Brit. J. Pharmacol. (1960), **15**, 260.

THE EXCRETION AND STABILITY TO METABOLISM OF BRETYLIUM

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(RECEIVED DECEMBER 18, 1959)

Bretylium (*o*-bromobenzylethyldimethylammonium) is a new type of hypotensive drug. It was estimated in extracts of human urine as the methyl orange complex. From 7 to 45% of a single oral dose was found in human urine within 9 hr. The [^{14}C] labelled drug was used to investigate excretion by cats. A minor proportion of a subcutaneous dose was eliminated by cats in the faeces, probably after secretion into the bile. Most of the dose was excreted unchanged in the urine. No products of metabolism were found in either human or cat urine. The drug suffered negligible change when incubated with rat liver tissue *in vitro*.

Bretylium tosylate (Darenthin ; *o*-bromobenzyl-ethyldimethylammonium toluene - *p* - sulphonate) is a quaternary salt described by Boura, Copp, and Green (1959) that selectively impairs the function of adrenergic neurones and lowers the blood pressure of hypertensive patients (Boura, Green, McCoubrey, Laurence, Moulton, and Rosenheim, 1959). It is notable for its freedom from central effects and for lack of actions on the parasympathetic nerves which mar the use of ganglion blocking agents. For a quaternary salt it is fairly well absorbed as judged by its effects in both man and animals. By the same criterion its absorption by any one patient was found to be fairly constant, but there was considerable variation in the effective dose needed by different patients. This paper gives information on the excretion of the drug and its stability to metabolism.

METHODS

Assay of Bretylium in Human Urine.—A satisfactory specific method of chemical assay was not devised ; bretylium is stable to chemical reagents under mild conditions and lacks physical properties suitable for assay purposes. Moreover, the drug is very hydrophilic, its reineckate rather soluble (about 20 mg./l.) and continuous extraction very slow. The following two methods, based on the well-known association of bases with sulphonic acid dyes, were intended to provide approximate values for assessing the excretion of the drug in human urine.

A preliminary estimate was gained by chromatography of 100 μl. portions of urine in the organic phase of n-butanol/acetic acid/water (4:1:5). The size of the spot revealed at Rf 0.75 by spraying with Dragendorff's reagent was compared with standard spots of 2 to 10 μg. of the cation. Rf values in other solvent mixtures were 0.75 in s-butanol/acetic acid/water (12:5:3) and 0.67 in n-butanol/pyridine/water (1:1:1).

Method A.—The sample was diluted with an equal volume of ethanol and filtered. An aliquot of the filtrate, usually 2 ml., was diluted with 5 vol. of 0.05M phosphate buffer, *p*H 7, and the solution passed down a column (10 × 1 cm.) of Amberlite IRC-50 resin buffered at *p*H 7. The column was washed to *p*H 5 by 300 ml. 0.05M phthalate buffer, and the eluate, containing interfering bases, rejected. Bases retained on the column were eluted by 150 ml. warm 0.5N hydrochloric acid. The eluate was neutralized, evaporated under reduced pressure, and extracted with 3 × 20 ml. portions of ethanol. Ethanolic methyl orange (0.05%, 3 ml.) was added, and the solution dried under reduced pressure. The base-dye complexes were extracted with several portions of warm ethylene dichloride until the washings were colourless. The extract was adjusted to volume, usually 25 ml., and the red colour produced by dilution with an equal volume of ethanolic hydrochloric acid (1%) was measured at 525 mμ.

Recovery of 1 mg. amounts of bretylium iodide from normotensive urine averaged 98 ± 11% in four trials. A major source of error arose from colloidal methyl orange carried over in the ethylene dichloride if evaporation of water was not sufficiently thorough. The lengthy procedure could be shortened for urines containing more than 10 mg. of drug/100 ml. (as shown by chromatography). The neutralized acid eluate from the resin was evaporated to a few ml. and 1 ml. 0.5N sulphuric acid and 5 ml. sodium

phosphate (5%) added. A blue complex was formed after shaking with 10 ml. of a cobalt thiocyanate reagent (Ashbrook, 1959); this complex was extracted by thorough shaking with 10 ml. ethylene dichloride. Emulsions were troublesome. The blue colour was read at 620 mμ.

Method B.—In single-dose experiments, blank values were obtainable from urine specimens collected from the patients at the beginning and end of the experiment and it was convenient to obtain a result rapidly by distilling 1 to 2 ml. of the sample with 3 ml. ethanolic methyl orange (0.05%) and 50 ml. ethylene dichloride, until the distilling temperature had reached 85°, adding more solvent if necessary. The solution in the flask was cooled, filtered, adjusted to 25 ml. and read as in Method A. Recovery of 1 mg. amounts of drug from normotensive urine averaged 92±13% in three trials.

Assay of [^{14}C]-labelled Bretylium. — Urines in appropriate amounts were dried on lens paper on planchettes and counted under an end window counter. The results were referred to standards prepared by adding known amounts of labelled drug to similar urines. Faeces were digested with hot 2N hydrochloric acid, cooled and filtered. The volume was adjusted to 100 ml. and aliquots counted as described for urine. Similar methods were used in some experiments with tissues *in vitro*, but where greater accuracy was desired or where the level of radioactivity was low, the sample was dried and combusted in oxygen, with added glucose if necessary, and the carbon dioxide was counted as gas (Glascock, 1954).

Identity of the Excreted Material.—The mobilities of spots revealed by Dragendorff's reagent and autoradiography of paper chromatograms of whole urines were compared in the solvent systems mentioned above with authentic material dissolved in the same samples.

Attempts to isolate pure specimens of bretylium iodide or bromide after precipitation of urinary bases as the reineckates were unsuccessful, and an isotope dilution method was applied. A cat received 22 mg. of [^{14}C]-labelled bretylium iodide subcutaneously. The 1 to 2 and 3 to 5 day urines were collected separately, made up to volume, and aliquots retained for [^{14}C] assay. Carrier bretylium bromide (400 mg.) was added to each sample followed by evaporation to dryness and extraction with ethanol. A portion of each extract was retained for [^{14}C] assay before evaporation and precipitation of the reineckates. These were crystallized once from dilute acetone, and the bases were liberated by silver sulphate. The bases were chromatographed on Whatman 3 MM paper in n-butanol/acetic acid/water. The region indicated on small strips sprayed with Dragendorff's reagent was eluted with water and the eluate evaporated. Bretylium was finally isolated as the reineckate for analysis of [^{14}C] by combustion to carbon dioxide and counting as gas. The reineckates were crystallized from dilute acetone, m.p. 175–178° (decomp.). (Found: C, 32.0; H, 4.0; N, 17.5. $C_{15}H_{23}N_7S_4CrBr$ requires C, 32.1; H, 4.1; N, 17.5%).

Search for Possible Products of Metabolism of Bretylium

Carbon Dioxide.—A rat (100 g. wt.) received 1 mg. of the labelled drug subcutaneously. It was kept in a metabolism chamber in a slow stream of air, and the issuing gas was scrubbed in two towers containing glass beads moistened with 5N potassium hydroxide. Trapped carbon dioxide was liberated into a vacuum line and counted as gas.

Bromide Ion.—A portion of a 24 hr. specimen of urine from a patient who had received 2,250 mg. of bretylium tosylate the day before was examined for bromide ion by the method of Belote (1927).

o-Bromobenzoic Acid.—A 24-hr. specimen of urine from a patient who was receiving 900 mg. of bretylium iodide daily was evaporated to a syrup and hydrolysed by concentrated hydrochloric acid for 5 hr. at 100°. The solution was diluted to 250 ml. and extracted with ether. Organic acids were dissolved out by alkali and transferred back to ether. Removal of the solvent gave 450 mg. of dark material which yielded 350 mg. of white crystals, m.p. 153 to 156°, to hot ligroin. Neither these nor the crude material contained bromine. The crystals were identified by conventional methods as salicylic acid.

Experiments with Liver Tissue in vitro.—Rat liver slices were incubated for 2 to 3 hr. in Krebs-Ringer glucose phosphate saline containing [^{14}C]-labelled bretylium iodide (10^{-3} or 2×10^{-4}M). The rate of oxygen uptake, 46 μ-moles/g. tissue/hr., did not differ from similar experiments in which bretylium was omitted. Evolved carbon dioxide was recovered either from the pooled filter papers containing potassium hydroxide from the centre wells of manometric vessels or from baryta used to wash the issuing gas from aerated mixtures. Trichloroacetic acid extracts of homogenized incubation mixtures were chromatographed in n-butanol/acetic acid/water for autoradiography. The spots on chromatograms, corresponding to controls where the drug was added after completion of incubation, were excised and counted directly on the paper by a scintillation technique (Roucayrol, Oberhauser, and Schussler, 1957) using a non-volatile scintillator (Buck and Swank, 1958).

Liver homogenates were incubated in the fortified medium containing semicarbazide to trap formaldehyde as described by Axelrod (1956). Any formaldehyde produced during metabolism was distilled into phenylsemicarbazide solution for combustion and counting.

RESULTS

Excretion by Cats.—Estimation of the radioactivity in cat urine after subcutaneous doses of [^{14}C]-labelled bretylium iodide (10 mg./kg.) showed

TABLE I

EXCRETION OF [^{14}C]-LABELLED BRETYLIUM IN CATS

Cats were injected subcutaneously with bretylium tosylate 10 mg./kg. Figures are % of dose excreted. Serial estimations in single cats except the first row of urine figures and the bile figures.

	Hours			Days						
	1	12	18	1	2	3	4	5	6	7
Urine	15	30	74	—	—	—	—	—	—	—
,, ...	—	—	—	46·5	10·2	5·7	4·2	1·3	1·7	1·0
,, ...	—	—	44·7	—	14·1	4·7	—	—	—	—
Faeces	—	—	0	0·5	5·5	6·4	0·2	—	—	—
,, ...	—	—	—	—	1·5	—	—	5	—	—
Bile	1·3	0·5	0·1	—	—	0·006	—	—	—	—

that excretion began within 1 hr. and that about half the dose was excreted by this route within 24 hr. (Table I). In one cat, 83% of the dose was accounted for in urine and faeces within 7 days, though detectable radioactivity was still present in urine on the seventh day. The drug was secreted into bile and this may account for the minor amounts found in the faeces after the subcutaneous dose. In another experiment, in which 50 mg./kg. of bretylium iodide was given subcutaneously, 12.7 mg. was found in the bile after 18 hr.

Excretion in Human Urine.—Because of the unsatisfactory nature of the assay used, the results shown in Tables III and IV can only be regarded as approximations. The standard deviations in recovery experiments from normotensive urines were rather high, but the greatest source of error arose from the high blanks given by urines from hypertensive subjects. Normotensive subjects appeared to excrete less basic material, and their urines by Method B gave values that ranged from the equivalent of 1.4 to 8.3 μg./ml. bretylium tosylate (mean of 6 experiments = 4.1 μg./ml.). However, one further specimen of urine from a normotensive subject gave a value equivalent to 167 μg./ml. bretylium tosylate. Urines from 4 hypertensive subjects gave values equivalent to 50, 158, 214, and 368 μg./ml. These high values could not be traced to previous drug regimens. The resin treatment of Method A reduced these values to an equivalent of between 8 and 32 μg./ml. bretylium tosylate.

Table II gives a comparison of the values obtained by three variants of the methyl orange method on the same series of specimens. The third set of figures refers to the application of Method B to the eluate after an initial purification by chromatography.

The figure for the second day by Method A appears to be high, and recovery of small amounts of drug from paper was unsatisfactory, otherwise there was reasonable agreement considering the very high blank values given by this patient. High blank values were also found in a specimen from the same patient before bretylium treatment. The result suggests that about one third of the total dose was excreted in urine by this patient.

TABLE II

COMPARISON OF METHODS FOR ESTIMATING BRETYLIUM IN URINE

24 hr. urine specimens were collected on 5 successive days from a patient. During the first 2 days the patient received 900 mg. bretylium iodide and for the last 3 days a placebo only. The specimens were assayed by 3 different methods. Each figure represents the total 24 hr. excretion in mg. of total bases or bretylium iodide, and is the mean of two determinations. Total bases were calculated in terms of bretylium iodide. The mean of days 4 and 5 was taken as the blank for calculating the amount of bretylium excreted.

Day No.	Method A		Method B		Method B with Chromatography	
	Total Bases	Bretylium Iodide	Total Bases	Bretylium Iodide	Total Bases	Bretylium Iodide
1	980	505	595	496	448	424
2	707	232	216	117	127	103
3	536	61	168	69	20	0
4	456	—	106	—	24	—
5	494	—	92	—	—	—
Total		798		682		527

Table III shows that excretion of single oral doses reached a peak at 2 to 4 hr. after the dose, and that excretion had virtually ceased after 9 hr. The degree of absorption by different patients was variable. In the one instance studied (Table III), total absorption did not appear to be influenced by a heavy meal though the scatter of the figures after a meal contrasts with the smooth rise and fall seen in the same patient when fasting. Table IV shows excretion of total bases while on continued dosage with bretylium; it indicates that excretion became appreciable only when the total daily dose had exceeded 1 g. The very high value for the 15th day (confirmed by chromatography) shows that sudden absorption may occur for no apparent reason.

Metabolism in Vivo.—Chromatograms and autoradiograms of urine and bile from cats that had received the labelled drug showed spots with the same mobilities as bretylium. Trichloroacetic acid extracts of heart, spleen, lung, and superior cervical ganglion also contained unchanged drug. Examination of these chromatograms, and numerous chromatograms of whole human urine, by ultraviolet light and conventional spray reagents, failed to disclose anything that could be interpreted as a metabolic product of the drug.

A rat that had received labelled bretylium produced radioactive carbon dioxide equivalent to 0.03% of the dose during 6 hr. but none in the next 17 hr. No bromide ion was found in the human urine examined.

An isotope dilution method failed to disclose the presence of metabolic products in the urine of a cat. The reineckate was used as a convenient means of purifying the bretylium. The dilution factor calculated from estimation of [^{14}C] in the purified reineckate did not exceed that calculated from estimation of total [^{14}C] in an alcoholic extract of the urine. For a 1 to 2 days sample, the reineckate and alcohol extract factors were 113 and 116 respectively, and for the 3 to 5 days samples, 137 and 163.

Metabolism in Vitro.—Rat liver slices suspended in saline containing labelled bretylium evolved small amounts of labelled carbon dioxide at a rate of 0.15% added drug/g. tissue/hr. The result parallels the slow rate of evolution of carbon dioxide by the rat after dosage with the labelled drug. Attempts to detect formaldehyde such as could arise by *N*-demethylation were negative. Recovery of labelled drug after incubation with liver tissue was assessed by chromatography of protein-free extracts and

TABLE III

URINARY EXCRETION OF BRETYLIUM DURING A 24 HR. PERIOD

Patients were given oral doses of bretylium at 9 a.m. Thereafter urine samples were collected at intervals and assayed for total bases. Values represent mg./hr. of total bases in terms of bretylium. The % excretion was calculated as the total bases excreted from 9 a.m. to 6 a.m., less the mean of the 6 a.m. to 9 a.m. and 6 p.m. to 5 a.m. values. I=bretylium iodide, T*=bretylium tosylate with a heavy meal. T†=bretylium tosylate in the same patient while fasting.

	Method B			Method A	
Dose:	500 mg. I	300 mg. I	500 mg. I	500 mg. T*	500 mg. T†
Time	mg./hr.	mg./hr.	mg./hr.	mg./hr.	mg./hr.
6 a.m.– 9 a.m. ..	1·2	5·5	2·5	8·8	2·4
9 ,, 10 ,,	41·3	7·3	12·0	10·6	3·8
10 ,, 11 ,,				5·5	6·2
11 ,, –12 noon	41·5			26·4	9·8
12 noon–1 p.m.		21·3	7·3	3·5	7·3
1 p.m.– 3 ,, ..	24·3				
3 ,, – 6 ,, ..	6·8	13·3	9·0	8·0	5·3
6 ,, – 5 a.m. ..	1·3	2·4	3·3	1·0	1·9
% of dose excreted	45%	30%	12%	7%	8%

TABLE IV

URINARY EXCRETION OF TOTAL BASES BY TWO PATIENTS ON CONTINUED DOSAGE WITH BRETYLIUM TOSYLATE

The total bases were assayed by Method A and expressed in terms of bretylium tosylate, mg./24 hr.

Day	Dose	Total Bases	Dose	Total Bases
1	—	12·6	—	—
2	—	10·3	—	20·5
3	375	15·4	—	—
6	750	19·5	875	30·6
7	1,000	13·2	1,125	40·0
8	1,375	36·8	1,125	34·9
9	1,750	43·6	1,500	33·2
14	2,250	49·2	—	—
15	2,250	285·0	—	—
16	2,250	61·5	—	—
17	2,250	40·0	—	25·6
18	2,250	61·5	—	8·3
20	2,250	79·2	—	7·1

counting the appropriate areas of paper directly. The chromatograms from incubated mixtures gave $1,690 \pm 265$ counts/min., those from mixtures where labelled bretylium was added after incubation $1,600 \pm 160$ counts/min. (means of 6 results).

Discussion

No evidence was obtained to suggest that bretylium is metabolized in the body to any marked extent, though evolution of labelled carbon dioxide by rats and from liver incubated with the drug indicates that it is not completely immune from metabolic attack. Complete oxidation or extensive debromination by a dehalogenase would have been revealed by presence of bromide ion in urine. The evidence from chromatography and autoradiography can only apply to products with the quaternary group intact, but removal of this group would be expected to give a benzoic acid. The salicylic acid found in one urine almost certainly arose from ingested acetylsalicylic acid. Metabolites that retain the quaternary nitrogen such as a hypothetical mercapturic acid seem to be unlikely since isotope dilution give no hint of other products. Substituted bromobenzenes do not give rise to mercapturic acids (Williams, 1947).

Compared with many other quaternary salts, bretylium is absorbed fairly readily from the alimentary tract. In unpublished work it has been shown to be absorbed from the intestine and not from the stomach, but the mechanism is obscure. Current theories on drug absorption, that assume solution of unionized bases in lipid, are difficult to apply to quaternary salts, unless they form an unionized complex analogous to the complexes with sulphonic acid dyes, with some constituent of the intestine.

The authors gratefully acknowledge assistance with animal experiments by Mr. A. L. A. Boura, the provision of urine specimens by Dr. D. R. Laurence and Dr. R. Moulton, of University College Hospital Medical School, analyses by Mr. P. R. W. Baker, and technical assistance by Mr. S. W. Stock, Mr. A. E. Lakeman, and Mr. D. W. Richardson.

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