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GLC Determination and Urinary Recovery of Bumetanide in Healthy Volunteers

P. W. FEIT▲, K. ROHOLT, and H. SØRENSEN

Abstract □ A GLC determination of bumetanide (3-*n*-butylamino-4-phenoxy-5-sulfamylbenzoic acid), a potent "high ceiling" diuretic, was developed using flash-heater methylation. A mixture of tetramethylammonium hydroxide and trimethylanilinium hydroxide was advantageously used as the methylation reagent. The flash-heater methylation product proved to be methyl 3-(*N*-*n*-butylanilino)-5-dimethylsulfamyl-4-methoxybenzoate by spectroscopic comparison with authentic material achieved by a corresponding gram scale model experiment. The probable sequence of reactions involving methylation under simultaneous Smiles rearrangement is outlined. The GLC determination was found to be accurate at concentrations as low as 0.1 mcg./ml. human urine. In six healthy volunteers, the urinary recovery of bumetanide, the urinary excretion of sodium, potassium, and chloride ions, and the urine volume were determined after oral administration of 0.5 and 1 mg. of the drug. A parallelism between bumetanide excretion and saluretic action over the total period of response is shown.

Keyphrases □ Bumetanide—urinary excretion, man, relationship to saluretic action, GLC analysis after flash-heater methylation □ Urinary excretion, bumetanide—relationship to saluretic action, man □ Saluretic activity, bumetanide—relationship to urinary excretion, man □ GLC—analysis, bumetanide, after flash-heater methylation

Bumetanide (3-*n*-butylamino-4-phenoxy-5-sulfamylbenzoic acid) (I, Scheme I) was recently described as a new "high ceiling" diuretic in the experimental animal (1, 2) and in man (3, 4). In patients suffering from congestive heart failure, bumetanide has shown a diuretic profile and dose response comparable to that of furosemide, but at doses approximately one-fortieth of the latter drug (4). The aim of the present investigation was to develop a sensitive GLC assay for the determination of bumetanide in biological material. Special interests were to evaluate the urinary recovery after oral administration of the drug to healthy volunteers and to obtain information relating the renal drug excretion with the diuretic–saluretic activity.

Since functional groups of bumetanide make the drug unsuitable for direct GLC determination, it was necessary to find a suitable derivative. A relatively simple way of making derivatives of anionic compounds is by methylation in the injection port of the gas chromatograph, using a solution of tetramethylammonium hydroxide or trimethylanilinium hydroxide as the methylation reagent. The principle of flash-heater methylation was first utilized in preparation of methyl esters of carboxylic acid (5) with the aid of tetramethylammonium hydroxide. The advantage of using trimethylanilinium hydroxide was recently shown for barbiturates, phenolic alkaloids, and xanthine bases (6).

EXPERIMENTAL

GLC Determination—*Instruments and Conditions*—The GLC analyses were performed using a gas chromatograph[1] equipped with a flame-ionization detector and the following experimental conditions: column, 2 m. × 3.3-mm. o.d., stainless steel; packing, 1.5% OV-17 silicone[2] on 100-120-mesh diatomaceous earth[3]; column temperature, 270°; injection port temperature, 370°; detector temperature, 300°; carrier nitrogen flow, 30 ml./min.; and recorder chart speed, 0.3 cm. (0.1 in.)/min.

Reagents—For the methylation reagent, two parts of an approximately 1 *M* aqueous solution of trimethylanilinium hydroxide (6) were mixed with one part of a 10% methanolic solution of tetramethylammonium hydroxide. The latter was obtained by evaporating an aqueous tetramethylammonium hydroxide solution[4] in a rotating evaporator and redissolving the residue in the appropriate amount of methanol.

The internal reference solution consisted of 25 mcg. of 4-benzyl-3-*n*-butylamino-5-sulfamylbenzoic acid (7)/ml. in ether.

Procedure—Urine, 5–10 ml., was adjusted to pH 2 by addition of 1 *N* hydrochloric acid. Then 200 μl. of internal reference solution and 15 ml. of ether were added, and the mixture was shaken in a

[1] Perkin Elmer model 990.
[2] Applied Science.
[3] J.J.'s Chromatography Ltd., Diatomite CQ.
[4] Merck, Darmstadt, Germany.

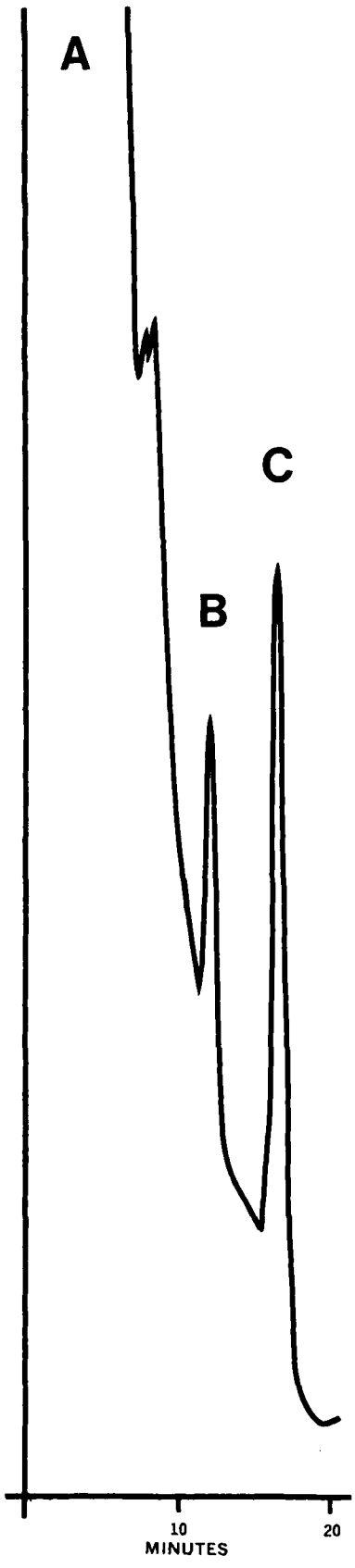
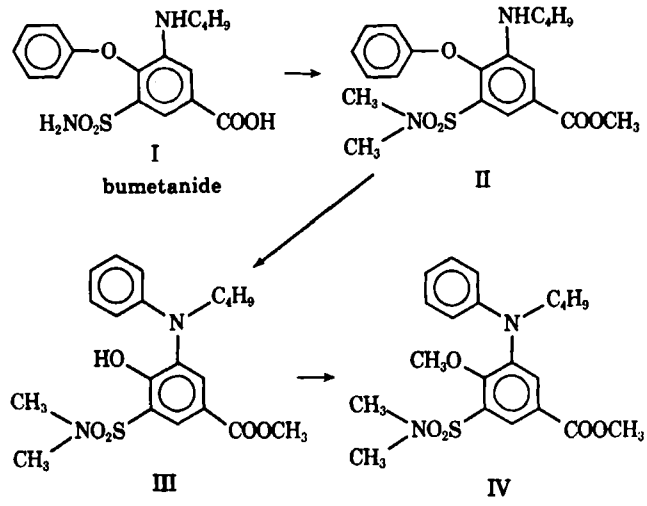


Figure 1—*Gas chromatogram of bumetanide from urine. Key: A, reagent peak; B, peak originated by bumetanide; and C, peak originated by the reference compound.*

separator for 2 min. The organic layer was washed with three 5-ml. portions of water and then filtered into a tapered centrifuge tube (10 ml.) through a small cotton plug, previously washed with ether and covered with a thin layer of anhydrous sodium sulfate. Then 50 μl. of methylation reagent was added, and the tube



Scheme I—Sequence of reactions during flash-heater methylation of bumetanide

was shaken for 2 min. After centrifugation, 1–2 μl. of the aqueous layer was injected into the gas chromatograph. The amount of bumetanide, in micrograms per milliliter of urine, was calculated from the formula:

$$\text{bumetanide} = \frac{A \cdot 2.5}{B \cdot u} \qquad \text{(Eq. 1)}$$

where A is the peak height ratio (bumetanide/internal reference), B is the peak height ratio in a corresponding standard determination in which 2.5 mcg. of bumetanide is added to 5 ml. of blank urine, and u is the quantity of the urine sample in milliliters. The peak heights were measured from a baseline drawn with the aid of a large French curve.

When the apparatus had been in disuse for some time for the determination of bumetanide, the peaks resulting from the first injections tended to be either very small or absent. This could be rectified by saturating the system with an injection of a concentrated solution of bumetanide and the reference compound in methylation reagent.

Synthesis: Methyl 3-*n*-Butylamino-5-dimethylsulfamyl-4-phenoxybenzoic Acid (II, Scheme I)—An alkaline (sodium hydroxide) solution of bumetanide (3-*n*-butylamino-4-phenoxy-5-sulfamylbenzoic acid) (I) was methylated with excess methyl iodide at room temperature while stirring for 24 hr. The excess methyl iodide was distilled off, and 3-*n*-butylamino-5-dimethylsulfamyl-4-phenoxybenzoic acid was precipitated from the reaction mixture by addition of 4 *N* hydrochloric acid until pH 2 (m.p. 187–188° after recrystallization from aqueous ethanol). The esterification was performed in methanolic hydrochloric acid by standing at room temperature for 24 hr. and was worked up as usual to yield II, m.p. 135–136°, after recrystallization from acetone–benzene.

Anal.—Calc. for $C_{20}H_{26}N_2O_6S$ (mol. wt. 406.49): C, 59.09; H, 6.45; N, 6.89. Found: C, 58.92; H, 6.42; N, 6.93.

Model Experiment: High Temperature Reaction of Bumetanide with Tetramethylammonium Hydroxide—A solution of bumetanide (3 g.) in tetramethylammonium hydroxide (45 ml., 10% in water) was introduced into a glass flask placed in an oil bath at 220–235° for 3 hr. while evaporating *in vacuo*. After heating for an additional 15 min., the reaction flask was cooled and the reaction product was dissolved in a mixture of 1 *N* hydrochloric acid (50 ml.) and ether (50 ml.). The organic layer was dried (magnesium sulfate) and evaporated. Two grams of the resulting residue (approximately 3 g.) was redissolved in ether (50 ml.) and extracted twice with 10-ml. portions of 1 *N* sodium hydroxide while ice cooling. The alkaline extract was immediately acidified with 1 *N* hydrochloric acid (30 ml.). The separated oil was reextracted with ether. After washing with water, drying (magnesium sulfate), and evaporating, 1.6 g. of oily material was obtained. After redissolving in ether, the solution was washed with diluted sodium hydrogen carbonate and dried (magnesium sulfate). After evaporation, oily methyl 3-(*N*-*n*-butylanilino)-5-dimethylsulfamyl-4-hydroxybenzoate

Table I—Mean Urinary Excretion in Healthy Volunteers during the Periods of Collection following Oral Administration of 0.5 and 1 mg. Bumetanide

Excretion[a]	Collection following 0–6 hr. Control[b]	0–6 hr. 0.5 mg.	0–6 hr. 1 mg.	6–24 hr. Control[b]	6–24 hr. 0.5 mg.	6–24 hr. 1 mg.	0–24 hr. Control[b]	0–24 hr. 0.5 mg.	0–24 hr. 1 mg.
Bumetanide, mcg.	—	166.7 ±9.9	404.3 ±39.5	—	10.0 ±4.2	23.2 ±6.0	—	176.7 ±8.9	427.5 ±35.1
Sodium, meq.	53.7 ±12.6	131.1 ±23.7	188.5 ±22.7	99.7 ±11.1	62.5 ±11.0	41.7 ±5.7	153.3 ±21.6	193.5 ±34.2	230.2 ±23.0
Potassium, meq.	28.2 ±2.0	45.7 ±3.4	51.0 ±5.1	40.0 ±5.7	41.0 ±7.2	34.0 ±7.4	68.2 ±6.3	86.7 ±8.1	85.0 ±11.3
Chloride, meq.	65.0 ±11.8	159.0 ±20.4	219.7 ±18.8	80.2 ±11.4	49.2 ±10.7	24.7 ±3.4	145.2 ±22.4	208.2 ±28.9	244.3 ±20.0
Volume, ml.	442.8 ±49.5	1469.2 ±90.6	2042.5 ±156.2	834.2 ±93.4	805.0 ±134.7	546.7 ±69.9	1277.0 ±113.1	2274.2 ±135.9	2589.2 ±177.9

[a] Values represent the mean ± *SD* for six volunteers. [b] Mean values calculated from the day before administration.

(III, Scheme I) was obtained. Crystallization was afforded from ethanol by slowly adding water. Recrystallization from ethanol–water yielded the analytically pure compound (0.64 g.), m.p. 75–77°; NMR [10% in $(CD_3)_2SO$, tetramethylsilane]: 0.88 (m, 3H, CH_3C), 1.0–1.8 (m, 4H, CH_2CH_2), 2.80 (s, 6H, CH_3NSO_2), 3.55 (m, 2H, CH_2N), 3.84 (s, 3H, CH_3OOC), 6.5–7.4 (m, 5H, C_6H_3N), 7.91 (d, J = 2.2 Hz., 1H, aromatic H), 8.29 (d, J = 2.2 Hz., 1H, aromatic H), and 10.85 (broad s, 1H, HO) p.p.m.

Anal.—Calc. for $C_{20}H_{26}N_2O_5S$ (mol. wt. 406.49): C, 59.09; H, 6.45; N, 6.89. Found: C, 58.83; H, 6.44; N, 6.89.

The remaining ether solution, after sodium hydroxide extraction, was washed with 1 *N* hydrochloric acid and water. Drying and evaporation resulted in crude methyl 3-(*N*-*n*-butylanilino)-5-dimethylsulfamyl-4-methoxybenzoate (IV, Scheme I) (0.45 g.). Recrystallization from ethanol (4 ml.) yielded the analytically pure compound (0.19 g.), m.p. 97–98°; NMR [10% in $(CD_3)_2SO$, tetramethylsilane]: 0.86 (m, 3H, CH_3C), 1.0–1.9 (m, 4H, CH_2CH_2), 2.78 (s, 6H, CH_3NSO_2), 3.72 (m, 2H, CH_2N), 3.75 (s, 3H, CH_3O), 3.88 (s, 3H, CH_3OOC), 6.7–7.5 (m, 6H, C_6H_3N), 8.01 (d, J = 2.2 Hz., 1H, aromatic H), and 8.23 (d, J = 2.2 Hz., 1H, aromatic H) p.p.m.

Anal.—Calc. for $C_{21}H_{28}N_2O_5S$ (mol. wt. 420.56): C, 59.98; H, 6.71; N, 6.66. Found: C, 59.78; H, 6.66; N, 6.54.

Human Volunteer Studies—The study was performed at dose levels of 0.5 and 1 mg. bumetanide. Scored tablets containing 1 mg. of bumetanide were used.

The trial was comprised of six volunteers who were members of the staff and 30–44 years old. All had normal blood urea and electrolyte levels. They took a normal diet and consumed, as far as possible, the same volume of fluid each day. On the 1st day of trial, each subject emptied his bladder at 7 a.m. and the urine was discarded. On the two pretreatment days, urine was collected over the 0–6- and 6–24-hr. periods. On the day of the test, urine was collected over the 0–1-, 1–2-, 2–4-, 4–6-, 6–8-, and 8–24-hr. periods following administration of the drug. On the day following treatment, urine specimens were collected over a 24-hr. period.

The content of bumetanide in urine was measured using the GLC determination as described. Blank urine samples taken before administration of bumetanide were analyzed to exclude the presence of other drugs that might interfere with the method. The presence of furosemide would interfere with the analysis, because it shows a peak with almost the same retention time as the peak originating from bumetanide. Consequently, it was found that the method could be adapted to furosemide.

The urinary excretion of electrolytes was determined using standard procedures.

RESULTS AND DISCUSSION

GLC Determination—A sensitive method was necessary for measuring bumetanide in urine at the low levels estimated to be present in urine. The described procedure is convenient for the determination of the drug in urine at levels as low as 0.1 mcg./ml. The use of the quaternary ammonium bases for the extraction of bumetanide from the organic solvent phase simplified the extraction procedure. No time-consuming evaporation steps were necessary to achieve the concentration of the drug required for GLC analysis. The accuracy of the analysis was tested by adding known amounts of bumetanide (between 0.1 and 0.5 mcg./ml.) to urine samples. The recovery was between 90 and 105%. A chromatogram from urine containing 0.19 mcg. bumetanide/ml. is shown in Fig. 1. A mixture of tetramethylammonium hydroxide and trimethylanilinium hydroxide (1:2) was used for methylation and extraction. Used alone, trimethylanilinium hydroxide resulted in an additional peak in the chromatogram, with a peak height dependent on the surplus of the reagent. Tetramethylammonium hydroxide gave only one peak; but when the reagent was used in a concentration sufficient for extraction from urine samples, a very broad reagent peak disturbed the analysis.

From a theoretical point of view, the target of the flash-heater methylation of bumetanide should be the carboxyl group and the sulfonamide nitrogen resulting in methyl 3-*n*-butylamino-5-dimethylsulfamyl-4-phenoxybenzoate (II). Compound II was prepared by synthesis and proved, by its different retention time, not to be identical with the flash-heater methylation product of bumetanide. Compound II, however, resulted in the same retention time as the bumetanide derivative in question when injected together with the methylation reagent. This indicated that the flash-heater methylation of bumetanide is rather more complicated. The gram scale investigation of the high temperature reaction of bumetanide with tetramethylammonium hydroxide resulted in the formation of methyl 3-(*N*-*n*-butylanilino)-5-dimethylsulfamyl-4-hydroxybenzoate (III, Scheme I) and methyl 3-(*N*-*n*-butylanilino)-5-dimethylsulfamyl-4-methoxybenzoate (IV, Scheme I).

IR, TLC, and GLC analyses of IV, together with the IR and TLC analyses of the peak collected from the GLC effluent of bumetanide in tetramethylammonium hydroxide, showed the derivative measured in this GLC method to be identical with Compound IV. Formation of IV involves Smiles rearrangement (8) and methylation of the intermediate phenolic hydroxyl of III. The probable sequence of reactions is outlined in Scheme I.

Human Volunteer Studies—The mean urinary recovery of bumetanide, excretion of electrolytes, and urinary volume in six volunteers after oral administration of 0.5 and 1.0 mg. of the drug are presented in Table I. The values during 0–6, 6–24, and 0–24 hr. after administration and control mean values are given. The differences for the 0–6-hr. interval are statistically significant except for potassium.

The histograms in Figs. 2 and 3 depict the excretion of bumetanide and corresponding urinary sodium in the urine samples over a 24-hr. period following 0.5 and 1 mg. of drug administration, respectively.

The present data show that the urinary excretion of bumetanide is rapid and attains its maximum, in the majority of cases, between 1 and 4 hr. after administration. Excretion is virtually complete after 8 hr. Apart from two subjects receiving 1 mg. bumetanide, no drug was detectable in the urine after 24 hr. The total 48-hr. urinary excretion of bumetanide amounted to 35 and 44% for the 0.5-and 1-mg. doses, respectively.

After enzymatic hydrolysis of the 1–2-hr. urine samples using β-glucuronidase-arylsulfatase[5], the concentration of bumetanide

[5] C. F. Boehringer GmbH., Mannheim, Germany, Catalog No. 15247 EGAF.

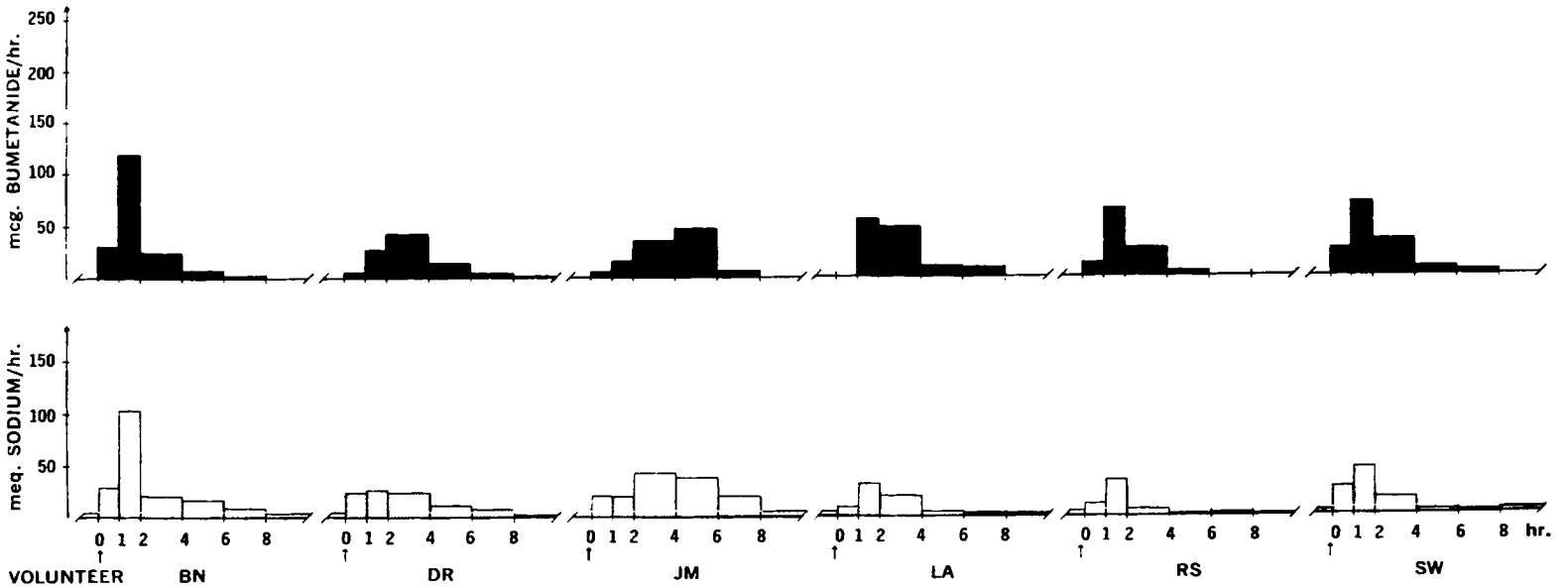


Figure 2—*Urinary excretion of bumetanide (bars upper diagram) and sodium (bars lower diagram) in six healthy volunteers following oral administration of 0.5 mg. of bumetanide.*

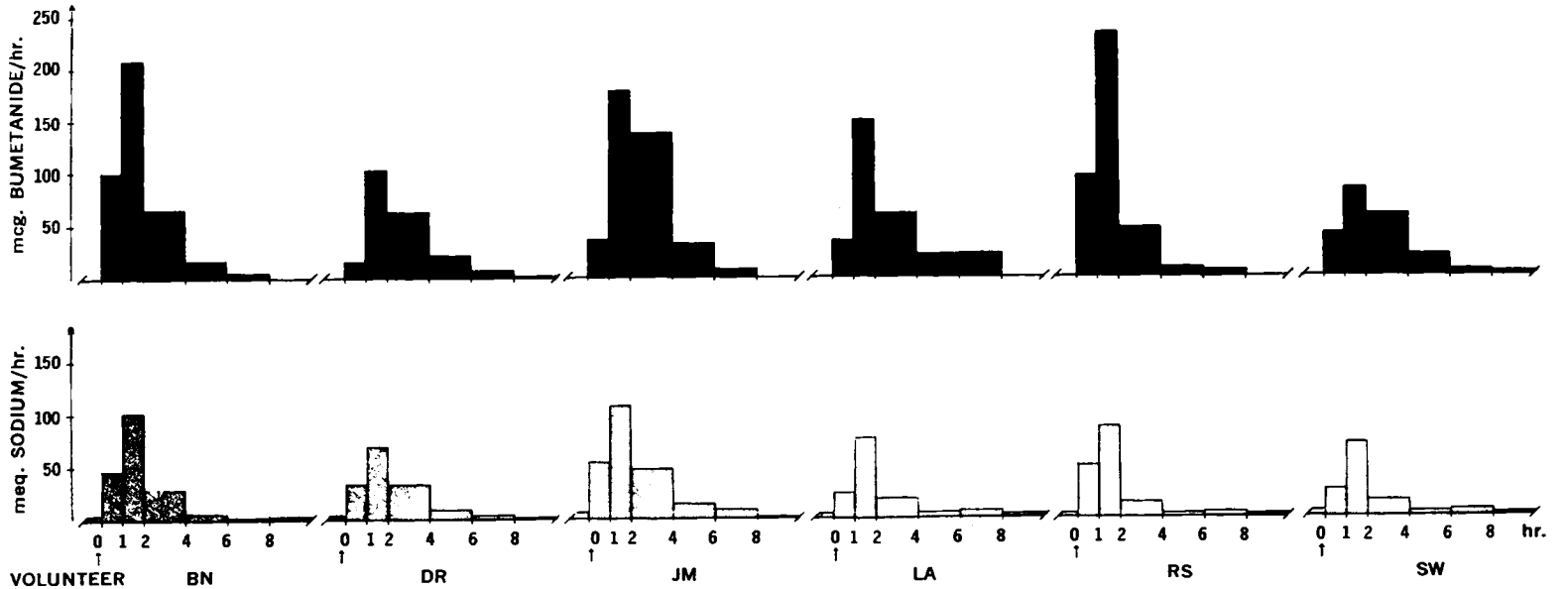


Figure 3—*Urinary excretion of bumetanide (bars upper diagram) and sodium (bars lower diagram) in six healthy volunteers following oral administration of 1 mg. of bumetanide.*

found did not exceed the original level. This indicates that bumetanide is excreted unconjugated in human urine.

However, the urinary excretion of bumetanide cannot be considered as a measure of orally absorbed drug since a biliary excretion of the drug cannot be excluded. Biliary excretion would make the results in accordance with the low dose dog assay, where an almost equal response to the drug was obtained after oral and intravenous administration.

The results confirm the marked saluretic and diuretic effect of bumetanide after oral administration of both 0.5 and 1 mg. to human volunteers and show clearly a parallelism between bumetanide excretion and saluretic action over the total period (Figs. 2 and 3).

SUMMARY

A GLC determination of bumetanide, using flash-heater methylation by means of a mixture of tetramethylammonium hydroxide and trimethylanilinium hydroxide, was developed. The flash-heater methylation product of bumetanide proved to be methyl 3-(*N*-*n*-butylanilino)-5-dimethylsulfamyl-4-methoxybenzoate, formed by methylation under simultaneous rearrangement. The GLC determination was found to be accurate at concentrations as low as 0.1 mcg./ml. human urine. In six healthy volunteers, the urinary excretion of sodium, potassium, and chloride, the urine volume, and the urinary recovery of bumetanide were determined after oral administration of 0.5 and 1 mg. of the drug. A parallelism between bumetanide excretion and saluretic action over the total period of response is shown.

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Influence of Solution Electrolyte Content and Dielectric Constant on Drug Adsorption by Kaolin

N. A. ARMSTRONG▲ and C. D. CLARKE*

Abstract □ The adsorption of benzoic acid and crystal violet on kaolin was investigated to elucidate the influence of the system dielectric constant and the electrolyte content on this example of drug–adjuvant interaction. The differing results obtained with the two adsorbates reflect the dissimilar adsorption sites on the kaolin platelet and the different mechanisms involved in the uptake of acids and bases on clays. The experimental data presented are satisfactorily explained by a consideration of the effect of both the dielectric constant and the electrolyte concentration and valency on adsorbent and adsorbate characteristics.

Keyphrases □ Kaolin, adsorption of benzoic acid and crystal violet—location of adsorption sites, influence of dielectric constant and electrolyte content □ Adsorption of benzoic acid and crystal violet by kaolin—influence of dielectric constant and electrolyte content □ Drug–adjuvant interactions—influence of dielectric constant and electrolyte content, drug adsorption by kaolin □ Dielectric constant, electrolyte content—effect on drug adsorption by kaolin □ Electrolyte content, dielectric constant—effect on drug adsorption by kaolin

Previous papers (1, 2) showed that the Langmuir adsorption isotherm represents the adsorption of benzoic acid and crystal violet on kaolin, and they discussed the effect of environmental pH on this equilibrium process. From the results obtained, the probable adsorption sites for acidic and basic materials on clays were suggested, namely that anionic materials were adsorbed on the edge and cationics on the cleavage surface of the clay. The significance of such an evident drug–adjuvant interaction on preservative efficiency or therapeutic potency was briefly discussed. The presence of soluble material in a suspension formulation, for example as active principle, flavoring, coloring, buffering, or peptizing agents will not only influence the formulation's pH but also contribute to the electrolyte content of the system. This increase in electrolyte concentration will also result in an increase in the dielectric constant of the system (3). Both of these factors influence the characteristics of the electrical double layer of the suspended phase, this double layer constituting the area where adsorption and/or ion exchange takes place. It was, therefore, considered desirable to determine the effect of both the system dielectric constant and the electrolyte content (with respect to concentration and valency) on the problem of drug–adjuvant interaction through investigating the influence of these factors on the uptake of benzoic acid and crystal violet on kaolin.