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EXCRETION OF BEPHENIUM SALTS IN URINE OF HUMAN VOLUNTEERS

BY

E. W. ROGERS

Wellcome Laboratories of Tropical Medicine, London

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 ("alcopar") 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 mμ. "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

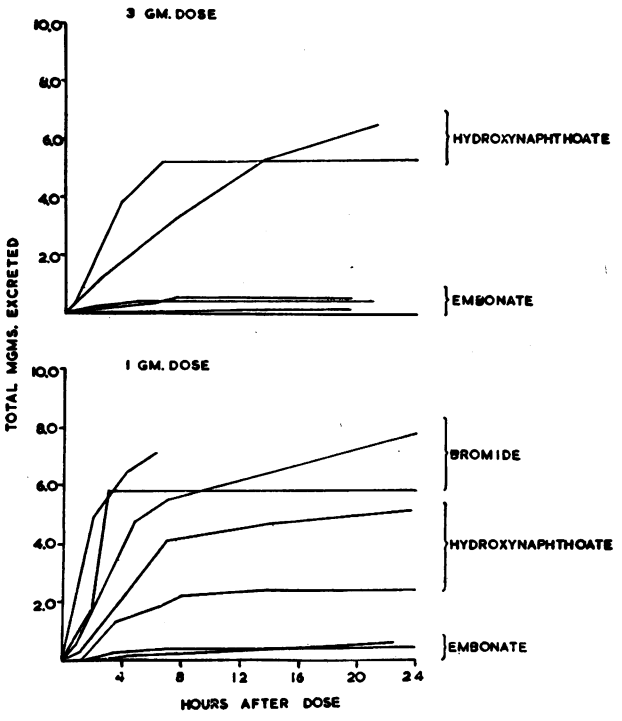

FIG. 1.—Cumulative excretion rates of bephenium bromide, embonate, and hydroxynaphthoate in human volunteers after a single dose.

metabolites in man is low. The bromide, which is soluble to the extent of 30% in water, was excreted in larger amounts than the less soluble hydroxynaphthoate. The almost insoluble embonate was excreted in still smaller amounts.

As found by Goodwin *et al.* (1958) in Ceylon, the bromide gave rise to nausea and vomiting. Side-effects from the hydroxynaphthoate and embonate were slight; mild diarrhoea sometimes occurred after a single dose, and some volunteers complained of borborygmi. The same effects occurred daily after repeated doses of hydroxynaphthoate. Some subjects did not complain at all, and the intensity of side-effects was not correlated with the amount of drug excreted in the urine. Side-effects ceased completely 24 hours after the dose. It is interesting to note that no side-effects were observed after treatment with embonate or hydroxynaphthoate in Ceylon, where many of the subjects were already suffering from gastro-enteritis. This might perhaps be explained by dietary differences; the digestive tracts of those accustomed to hot curries may well be less sensitive than those of subjects who partake of less-stimulating foods. It is also of interest that no such side-effects were encountered among mentally defective children in an institution in Britain where the drug was tested for its action on whipworm infection.

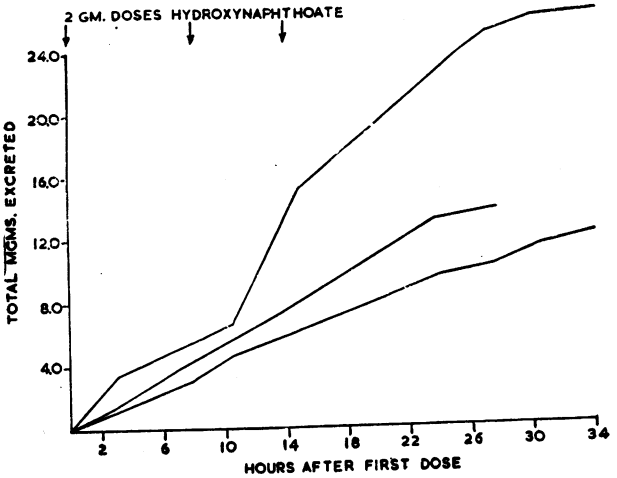

FIG. 2.—Cumulative excretion rates of bephenium hydroxynaphthoate in human volunteers after three single doses in one day.

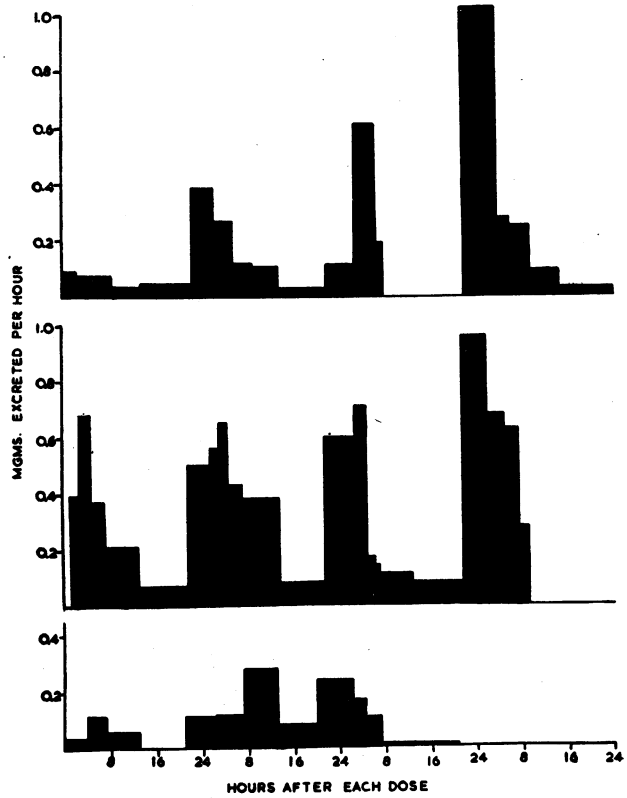

FIG. 3.—Hourly excretion rates of bephenium hydroxynaphthoate in human volunteers after successive daily doses.

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Medical Memoranda

Thrombocytopenic Purpura Apparently Due to Tablets Containing Salicylamide and Mephenesin ("Arthripax")

A woman aged 64 was seen by me on October 16, 1958, suffering from widespread purpura with subcutaneous ecchymoses and haemorrhages into the tongue. The rash had begun to appear ten days before and had gradually increased in severity. On inquiry it was found that she had been taking two tablets of arthripax three times daily for about three weeks on account of a painful arthritis of the left knee.

Apart from the haemorrhages physical examination was negative, and there was no previous history of important illness. The blood count was as follows: total red cells 2,977,000 per c.mm.; haemoglobin, 56% (8.3 g./100 ml.): colour index, 0.94; total white cells per c.mm., 6,900. Differential count: polymorphonuclears, 63.5%; lymphocytes, 30.0%; large mononuclears, 6.5%; eosinophils, nil: basophils, nil; aniso- and poikilocytosis, slight; nucleated red cells, nil; platelets, less than 10,000 per c.mm.

The patient, who was ambulant, was told to stop taking the tablets and sent home to rest. When the result of the blood count was received a few hours later her doctor was warned by telephone to report at once if her condition deteriorated, so that she could be admitted immediately. In fact the patient made an uninterrupted recovery; no new haemorrhages appeared and the existing ones gradually resolved.

On October 30 she was well except for some breathlessness on exertion. The blood count then was: total red cells, 2,730,000 per c.mm.; haemoglobin, 58% (8.6 g./100 ml.); colour index, 1.04; total white cells, 6,300 per c.mm. Differential count: polymorphonuclears, 61%; lymphocytes, 29%; large mononuclears, 10%; eosinophils, nil; basophils, nil; aniso- and poikilocytosis, fairly marked; nucleated red cells, nil; platelets, 173,000 per c.mm.

On November 28 the blood count was as follows: total red cells 3,740,000 per c.mm.; haemoglobin 70% (10.4 g./100 ml.); colour index, 0.94; total white cells 5,900 per c.mm. Differential count: polymorphonuclears, 70.0%; lymphocytes, 23.5%; large mononuclears, 5.0%; eosinophils, 1.5%; basophils, nil; aniso- and poikilocytosis, nil; nucleated red cells, nil; platelets, 179,000 per c.mm.

Arthripax tablets contain salicylamide 5 gr. (0.32 g.), mephenesin 1 gr. (65 mg.), aluminium glycinate 1½ gr. (0.1 g.). The dose of both drugs is well within that which is considered to be safe, and the makers tell me that they have not heard of similar cases.

The question arises whether mephenesin or salicylamide was responsible, it being assumed that the patient had some idiosyncrasy. Both drugs contain a benzene ring. An orthopaedic colleague has told me of a case of purpura following mephenesin administration some years ago, but