up surveys have shown variable results in the reduction of mf rates and densities, but on occasion[8-10] have indicated that eradication can be attained after prolonged administration of relatively large doses of DEC to a small population.

However, filariasis-control programs using DEC have demonstrated three practical problems: 1) adverse reactions are frequently experienced by carriers when first taking DEC, and those experiencing the more severe reactions may object to the continued administration of the drug,[3, 5, 11, 12] 2) some carriers, although showing considerable reduction of mf density, persistently remain positive after treatment, and 3) other carriers, who become temporarily negative after treatment, later relapse to carrier status,[2, 7, 11, 13] although maintaining low mf counts.

As these problems threaten the successful outcome of a control program, it was decided to investigate the relation of age, sex, and mf density to the immediate and long-term results of a chemotherapeutic filariasis-control program, which was inaugurated in American Samoa in 1962.[14] It was hoped that the study would yield information concerning the mechanisms involved in the primary reduction of mf rates and subsequent recurrence rates, thus defining more clearly the characteristics of those mf carriers who are more likely to have reactions or to remain positive after an apparently adequate course of DEC therapy.

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MATERIALS AND METHODS

The present study was based on data collected from 175 carriers of sub-periodic *Wuchereria bancrofti*; the distribution of age, sex, and mf density in these persons before treatment is shown in Table 1. Patients were carefully weighed before the initial administration of DEC and thereafter received a single daily dose of the drug in the amount of 6 mg per kg of body weight, given for 5 to 6 consecutive days, *i.e.*, a total of 30 or 36 mg per kg of body weight. This treatment was repeated after 6 months, bringing the total dose of DEC to 60 or 72 mg per kg of body weight.

Adverse reactions to DEC were recorded daily by questioning and examining all patients as they returned for their daily dose of DEC, which was always administered under strict supervision. The presence of one or more of the customary adverse manifestations to DEC, (*e.g.*, chills, fever, arthralgia, myalgia, vomiting, lymphangitis, adenitis) reported by a carrier during the first 48 hours constituted one positive adverse reaction and was thus tabulated by age, sex and pretreatment mf density. Reactions were not separated into toxic or "allergic" types,[6] because toxic reactions with this dosage were relatively slight.

Carriers were tested for mf each time by the examination of two 20-cmm samples of blood, obtained by finger-puncture, between the hours of 8 a.m. and 8 p.m. Densities were reported as the average (mean) count of the two samples. These counts were made shortly before treatment, 1 week and 6 months after the first treatment, and 18 and 28 months after the second treatment, that is, 24 and 36 months after the beginning of

TABLE 1

Distribution before treatment of age, sex, and microfilaria density in 175 microfilaria carries in American Samoa, 1962*

Age group (years)	Sex	Microfilaria density						Subtotal	Total/ age group	% by age group
		1-10	11-20	21-50	51-100	100-200	201+			
0-4	M	0	0	0	0	0	0	0		
	F	0	0	1	0	0	0	1	1	0.5
5-9	M	5	0	2	0	2	0	9		
	F	4	2	2	1	4	0	13	22	12.0
10-14	M	7	1	3	3	1	0	15		
	F	5	3	1	3	0	2	14	29	17.0
15-19	M	5	2	3	0	4	0	14		
	F	1	1	1	1	0	0	4	18	10.0
20-24	M	1	0	3	0	0	1	5		
	F	5	0	4	1	0	0	10	15	8.5
25-29	M	1	2	3	2	1	0	9		
	F	1	0	0	2	0	0	3	12	7.0
30-34	M	0	0	1	0	1	1	3		
	F	5	0	1	1	0	0	7	10	5.5
35-39	M	0	1	0	3	0	0	4		
	F	3	2	1	1	1	0	8	12	7.0
40-44	M	4	2	2	0	2	2	12		
	F	1	1	0	0	1	0	3	15	8.5
45-49	M	3	0	0	0	1	0	4		
	F	1	0	0	0	1	1	3	7	4.0
50-54	M	2	1	1	0	1	1	6		
	F	2	1	0	1	2	0	6	12	7.0
55-59	M	0	1	0	1	1	2	5		
	F	1	0	1	1	0	1	4	9	5.0
60 +	M	2	0	2	1	1	1	7		
	F	2	0	1	1	1	1	6	13	7.5
Totals.....	M							93		
	F							82		
	All	61	20	33	23	25	13		175	
Total %		35	11	19	13	14	8			
Total cumulative % ...		35	45	64	78	92	100			

* Average of two 20-cmm samples of blood.

therapy. The data from carriers found still to be positive at these intervals of time were tabulated by age, sex, and mf density, in order that the relations between these factors and the reduction of mf rates could be determined. The results were statistically analyzed by calculating the standard error of the difference between two proportions:

$$\text{S.E.} = \sqrt{p(1-p)(1/n_1 + 1/n_2)}$$

In comparing low with high mf densities, calculations were made by comparing carriers having counts under 20 mf with those having counts over 200 mf per 20 cmm of blood.

The groups of mf density shown in the tables were arbitrarily selected in such a way as to clarify the presentation of the data. Any other group distribution, such as those suggested by the Japanese workers Sasa *et al.*[6] and Sasa[11] probably would have been equally acceptable and useful.

RESULTS

Reactions to DEC

The frequency distribution of adverse reactions to the first administration of DEC is presented

TABLE 2

Distribution of age, sex, and microfilaria density in carriers with adverse reactions to diethylcarbamazine*

Age			Sex		
Age group	Reactors/no. Examined	Reactors	Sex	Reactors/No. Examined	Reactors
(*years*)		(*percent*)			(*percent*)
0–4	0/1	0	M	44/87	50
5–9	10/22 } 27/70	45 } 39	F	53/88	60
10–14	12/29 }	41 }			
15–19	5/18 }	28 }			
			Microfilaria density		
			MF Density Groups	Reactors/No. Examined	%
20–24	8/15	53			
25–29	7/12 } 31/49	58 } 63	1–10	21/61	34
30–34	8/10 }	80 }	11–20	11/20	55
35–39	8/12 } 70/105	66 } 67	21–50	19/33	58
40–44	10/15 }	66 }	51–100	17/23	74
45–59	4/7 } 39/56	57 } 70	101–200	19/25	76
50+	25/34 }	74 }	201+	10/13	77

* Average of two 20-cmm samples of blood.

in Table 2. This distribution is shown by age, by sex, and by groups of mf density before treatment.

The data indicated that the percentage of carriers reporting adverse reactions to DEC in the age group 0 to 19 years was significantly lower, *i. e.*, 39%, than in the adult or older age groups, where it was 63% and 70%, respectively. The combined percentage of reactions reported by the latter two groups was 67%. The difference of 28% between the younger age group and the older is highly significant (S.E. = 7.7%, $p < .001$).

The distribution by sex of carriers reporting reactions to the first administration of DEC did not show any significant difference between the sexes in the proportion reacting to DEC, as the difference of 10% between the two groups was not statistically significant (S.E. = 7.1%, $p = .17$).

The distribution of reactors to DEC by their pretreatment mf density, however, showed that the frequency of reported reactions increased from a low of 34% in the group with pretreatment mf density between 1 and 10, to a high of 77% in the group having more than 200 mf per 20 cmm of blood before treatment. The difference between these two groups is statistically highly significant (S.E. = 11.7%, $p < 0.001$).

Reduction of Microfilaremia

Table 3 presents the distribution of those mf carriers who were found to be positive at various intervals of time after the administration of DEC. These are arranged by age, sex, and by groups of mf density before treatment.

The percentage of carriers who remained positive 1 week after the administration of 30 or 36 mg per kg of body weight was similar in each age group. Also, no significant difference between sexes was apparent, as the proportion of males and females remaining positive were 31% and 33%, respectively. Of the carriers originally showing between 1 and 10 mf per 20 cmm of blood, only 1.6% remained positive 1 week after treatment, whereas 30% remained positive in the group with 11 to 20 mf and 62% in the group with original counts over 200 mf. The difference between the percentage of carriers who remained positive in the low density and in the higher density groups is highly significant ($p < .0001$).

Six months after the first round of treatment, data were obtained from 103 of the original car-

TABLE 3

Distribution of age, sex, and microfilaria density in carriers positive after treatment with diethylcarbamazine*

Epid. Variables	†After first treatment				‡After second treatment			
	1 week		6 months		18 months		28 months	
	No. +/No. Examined	%	No. +/No. Examined	%	No. +/No. Examined	%	No. +/No. Examined	%
Age group (*years*)								
0–4	1/1	100	0/1	0	0/1	0	0/1	0
5–9	7/22	32	3/14	21	2/18	11	5/20	25
	24/70 (34%)		15/43 (35%)		12/57 (21%)		18/59 (31%)	
10–14	9/29	31	6/19	32	7/26	27	9/25	36
15–19	7/18	39	6/9	66	3/12	25	4/13	31
20–24	5/15	33	6/9	66	2/12	17	2/13	15
25–29	5/12	42	1/6	17	2/6	33	4/10	40
	15/49 (31%)		12/25 (48%)		6/34 (18%)		8/41 (19%)	
30–34	2/10	20	2/5	40	1/7	14	1/9	11
35–39	3/12	25	3/5	60	1/9	11	1/9	11
40–44	5/15	33	5/10	50	1/9	11	5/12	42
	17/56 (30%)		15/35 (43%)		10/36 (28%)		14/46 (30%)	
45–49	1/7	14	0/3	0	0/4	0	1/6	17
50+	11/34	32	10/22	45	9/23	39	8/28	28
Sex								
Males	29/93	31	22/55	40	18/62	29	26/80	33
Females	27/82	33	20/48	42	10/65	15	14/66	21
Pretreatment MF Density*								
1–10	1/61	1.6	4/37	11	1/45	2.2	7/50	14
11–20	6/20	30.0	6/12	50	3/11	27.0	6/14	43
21–50	16/33	48.0	13/20	65	5/24	21.0	7/31	22
51–100	9/23	39.0	8/12	67	6/21	28.0	4/20	20
101–200	16/25	64.0	6/14	43	8/19	42.0	11/21	52
201+	8/13	62.0	5/8	62	5/7	71.0	5/10	50

* Per 20-cmm sample of blood.

† Point at which first treatment began.

‡ Point at which second treatment began.

riers. Forty-two of these were positive for mf, 21 having remained so since treatment, and 21, who were temporarily negative after treatment, having reverted to a positive state. Their distribution is given in the third column of Table 3. No significant differences were observed by age groups in the percentage of carriers who were found positive at this time, as the difference of 13% between the age groups 0 to 19 and 20 to 39 is not statistically significant (S.E. = 12.6, p = .30). Likewise, there was no significant difference by sex in this regard.

Among the carriers with pretreatment counts between 1 and 10 mf per 20 cmm of blood, 11% were positive 6 months after treatment, while 50% in the group 11 to 20 and 62% in the group with more than 200 mf remained positive. These differences are statistically significant (p = .02).

Upon retesting 127 of the original carriers 18 months later, *i.e.*, 18 months after the second treatment and 24 months after therapy was begun, 28 were found to be positive for mf. Of these, 17 had remained positive since the previous survey, while 11 had relapsed to a carrier state in

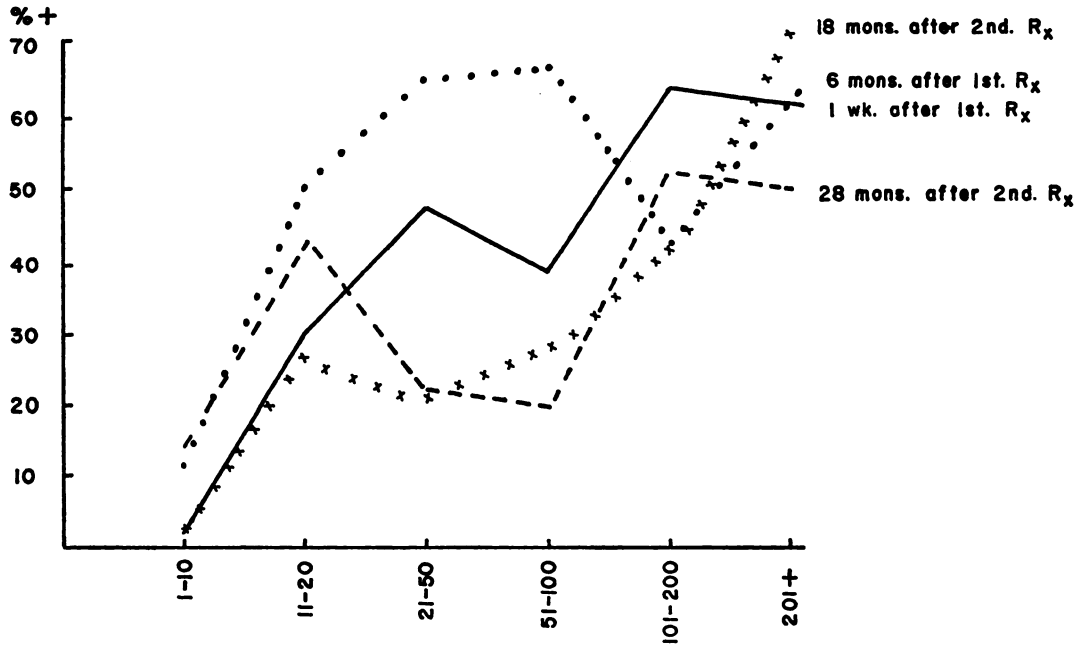

FIGURE 1. Percentage of persons with microfilaremia at four periods of time after treatment with diethylcarbamazine, correlated with the density of microfilariae before treatment. The abscissa figures are for numbers of microfilariae found before treatment.

the interim. Their distribution by age, sex, and initial mf density is shown in the fourth column of Table 3. The percentage of carriers found positive at this time did not vary significantly either by age or by sex. The difference of 14% noted between males and females is just short of being statistically significant (S.E. = 7.1, p = .054). Of the carriers with original counts between 1 and 10 mf, only 2.2% were still positive, whereas 36% of those with initial counts between 11 and 20 and 71% of those with counts over 200 remained positive. These differences are highly significant ($p < .0001$).

Of the original carriers, 146 were re-examined 34 months after the initiation of therapy, that is, 28 months after administration of the second round of treatment with DEC, and 40 were found to harbor mf. Of these, 12 had remained positive since the last survey, 22 had become positive during the interval, and 6 were unknown, as they had not been available at the previous survey. Their distribution is shown in the last column of Table 3. Again, no significant differences were observed either by age or sex, whereas significant differences were present in the distribution of positives by original mf density groups. These results are summarized in Figure 1, which shows in graphic form the percentages of mf carriers found positive at various intervals of time since the start of treatment, arranged by their pretreatment mf density groups.

DISCUSSION

Reactions to DEC

Kenney and Hewitt[15] believed that the degree of sensitization to released mf proteins, developed during varying periods of exposure in different persons, governed the extent of systemic reactions to DEC, as they were unable to find any measurable relation between the size of the dose and the number and degree of systemic reactions.

Edeson and Wharton[12] in Malaya, found that, although there was apparently some relation between reactions and mf counts, it was neither absolute nor constant. Sasa *et al.*,[6] however, have demonstrated a direct correlation between the frequency and the severity of the febrile reactions to DEC and the mf density of carriers infected with *W. bancrofti*.

Our observations show that, in sub-periodic filariasis, the proportion of carriers with initial reactions to DEC, whether toxic or "allergic,"

was directly related to the pretreatment mf counts of the carriers. Carriers with low initial mf counts exhibited significantly fewer reactions than those with high original counts, when DEC was administered for the first time, in equal amounts, to all carriers. As most of the observed reactions were of the so-called "allergic" type, it would seem natural to assume that their frequency is related to the worm load and to the amount of antigenic material that is liberated by their destruction.

Burnett and Mataika[16] noted fewer reactions among children treated with DEC in Fiji. Our observations show that there was a statistically significant difference in the percentage of adverse reactions between the younger age group and the older. This observation cannot be attributed to under-reporting, because the age group 5 to 9 reported slightly more reactions than the group 15 to 19, whereas the opposite should have held true if under-reporting had been the case. As DEC was administered to all carriers in proportion to body weight, each carrier received a proportionately equal amount of the drug; therefore, the observed difference cannot be attributed to differences in the dosage of DEC.

As densities were distributed evenly by age groups in this sample (Table 1), the observation cannot be explained by age differences in the pattern of distribution of mf counts. Thus it appears that the difference observed may be related to actual age differences in the susceptibility of carriers to liberated mf products.

It is interesting to recall that Beye *et al.*,[17] in Tahiti, and Ciferri *et al.*,[18] in American Samoa, found a similar pattern in the age distribution of positive skin reactions to antigens extracted from *Dirofilaria*, namely that, among persons with microfilaremia, fewer positive reactions were present in younger age groups than in older. It would seem likely, therefore, that hypersensitivity to mf products may be several years in developing, and thus increases with exposure. As fewer sensitized persons can be expected in the younger age groups, fewer adverse reactions to DEC may be expected to occur in this group. This observation may be relevant to mass control programs, for it narrows the spectrum of the population in which high frequencies of reactions can be anticipated and offers some understanding of the potential immunologic mechanisms involved in these reactions.

Microfilaremia

Although Hewitt *et al.*[19] found no definite relation in the experimental animal between the height of the initial counts and the rapidity with which mf disappeared or recurred in the blood after treatment with DEC, Sasa *et al.*[20] stated that, in their experiments, animals infected with small numbers of worms showed a complete cure more often than those infected with large numbers of worms. In infections in man Kenney and Hewitt[15] observed that initially low mf counts became negative more quickly than high counts, regardless of the dosage of DEC administered, and Jordan[21] stated that at the end of a treatment period, there was a lower percentage of positive blood films in the 1 to 50 mf group than in that with more than 50 mf, suggesting that persons with lower counts responded better to a given dose of the drug than those with high counts. Several other observers[2, 3, 13, 15] have commented briefly on the difficulty of eradicating either *W. bancrofti* or *B. malayi* infections from human carriers who showed initially high counts. Recently a statistically significant difference was demonstrated[2, 3] between the pretreatment total number of mf in those carriers who become negative after only one course of DEC and those who require several courses of treatment. Thus Jordan's hypothesis that a group of carriers with high mean mf counts may require more drug than a group with lower mean counts may be correct.

The present studies show a persistent positive correlation between the initial mf counts of the carriers and the positive rates found at four intervals after the administration of an identical amount of DEC per kg of body weight to each carrier. This relation was present not only at 1 week after treatment, but also at all subsequent periods when determinations were made. The observation suggests that the "hard core" mf carriers of which Jordan spoke[21] are those with the highest mf counts; these could be of considerable importance of terms of individual treatment and mass control programs. It is consistent with the hypothesis that the drug may act upon both the circulating mf and on the adults on a weight-by-weight basis, and that either larger or more frequent doses, or both, of DEC may be required to eradicate the heavier infections.

Failure to consider the original mf density of treated populations when evaluating the results of various dosages and schedules of DEC admin-

istration has probably in part caused the apparent variability of therapeutic success reported in the literature. It would appear advisable, therefore, to arrange experimental and treatment groups by pretreatment mf densities in order to obtain statistically comparable data for the evaluation of the results of antifilarial drug therapy in control programs.

SUMMARY

A study group of 175 mf carriers of varying ages and mf densities, were treated for 5 or 6 days at 6 mg/kg and again after 6 mo. with a total dose of DEC between 60 and 72 mg per kg of body weight and examined regularly for microfilariae by two 20 cmm samples of blood at 1 week, 6 months, and 2 and 3 years after the beginning of therapy.

The frequency of adverse reactions to the first administration of DEC varied directly with the pretreatment mf density of the carriers and with the age group receiving treatment: carriers with initially low counts and in the younger age groups, 0 to 19 years, experienced significantly fewer reactions to DEC than those with higher counts or in the older age groups, suggesting that, whatever the nature of these reactions, they appear to be related to the intensity of the infection and to the length of exposure to mf products.

No correlation could be found between age groups or sex distributions and the percentage of carriers who remained positive 1 week after therapy or several months or years later. A significant positive correlation, however, was consistently found between pretreatment mf density and percentage of carriers found positive at various intervals after treatment, in that the highest positive rates after treatment were found among the carriers with highest initial counts, indicating that the dosage of DEC may have to be gauged to the pretreatment mf density of the individual person or to the population at risk, in order to insure optimal therapeutic results.

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