Diuretic Activity of Three Primary Fractions of Total Nondialyzable Solids From Human Urine.* (27778)

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We have reported previously(1) that when normal dog and human urine are infused intravenously into normal unanesthetized dogs there is a marked increase in rate of urine flow over the pre-injection rate. The volume of urine recovered, in excess of that infused plus that which would have been excreted without diuresis, represented a reduction in total body water of the animal equivalent to approximately 1.0-1.5% of body weight. When concentrated dialyzed pooled human urine(2) is assayed in the dog there is an average loss of body water of 13 ml/kg during a 5-hour period. The maximum dry weight of material injected was 1.76 mg/kg. Lyophilized pregnant mare urine also produces a significant diuresis but less than with pooled human urine. Spray dried pregnant mare urine was fractionated by ammonium sulphate and acetone precipitation(3). With neither procedure was there a complete separation of the diuretic factor, although the diuretic factor was more concentrated in the fraction obtained by half saturation with ammonium sulphate and also in the fraction obtained at pH 10 with an acetone concentration between 63% and 75% after prior refluxing of the sample for 30 minutes at pH 1.0.

Also, there have been reports of the appearance of a diuretic factor in blood and urine following hemorrhage in the dog(4,5,6) and following the experimental production of edema(7). Milies(8) has reported a protein diuretic factor in a perfusate of the dog liver.

This paper concerns the diuretic activity of 3 primary fractions of the total nondialyzable solids (TNDS) isolated from pooled human urine samples and samples obtained from individual persons.

Methods. Source of preparations. Twenty-four-hour urine samples were collected in clean bottles with a small volume of chloroform as a preservative. Samples FlDB, 4F and 5F were from pregnant women, and in the case of the last 2 pre-eclampsia was a tentative diagnosis although there was no evidence of renal disease. Sample FlE was from a female with calculous disease whose urine showed a low albumin and a markedly increased gamma globulin concentration. Samples FlJ, 33 and 34 were individually pooled samples respectively from 3 normal male subjects. All other samples were mixed pooled samples from normal male and female subjects.

Fractionation of total nondialyzable solids. In the case of each preparation, the 24-hour urine samples were mixed and placed in cellulose dialyzer tubing† of 2.8 cm inflated diameter. The sacs were knotted to include an approximate volume of 35 ml of air in order to keep the sacs upright during dialysis. The samples were dialyzed against tap water and distilled water at 5°C for 48 to 72 hours. The water was changed at least daily, and in some instances more frequently.

The sacs were emptied being careful to flush out any precipitate that may have formed during dialysis. The dilayzed samples were fractionated by the procedure of Boyce *et al.*(9). The samples were subjected to ultrafiltration at 5°C under an air pressure of 40 lb per square inch. The filters had a pore diameter of 20 (range 14 to 26) mμ. The solids in the ultrafiltrate were recovered by lyophilization and are designated as fraction UFO. The residue on the membrane fraction RO, was removed and extracted 3 to 5 times with 5 volumes of either 0.1M NaCl or a barbital-sodium barbital (Veronal) buffer, ionic strength 0.1N, pH 8.6. The Veronal insoluble fraction, designated as fraction Rl, was recovered by centrifugation, and immediately suspended in

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† A. H. Thomas Co., dialyzer tubing No. 4465-A2, 4.8 mμ pore size.

distilled water and dialyzed against cold distilled water for at least 24 hours to remove any Veronal. The solids were recovered by lyophilization. The extract of the residue from ultrafiltration, designated as fraction RSl, was dialyzed against cold distilled water and recovered by lyophilization.

Assay procedure for the dog. Ten trained female dogs, prepared for easy catheterization by an episiotomy were used repeatedly as an assay pool. The dogs were replaced at approximately 6-month intervals or more frequently if there were evidence of urinary tract infection.

At 4 p.m. on the day prior to assay the dogs were hydrated by giving 25 ml/kg of 0.9% NaCl solution by stomach tube, and food and water were removed. At 9:45 a.m. on the day of assay the dogs were catheterized, their bladders emptied, and they were given a load of 24 ml/kg of 0.9% NaCl solution by stomach tube. One hour later the urine volume was recorded but not included in the calculations. At this time the sample to be assayed or the solvent was injected intravenously in a volume of 0.5 ml/kg dissolved in either distilled water or a universal buffer.‡ The dogs were returned immediately to metabolimeter cages. Five hours after the injection, the dogs were catheterized and total volume of urine excreted during this 5-hour period was recorded. The diuretic response (DR) is expressed as ml/kg/5 hr. On a given day, 5 dogs were run with the material to be assayed being given to 3 dogs and the solvent being given to 2 dogs to determine the control response (CR). After a lapse of one day, these same dogs were used for assay of the same preparation, but the particular dogs receiving unknown and solvent were reversed. Each assay consisted, usually, of unknown and control measurements of urine output on 10 dogs. The dogs were kept in a room at 24°-25°C from the time of initial hydration.

Assay procedures for the rat. Male Sprague-Dawley rats weighing 125-275 g were run in groups of 8 rats per dose (subgroups of 4 rats per cage). The rats were kept in a room at 24°-25°C during the assay procedures. Two procedures were used:

(A) At 3:00 p.m. on the day prior to assay both food and water were removed, and the rats were hydrated orally with 5% of body weight of 1% NaCl solution. At 8:30 a.m. on the day of assay the rats were hydrated orally with 2.5% body weight of distilled water. Two hours later an oral load of 5% of body weight of 1% NaCl solution was given, and the bladders were emptied; the preparation, or solvent as control, was given intraperitoneally in a volume of 0.5 ml/100 g, and the rats were placed in metabolimeter cages. Five hours later the urine volume was recorded for each subgroup and average volume for the group was calculated. The urine obtained from a group (8 rats) was mixed, and sodium and potassium concentrations were determined by flame photometry.

(B) This procedure is similar to the one given above. Exceptions were: on the afternoon prior to assay, only food was removed, and the rats were hydrated with distilled water. At 8:30 a.m. on the day of assay, water was removed, and the water hydration was omitted. At 10:00 a.m. the rats were loaded, as above, and Procedure A was followed.

Results. The assay results in dogs obtained with 10 Veronal buffer extracts of the residue from ultrafiltration (fraction RSl) and 6 ultrafiltrates (fraction UFO) are presented in Table I. Seven of the RSl fractions resulted in a significant increase in rate of urine flow when tested by a calculation of Student's t value(10). The range of increased mean urine excretion, expressed as ml/kg/5 hr, was 39%-96% of the mean control value. These results were obtained with a range of dose of 0.5-10 μg/kg of the preparation.

Also, in Table I are given the results obtained with 6 preparations of fraction UFO. In no instance was there a significant increase in the rate of urine flow in a dose range of 0.5-2.0 μg/kg.

For comparison, the results obtained with 2 known diuretics, acetazolamide and lyophilized chlorothiazide sodium, are given. The

‡ Each of the following in 0.1M concentration: NaH_2PO_4, sodium citrate, glycine, and dl-alanine. pH is approximately 6.2.

TABLE I. Diuretic Activity of 2 Fractions of Human Urine Total Nondialyzable Solids Given Intravenously. The solvent was distilled water or universal buffer.

Preparation	Dose, μg/kg	No. of dogs, C/E*	Mean control response (CR), ml/kg/5 hr	DR−CR,† ml/kg/5 hr	Probability
		Fraction: RS1			
2F	5.0	10/10	10.6	7.9	<.001
4F	5.0	10/10	8.5	8.1	<.001
5F	5.0	10/10	10.8	2.4	>.2
F1E	5.0	10/10	12.1	11.5	<.001
6F (2)	.5	10/10	11.0	8.9	<.001
6F (2)	1.0	12/9	12.1	9.6	<.001
7F	1.0	7/13	10.8	10.4	.01
12F	.5	10/10	10.6	4.1	<.05
13F	.5	8/9	12.8	2.3	>.4
19F (Ly)	10.0	9/10	14.3	11.8	<.001
		Fraction: UFO			
9F	1.0	10/10	12.2	1.7	>.4
33	1.0	9/8	15.3	2.2	>.5
34	.5	10/10	14.6	.8	>.7
HP	1.0	8/9	12.8	2.3	>.4
20F	2.0	10/10	15.9	1.2	>.5
26F	2.0	10/10	13.3	.1	>.9
	(mg/kg)	Standard diuretics			
Acetazolamide‡	15	9/10	13.7	9.1	<.02
Chlorothiazide	5	10/9	11.4	10.8	<.001

* Control/Experimental.
† DR is diuretic response in ml/kg/5 hr; CR is control response in same units.
‡ Given orally in capsule.

former was administered orally in a dose of 15 mg/kg, and the latter intravenously in a dose of 5 mg/kg. Under these conditions of assay, the 2 diuretics gave results equivalent to those obtained with 1-5 μg/kg of fraction RSI.

In Table II the results obtained in the rat with the Veronal buffer insoluble fraction (R1) of 2 preparations are given. The fractions were given intraperitoneally as a suspension in Veronal buffer. It is doubtful if the increased rate of excretion for urine or sodium with 10 μg/100 g of fraction 19F-R1 is significant, but the increases noted with 20 μg/100 g of fraction 19F-R1 and 25 μg/100 g of fraction 24F-R1 probably are significant.

In Fig. 1 are given regression lines representing the dose-response relationship in the dog for 2 preparations of fraction RSI. For preparation F1DB the intravenous dose range was 0.4-20 μg/kg; and the correlation coefficient for the data was 0.90 with $S_{y \cdot \log x} = 1.356(10)$. For preparation F1J the intravenous dose range was 5-25 μg/kg; and the correlation coefficient for the data was 0.99 with $S_{y \cdot \log x} = 0.357$.

Discussion. The results of the experiments reported here confirm earlier reports(1,2)

TABLE II. Diuretic Activity of Fraction R1 in Rats.

Preparation	Dose,† μg/100 g	Urine vol ml/kg/5 hr	% change	Sodium excretion meq/kg/5 hr	% change	Potassium excretion meq/kg/5 hr	% change
Control		34.7		4.39		.373	
19F − R1*	10	39.9	+15	5.18	+18	1.117	+300
19F − R1	20	52.4	+51	7.05	+61	1.100	+295
Control		24.3		3.29		.965	
24F − R1	25	44.2	+82	4.75	+44	1.560	+ 62

* Fraction 19F − R1 was assayed using procedure (A), and for fraction 24F − R1 procedure (B) was used.
† Preparation was given by intraper. inj.

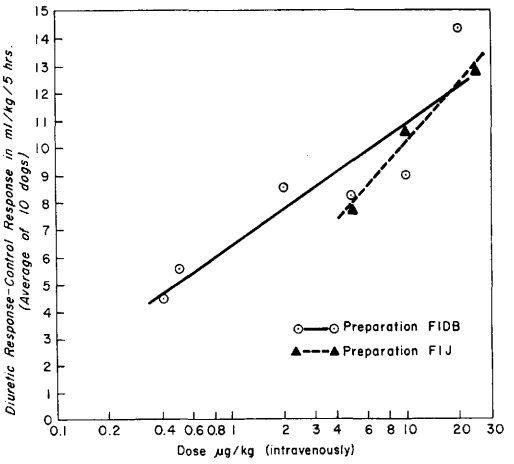

FIG. 1. Diuretic dose-response relationships for 2 RS1 fractions of total nondialyzable solids of human urine.

that normal human urine contains a diuretic factor when assayed in the dog. However, significant diuresis was obtained in these experiments with much lower doses (0.5-10 µg/kg) than in the previous experiments.

A simple fractionation of the total nondialyzable solids of human urine by ultrafiltration and extraction of the residue with Veronal buffer has shown that the diuretic factor is not present in the ultrafiltrate, at least when given in doses comparable to other active fractions. The Veronal buffer extract does contain the diuretic factor in a very active form. With respect to volume excretion and during the time limits of the assay, fraction RSl appears to be from 1000 to 5000 times more potent on a weight basis than intravenous chlorothiazide.

Assay of the residue from Veronal buffer extraction has not been possible in the dog for it appears to be quite toxic in this animal. When this fraction (Rl) has been administered intravenously to the dog, in dilute suspension, it produces copious vomiting, diarrhea and prostration. In fact, care must be exercised that fraction Rl does not contaminate fraction RSl, for vomiting will occur if contamination is present. However, fraction Rl does have some diuretic activity, at least in the rat, as shown in Table II. It appears that Veronal buffer extraction of fraction RO does not extract completely the diuretic factor. This may be due to the conditions of the extraction, for in one experiment with continuous stirring during extraction for 1 hour at 5°C a much greater proportion of fraction RO was found to be soluble in Veronal buffer than in the experiments reported here, where the mixture was stirred for only 5-10 minutes per extraction. With the longer stirring, however, the extract was more toxic to dogs. In some experiments we have found that fraction RO is almost completely soluble in distilled water. The diuretic and toxic effects of the water solution of fraction RO have not yet been studied.

The results reported here do not eliminate the possibility that the diuretic factor may be present in fraction UFO, but if this is the case one must conclude that the specific diuretic activity is considerably lower than in fraction RSI. Although the results with these simple fractionation procedures do not indicate a complete isolation of the urinary diuretic factor in any one fraction, they do indicate a rather consistent appearance of the factor in fraction RSl with a relatively high specific activity.

Within the dose range of 0.4-10 µg/kg with one preparation and in a range of 5-25 µg/kg with a second preparation there is a rather good dose response relationship, as far as volume excretion is concerned. At higher doses the relationship approaches a plateau probably due to an approximation of the physiological limits of response under the conditions of hydration in the assay.

The effect of the diuretic factor on electrolyte excretion has not been studied in the dog. The design of the dog assay would result in gross electrolyte contamination of the collected urine. However, in the rat assay of fraction Rl there were considerable increases in sodium and potassium excretion with the two preparations tried at doses of 20 and 25 µg/100 g.

Summary. The total nondialyzable solids of normal human urine have been partially fractionated by ultrafiltration yielding an ultrafilterable fraction (UFO) and a residue, fraction RO. The latter has been fractionated further by extraction with barbital-sodium barbital buffer, pH 8.6, yielding a soluble fraction RSl and an insoluble residue

fraction Rl. A significant increase in rate of urine flow in dogs was obtained by intravenous administration of fraction RSl in a dose range of 0.5-10 μg/kg. On a weight basis fraction RSl is much more potent than acetazolamide or chlorothiazide, under the conditions of the assay. Fraction UFO did not result in an increase in urine flow when given in a dose range of 0.5-2 μg/kg. Fraction Rl could not be assayed in the dog because of toxicity, but when administered as a suspension intraperitoneally in doses of 20-25 μg/100 g to the rat it resulted in appreciable increases in rate of urine flow and electrolyte excretion.

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Mucosal Adsorption and Absorption of Vitamin B_{12} in the Intestine of the Rat. (27779)

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It is established that intrinsic factor (IF) mediates the process of physiological absorption of Vit. B_{12} (B_{12}) in the intestine. Recent *in vitro* studies(1,2) have indicated that IF first brings about adsorption of B_{12} to the mucosal surface with the aid of calcium ion. The subsequent steps for absorption of B_{12} have not yet been elucidated. One of the difficulties in such studies lies in the lack of a means to differentiate surface adsorption and absorption past the mucosal barrier.

The present investigation was undertaken to study quantitatively the adsorption of Co^{60}-labeled cyanocobalamin (Co^{60}-B_{12}) and its transport past the intestinal serosa in relation to time, using the intestinal loop technic(3). This *in vivo* system lends itself to such differential measurement with a functioning bowel and controlled amounts of IF. The results indicate that not all B_{12} molecules that are first adsorbed to the surface pass the mucosal barrier, and that there is a nonspecific adsorption of B_{12} that does not represent the absorptive process.

Methods. Young adult rats of the Wistar strain were used. The preparation of a washed loop with the entire jejunum and ileum by operation, and of rat intrinsic factor concentrate (IFC) from the rat stomach mucosae has been described(3). Such a loop has no more access to endogenous IF. Co^{60}-B_{12} having a specific activity of 1 μc/μg* was applied into the loop with or without IFC, and the animal was killed after certain intervals. The dose of IFC was so chosen as to yield a maximum absorption.

The transserosal transport of Co^{60}-B_{12} is herein referred to as "absorption" and was calculated directly from the difference between the dose given and the radioactivity recovered in the loop including the content, and indirectly from the radioactivity in the liver and kidneys. The adsorption was assessed by washing the loop lumen a short time after administration of the dose and determining the radioactivity that was not washed away.

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