DATA EVALUATION RECORD

(1) CHEMICAL: Trichlorfon

(2) TYPE OF FORMULATION: Tablets (100 mg active ingredient/tablet)

(3) CITATION: Plestina, R., Davis, A., and Bailey, D.R. 1972. Effect of metrifonate on blood cholinesterases in children during the treatment of schistosomiasis. Bull. WHO 46:747-759

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(6) TOPIC: Case History

(7) CONCLUSION: In a controlled clinical trial with Tanzanian school children, trichlorfon was an effective schistosomicide in Schistosoma haematobium infections and showed moderate activity against hookworm. Within the range used (7.5-12.5 mg/kg, up to 3 doses at 14-day intervals), the compound was well tolerated despite its inhibiting effect on erythrocyte and plasma cholinesterases. Plasma cholinesterase was more strongly inhibited than the erythrocyte enzyme, but recovered more rapidly (in 4 weeks), with repeated dosing, unreactivated erythrocyte cholinesterase apparently accumulated; recovery required 8-15 weeks.

CORE CLASSIFICATION: Not applicable

(8) MATERIALS AND METHODS:

Trial Design: In a primary school in the town of Tanga, Tanzania, 63 children were identified, by urinary miracidial and egg counts, as having Schistosoma haematobium infections. Their age ranged from 7 to 18 years, and after a medical examination, they were considered fit to receive trichlorfon. The patients were stratified into three groups representing mean miracidial counts of 1-199, 200-999, and over 1,000 per 10-ml sample of urine. From these strata, patients were randomly assigned to one of three treatment groups, (A, B, or C) by means of random numbers. The selected doses were randomly assigned to the treatment groups, and the order in which the groups were to be treated was

also randomly allotted. Before the trial commenced, 2 of the 63 children left school. Only one child refused to take the drug a second time. No absenteeism on the days following the treatment was recorded. The following groups were dosed:

Group	Number of Children	Dose	Order of Treatment
A	21	7.5 mg/kg	1
B	19	12.5 mg/kg	3
C	19	10 mg/kg	2

These doses were decided upon after consideration of previous trials of 5-15 mg/kg. In each treatment group, one-half of the patients were given three doses of the drug at intervals of 14 days, regardless of their parasitological state on follow-up, while the remaining ones were treated only if urine samples showed viable miracidia 2 weeks after the preceding dose.

All doses were rounded off to the nearest 25 mg (from 100-mg tablets). The tablets were swallowed with water, and mouths were inspected to insure that all doses were taken. All doses were given by a physician, and all were interviewed and examined, if necessary, after each treatment.

One to two weeks before treatment, two blood samples were taken from each child to establish his pretreatment

cholinesterase level. After each dose, blood samples were taken from those in each treatment group at different intervals to show the spectrum of cholinesterase activity.

For Group A, blood samples were obtained at 0.5, 3, 6, 24, 48, 72, and 96 hours, and 1 and 2 weeks after dose 1. After dose 2, a sample was taken at 14 days. After dose 3, samples were obtained after 1, 14, 28, 56, and 106 days.

In Group C, sampling occurred at 1, 3, 7, and 14 days after dose 1, at 1 and 14 days after dose 2, and at 1, 14, 28, and 93 days after dose 3.

In Group B, samples were taken at 0.25, 1, 3, 7, and 14 days after dose 1, at 1, 3, and 14 days after dose 2, and at 1, 3, 28, 48, and 97 days after dose 3.

In those treated with one or two doses only, sampling was performed at different intervals. Sampling in outpatients was frequent.

All blood samples were coded, and estimations of enzyme activity were performed blind.

Parasitological Controls: Previous evidence from Bull. WHO (1966. 35:827; 1968. 38:197; 1969. 41:209) indicates that urinary miracidial output in untreated patients, both adults and children, does not decline over a period of several months to an extent liable to confuse therapeutic assessment of a schistosomicide. Hence, no direct concurrent parasitological controls were used.

Monitoring of blood cholinesterase: Samples of whole blood were usually taken between 8:30 and 10:30 a.m. After a finger prick, the first drop of blood was discarded and the puncture was wiped. For determination of whole blood cholinesterase, 20 µl of blood was taken into a Sahli hemoglobin pipette rinsed with 2% heparin immediately before use. The tip was wiped, blood was sucked about 2 cm up the pipette, and the pipette was sealed with a rubber band, and stored cold until tested. A further blood sample was taken in a dry, heparinized, capillary tube described by Stubbs and Fales (1960. Am. J. Med. Technol. 26:25). After one end was sealed, the tubes were stored in a vacuum flask packed with ice until they were centrifuged for 30 minutes at 2,000 rpm. One such tube containing about 100 µl of blood was sufficient for determination of both erythrocyte acetylcholinesterase and plasma cholinesterase activity. Hematocrit measurements were made with these tubes to check the contribution of erythrocyte cholinesterase to the total activity in the blood.

Cholinesterase Activity: The spectrophotometric method of Ellman et al. (1961. Biochem. Pharmacol. 7:88), as modified by Wilhelm (1968. Arh. Hig. Rada. 19:199) and Vandekar (1969. Bull. WHO 38:609) was used. Determinations were made within a few hours after sampling in an air-conditioned laboratory at 22-24°C. A Unicam

SP600, series 2 spectrophotometer with constant voltage transformer and power supply unit was used. The same buffers and reagents were used in measurement of each cholinesterase activity. Double distilled water was used in all experiments.

Phosphate buffers (0.1 M, pH 7.4) were prepared weekly or twice a week. The stock solution of S-acetylthiocholine iodide ($6x10^{-2}$ M) was used for no longer than 2 successive days. A mixture containing 10 ml of 5,5'-dithiobis(2-nitrobenzoic) acid (DTNB) (3.96 mg/ml) and 1 ml of a solution of $NaHCO_3$ (15 mg/ml) was prepared once or twice a week. All solutions were stored at 4°C.

For measurement of enzyme activity, 20 µl of blood or 10 µl of packed erythrocytes were expelled into 12 ml of buffer. After mixing, aliquots of 3 ml were transferred into each of three test tubes containing 100 µl of DTNB solution. The reaction was started in two tubes by adding substrate; the third served as a blank for measurement of the change of extinction at 412 nm in 2 minutes. For measurement of plasma cholinesterase activity, 20 µl of plasma was transferred into each of three tubes containing 3 ml of buffer and 100 µl of DTNB solution. The subsequent procedure was the same as described above. The results were calculated as the mean of two replicate readings and expressed as changes of extinction/minute/ml.

Parasitological Methods: From the total bladder content of each patient, collected between 10:00 a.m. and 2:00 p.m. the eggs in a random sample of 10 ml were hatched under artificial light. Miracidia were then killed, fixed with ethanol, and stained with eosin. After centrifugation and withdrawal of the super natant, the miracidial and dead egg contents of the final 0.1 ml were counted. These studies were conducted 12, 13, and 14 days after the first and second dose and 4 and 12 weeks after the third dose.

To examine for hookworm infections by egg counts, a part of each stool was used to prepare a suspension by the method of Stoll (1923. Am. J. Hig. 3:59). Four replicate samples of 0.075 ml of the suspension were examined, and the number of eggs in each was counted. If a count revealed hookworm infection, another dilution count was performed on a second stool specimen passed on another day. The mean number of eggs/g of stool was calculated.

A second part of each stool sample was used for the cultivation of larvae by the method of Sasa et al. (1958. Jpn. J. Exp. Med. 28:129). About 0.5 g of fresh stool was smeared on a strip of filter paper, which was then inserted into a test tube containing 5 ml of water, so that the end was submerged. The tubes were sealed and incubated for 8 days, at which time any eggs hatched,

and the larvae fell into the water. After removal of the strips, the tubes were heated at 56°C for 30 minutes to kill the larvae. The water was centrifuged, and the deposit was examined by microscopy.

Follow-up studies were performed 1 month after the last dose of trichlorfon. If the first dilution count failed to reveal ova, the second stool was examined by the formol-ether concentration method of Ridley and Hawgood (1956. J. Clin. Pathol. 9:74).

(9) REPORTED RESULTS:

Clinical Observations: Minor complaints, transient and needing no treatment, were reported from 5 of 65 treated children. These are detailed in Table 1.

Cholinesterase Activities: Although hematocrit readings ranged from 25 to 48 among the children, the variation for repeated estimations for individuals was small. Thus results of whole blood cholinesterase in each child were comparable.

At all dose levels, whole blood cholinesterase activity was markedly reduced. The effect approved to be dose related, since a linear contrast (estimator of linear trend) of mean values at the same posttreatment times, averaged over those times with data for all three doses, gave a "t" value of 5.54 ($p<0.001$).

Complete recovery of whole blood enzyme activity had not occurred at 2 weeks after the initial dose; a subsequent dose depressed the activity further. After the third dose, recovery was complete at 8 weeks in Group A children (7.5 mg/kg), at 13 weeks in Group C children (10.0 mg/kg), and at 14 weeks in Group B children (12.5 mg/kg).

The pattern of cholinesterase activity in packed erythrocytes was parallel to that in whole blood.

Plasma cholinesterase activity, regardless of the dose given, was extremely low 24 hours after treatment. This activity recovered more rapidly than erythrocyte cholinesterase.

The pattern of blood cholinesterase activities in a few adult outpatients was similar to that in the children. The most pronouced inhibition occurred at 6 hours after dosing. Again, inhibition and recovery of enzyme activity was more rapid in plasma than in erythrocytes.

Parasitological Data: The relevant variables for each group are compared in Table 2. Analyses of variance using a logarithmic transformation of the egg count data supported the null hypothesis that the sample variables were drawn from the same or similar populations ($P>0.05$). There was a difference, significant at the 5% level, between the means of body weight.

The favorable results of treatment of urinary schistosomiasis are shown in Table 2. Children who received 1, 2, or 3 doses are included in this table.

The results of treatment on hookworm infections at 1 month are shown in Table 3. About one-half of those treated could be classified as cured.

(9) TECHNICAL REVIEW TIME: 8.0 hours