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Metrifonate in Urinary Schistosomiasis *

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This paper describes the effect of oral metrifonate, an organophosphorus cholinesterase inhibitor, on Schistosoma haematobium *infections.*

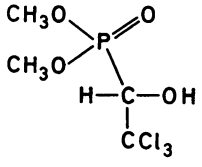
The methodology of initial studies in hospital patients and 3 field trials in schoolchildren, using spaced doses ranging from 5 mg to 15 mg per kg of body-weight is detailed.

The expected fall in plasma cholinesterase was confirmed. No major side-effects were encountered and minor symptoms were associated with high doses only.

The optimum dose was 7.5 mg per kg, given once every 14 days or once monthly to a maximum of 3 doses if necessary. About two-thirds of patients were cured after 1 or 2 doses, but some 10 % of these relapsed within 6 months of treatment. In 2 trials of this dose, cure rates of 71 % and 79 % were obtained.

The authors conclude that metrifonate is a useful addition to oral schistosomicides for the treatment of S. haematobium *infections in children, but suggest that more trials with spaced dose regimes are indicated and that further experimental studies are needed to define its mode of action.*

The organophosphorous cholinesterase inhibitor, *O,O*-dimethylhydroxy-2,2,2-trichlorethyl-phosphonate, has been used sporadically in the treatment of human schistosomiasis for a decade. This compound, the structural formula of which is shown as follows,



has insecticidal properties and was originally used for plant protection in technical grade purity as Dipterex (Bayer). It was issued in pure form for human trials as Vermicide Bayer 2349; the accepted generic name became trichlorphone which was changed later to metrifonate (International Non-proprietary Name).

The insecticidal action of organophosphorous esters depends on enzymic inhibition of specific esterases in ganglionar synapses and neuromuscular junctions. Cerf, Lebrun & Dierickx (1962) considered the extension of this property to other invertebrates including the helminths. After *in vitro* experiments, Dipterex was selected for human trials and preliminary results showed evidence of therapeutic activity in schistosomiasis, hookworm, ascariasis, trichuriasis and creeping eruption. Recent work suggests activity in onchocerciasis (Salazar-Mallen, González-Barranco & Mitrani-Levy, 1969). Extensive biochemical monitoring during the initial clinical trials revealed the expected fall in plasma and red cell cholinesterase but no abnormal findings referable to the haemopoietic tissues, liver, spleen, kidneys, pancreas or cardiovascular system (Beheyt et al., 1961). Tolerance was good, although subjective gastrointestinal disturbance occurred. The enzyme reactivator, pralidoximine iodide, also known as 2-pyridinium aldoxime methiodide or 2-PAM, was an effective antidote and atropine could be used for symptomatic relief.

Later clinical trials were characterized by pronounced variation in dosage and in length of treatment, which ranged from 2 to 20 days. Therapeutic results in schistosomiasis were predictably variable but *Schistosoma mansoni* infections responded much less favourably than those due to *S. haematobium*. It appeared that the optimum dose compatible with maximum cure and minimum side-

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effects lay between 5 mg and 15 mg per kg of body-weight but the preferred length of treatment was not clear.

In an investigation involving 4 schistosomicides given at spaced intervals of one month to children with *S. haematobium* infections, Forsyth & Rashid (1967a) treated 10 Zanzibari schoolboys with metrifonate at a dose of 10 mg per kg, for 1–6 doses. One patient was cured after 1 dose, another after 2 doses, and the others showed a fall in urinary egg output. Later the same authors treated 76 patients with metrifonate in doses ranging from 5 mg per kg, given fortnightly, to 10 mg per kg given monthly. Varying numbers of doses were given until all patients were cured, and remained so after prolonged follow-up, although it was noted that the pre-treatment egg output of these patients was low (Forsyth & Rashid, 1967b).

These results encouraged us to undertake trials of metrifonate [1] in urinary schistosomiasis. We describe three investigations into different doses and times of administration.

MATERIAL

In preliminary studies male and female African adults and children, aged from 5 to 65 years, were treated. In field trials the patients were male and female African children, and a few Asian and Arab children, of 5–15 years of age, who attended local authority schools in the areas of endemic *S. haematobium* infection surrounding Tanga, Tanzania.

METHODS

The methods used have been described in detail previously (Davis, 1966, 1968). Urines were examined after the hatching of a 10-ml random sample of the total bladder content of each patient, collected between 10.00 hours and 14.00 hours. Miracidia were killed and fixed with alcohol, then stained with eosin. Following centrifugation and withdrawal of the supernatant the miracidial and dead egg content of the final 0.1 ml was counted in a cover-slip preparation under the 16-mm objective lens.

The details of follow-up procedures have also been described previously in the same papers, but, in brief, for 3 consecutive days urines were subjected to hatching tests and examined for miracidia and eggs following sedimentation for 30 minutes, after which 10 ml from the bottom of the container were withdrawn and processed. Each urine jar and each piece of apparatus for the processing of urine was labelled with the patient's name and trial number and were used throughout the follow-up period. The total bladder content from each patient was collected between 10.00 hours and 14.00 hours.

INITIAL STUDIES

Patients attending a schistosomiasis reference clinic were selected. All had *S. haematobium* infection; none had any other serious condition, and, in particular, none had a previous history of asthma. After urinary miracidial counts, parasitological and haematological screening, chest X-ray and electrocardiography, 12 patients were given metrifonate as a single dose of 7.5 mg per kg of body-weight. If hatched miracidia were detected in the urine after 14 days this dose was repeated and urines were re-examined after a further 2 weeks. If necessary, a third dose was given.

Six patients were given 1 dose, 4 patients were given 2 doses and 2 patients were given 3 doses. Their pretreatment plasma cholinesterase values, estimated with the Tintometer Ltd field kit, based on a modification of Edson's method (1955), ranged from 87.5% to 100%, which fell to 25% in 1–24 hours after 1 dose of drug. The levels gradually regained their pretreatment values over 14–21 days but were depressed again if further doses were given. There were no significant changes in serial estimations of

[1] Supplied by courtesy of Farbenfabriken Bayer A.G.

haemoglobin, blood urea, total and differential white cell counts, and repeated electrocardiograms showed no alterations of note. Four patients complained of abdominal pain, 3 patients had diarrhoea and 2 patients were nauseated. These symptoms were mild and no medication was needed.

Three patients were lost to follow-up and 8 were cured 6 weeks after treatment. One was a failure. Five of 7 patients with pretreatment hookworm infection were negative at post-treatment examinations.

The drug was considered promising and a further 11 patients were given 10 mg metrifonate per kg, 12 patients were given 12.5 mg per kg and 14 patients were given 15 mg per kg of body-weight. These doses were repeated after 14 days if hatched miracidia were isolated at that time. The maximum number of doses was fixed at 3. Most patients were no longer passing viable ova after 2 doses. Mild abdominal pain, vomiting, diarrhoea, or weakness on the day of dosage were noted by the majority of patients but in all cases except one these symptoms were not marked, had disappeared on the day following treatment, and required no medication. One boy aged 11 years was said to have collapsed 1½ hours after his first dose. Examination soon afterwards revealed no evidence of severe intoxication and his plasma cholinesterase was 25%. He was given intramuscular atropine and was symptom-free the following day.

With the exception of the fall in plasma cholinesterase, clinicopathological investigations again revealed no significant changes after treatment. It was concluded that the drug was safe, given in spaced dosage, and that 15 mg per kg should be the maximum single dose.

FIELD TRIALS

TRIAL 1

In Trial 1, doses of 5.0 mg or 7.5 mg metrifonate per kg of body-weight were given at monthly intervals for a maximum of 3 doses if necessary.

Following primary screening and 2 consecutive daily pretreatment urinary miracidial and egg counts, 85 schoolchildren were available, 56 boys and 29 girls, aged 5–12 years. All were examined clinically and all were fit to receive metrifonate. Using a table of random numbers, patients were simply randomized into one of two groups, A or B, and doses were randomized to group.

Comparison of pretreatment variables

Comparative data on the 3 main variables in each treatment group—the pretreatment miracidial output per standardized volume of randomly sampled urine, body-weights and sex—are shown in Table 1.

The urinary miracidial output was dealt with by a transformation of the 2 pretreatment counts to the logarithm of the geometric mean (log M_G) which stabilized the variance and corrected the skewed frequency distribution of the original units to a normal distribution (Table 2). Body-weights were treated in the original kilogram units.

The variances of the transformed miracidial counts in each treatment group were similar ($F=1.064$; 42 and 41 degrees of freedom; non-significant at $F_{0.05}$ 2-tail level), and so were the means of the counts ($t=0.306$; 83 degrees of freedom; $0.8>P>0.7$).

Although the variances of the body-weights in each group were similar ($F=1.196$; 42 and 41 degrees of freedom; non-significant at $F_{0.05}$ 2-tail level),

TABLE 1
PRETREATMENT VARIABLES IN 2 GROUPS OF SCHOOLCHILDREN WITH URINARY SCHISTOSOMIASIS: TRIAL 1

	No. of patients			Pretreatment miracidial counts per random 10-ml specimen of urine		Median miracidial density per 10 ml of urine	Mean body-weight (kg)
	Total	Boys	Girls	Arith. mean	Geom. mean		
Group A	43	26	17	279	83.5	83.5	29.9
Group B	42	30	12	283	92.6	81.5	34.3

TABLE 2

CUMULATIVE FREQUENCY DISTRIBUTION OF THE LOGARITHMS OF THE GEOMETRIC MEANS OF 2 PRETREATMENT MIRACIDIAL COUNTS ON RANDOM 10-ml URINE SAMPLES IN 85 SCHOOLCHILDREN WITH URINARY SCHISTOSOMIASIS: TRIAL 1

Class interval of log. of geom. mean of 2 pretreatment miracidial counts per 10 ml of urine	Frequency	Cumulative frequency	
		No.	%
.0 –0.2499	1	1	1.2
0.25–0.4999	0	1	1.2
0.50–0.7499	1	2	2.4
0.75–0.9999	3	5	5.9
1.00–1.2499	6	11	12.9
1.25–1.4999	8	19	22.4
1.50–1.7499	11	30	35.3
1.75–1.9999	17	47	55.3
2.00–2.2499	14	61	71.8
2.25–2.4999	8	69	81.2
2.50–2.7499	6	75	88.2
2.75–2.9999	6	81	95.3
3.00–3.2499	2	83	97.6
3.25–3.4999	1	84	98.8
3.50–3.7499	1	85	100.0

there was a difference between the mean body-weights just significant at the 0.05 level ($t=2.156$; 83 degrees of freedom; $0.05>P>0.02$).

The proportions of males and females in each group were similar.

Dosage

Group A (43 patients) was randomly allocated a dose of 7.5 mg metrifonate and Group B (42 patients) 5.0 mg metrifonate per kg body-weight per month to a maximum number of 3 doses if necessary.

The drug was supplied in capsules containing 50 mg metrifonate. Each group was treated simultaneously and, in all trials, the dose was rounded to the nearest 25 mg. The capsules were swallowed with water and mouth inspection ensured that all doses were taken.

Side-effects

No side-effects were seen in either group given metrifonate at a dose of 5.0 mg or 7.5 mg per kg body-weight, monthly, to a maximum of 3 doses.

Follow-up

Urines were examined by hatching and miracidial counting at 14, 15 and 16 days, and at 38, 39 and 40 days after the first dose, when a second dose was given if necessary (holidays having prolonged the projected one-month period). At 26, 27 and 28 days after the second dose, urines were again examined and a third dose given if necessary. Thereafter all patients were followed up by 3 consecutive daily urine examinations at 1, 2, 3, 6, 10 and 12 months after treatment. If miracidia were hatched from any specimen during the 3-day follow-up, a further dose of drug was given; if urine showed no miracidia over 3 consecutive days 1 month after dose 1 or dose 2, no further treatment was given but follow-up continued. If miracidia were found in the sedimented urine on the first day of follow-up, 2 further daily random samples of the total bladder content were examined so that the post-treatment miracidial output could be validly compared with the pretreatment miracidial output.

Nomenclature used in evaluation of results

Much of the literature on chemotherapeutic trials in schistosomiasis suffers from variation in nomenclature. In these trials the following working definitions were adopted.

A cure was the term applied to a patient treated for urinary schistosomiasis who had negative urines on 3 successive days at 2, 4 and 6 months after treatment, or at some similar post-treatment time sequence. A negative urine meant the absence of hatched miracidia although dead eggs may have been present.

A failure was a patient from whom viable miracidia were demonstrated in any urine at any follow-up after treatment. We attributed a failure occurring during the first 6 months after treatment to therapeutic or drug failure, realizing that occasional maturing preinfections may have occurred. It was known, however, from previous experience with both antimony and niridazole in schistosomiasis in this area, that the major proportion of therapeutic failures became manifest in the first 6 months after treatment and that most occurred in the first 4 months.

A relapse was a failure occurring *within* 6 months of treatment *following* previously negative urines.

A reinfection referred to a failure at 9 or 12 months after treatment when all previous urine examinations to 6 months had been negative.

A point failure estimate was the number of failures occurring in a treatment group *at* a specific point in

time, usually at 2, 4, 6 and 12 months after treatment. This figure may be expressed as a proportion of the total number in the group or as a percentage.

A point cure estimate was similarly defined as the number of cures at a specific point in time after treatment.

A cumulative failure estimate was the total number of failures occurring during a specified time period after treatment. This number was usually expressed as a proportion of the total number treated or, occasionally, as a percentage.

A cumulative cure estimate was similarly the total number of cures occurring during a specified time period after treatment.

The failure rate in different intervals after treatment was the number of cases failing during the particular period, expressed as a proportion of the number at risk at the beginning of that period. It was thus a conditional probability, the probability of failing in a specified time period, given that failure had not occurred at the beginning of that period. This excluded those failures detected initially during a previous period. A similar definition applied to a *cure rate*.

Results

The proportion of patients cured, the number of relapses to 6 months, and the number of reinfections at 10 months or 1 year after treatment are all shown in relation to the number of doses given in each treatment group in Table 3.

Reinfections. One year after the last dose of the treatment schedule there were no reinfections in Group B (5.0 mg per kg) and 6 in Group A (7.5 mg per kg), a proportion of 0.140 of those treated.

Comment

There were 4 outstanding features of this trial:

(1) The complete absence of side-effects.

(2) The high proportion of those given 7.5 mg per kg who were cured after 1 or 2 doses; roughly one-third were cured after 1 dose and one-third after 2 doses. About 5% of these patients relapsed within 6 months of treatment (see Table 3).

(3) The 3-fold to 5-fold increase in cure rates achieved with an increase of unit dosage of only 2.5 mg per kg (Table 3). The lower limit of curative efficiency was 5 mg metrifonate per kg of body-weight but why the small increase in unit dose produced such disparity in the cure rates of the two groups was unknown. For a 30-kg schoolboy an increase in unit dose from 5.0 mg to 7.5 mg per kg represented an increase from 150 mg to 225 mg of drug.

(4) In those given 7.5 mg per kg the cumulative proportion of cures to 6 months was about 0.79, or 79%, and in those given 5.0 mg per kg the cumulative proportion of cures to 6 months was 0.357, an obviously statistically significant difference. One year after treatment the corresponding proportions of cures were 0.581 in Group A (7.5 mg per kg) and 0.310 in Group B (5.0 mg per kg), the difference remaining significant at the 5% level. The proportions of failures in Group B was unacceptably high at 0.595. Only 6 patients in Group A, about 7% of the total trial, were considered as reinfections, i.e., positive urines at 10 months or 1 year, having been negative to 6 months.

In the failures at 6 months the mean percentage reduction of miracidial output, calculated by methods previously detailed (Davis, 1966) was 93% in those given 7.5 mg metrifonate per kg and 69% in the group treated with 5.0 mg per kg.

TRIAL 2

In Trial 2, doses of 5.0 mg, 7.5 mg or 10.0 mg metrifonate per kg were given at fortnightly intervals for a maximum of 3 doses if necessary.

From 3 large schools within the town of Tanga, 1100 children were screened for urinary schistosomiasis. There were 241 positive cases (21.9%) but 29 children left school between screening and the start of the trial and were not available for treatment.

The remaining 212 children were allotted to 1 of 3 groups, A, B or C, from a table of random numbers and 3 different dosage regimes of metrifonate were randomized to the treatment groups.

Group A consisted of 72 children, who received 5 mg metrifonate per kg body-weight; Group B was composed of 71 children, who received 10.0 mg per kg; and in Group C there were 69 children, who received 7.5 mg per kg; all these were given at fortnightly intervals for 1, 2 or 3 doses as indicated by the success or failure of the previous dose to achieve cure.

In this trial it was necessary to stagger treatment times in order to ensure adequate clinical supervision and the groups were treated at weekly intervals. The order for the treatment of the groups was randomized and in the event Group A (5.0 mg per kg) was treated first, followed by Group B (10.0 mg per kg)

TABLE 3

PROPORTIONS OF CURES, FAILURES, RELAPSES AND REINFECTIONS WITHIN 1 YEAR OF METRIFONATE TREATMENT OF URINARY SCHISTOSOMIASIS IN RELATION TO NUMBER OF DOSES GIVEN AT MONTHLY INTERVALS AT A LEVEL OF 5.0 mg/kg OR 7.5 mg/kg: TRIAL 1

	1 Dose		2 Doses		3 Doses		Total	
Group	A	B	A	B	A	B	A	B
Total no. treated in group	43	42	43	42	43	42	43	42
Unit dose (mg metrifonate per kg)	7.5	5.0	7.5	5.0	7.5	5.0	7.5	5.0
No. needing 1, 2 or 3 doses [and proportion] [a]	14 [0.326]	3 [0.072]	15 [0.348]	4 [0.095]	14 [0.326]	35 [b] [0.833]	43 [1.000]	42 [1.000]
Early follow-up								
No. cured one month after 1, 2 or 3 doses [and proportion] [a]	13 [0.302]	3 [0.072]	15 [0.349]	4 [0.095]	8 [0.186]	5 [0.119]	36/43 [0.837]	12/42 [0.286]
No. at risk	43	42	29	39	14	35	—	—
Proportional cure rate for no. of doses (No. cured/ No. at risk)	0.302	0.072	0.517	0.102	0.571	0.143	—	—
No. of relapses to 6 months [and proportion] [a]	0 [0.000]	1 [0.024]	2 [0.046]	0 [0.000]	0 [0.000]	0 [0.000]	2/43 [0.046]	1/42 [0.024]
Follow-up at 6 months after treatment								
No. cured [and proportion] [a]	13 [0.302]	2 [0.048]	13 [0.302]	4 [0.095]	8 [0.186]	9 [0.214]	34/43 [0.790]	15/42 [0.357]
No. of failures [and proportion] [a]	0 [0.000]	1 [0.024]	2 [0.046]	0 [0.000]	6 [0.140]	25 [0.595]	8/43 [0.186]	26/42 [0.619]
No. missing at 6 months follow-up [and proportion] [a]	1 [0.024]	0 [0.000]	0 [0.000]	0 [0.000]	0 [0.000]	1 [0.024]	1/43 [0.024]	1/42 [0.024]
							1.000	*1.000*
Late follow-up: 1 year after treatment								
No. remaining cured at 10 months and 1 year [and proportion] [a]	10 [0.232]	2 [0.048]	9 [0.209]	4 [0.095]	6 [0.140]	7 [0.167]	25/43 [0.581]	13/42 [0.310]
No. of remaining failures [and proportion] [a]	0 [0.000]	1 [0.024]	2 [0.046]	0 [0.000]	6 [0.140]	24 [0.571]	8/43 [0.186]	25/42 [0.595]
Reinfections, i.e., positive at 10 months or 1 year after previously negative urines [and proportion] [a]	3 [0.070]	0 [0.000]	2 [0.046]	0 [0.000]	1 [0.024]	0 [0.000]	6/43 [0.140]	0/42 [0.000]
No. missing at 10 months and 1 year [and proportion] [a]	1 [0.024]	0 [0.000]	2 [0.046]	0 [0.000]	1 [0.024]	4 [0.095]	4/43 [0.093]	4/42 [0.095]
							1.000	*1.000*

[a] Any slight inaccuracy in the third digit of a proportion of total in group is due to rounding-off error.

[b] Includes 3 patients needing 3 doses but absent for last dose.

and lastly Group C (7.5 mg per kg). In the presentation of results it was considered preferable to tabulate data in an ascending range of dosage of 5.0 mg, 7.5 mg and 10.0 mg per kg, representing Groups A, C and B.

Follow-up urine examinations were conducted at 11, 12 and 13 days after the first dose, 12, 13 and 14 days after the second dose, 26, 27 and 28 days after the third dose, and at 3, 7 and 12 months thereafter. As in Trial 1, if 3 successive daily urines were negative, no further dose was given but follow-up continued. Only those positive at the first 2 follow-ups received 3 doses.

The frequency distribution of the pretreatment urinary miracidial counts and their relation to their class at school, as a rough reflection of age, are given in Table 4.

TABLE 4

FREQUENCY DISTRIBUTION OF THE ARITHMETIC MEANS OF 2 PRETREATMENT MIRACIDIAL COUNTS IN URINE IN RELATION TO CLASS AT SCHOOL AND HENCE AGE: TRIAL 2

Miracidia per random 10 ml of urine [a]	Class at school [b]							Total
	1	2	3	4	5	6	7	
1– 2	0	0	1	0	1	2	2	6
3– 4	0	0	0	0	0	2	1	3
5– 8	1	0	0	3	0	2	5	11
9– 16	0	1	2	4	4	5	2	18
17– 32	4	4	4	2	4	9	6	33
33– 64	1	4	4	2	3	4	9	27
65– 128	3	5	3	4	8	11	5	39
129– 256	2	3	2	5	4	11	4	31
257– 512	1	4	0	7	4	2	6	24
513–1 024	1	2	1	0	1	2	3	10
1 025–2 048	0	1	1	2	2	0	1	7
2 049–4 096	2	0	1	0	0	0	0	3
Total positive in school class	15	24	19	29	31	50	44	212

[a] Mean of 2 pretreatment counts; log. scale grouping.
[b] The age-range is from 5 years in Class 1 to 15 years in Class 7.

Comparison of pretreatment variables

The comparative data for the 3 pretreatment variables—miracidial output per random 10 ml of urine, body-weights and sex—are shown in Table 5.

Analyses of variance on a transformation of the pretreatment miracidial counts to the logarithm of the geometric mean which normalized the distribution, and on body-weights in the original units, gave in each instance, non-significant results at a 2-tailed 5% level. There was no significant difference in the proportion of the sexes in each group. The groups were considered comparable in respect of these variables.

Controls

Of the original 241 patients found on primary screening it was possible to re-examine 225 over a period of 3 months before commencing the trial, thus providing within-patient controls for the assessment of secular variation in urinary miracidial output.

Of these 225 control observations, 57 were made by examining urine by the constant technique

TABLE 5

PRETREATMENT VARIABLES IN 3 TREATMENT GROUPS OF SCHOOLCHILDREN WITH URINARY SCHISTOSOMIASIS: TRIAL 2

	No. of patients			Pretreatment miracidial counts per random 10-ml specimen of urine		Median miracidial density per 10 ml of urine	Mean body-weight (kg)
	Total	Boys	Girls	Arith. mean	Geom. mean		
Group A	72	53	19	166.8	48.8	62.4	35.4
Group B	71	53	18	293.2	55.2	74.3	34.6
Group C	69	47	22	184.8	48.6	94.3	37.3

102 days (ca 3½ months) after the first examination. In the remaining 168 patients, control urine examinations were made 42 and 96 days after the initial urine examination. No treatment was given during this period.

In the first group of 57, the second miracidial count was subtracted from the first. The sample of differences gave a normal distribution on a histogram and a parametric *t*-test was applied on the null hypothesis that the mean of the sample of differences did not differ from zero, as would be expected if there was no mean difference between the 2 sets of paired observations. The null hypothesis was maintained ($t=0.324$; 56 degrees of freedom; $0.8>P>0.7$), and it was concluded that there was no significant difference between the 2 sets of miracidial outputs from the same patients at an interval of 102 days in the absence of treatment. Egg output had not fluctuated to an extent liable to confuse therapeutic assessment, and repeatedly negative post-treatment urines could justifiably be attributed to the drug and not to secular variation in egg excretion.

In the larger series of controls subject to 3 pretreatment urine counts, the replicated observations were analysed by a non-parametric ranking procedure (Friedman, 1937, 1940). The test statistic χ_r^2 was 5.306 on 2 degrees of freedom, $0.1>P>0.05$, maintaining the null hypothesis of no difference between the urinary miracidial output of the same patients over a 3-month period in the absence of treatment.

Results

There were no serious side-effects. Occasional minor symptoms of doubtful significance were elicited but did not contraindicate continuance of treatment. Any incomplete treatment was always due to irregular school attendance.

The proportion of cures, failures, relapses and reinfections in each treatment group in relation both to the dose level and to the number of doses is given in Table 6.

Comment

There was a correlative trend towards increased proportions of cures with increase in unit dose and with the number of doses given. The trend was non-linear, as reflected in the proportions of the totals shown in Table 6. The increase in proportion of cures was marked between doses of 5.0 mg and 7.5 mg per kg but diminished slightly between 7.5 mg and 10.0 mg per kg.

Table 7 demonstrates the relationship between pretreatment variables and the number of doses needed to achieve cure. The greater the mean pretreatment urinary miracidial count, the more doses required to cure. Formal testing by an analysis of variance on a logarithmic transformation of miracidial counts confirmed a highly significant difference, at less than a 1% level, between patients needing 1, 2 or 3 doses. The need for multiple doses is dependent on the intensity of infection, and in this trial 3 doses failed to cure some high-intensity infections. Whether an increase to 4 or 5 doses would confirm a direct-infection intensity-dependence on numbers of doses is unknown.

The data in Table 7 show that there was no relationship between body-weight and the need for multiple doses, nor was any relationship discovered between body-weight and the proportion of cures or failures.

At 7 months after treatment the mean percentage reduction of miracidial output in the failures was 78% in Group A (5.0 mg per kg), 94% in Group C (7.5 mg per kg) and 84% in Group B (10.0 mg per kg).

Estimates of cure, immediately after treatment and at 7 months, in those given 7.5 mg per kg were almost identical with those of Trial 1 when the same dose was given at monthly intervals. A comparison of Tables 3 and 6 reveals that, at this dose, the proportions cured after 1, 2 or 3 doses at fortnightly or monthly intervals were similar.

TRIAL 3

In Trial 3, doses of 10.0 mg or 15.0 mg metrifonate per kg were given every fortnight for 3 doses.

It was unknown whether the failure to maintain an upward trend in proportions of cures at a dose of 10.0 mg per kg was a property of a relatively small sample. Nor was it known whether the results of treatment would improve if all patients were given 3 doses regardless of their follow-up status after 1 or 2 doses. Trial 3 was designed to try to answer these questions.

A rural school was screened and 69 children, 54 boys and 15 girls, were available for treatment. Their comparative pretreatment data are given in Table 8. The urinary miracidial output was considerably higher than that in the urban schools which participated in Trials 1 and 2.

TABLE 6

ESTIMATES OF PROPORTIONS OF CURES, FAILURES, RELAPSES AND REINFECTIONS WITHIN 1 YEAR OF METRIFONATE TREATMENT OF URINARY SCHISTOSOMIASIS IN RELATION TO NUMBERS OF DOSES OF 5.0 mg/kg, 7.5 mg/kg OR 10.0 mg/kg GIVEN AT INTERVALS OF 14 DAYS TO A MAXIMUM OF 3 DOSES IF NECESSARY: TRIAL 2

	1 Dose			2 Doses			3 Doses			Total		
Group	A	C	B	A	C	B	A	C	B	A	C	B
Total no. treated in group	72	69	71	72	69	71	72	69	71	72	69	71
Unit dose (mg metrifonate per kg)	5.0	7.5	10.0	5.0	7.5	10.0	5.0	7.5	10.0	5.0	7.5	10.0
No. needing 1, 2 or 3 doses for early cure [and proportion] [a]	13 [0.181]	18 [0.261]	20 [0.282]	13 [0.181]	26 [0.377][b]	32 [0.451][c]	46 [0.638][d]	25 [0.362]	19 [0.267]	72 [1.000]	69 [1.000]	71 [1.000]
Early follow-up												
No. cured 2 weeks after dose 1 [and proportion] [a]	12 [0.167] (1 M)[e]	18 [0.261]	20 [0.282]							12/72 [0.167]	18/69 [0.261]	20/71 [0.282]
No. cured 2 weeks after dose 2 [and proportion] [a]				13 [0.181]	24 [0.348] (2 M)[e]	28 [0.394]				13/72 [0.181]	24/69 [0.348]	28/71 [0.394]
No. cured 4 weeks after dose 3 [and proportion] [a]							16 [0.222] (1 M)[e]	16 [0.232]	10 [0.141]	16/72 [0.222] *41/72 [0.570]*	16/69 [0.232] *58/69 [0.841]*	10/71 [0.141] *58/71 [0.817]*
No. at risk at time of dose	72	69	71	59	51	51	46	25	19			
Proportional cure rate for no. of doses (No. cured/No. at risk at time of dose)	0.167	0.261	0.282	0.220	0.470	0.549	0.348	0.640	0.526			
No. of relapses to 7 months [and proportion] [a]	1 [0.014]	1 [0.014]	2 [0.028]	4 [0.056]	2 [0.028]	7 [0.098]	5 [0.069]	4 [0.058]	2 [0.028]	10/72 [0.139]	7/69 [0.100]	11/71 [0.154]

TABLE 6 (*continued*)

	1 Dose			2 Doses			3 Doses			Total		
Group	A	C	B	A	C	B	A	C	B	A	C	B
Follow-up at 7 months after treatment												
No. cured [and proportion] [a]	12 [0.167]	16 [0.232]	14 [0.197]	9 [0.125]	21 [0.304]	19 [0.268]	11 [0.153]	12 [0.174]	8 [0.113]	32/72 [0.445]	49/69 [0.710]	41/71 [0.578]
No. of failures [and proportion] [a]	1 [0.014]	1 [0.014]	2 [0.028]	4 [0.056]	1 [0.014]	7 [0.099]	28 [0.389]	11 [0.159]	11 [0.155]	33/72 [0.458]	13/69 [0.188]	20/71 [0.282]
No. missing at 7-month follow-up [and proportion] [a]	0 [0.000]	1 [0.014]	4 [0.056]	0 [0.000]	4 [0.058]	6 [0.084]	7 [0.097]	2 [0.029]	0 [0.000]	7/72 [0.097] *1.000*	7/69 [0.101] *1.000*	10/71 [0.140] *1.000*
Late follow-up: 1 year after treatment												
No. remaining cured at 1 year [and proportion] [a]	12 [0.167]	17 [0.246]	15 [0.211]	10 [0.139]	21 [0.304]	20 [0.282]	13 [0.180] [f]	10 [0.145]	4 [0.056]	35/72 [0.486]	48/69 [0.695]	39/71 [0.549]
No. remaining failures at 1 year [and proportion] [a]	1 [0.014]	0 [0.000]	2 [0.028]	3 [0.041]	1 [0.014]	6 [0.084]	30 [0.417]	11 [0.159]	11 [0.155]	34/72 [0.472]	12/69 [0.173]	19/71 [0.267]
Reinfections, i.e., positive 1 year after previously negative (and proportion] [a]	0 [0.000]	0 [0.000]	0 [0.000]	0 [0.000]	0 [0.000]	1 [0.014]	0 [0.000]	0 [0.000]	1 [0.014]	0/72 [0.000]	0/69 [0.000]	2/71 [0.028]
Missing at 1 year [and proportion] [a]	0 [0.000]	1 [0.014]	3 [0.042]	0 [0.000]	4 [0.058]	5 [0.070]	3 [0.042]	4 [0.058]	3 [0.042]	3/72 [0.042] 1.000	9/69 [0.130] 0.998	11/71 [0.155] 0.999

[a] Any slight inaccuracy in the third digit of a proportion of total in group is due to rounding-off errors.

[b] Includes 2 patients needing 3 doses but who were absent for the third dose.

[c] Includes 3 patients needing 2 doses. Each received only 1 dose, were absent for the second dose, and became cures at subsequent follow-up. Also includes 4 patients given 2 doses at an interval of one month having been absent for their second dose 14 days after the first dose; 2 were subsequently cured.

[d] Includes 4 patients needing 3 doses. All were absent for the last dose.

[e] 1 M and 2 M indicate that 1 or 2 patients of tha tsubgroup were missing at that follow-up

[f] Includes 2 patients who were missing at the 7-month follow up.

TABLE 7

RELATIONSHIP BETWEEN PRETREATMENT VARIABLES AND NUMBER OF DOSES OF METRIFONATE NEEDED IN 3 GROUPS OF SCHOOLCHILDREN: TRIAL 2

	Mean miracidial density per 10 ml urine[a] (replicated pretreatment urine counts)			Mean body-weight (kg)[a]		
Group	A	C	B	A	C	B
Unit dose (mg metrifonate per kg)	5.0	7.5	10.0	5.0	7.5	10.0
Needing 1 dose for cure	19.11 (13)	20.42 (18)	26.84 (20)	33.35 (13)	38.89 (18)	35.15 (20)
Needing 2 doses for cure	31.15 (13)	180.18 (26)	178.54 (32)	33.85 (13)	35.31 (26)	36.26 (32)
Needing 3 doses for cure	250.34 (46)	332.32 (25)	763.63 (19)	36.42 (46)	38.14 (25)	31.29 (19)

[a] Figures in parentheses denote the number of patients in each category.

The children were randomized to treatment groups and dose was randomly allocated to group.

Comparison of variances and means of pretreatment miracidial counts after transformation to log M_G, and body-weights in the original units, maintained the null hypothesis of equality and the groups were considered comparable in respect of these variables.

Dosage

Group A, 35 patients, received a dose of 10.0 mg per kg of body-weight, and Group B, 34 patients, received 15.0 mg per kg of body-weight. In Group A 1 patient received only 1 dose; he has been retained in the analysis although it is doubtful whether this is justifiable.

All children were treated with 3 fortnightly doses regardless of their follow-up status. Follow-up was performed for 3 consecutive days, 2 weeks after the first dose, 2 weeks after the second dose and 4 weeks after the third dose, in order to estimate the proportions of cures after each dose.

Side-effects

At these higher doses side-effects began to appear. In Group A, 10.0 mg per kg, there were 4 complaints and in Group B, 15.0 mg per kg, there were 13 complaints, of abdominal pain, nausea, diarrhoea and vomiting. On only 1 occasion was it considered that part of a dose may have been lost from vomiting. In no instance did these symptoms require medication.

Results

The point estimates of cure on follow-up, 2 weeks after 1 dose, 2 weeks after the second dose and

TABLE 8

PRETREATMENT VARIABLES IN 2 GROUPS OF SCHOOLCHILDREN WITH URINARY SCHISTOSOMIASIS: TRIAL 3

	No. of patients			Pretreatment miracidial counts per random 10-ml specimen of urine		Median miracidial density per 10 ml of urine	Mean body-weight (kg)
	Total	Boys	Girls	Arith. mean	Geom. mean		
Group A	35	30	5	1 217.9	321.4	449	28.7
Group B	34	24	10	690.8	177.8	238	26.4

TABLE 9

POINT ESTIMATES OF CURE OF SCHOOLCHILDREN WITH URINARY SCHISTOSOMIASIS TREATED WITH 3 DOSES, AT INTERVALS OF 14 DAYS, OF METRIFONATE AT 10.0 mg/kg OR 15.0 mg/kg: TRIAL 3

	3 Negative urines (cure)		Miracidia in urine (failure)		Missing at follow-up		Proportion of cures [a]	
Group	A	B	A	B	A	B	A	B
Dose (mg metrifonate per kg)	10	15	10	15	10	15	10	15
No. treated	35	34	35	34	35	34	35	34
Time of follow-up:								
2 weeks after dose 1	6	8	27	26	2	0	0.171 [b]	0.235 [b]
2 weeks after dose 2	19	24	15	10	1	0	0.543 [b]	0.701 [b]
4 weeks after dose 3	24	29	8	3	3	2	0.686 [b]	0.853 [b]
3 months after dose 3	18	22	12	6	5	6	0.514 [b]	0.647 [b]
6 months after dose 3	19	23	12	8	4	3	0.543 [b]	0.676 [b]
9 months after dose 3	15	20	15	9	5	5	0.428 [b]	0.588 [b]

[a] No. of cures/Total in group.

[b] None of these differences was significant at the 0.05 level on χ^2 testing with Yates' correction. Nor did loading missing cases on to extreme ends of the arrays under test produce a significant difference, except for one weighting procedure at 9 months.

4 weeks after the third dose and at 3, 6 and 9 months after treatment are given in Table 9.

Comment

One of the defects of small groups in clinical trials is the wide sampling distribution of a proportion from a binomial population. Taking the results in Table 9 at 1 month after treatment as a fair reflection of drug efficacy, the 3 doses of 10 mg per kg cured 69% of the sample and 3 doses of 15.0 mg per kg cured 85%. But the 95% confidence limits of a proportion of 69% in a sample of 35 are 51%–84%, and the 95% confidence limits of a proportion of 85% in a sample of 34 are approximately 68%–95%, producing a considerable overlap. Demonstration of a real difference in cure from 69% to 85% with a 5% probability of type I error and a 10% probability of type II error would require a sample size of about 300 patients. Side-effects appearing at the dose levels of 10.0 mg and 15.0 mg per kg may render such a trial impracticable. The evidence did not suggest that an increase in unit dose above 10.0 mg per kg was likely to be associated with steadily rising proportions of cures. Nor did it seem that the routine administration of 3 doses carried any advantages. Very few relapses were encountered when cure had been attained after 1 or 2 doses at any unit dose level.

Nine months after treatment the mean reduction of miracidial output in the failures was 97% in Group A (10.0 mg per kg) and 82% in Group B (15.0 mg per kg).

COMPARISON OF ALL TRIALS

A comparison of the proportions of cures obtained in all trials is shown in Table 10.

Because the highest miracidial outputs were treated with the highest fixed dose schedule a comparison was not truly valid but it provided an overall view of the effect of the drug in juvenile urinary schistosomiasis. The probability of a linear relationship between increasing doses and proportions cured was calculated, using the methods described by Armitage (1955) for those trials on fortnightly administration ranging from unit dose of 5.0 mg to 15.0 mg per kg. The results confirmed the suspicion

TABLE 10

ESTIMATED PROPORTIONS OF CURES IN CHILDREN GIVEN METRIFONATE IN VARYING DOSE SCHEDULES IN ALL 3 TRIALS [a]

	Unit dose																							
	5 mg/kg						7.5 mg/kg						10 mg/kg						15 mg/kg					
	Monthly to maximum of 3 doses if necessary (42 treated)			Every 14 days to maximum of 3 doses if necessary (72 treated)			Monthly to maximum of 3 doses if necessary (43 treated)			Every 14 days to maximum of 3 doses if necessary (69 treated)			Every 14 days to maximum of 3 doses if necessary (71 treated)			Every 14 days for 3 doses (35 treated)			Every 14 days for 3 doses (34 treated)					
	Proportion cured [b] at:			Proportion cured [b] at:			Proportion cured [b] at:			Proportion cured [b] at:			Proportion cured [b] at:			Proportion cured [b] at:			Proportion cured [b] at:					
	1 month	6 months	1 year	1 month	6 months	1 year	1 month	6 months	1 year	1 month	6 months	1 year	1 month	6 months	1 year	1 month	6 months	9 months	1 month	6 months	9 months			
After 1 dose	0.072	0.048	0.048	0.167	0.167	0.167	0.302	0.302	0.232	0.261	0.232	0.246	0.282	0.197	0.211	0.171	—	—	0.235	—	—			
After 2 doses	0.095	0.095	0.095	0.181	0.125	0.139	0.349	0.302	0.209	0.348	0.304	0.304	0.394	0.268	0.282	0.372 [c]	—	—	0.466 [c]	—	—			
After 3 doses	0.119	0.214	0.167	0.222	0.153	0.180	0.186	0.186	0.140	0.232	0.174	0.145	0.141	0.113	0.056	0.143	0.543	0.428	0.152 [c]	0.676	0.588			
Cumulative proportion of cures for all 3 doses i.e. for whole trial	0.286	0.357	0.310	0.570	0.445	0.486	0.837	0.790	0.581	0.841	0.710	0.695	0.817	0.578	0.549	0.686	0.543	0.428	0.853	0.676	0.588			

[a] Derived from Tables 3, 6 and 9.

[b] Proportion cured = No. of cures/Total no. in group.

[c] By subtraction from point proportion at that time.

from the crude data that there was no true linear relationship between increased unit dose and proportion cured and the optimum dose was 7.5 mg per kg of body-weight. At all levels and in all trials, however, the comparison of pretreatment miracidial output in the cures and in the failures showed highly significant differences. The failures always showed a markedly higher pretreatment output than the cures. Within each regime intensity of infection was inversely related to cure rate but between dose regimes there was no linear relationship between cure rate and increase in unit dose.

DISCUSSION

The results of these trials confirmed that metrifonate is an effective drug in the treatment of urinary schistosomiasis. There was little difference in therapeutic results whether the drug was given every 14 days or once monthly except at the lower limit of effective dosage, 5 mg per kg, where administration every 14 days was superior to administration once monthly. A fixed dose schedule of 3 doses at fortnightly intervals carried no major advantage. The optimum dose is 7.5 mg per kg, which can be given either fortnightly or monthly. The drug should not be given at intervals of less than 14 days because of cholinesterase depression and slow recovery. At a unit dose of 7.5 mg per kg, side-effects were negligible and up to 6 months a cumulative cure rate of about 75% can be expected. It is possible that, by increasing the number of doses to 4 or 5, increased cure rates may ensue, but we have not explored this factor. In those cures after 1 or 2 doses, about two-thirds of patients, the relapse rate within 6 months of treatment was low, of the order of 1 in 10. It is impossible to estimate the true relapse rate as some cases classified as relapses may have had maturing preinfections at the time of treatment. Commonly, maturing parasites are relatively unaffected by drugs which have a marked schistosomicidal action on adult worms.

The mode of action of the drug is unknown. Barker, Bueding & Timms (1966) presented evidence suggesting the presence of cholinesterase receptors in *S. mansoni* which were not identical with the human types of receptor. *In vitro* experiments have shown that the concentration of Dipterex required to produce paralysis of worms is high when compared with effective chemotherapeutic doses (Barker, Bueding & Timms, 1966). Presumably the action of the drug is due to enzyme inhibition in the adult worms, the enzymes being closely related to cholinesterase and affected by metrifonate in a similar fashion to human cholinesterase.

It has been suggested that adult schistosomes, under the influence of the drug, drift to the small arterioles of the lungs where they are trapped, encased and die (Forsyth & Rashid, 1967b). There is no experimental or clinical evidence supporting this hypothesis and it fails to explain the phenomenon of clinical relapse unless it is postulated that relapses are, in fact, maturing preinfections. Evidence on this hypothesis could be gathered by treating a series of *S. haematobium* infections where preinfection and reinfection risk can be definitely excluded.

Reduction of sperm count, striking effects on motility and viability of sperms and an increase in abnormal forms have been noted after treatment of schistosomiasis with Dipterex (Hanna et al., 1966). In that trial, however, a total dose of 60 mg per kg given over 12 days was used. It is much less probable that such unacceptable effects would be seen after 7.5 mg per kg given to a maximum of 22.5 mg per kg over 6 weeks or 3 months. The point must be investigated and clarified. Until then treatment should be restricted to children.

The paradox of human therapeutic activity, yet failure of action in experimental *S. mansoni* infections in mice, hamsters and monkeys has been noted previously (Abdallah et al., 1965; Katz, Pellegrino & Pereira, 1968), and it has been suggested that because of the poor clinical response of *S. mansoni* in humans treated with metrifonate, and the known toxicity of the organophosphorus compounds, further clinical trials are unlikely to yield positive information (Katz, Pellegrino & Pereira, 1968). Yet the undoubted efficacy of the drug in *S. haematobium* infections in children and its virtually complete lack of short-term toxicity in these trials would suggest that further trials are indicated. The striking contrast between the therapeutic results of the metrifonate treatment of human *S. haematobium* and *S. mansoni* infections may be due to differences in the structure of the cholinoceptive receptors of the two

species, although anatomical differences in parasite habitat and response to schistosomicides can equally be held to be productive of this phenomenon.

A large number of organophosphorus compounds are in regular use in agriculture and public health. There is much accumulated knowledge of their toxicology. The concept of *specific* enzyme inhibition as a mode of action is attractive and could stimulate a search for new compounds with predictable structure–activity relationships. The use of a stable reproducible model infection with *S. haematobium* may be helpful in explaining the numerous puzzling points of the action of metrifonate. The evidence available to date suggests that the drug would have little future in the treatment of *S. mansoni*. There would appear to be justification for continuance of the search for schistosomicides among the organophosphorus compounds.

RÉSUMÉ

TRAITEMENT DE LA SCHISTOSOMIASE URINAIRE PAR LE MÉTRIFONATE

Le métrifonate, composé organo-phosphoré et inhibiteur de la cholinestérase, fait preuve de propriétés antihelminthiques qui ont été mises à profit à plusieurs reprises pour le traitement de la schistosomiase humaine. Le présent article expose les résultats obtenus en Tanzanie par l'administration de doses espacées du produit dans l'infection à *Schistosoma haematobium*.

Lors de chacun des essais, on a procédé, avant et après chaque traitement, à de multiples examens et à des analyses quantitatives minutieuses de l'urine par le test d'éclosion des œufs, suivi de la numération des miracidiums et des œufs morts. La tolérance au médicament, recherchée au préalable sur des malades hospitalisés, a été satisfaisante après administration de doses allant de 5 à 15 mg/kg. Les doses les plus élevées ont entraîné de légers troubles gastro-intestinaux. La cholinestérase plasmatique, après s'être abaissée à 25% de sa valeur normale en 1-24 heures, a recouvré son taux initial en 14-21 jours. Le phénomène s'est reproduit à chaque nouvelle prise du médicament. Les examens hématologiques et électrocardiographiques n'ont décelé aucun trouble. Les résultats thérapeutiques ayant été intéressants, trois essais de traitement ont été entrepris chez des écoliers africains.

Dans le 1er essai, 5 et 7,5 mg/kg de métrifonate ont été donnés une fois par mois, avec un maximum de 3 doses, à respectivement 42 et 43 enfants. Avec la dose de 5 mg/kg, le taux de guérison a été faible (36% au 6e mois); avec la dose de 7,5 mg/kg, les deux tiers des malades ont été guéris après une ou deux doses et le taux global de guérison a atteint 79% au 6e mois. Chez les malades guéris après avoir reçu moins de trois doses, on a observé 6% de rechutes durant les 6 premiers mois. Après un an, la proportion des guérisons était de 31% et 58% chez les enfants traités respectivement par des doses de 5 et 7,5 mg/kg. On notait à ce moment un taux de réinfection de 14%. Aucune réaction secondaire n'a été observée.

Au cours du 2e essai, on a administré le métrifonate à raison de 5 mg/kg à 72 enfants, de 7,5 mg/kg à 71 enfants et de 10 mg/kg à 69 enfants, tous les 14 jours, la dose étant renouvelée deux fois au maximum en cas d'échec du ou des traitements précédents. Les taux de guérison chez les enfants traités par 5, 7,5 et 10 mg/kg ont été respectivement de 17%, 26% et 28% après une dose, de 18%, 35% et 39% après deux doses et de 22%, 23% et 14% après trois doses. Il y a eu 10 à 15% de rechutes au cours des 7 premiers mois. Après 7 mois et un an, les taux de guérison étaient de 45% et 49% (dose de 5 mg/kg), de 71% et 70% (dose de 7,5 mg/kg) et de 58% et 55% (dose de 10 mg/kg). Aucune réaction secondaire notable n'a été signalée.

Le 3e essai a consisté en l'administration, à 14 jours d'intervalle, de 3 doses de métrifonate de 10 mg/kg à 35 enfants et de 15 mg/kg à 34 enfants. Durant le traitement, 4 des 35 enfants du 1er groupe ont présenté des douleurs abdominales, des nausées, de la diarrhée et des vomissements; les mêmes symptômes sont apparus chez 13 des 34 enfants du second groupe. Un mois après le traitement, les taux de guérison étaient de 69% (dose de 10 mg/kg) et de 85% (15 mg/kg). Ils n'étaient plus, après 6 mois, que de 54% et 68% respectivement.

L'analyse des résultats donne à penser qu'il existe une relation directe entre l'intensité de l'infection initiale et la nécessité d'administrer plusieurs doses du médicament pour obtenir la guérison. La dose optimale semble être de 7,5 mg/kg. Une dose de 15 mg/kg donne des taux de guérison légèrement supérieurs, mais cet avantage est contrebalancé par l'apparition de réactions secondaires. L'adoption d'un schéma fixe est utile en pratique.

Selon les auteurs, le métrifonate peut prendre place parmi les schistosomicides oraux actifs contre les infections à *S. haematobium* chez les enfants. Son efficacité contre *S. mansoni* est beaucoup plus faible. Son mode d'action reste encore mystérieux à de nombreux égards et de nouvelles recherches sont nécessaires.

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