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Osmotic Pressure and Electrical Conductivity of Urine Under Drug-Induced Diuresis.* (32244)

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Investigation of the electrical conductivity and relative salinity of normal human urine by Hull(1), prompted further study of these during the diureses of saluretic agents. Mercaptomerin (THIOMERIN) and ethacrynic acid† were selected to show how measurements of urinary concentrative properties (2), specifically osmotic pressure and electrical conductivity, may be used to analyze pharmacologic actions beyond the possibilities afforded by measurement of individual electrolytes alone.

Methods. Standard analytic technics were used: sodium and potassium by flame photometer; chloride by Cotlove titrimeter; osmotic pressure by Fiske osmometer; electrical conductivity by RC 16B2 bridge.‡

Six unanesthetized female dogs (10-20 kg) with implanted cystostomy tubes(3) were maintained on standard laboratory rations for at least seven days before experimentation. In addition, 6 other female dogs of similar weight were anesthetized with sodium pentobarbital, urine collections being made by catheter. A control urine sample was collected, followed by administration of either intravenous mercaptomerin (4 mg Hg per kg) or ethacrynic acid (1 mg per kg). Successive, timed collections were made and the urine analyzed.

Conductivity and osmotic pressure of urine were expressed, respectively, as condosity (T = millimolarity or millinormality of NaCl solution having the same specific conductance as the urine) and osmosity (S = millimolarity or millinormality of NaCl solution having the same freezing point), the conversions being obtained by standard tables(1,4,5). The ratio of these, the relative salinity ($\Sigma = T/S$), measures the fraction of the total number of dissolved particles in the urine which behave as if they were NaCl. The difference, S — T, is a defined measure of the total nonelectrolyte of the urine (noncondosity); and by subtracting actual urinary sodium concentration (in equipollent units of mEq/l) from S or T, we obtain defined measures of the total "nonsodium" solute concentration of the urine and the total "nonsodium electrolyte" concentration, respectively.

Results. With no significant differences in pharmacologic action manifest between anesthetized and unanesthetized dogs, the results in these series were combined as averages. Fig. 1 and 2 illustrate the flow and concentrative changes in the urine. Tables IA and IIA provide average excretion rates; Tables IB and IIB, ratios of electrolytes and concentrative properties. Statistically significant changes between control and experimental values with both drugs were determined by t-test ($P < .05$) for urine flow and all excretion rates except for total nonelectrolytes; among ratios, for relative salinity (T/S) and relative nonsalinity, (S — T)/S. Significant changes with mercaptomerin were Na/S and (Na + K)/S; and K/T with ethacrynic acid. Other ratios changed as shown in the Tables but too few measurements were available to decide statistical significance. In the case of

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‡ Industrial Instruments, Inc., (now Cedar Grove Operations, Beckman Instruments, Inc.).

ratios such as Na/T and (Na + K)/T, their relative constancy is the significant fact, not statistical but physiologic.

Discussion. Although well known differences in pharmacologic action between these drugs can be distinguished in the Tables and Figures (*e.g.*, more rapid action of ethacrynic acid), we are less concerned with these than to exemplify new quantitative descriptions of the saluresis phenomenon.

Thus, in saluresis with both drugs, urinary concentrations are similar. Relative salinity rises until over 70% of the total solute particles are (or behave as if they were) ionic; and, at the height of saluresis, half or more of the total urinary solute particles are rep-

TABLE I A. Effects of Mercaptomerin upon Urinary Excretion.

Time (min)	Urine flow (ml/min)	Excretion rate (μEq/min)						
		Na	K	Na + K	Cl	Electrolytes	Non-electrolytes	Total solutes
0	.48	52	32	84	46	70	148	218
60	2.6	315	52	367	281	343	281	624
90	3.0	429	39	468	444	426	204	630
120	2.8	316	34	350	291	336	134	470

Average values for 6 dogs. Excretion rates in μEq/min were obtained from product of urine flow (ml/min) and concentration (mEq/l). Excretion rates for electrolytes, nonelectrolytes, and total solute are obtained from the products of urine flow and T, S—T, and S, respectively, where T is condosity, S—T is noncondosity, and S is osmosity, all in identical units of millinormal (= millimolar) NaCl solutions. Osmolalities and osmolal excretion rates may be approximated by multiplying values of T, (S—T), S, and their corresponding rates, respectively, by 1.86. More exact conversions may be obtained from appropriate tables(1).

TABLE I B. Effects of Mercaptomerin upon Concentrative Ratios.

Time (min)	Na/T	K/T	(Na+K)/T	Na/S	K/S	(Na+K)/S	(S—T)/S	T/S
0	.74	.46	1.20	.24	.15	.39	.679	.32
60	.918	.15	1.07	.505	.08	.588	.450	.550
90	1.01	.09	1.10	.681	.06	.743	.324	.676
120	.940	.10	1.04	.672	.07	.745	.285	.715

Average values for same animals as Table I A. T, condosity; S, osmosity; T/S, relative salinity; (S—T), noncondosity; (S—T)/S, relative nonsalinity.

TABLE II A. Effects of Ethacrynic Acid upon Urinary Excretion.

Time (min)	Urine flow (ml/min)	Excretion rate (μEq/min)						
		Na	K	Na + K	Cl	Electrolytes	Non-electrolytes	Total solutes
0	.27	43	30	73	26	76	116	192
15	6.0	582	162	744	606	858	294	1152
45	4.3	301	125	426	335	529	189	718

Average values for 6 dogs. Excretion rates in μEq/min were obtained from product of urine flow (ml/min) and concentration (mEq/l). Excretion rates for electrolytes, nonelectrolytes, and total solute are obtained from the products of urine flow and T, S—T, and S, respectively, where T is condosity, S—T is noncondosity, and S is osmosity, all in identical units of millinormal (= millimolar) NaCl solutions. Osmolalities and osmolal excretion rates may be approximated by multiplying values of T, (S—T), S, and their corresponding excretion rates, respectively, by 1.86. More exact conversions may be obtained from appropriate tables(1).

TABLE II B. Effects of Ethacrynic Acid upon Concentrative Ratios.

Time (min)	Na/T	K/T	(Na+K)/T	Na/S	K/S	(Na+K)/S	(S—T)/S	T/S
0	.57	.39	.96	.22	.16	.38	.604	.40
15	.678	.189	.867	.505	.141	.646	.255	.745
45	.569	.236	.805	.419	.174	.593	.263	.737

Average values for same animals as Table II A. T, condosity; S, osmosity; T/S, relative salinity; (S—T), noncondosity; (S—T)/S, relative nonsalinity.

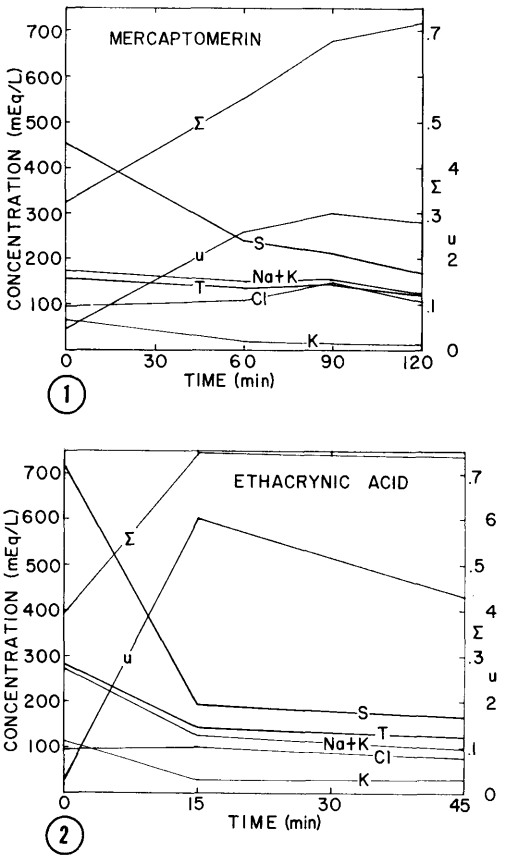

FIG. 1. Time course of urinary flow rates, concentrations and relative salinities in 6 dogs (averaged) receiving mercaptomerin. Zero time values reflect the preceding control period; later values are plotted at the end of preceding time period of collection. Concentration in mEq/l: S, osmosity; T, condosity; Na, sodium; K, potassium; Cl, chloride. Σ, relative salinity (= T/S); u, urine flow (ml/min).

FIG. 2. Time course of urinary flow rates, concentrations and relative salinities in 6 dogs (averaged) receiving ethacrynic acid. Zero time values reflect the preceding control period; later values are plotted at the end of preceding time period of collection. Concentration in mEq/l: S, osmosity; T, condosity; Na, sodium; K, potassium; Cl, chloride. Σ, relative salinity (= T/S); u, urine flow (ml/min).

resented in effect by sodium salts (Na/S). Ordinarily relative salinity of urine appears little affected by states of hydration or dehydration, water diuresis or oliguresis(1); it may therefore be a singularly useful and convenient property by which to measure saluresis, a condition in which it is augmented. In these studies, relative salinity parallels diuresis as well (Fig. 1 and 2).

The ratios of Na, K and (Na + K) concentrations to condosity are new to the literature. Fuller interpretation awaits more detailed electrolyte analysis of urine because compounds such as Na_2SO_4, KCl, etc., in aqueous solution, are known to have relative salinities in excess of 1 (by definition, the relative salinity of binary aqueous solutions of NaCl of any concentration is exactly 1). This fact is related to the heavier charge carried by bivalent ions and to the greater mobility of K or H over Na ions.

The observed high values of (Na + K)/T suggest that most of the conductivity of urine in these experiments is derived from the presence of uniunivalent Na and K salts; the excretion of these cations in saluresis is paralleled by that of Cl (Tables IA, IIA). The increase in the ratio (Na + K)/S during saluresis, *i.e.*, in the fraction of total solute particles represented by Na and K salts, is another characteristic measure of saluresis.

A novel feature of this analysis is the estimation of nonelectrolyte concentrations or excretion rates from the noncondosity, S — T. Although its units are somewhat paradoxically given in terms of millimolarity or millinormality of NaCl, any such value has an exact conjugate value of freezing point depression and, therefore, of milliosmolality. Approximation may be made by multiplying by 1.86: thus, 1.86 × S = milliosmolality of total solute of urine; 1.86 × T = milliosmolality of total electrolyte of urine; and 1.86 × (S — T) = milliosmolality of total nonelectrolyte of urine. While use of conversion tables may be theoretically more exact than the use of the factor 1.86, a special kind of error afflicts the two latter calculations, one which is augmented by the presence of salts with relative salinities different from 1. Nevertheless, in a system defined in this way, it is simple, rapid and convenient to estimate total urinary electrolyte and nonelectrolyte independently, if need be, of analytic determinations of specific ions.

Summary. The saluresis of mercaptomerin and ethacrynic acid in dogs was measured in terms equivalent to urinary osmotic pressure and electrical conductivity. When converted into identical units (millimols or millie-

quivalents of NaCl per liter), these concentrative properties are shown to provide unique pharmacologic parameters of renal function such as the relative salinity or ionic fraction of the total solute concentration. In conjunction with specific electrolyte analyses, *e.g.*, Na, K, and Cl, expressed in similar equipollent units (mEq/l), other singular relationships in urinary function are demonstrable.

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Passing of Insulin from Plasma into the Bile.* (32245)

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Previous studies have shown that insulin is present in the bile of different species and that the concentration of insulin in the bile flow increases after stimulation of endogenous insulin production(1,2). These findings suggest the possibility that the bile is an avenue for the loss of insulin from the circulation. This possibility is confirmed in the present paper as insulin injected in circulating blood was recovered in the bile.

Material and methods. Insulin in the bile was determined according to method C of Hales and Randle(3). The bovine insulin used for standards in the immunological test was obtained from Eli Lilly Co. Iodinated insulin-I-125 was supplied by the Radiochemical Centre, Amersham, Buckinghamshire, England. Insulin binding serum was obtained from Wellcome Laboratories. Insulin concentrations are expressed as bovine equivalent, since bovine insulin was used as a standard.

Rabbits (1.5 to 2.0 kg) were deprived of food for 18 hours. To obtain the bile the animals were anesthetized with ether and then a catheter was placed in the choledochus through a medial abdominal incision. The cystic duct was ligated and the gall bladder removed, the catheter was guided to the exterior, where the bile could easily be collected in a test tube. Insulin was injected rapidly into the abdominal vena cava and samples of bile were taken at different time intervals. The hepatic bile flow, up to 7 hours following cannulation of the choledochus, was 7-9 ml per hour. Blood glucose(4) and plasma insulin were determined at the end of each experiment.

Results. The results obtained by analysing the presence of insulin in the bile flow after i.v. administration of 2 mg (50 IU) of bovine insulin in normal rabbits are summarized in Fig. 1. Large concentrations of insulin appeared immediately in the biliary flux; highly significant concentrations of insulin were still found in bile even 3½ hours after injection of the hormone. It is very possible that the significance of these experiments lies in the shape of the recovery curve, the first part of which lasts approximately one hour. This may be related to the affinity of insulin for the liver.

In the experiments described above, blood glucose was very low from the beginning (25 ± 4 mg/100 ml) and the insulin concentration in plasma was very high, even at the end of the experiment (>500 μU/ml). These are abnormal conditions. It was there-

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