

CLINICAL TRIALS WITH BEPHENIUM HYDROXYNAPHTHOATE AGAINST HOOKWORM IN CEYLON

BY

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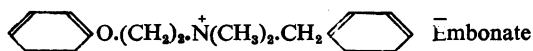
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Copp, Standen, Scarnell, Rawes, and Burrows (1958) showed that members of a series of new quaternary ammonium compounds were active against a broad range of parasitic nematodes in laboratory and domestic animals. One of the most active compounds, bephenium embonate (benzylmethyl-2-phenoxyethylammonium embonate), was shown by Rawes and Scarnell (1958) to be of value in the treatment of nematodiriasis in lambs.



The demonstration of high activity of bephenium salts against *Ancylostoma caninum* in dogs and cats (Copp *et al.*, 1958) led naturally to preliminary trials in London against hookworm infections in man. We are indebted to Professor A. W. Woodruff, of the Tropical Diseases Hospital, and Drs. D. H. Garrett and M. J. Andrewes, of the Dreadnought Hospital, for carrying out these trials. The results were encouraging and justified more extensive trials against heavier hookworm infections in Ceylon.

Tests in laboratory animals, including monkeys, showed that the toxicity of salts of bephenium was low. The less soluble salts, such as the 2-hydroxy-3-naphthoate and embonate, were poorly absorbed when given by mouth to human volunteers (Rogers, 1958).

Materials and Methods

Experiments with *A. caninum* in dogs and cats (Burrows, 1958), *Nippostrongylus muris* in rats, and *Nematospiroides dubius* in mice (Copp and Standen, 1958, in preparation) showed that bephenium halides, although more irritant, were appreciably more active against these organisms than were the less soluble hydroxynaphthoate and embonate. It was also found that equivalent anthelmintic activity was obtained when a proportion of the dose of halide was replaced by hydroxynaphthoate. On the basis of these observations, bephenium bromide, hydroxynaphthoate, and mixtures of these salts were selected for trial. The almost insoluble embonate was also tested.

Bephenium bromide and mixtures of bromide and hydroxynaphthoate were given as tablets, prepared so that they would disintegrate in the stomach within a few minutes of administration. The hydroxynaphthoate and embonate were given as tablets or as a sweetened and flavoured suspension in water. As a control group a proportion of the patients were treated with tetrachlorethylene. According to the general practice in Ceylon, it was administered together with a saline purge (Maplestone and Mukerji, 1939).

Faecal specimens were collected in ice-cream cartons. Patients whose stools contained hookworm ova on examination by direct smear were selected for treatment. Intercurrent infections with roundworm and whipworm, as seen by direct smear, were recorded. The number of

hookworm eggs per millilitre of faeces was determined before treatment by the dilution-count technique as described by Stoll and Hausheer (1926). Four days after treatment a further specimen was collected and examined by direct smear. If positive for hookworm ova, a dilution count was made. If negative, the specimen was examined after concentration by the M.I.F.C. method (Sapero and Lawless, 1953; Blagg, Schloegel, Mansour, and Khalaf, 1955). All counts were made in duplicate.

The effect of treatment was assessed by calculation of the percentage reduction in egg count. An arbitrary figure of 200 eggs/ml. was awarded to those specimens which were negative on direct smear but positive on concentration.

In some instances the stools passed after treatment were washed through a No. 60 sieve and the hookworms recovered for identification of species.

Patients were treated at the General Hospital and Lady Ridgeway Children's Hospital in Colombo, and at the General Hospital in Negombo. A group of Tamil labourers and their children were treated under field conditions at the Sapumalkande rubber estate, Dehiowita. Patients were selected for treatment with the different preparations of bephenium by a random method. A special group of children with infections which had proved resistant to successive treatments with tetrachlorethylene were given multiple doses of bephenium hydroxynaphthoate ("alcopar") at Ragama Convalescent Home.

Bephenium salts were given early in the morning on an empty stomach, and no food was allowed until at least two hours after the dose. No purge was given after the dose. One group of patients were given three doses on one day; the second and third doses were spaced between meals. Patients in hospital were treated with piperazine citrate syrup ("antepar") on the day before administration of a bephenium salt. Estate labourers were given no pre-treatment with piperazine. Throughout this report all doses of bephenium salts are expressed in terms of base: 1 g. of bephenium base is contained in 1.31 g. of bephenium bromide, 1.73 g. of bephenium hydroxynaphthoate, and 1.75 g. of bephenium embonate.

The weight of infection with hookworm before treatment varied greatly among the 284 patients treated. An analysis of the distribution of egg counts (Fig. 1) shows that patients admitted to hospital had a significantly higher mean rate of infection than the labourers at the rubber estate. The random selection of patients for the separate treatment groups resulted in fairly evenly distributed proportions of heavy, moderate, and light infections.

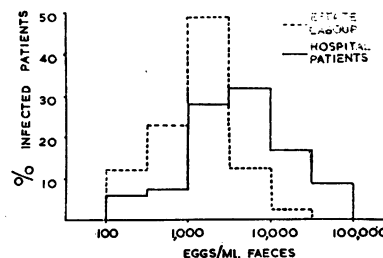


FIG. 1.—Distribution of the number of hookworm eggs/ml. of faeces from 132 estate labourers and 109 hospital patients in Ceylon.

Effect of Bephenium Salts in Treatment of Hookworm Infection

Bephenium Hydroxynaphthoate

The first few patients were treated with small doses of bephenium, the size of the dose being adjusted in accordance with body weight. It soon became apparent that the less soluble salts had no toxic side-effects even when given in doses as large as 200 mg./kg. It was also clear that the small doses administered to children on the mg./kg. basis had no appreciable effect on the egg count. As a result of these preliminary tests it was concluded that a single dose of less than 2 g. in the child and in the adult was insufficient, and subsequently a standard dose of 2 to 3 g. of bephenium

base was administered to all patients irrespective of weight and age. The drug has a bitter taste, but was accepted by both adults and children. Table I shows the results obtained with patients in hospital who were given single doses of bephenium hydroxynaphthoate. Hookworms were recovered from the stools of patients given as little as 1 g.

given daily for four successive days; those who still retained some degree of infection and were still in hospital were given a further seven-day course of treatment. The results are given in full in Table III.

The effect of four 2-g. doses on the faecal egg count of one 6-year-old child is shown in Fig. 2. This child had advanced cirrhosis of the liver (confirmed by biopsy) and gross ascites. He was considered by the physician to be unsuited for treatment with tetrachlorethylene. Bephenium greatly reduced the egg count, and no toxic side-effects of any kind were observed. After treatment, hookworms were recovered from the stools. Examination of this child 10 weeks after treatment showed that the stools were free from hookworm ova.

In order to study the effectiveness of bephenium hydroxynaphthoate under field conditions, trials were carried out in a group of 132 Tamil labourers and their

TABLE I.—Effect of Single Doses of Bephenium Hydroxynaphthoate or Tetrachlorethylene against Hookworm (*Necator americanus*)

	Bephenium Hydroxynaphthoate*			Tetrachlorethylene	
	0.5-1.5 g.	2 g.	2.5-3 g.	5 m. (0.3 ml.) Children	30-40 m. (1.8-2.4 ml.) Adults
No. of patients treated	6	16	29	5	11
„ in whom degree of infection was reduced	3	14 (88%)	27 (93%)	2	9 (82%)
„ in whom infection was reduced by	1 (100-75%)	11 (69%)	13 (45%)	0	8 (73%)
	2 (74-25%)	3 (19%)	11 (38%)	1	0
	2 (24-0%)	2 (12%)	5 (17%)	4	3 (27%)
Initial weight of infection (eggs/ml.)	37,000-1,200	44,000-200	44,400-200	6,400-400	31,700-100
Mean reduction in count	28%	78%	64%	12%	70%

* Dose in terms of base.

of bephenium even if the subsequent egg count showed no significant reduction. All hookworms isolated from the stools were identified as *Necator americanus*.

Twenty-seven patients were given 2 g. of hydroxynaphthoate three times on one day. The results are shown in Table II.

A group of children aged from 4 to 10 years were treated with multiple doses of bephenium hydroxynaphthoate. All had been admitted to hospital in a critical condition, suffering from malnutrition, anaemia, severe gastro-enteritis, and hookworm infection. Before treatment with bephenium their general condition had been improved by intravenous fluids, iron, and vitamins, but they were still passing several fluid stools each day. Some of the children had already received one or more treatments with tetrachlorethylene, but their stools still contained hookworm ova. Because of their precarious fluid balance, others were regarded by the physician as being too ill to be given tetrachlorethylene and subsequent purgation. In view of the persistent intestinal hurry, it was considered rational to administer more than one dose of bephenium. Doses of 2 g. were

TABLE II.—Effect of Multiple Doses of Bephenium Hydroxynaphthoate against Hookworm. Three Doses Each of 2 g. (Base) were Given on One Day

Sex	Age	Hookworm Eggs/ml. Faeces		
		Initial	Final	Reduction (%)
M	Adult	27,000	14,400	48
M	„	24,300	1,300	92
M	„	22,800	1,700	93
F	5	15,700	200	99
M	Adult	15,200	2,800	82
M	„	15,100	1,300	91
M	„	15,100	800	95
M	„	8,000	1,800	80
F	5	7,600	0	100
M	Adult	6,700	200	97
M	„	5,000	0	100
M	„	3,700	0	100
M	„	2,000	500	75
M	„	1,700	0	100
M	„	1,600	300	82
M	„	1,300	0	100
M	„	1,200	400	67
M	„	1,000	2,500	Failure
M	„	800	200	75
M	„	700	200	72
M	„	600	0	100
F	6	400	0	100
M	Adult	300	0	100
M	„	200	300	Failure
M	„	200	200	0
M	„	100	0	100
M	„	100	0	100

No. of patients treated .. 27
 „ in whom degree of infection was reduced .. 24 (89%)
 No. in whom infection was reduced by { 100-75% .. 21 (78%)
 { 74-25% .. 3 (11%)
 { 24-0% .. 0
 Initial weight of infection (eggs/g. faeces) .. 27,000-100
 Mean % reduction in count .. 79

TABLE III.—Effect of Multiple Doses of Bephenium Hydroxynaphthoate against Hookworm in Children. Doses of 2 g. (Base) were Given Daily for Four Days; Some Patients Received an Additional Course of Treatment for Seven Days

Sex	Age Years	Hookworm Eggs/ml. Faeces				
		Initial	After 4 Doses		After a Further 7 Doses	
			Final	Reduction (%)	Count	Total Reduction (%)
M	6	62,800	6,900	89		
F	10	50,000	800	98		
M	4½	14,800	0 conc.	100		
M	7	11,800	1,000	92		
M	6	9,000	+ direct (no count)	0 conc.	100	
F	7	8,400	200	98		
M	5	7,700	400	95		
F	7	6,400	900	86	0 conc.	100
M	7	5,000	900	82	300	94
F	6	4,700	2,800	40		
M	4	3,200	400	87	900	72
M	4	3,000	300	90		
M	6	2,800	1,900	32	900	68
F	4	2,400	0 conc.	100		
M	10	2,400	0	100		
M	8	1,600	400	75	300	82
F	4	1,200	200	83		
F	8	1,200	3,300	0	600	14
M	8	700	900	0		

No. of cases .. 18
 No. in which infection was reduced .. 7
 No. reduced by { 100-75% .. 16 (89%)
 { 74-25% .. 14 (78%)
 { 24-0% .. 2 (11%)
 Mean reduction in count .. 75%
 Total reduction in count .. 76%

"0 conc." indicates negative on concentration.

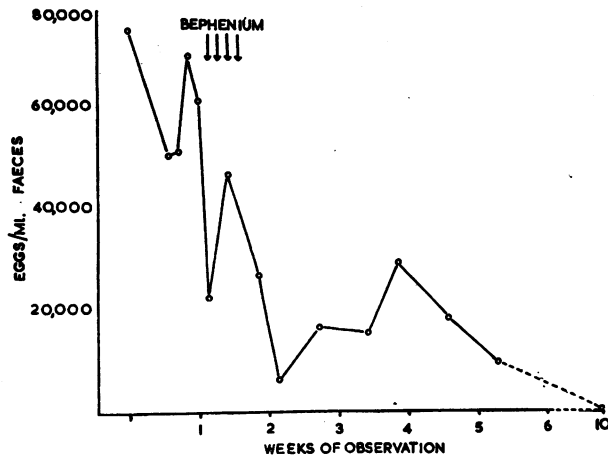


FIG. 2.—Effect of four doses each of 2 g. (base) of bephenium as hydroxynaphthoate on the faecal egg count. The drug was given to a child with heavy hookworm infection, advanced cirrhosis of the liver, and ascites.

families employed at the Sapumalkande rubber and tea estate near Dehiowita. The patients were divided into six treatment groups, which were given: (1) 3 g. of bephenium alone, as a suspension in water; (2) 3 g. of bephenium, as suspension, one hour after a dose of 1 oz. (30 g.) of magnesium sulphate in water; (3) 3 g. of bephenium, as suspension, together with the saline purge; (4) 3 g. of bephenium together with 1 fl. oz. (28 ml.) of piperazine citrate syrup; (5) 1 g. of bephenium, as suspension, daily for

majority of stools examined by direct smear were seen to contain numerous ova. No attempt was made to evaluate the number of roundworm ova per millilitre of faeces, and the effect of the drug was assessed by the presence or absence of eggs in direct smears at follow-up examinations.

Table V shows the results obtained against the intercurrent *Ascaris* infections in the patients treated for hookworm with bephenium. Whilst a marked effect was seen against roundworm, there was no significant action against whipworm following a single 3-g. dose of bephenium.

TABLE IV.—Effect of Bephenium Hydroxynaphthoate or Tetrachlorethylene against a Series of 132 Hookworm Infections in the Field

	Bephenium Hydroxynaphthoate*					T.C.E. (30 m. (1.8 ml.) with Purge)
	3 g.	3 g. Preceded by Purge	3 g. with Purge	3 g. with Piperazine 1 fl. oz. (28 ml.)	1 g. Daily for 4-5 Days	
No. of patients treated . . .	28	20	19	32	15	18
„ in whom degree of infection was reduced	24 (86%)	17 (85%)	15 (79%)	27 (84%)	14 (93%)	14 (78%)
No. in whom { 100-75% infection was { 74-25% reduced by { 24-0%	16 (57%) 7 (25%) 5 (18%)	10 (50%) 5 (25%) 5 (25%)	5 (26%) 6 (32%) 8 (42%)	20 (62%) 8 (25%) 4 (13%)	11 (74%) 2 (13%) 2 (13%)	10 (55%) 3 (17%) 5 (28%)
Initial weight of infection (eggs/ml.)	6,600- 100	22,300- 200	14,500- 200	11,500- 100	7,200- 400	11,900- 200
Mean reduction in count	66%	67%	41%	74%	72%	61%

* Dose in terms of base.

four or five days; (6) 30 minims (1.8 ml.) of tetrachlorethylene together with the saline purge. The results are shown in Table IV.

Bephenium Bromide

This salt has an extremely bitter taste, and was administered with difficulty because of its nauseating properties. Of the seven patients who were given 1.25 g. to 2.5 g. of the drug as tablets, two vomited almost immediately. Reduction of egg count in the stools was observed in only three of the patients, and the mean reduction for the series was 25%. The use of this salt was discontinued.

Mixtures of Bephenium Bromide and Hydroxynaphthoate

Small groups of patients were treated with doses of 2 to 2.5 g. of mixtures of bephenium bromide and hydroxynaphthoate in the proportions of 2:1, 1:1, and 1:2 respectively. The effectiveness of the dose was directly proportional to the amount of hydroxynaphthoate in the mixture. With a mixture containing 2 parts of bromide to 1 part of hydroxynaphthoate the mean reduction in egg count was 55% (4 cases); with equal proportions of salts this figure was 64% (5 cases); with a mixture of 1 part of bromide to 2 parts of hydroxynaphthoate the reduction was 70% (5 cases), a figure which is similar to that given by the hydroxynaphthoate alone. Mixtures containing the bromide caused nausea, and since they offered no advantage in effectiveness their use was discontinued.

Bephenium Embonate

Bephenium embonate was the least soluble of the compounds investigated. It was tested at doses of 0.75 to 5 g. in a series of 18 patients whose egg counts ranged from 83,800 to 200 eggs/ml. There was no correlation between anthelmintic effect and dose. Twelve of the patients (67%) showed a reduction of egg count, and the mean reduction for the series was 55%. The proportions of patients with reductions in egg counts of 100-75%, 74-25%, and 24-0% were 50%, 17%, and 33% respectively. These results compared unfavourably with those obtained with the hydroxynaphthoate.

Effect of Bephenium Hydroxynaphthoate Against Roundworm Infections

A high proportion of the persons at the Sapumalkande estate selected for treatment with bephenium for hookworm were intercurrently infected with roundworm and whipworm. Most of the roundworm infections were very heavy; the

Discussion

Tetrachlorethylene has been used for many years in the treatment of hookworm infection. It is cheap and effective and the toxicity is low. It is best administered in the morning, following a saline purge the night before. Two hours after the dose, a second purge is given to remove the tetrachlorethylene and thereby avoid absorption. This procedure rarely fails to remove most of the hookworms. For mass treatment in the field, in out-patient departments, and in crowded hospitals in the tropics with limited nursing staff, these optimal conditions for

treatment are often impracticable. Maplestone and Mukerji (1939) introduced a method in which the tetrachlorethylene is given together with the saline purge in a single dose; this is the method most widely used at present in Ceylon. Mass treatment for many years by this practical method has led to a considerable decrease in weight of infection in estate labour.

Tetrachlorethylene is unpleasant to take and often produces dizziness and nausea. These side-effects and the necessity for abstinence from food until the purge has taken effect usually involve absence from work on the day of treatment. In addition, roundworm infection is often

TABLE V.—Effect of Bephenium Hydroxynaphthoate or Tetrachlorethylene against a Series of 101 Cases of Ascariasis in the Field

Treatment (Dose in Terms of Base)	No. of Patients Positive on Direct Smear		
	Before Treatment	After Treatment	% Cleared
3 g. bephenium alone	22	5	77
3 „ „ preceded by saline purge	16	7	56
3 „ „ together with saline purge	13	6	54
3 „ „ 1 fl. oz. (28 ml.) piperazine citrate syrup	24	11	54
1 g. bephenium daily for 4 or 5 days	10	1	90
30 m. (1.8 ml.) tetrachlorethylene together with saline purge	16	12	25

found in association with hookworm. Tetrachlorethylene has little anthelmintic value against roundworms, although it stimulates them and may constitute a danger when massive infections are present.

In patients suffering from heavy hookworm infection with severe anaemia, malnutrition, and diarrhoea the condition of the circulation is sometimes such that it is inadvisable to endanger the precarious fluid balance by giving a saline purge. Treatment with tetrachlorethylene must be withheld until the condition of the patient can be improved. This is an important consideration in the treatment of children, and the small dose of tetrachlorethylene which can be given safely is often valueless for the expulsion of the hookworms (Table I). These characteristics of tetrachlorethylene have stimulated the search for new remedies. The results obtained in the present clinical trial with bephenium hydroxynaphthoate show that this drug overcomes many of the disadvantages associated with the use of tetrachlorethylene. However, in view of the bitter flavour of bephenium it is desirable to withhold treatment from patients with severe vomiting and dehydration until the fluid balance has been restored by intravenous infusion.

Table I shows that a single dose of 2 to 3 g. of bephenium hydroxynaphthoate was at least as effective as 30 to 40 min. (1.8 to 2.4 ml.) of tetrachlorethylene, the dose used for treatment in Ceylon hospitals. Adult male patients weighed about 100 lb. (45.4 kg.) and were given 40 min. (2.4 ml.); adult females weighed about 80 lb. (36.3 kg.) and were given 30 min. (1.8 ml.). The larger dose of 60 min. (3.6 ml.) recommended by Maplestone and Mukerji (1939) was not used because previous experience in Ceylon had shown that it often caused dizziness and other unpleasant side-effects. The children recorded in Table I were 5 to 6 years old and weighed between 17½ and 26 lb. (7.9 and 11.8 kg.). They were given 5 min. (0.3 ml.) of tetrachlorethylene, which was ineffective.

When three doses of bephenium hydroxynaphthoate, each containing 2 g. of base, were given on the same day to a group of 27 patients, the effect was similar to that produced by the single dose containing 2 to 3 g. when assessed by the mean percentage reduction in egg count. Some of the patients were children who were passing several fluid stools a day, and the three-dose treatment had a greater effect upon their hookworm infection and general condition than would have been expected from a single dose.

The results in Table III were obtained with 18 children who were also suffering from chronic diarrhoea. Most of the children had been given treatment with ineffective doses of tetrachlorethylene on several occasions. After four successive daily doses of 2 g. of bephenium hydroxynaphthoate, 16 (89%) showed considerable reduction in egg count. All the children were passing formed stools, and their general condition had much improved. Five of the seven children who remained in hospital showed a further reduction in egg count after an additional course of seven doses of 2 g. One child, who was still passing hookworm ova in his stools, subsequently had a recurrence of diarrhoea. This was controlled by a further course of treatment of three doses of 2 g. in one day, which reduced the egg count to insignificant proportions.

It is doubtful if the result in this group of children could have been achieved with any treatment other than bephenium. Multiple-dose treatment with bephenium would appear to be particularly advantageous in hookworm infection associated with intestinal hurry; it is reasonable to suppose that greater opportunity for contact of the drug with the worms is provided by a succession of doses. The effect of bephenium in controlling the diarrhoea of patients infected with hookworms is of special interest because the drug sometimes caused looseness of the bowel when given in equivalent doses to normal volunteers in England (Rogers, 1958).

Table IV shows that when a saline purge was given one hour before a dose of 3 g. of bephenium as hydroxynaphthoate the results obtained were the same as those after the drug was given alone. On the other hand, when the purge was given together with the bephenium the results were much less satisfactory. It is likely that the drug was removed from its site of action before it had exerted its full effect. It is possible that the purge induced conditions similar to those in children with diarrhoea (Table III) and reduced the opportunity for contact of the drug with the worms.

The results obtained when a dose of 1 g. of bephenium was given daily for four to five days were similar to those recorded for a single dose of 3 g.

In earlier experiments with *N. muris* in rats it was observed that piperazine exerted an effect antagonistic to the anthelmintic action of bephenium halides. It was not known whether this antagonism would occur in hookworm infections in man. In the group of patients treated with bephenium hydroxynaphthoate and piperazine (Table IV) it was clear that the activity of bephenium was unimpaired.

It has been shown (Table V) that bephenium hydroxynaphthoate has a marked effect against roundworms. No additive effect was observed when it was given together with piperazine.

For assessment of results in these trials one duplicate egg count was made before and after treatment. It was appreciated that daily egg counts in any individual would show a marked variation, and that comparison of counts at two points several days apart might be suspect. In trials of drugs of doubtful efficacy against hookworm the arbitrary selection of two points in the daily variation sequence may lead to erroneous conclusions (Goodwin and Standen, 1958). This would be true if results of treatment related to single or small numbers of cases, but where overall reduction can be achieved in a series of reasonable length the anthelmintic effect becomes apparent.

Our results show quite clearly that bephenium hydroxynaphthoate is highly effective in the treatment of hookworm infections. This is most apparent in those cases where very heavy infections were cured, or the egg count was reduced to insignificant proportions. However, the effect of drug treatment is not shown so satisfactorily in cases where the infection is light and where only partial reduction in egg count has been obtained. Daily variation apart, this is largely due to the fact that any dilution egg-count technique is insufficiently sensitive to provide accurate information where the number of eggs in the faecal suspension is small. It has been shown by Beaver (1955) that 200-500 ova per ml. of faeces may be produced by not more than two female *N. americanus*. When such small numbers of worms are present the opportunity for variation in daily egg count is much increased. In these instances the application of a multiplication factor of 100 or 200 to the number of eggs seen in a small measured volume affords too wide a margin of error for accurate conclusions to be drawn.

Summary

Bephenium, a new anthelmintic, was compared with tetrachlorethylene in the treatment of 284 cases of hookworm infection in Ceylon.

Bephenium hydroxynaphthoate was selected as the most suitable salt. A single dose of 2 or 3 g. of base, or multiple doses given on successive days or three times on the same day, compared favourably in effectiveness with tetrachlorethylene.

No toxic side-effects were observed when large doses of bephenium were given.

Bephenium hydroxynaphthoate was particularly suitable for the treatment of patients with advanced anaemia, diarrhoea, and heavy hookworm infections because of its low toxicity and because no purge was necessary.

Bephenium hydroxynaphthoate was also effective against roundworm, which was present as a concurrent infection in 85 of the patients treated with this drug. Tetrachlorethylene was ineffective against roundworm in 16 patients treated.

We are grateful to Dr. D. L. J. Kahawita, Director of Health Services, Dr. R. D. de A. Seneviratne, Director of the Medical Research Institute, Dr. Stanley de Silva, and Dr. C. Ratnavel for the facilities which were provided for this work in Ceylon. We are also grateful to the physicians who co-operated in the investigation, and permitted us to treat their patients, particularly Dr. R. P. Jayewardene, Professor C. C. de Silva, and Dr. A. F. S. Perera, of the Colombo hospitals, and Dr. G. Chinniah, of the hospital at Negombo. Work on the Sapumalkande Estate was greatly assisted by the co-operation of Dr. O. J. S. MacDonald and Dr. N. J. Nipps, of the Planters' Association Health Scheme. We are indebted to the staff of the Medical Research Institute, especially to Mr. Y. Wijayarajam, for technical assistance, to the nursing staff at the hospitals, and to the matron at Ragama Convalescent Home for their valuable help.

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EXCRETION OF BEPHENIUM SALTS IN URINE OF HUMAN VOLUNTEERS

BY

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Goodwin, Jayewardene, and Standen (1958) have shown that bephenium salts were effective for the treatment of hookworm in man. Of the various compounds used for clinical trials in Ceylon, bephenium hydroxynaphthoate ("alco-par") was the most active, the embonate and bromide being considered unsatisfactory for clinical use. No toxic side-effects were observed when large doses of the hydroxynaphthoate were given. The present paper describes the urinary excretion rates of bephenium salts in volunteers in Britain.

Determination of Bephenium in Urine

The concentration of bephenium in urine was measured by means of a modification of the methyl orange dye-lake procedure. This method was supplied by Dr. S. R. M. Bushby, of the Wellcome Research Laboratories, Beckenham.

Reagents.—*Ethylene dichloride*:—the solvent was shaken with activated carbon, filtered, washed with 1/5 of its volume of N/1 NaOH and then washed twice with an equal volume of distilled water; it was then filtered to remove residual water. *Methyl orange solution*:—a saturated solution of methyl orange was prepared in 0.5 M boric acid, and washed with an equal volume of ethylene dichloride. *Acid alcohol*:—2 ml. of concentrated HCl was added to 100 ml. of absolute ethanol. *Solid sodium chloride*. 40% w/v *Sodium hydroxide solution*. Glassware was washed in very dilute detergent, well rinsed, washed in dilute HCl, and then in distilled water. Acetone was not used for drying glassware. Failure to observe these precautions resulted in high "blank" values.

Procedure.—1 ml. of urine was pipetted into a tube and 300 mg. of solid sodium chloride was added. The tube was shaken to saturate the solution and 8 ml. of ethylene dichloride was added. The tube was stoppered, the contents were shaken for 10 minutes and then centrifuged; 6 ml. of the lower (solvent) layer was removed into a clean tube. 0.4 ml. of the methyl orange reagent was added and the tube shaken thoroughly. The excess of methyl orange solution was removed with a Pasteur pipette after centrifuging; 5 ml. of 40% sodium hydroxide was added and mixed by a brief shaking. The tube was centrifuged and 4 ml. of the upper (solvent) layer was removed into a clean tube. 0.4 ml. acid alcohol was added and the absorption was measured in an absorptiometer at 540 m μ . "Blanks" were prepared, using water instead of urine, and treated in the same way. A standard curve was prepared with solutions of bephenium hydroxynaphthoate of known strength, and the basic substances excreted in the urine were expressed as bephenium.

Excretion of Bephenium after Oral Administration in Man

The preparations used were: bephenium bromide as compressed tablets each containing 500 mg. of base, and

bephenium embonate and hydroxynaphthoate in the form of sweetened and flavoured emulsions containing 500 mg. of base per fl. dr. (140 mg. per ml.).

The volunteers were healthy normal adults. For each excretion study the subject was given the dose orally, followed by 100 ml. of diluted orange squash. Urine was collected at each emptying of the bladder, and the volume and time were noted. The urines were refrigerated until used for analysis; no preservatives were added. The Table shows the percentage of the dose recovered.

Figs. 1 and 2 show the cumulative excretion rates of the drug and its metabolites after doses of bephenium bromide, embonate, and hydroxynaphthoate. Fig. 3 shows the excretion rates of bephenium hydroxynaphthoate after the administration of 2.5 g. base daily on successive days. It will be seen that the excretion of bephenium and its

Dosage and Percentage Excretion of Bephenium in Human Volunteers

Sex	Age	Compound	Dose in g. Base	% of Dose Recovered in 24 Hours
M	29	Bromide	1	0.79
M	25	"	1	0.72
M	51	"	1	0.59
F	19	Embonate	1	0.04
M	19	"	1	0.06
M	26	"	3	0.05
M	27	"	3	0.06
M	40	"	3	0.06
M	32	Hydroxynaphthoate	1	0.52
F	25	"	1	0.25
M	43	"	3	0.18
M	38	"	3	0.22
M	39	"	3 doses of 2 g. in one day	0.44
M	36	"	" "	0.21
M	28	"	" "	0.23
M	32	"	2.5 g. daily for 4 successive days	1st day 0.05 2nd " 0.12 3rd " 0.10 4th " 0.26
M	46	"	" "	1st " 0.14 2nd " 0.27 3rd " 0.19 4th " 0.23
M	44	"	2.5 g. daily for 3 successive days	1st " 0.05 2nd " 0.14 3rd " 0.06

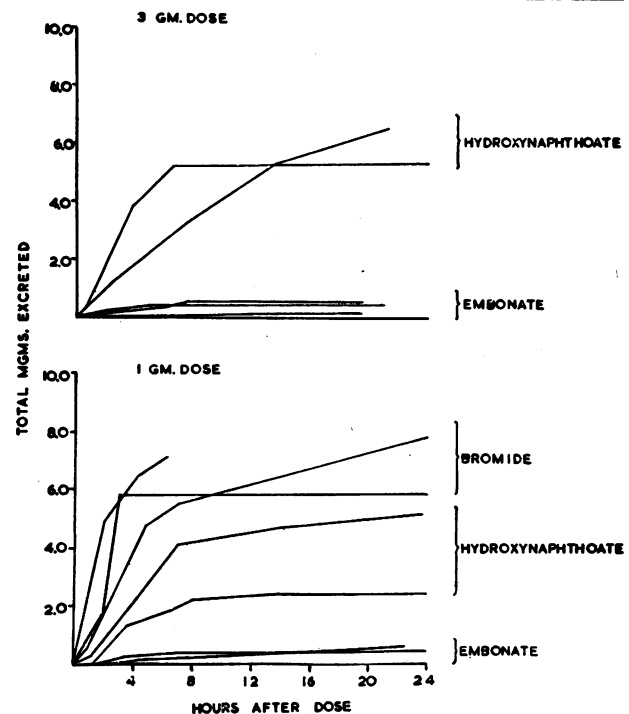


FIG. 1.—Cumulative excretion rates of bephenium bromide, embonate, and hydroxynaphthoate in human volunteers after a single dose.