

THE MECHANISM OF FORMATION OF OSMOTICALLY CONCENTRATED URINE DURING THE ANTIDIURETIC STATE¹

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The present study is concerned with the operation by which the kidney, during antidiuresis, conserves water for the body by elaborating a urine osmotically more concentrated than the blood.

It is appropriate to emphasize that in the mammals the osmotic pressure of the plasma and the interstitial fluid is one of the most closely guarded of all the homeostatic states, and that the kidney is chiefly responsible for the regulation of this osmotic pressure within narrow limits. Osmotic dilution of the plasma is normally offset by the excretion of a urine osmotically more dilute than the plasma, as in water diuresis, while an increase in osmotic concentration of the plasma is offset by the excretion of urine osmotically more concentrated than the plasma. The present paper is concerned only with the mechanism of the excretion of a hypertonic urine.

That no osmotic concentration is achieved in the separation of the glomerular filtrate is established, to the satisfaction of all investigators, by the well known micropuncture studies of Richards and his coworkers in the Amphibia, and Walker, Oliver, and their coworkers in the guinea pig, rat and opossum. These studies have also demonstrated, at least under the recorded experimental conditions, that the urine remains isosmotic with the plasma, or nearly so, throughout the length of the proximal segment, at a time when the bladder urine may be concentrated osmotically to a considerable extent. Unfortunately, such micropuncture studies as are

available have failed to explore the question of just where, in the more distal portion of the nephron, osmotic concentration is effected, whether in the distal segment (which can be differentiated cytologically into at least two portions), or in the collecting ducts, or both. We are here not primarily concerned with this anatomical question, but rather with the quantitative evaluation of the overall osmotic operation.

In the process of osmotic regulation as observed during antidiuresis (*i.e.*, in the hydropenic state or during the administration of antidiuretic hormone) the kidney separates from the plasma more osmotically active material than water, and thus produces a urine that is osmotically more concentrated than the plasma. The volume of plasma cleared of osmotically active material is calculated, as with any clearance, as the rate of excretion of osmols per minute, $U_{osm}V$, divided by the osmotic concentration of the plasma, P_{osm} . The osmotic concentrations in urine (U_{osm}) and plasma (P_{osm}) are measured by the reduction of freezing point or, alternatively, the reduction of vapor pressure of urine and plasma, respectively, and V signifies the urine flow in ml. per min. It is convenient to designate this *osmolar clearance*, $U_{osm}V/P_{osm}$, as C_{osm} , which has the same connotation as other familiar clearance expressions and refers to the rate of clearance from the plasma of all osmotically active material, irrespective of its nature.

Reasons will be given later to support the belief that an osmotically concentrated urine is elaborated by the subtraction of water from the isosmotic glomerular filtrate rather than by the addition of solutes. The quantity of water so abstracted per minute has been designated as T^cH_2O by Wesson and Anslow (1) the superfix *c* serving to identify it as the quantity of water abstracted in the concentrating operation.⁴ This moiety of water is

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³ The substance of this paper has been reported in a preliminary way by the senior author (Smith, *Federation Proc.*, 1952, 11, 701; also in *Symposium on renal function in infancy*, M and R Corporation, Buffalo, March 3 and 4, 1953).

⁴ This term was originally designated as T^cH_2O by Smith (2) but T^cH_2O is clearly better since it is indicative

given by the osmolar clearance minus the urine flow:

$$(1) \quad T^c_{H_2O} = C_{osm} - V$$

This study is concerned with the urinary concentrating operation, as represented by equation (1), during progressively increasing osmotic diuresis induced by mannitol in hydropenic and hydrated subjects, with and without the simultaneous administration of Pitressin.⁵

METHODS

Our observations have been made on subjects who had been admitted to Bellevue Hospital for minor or elective surgery or were about to be discharged after such surgery. They were selected with care to exclude cardiovascular or renal disease, and ranged in age from 15 to 46 years. Prior to observation they had been on the regular ward diet and unrestricted salt intake.⁶ Except where otherwise noted, they had taken no fluids during the previous 12 to 14 hours. In no case were they prehydrated in the sense of Ladd, 8 to 13 hours before the experiment (5), and it is assumed that their physiological state was that of individuals consuming only moderate quantities of fluid up to the time of fluid restriction.

Observations were made in the forenoon while the subjects were in the fasting state. To induce progressive and controllable osmotic diuresis, 10 per cent (hypertonic) mannitol solution was administered by a constant-rate infusion pump at a speed of 20 ml. per min., or a 5 per cent (isotonic) solution was administered at rates up to 40 ml. per min. Urine was collected by an indwelling bladder catheter at intervals of approximately 10 minutes and the bladder emptied with air and manual compression. Blood was drawn through a retention needle from the antecubital vein into heparinized syringes and

of the qualitative nature of the process. Brodsky, Rapaport, and West (3) have called this moiety the water economy of the kidney and indicated it as H_2O_{ec} . Brodsky (4) reports that during hydropenia and mannitol diuresis in man, this term reaches a level of 5 to 7 ml. per min. at urine flows exceeding 4 ml. per min., with small change at urine flows up to about 9 ml. per min.

⁵ The present investigation had its origin in Ladd's (5) observation that in subjects who have been prehydrated with a massive dose of water 8 to 13 hours before examination, the infusion of saline coupled with Pitressin leads to copious saline diuresis in which a constant quantity of water (ca. 2.0 ml. per 100 ml. of glomerular filtrate) was abstracted from an isosmotic urine.

⁶ Because of the widespread use of antibiotics pre- and postoperatively, it was unavoidable that over half of the subjects had received such medication, usually penicillin. No one, however, had received an antibiotic within 12 hours of study.

centrifuged immediately in rubber-capped tubes, and the plasma separated and stored in stoppered tubes.

The freezing-point method of Wesson (6), utilizing a thermistor, was used to measure the osmotic pressure of plasma and urine. To exclude a possible source of error, the osmotic pressure of arterial and venous blood drawn early during the infusion of 10 per cent mannitol at 20 ml. per min. was compared and found to agree within one per cent. The same osmotic pressure was observed when one part of a sample of blood was centrifuged under oil, the other in a capped tube but in contact with air.

The filtration rate was measured by the inulin clearance (C_{IN}). The inulin was administered at a constant rate, after an initial priming dose, by a second infusion pump. The resorcinol method of Roe as modified by Schreiner (7), was used for the determination of inulin in unyeasted urine and cadmium sulfate filtrates of plasma. In the calculation of the inulin and osmotic clearances a delay time of 2.5 minutes was allowed.

Sodium and potassium were determined with a Perkin-Elmer flame photometer (model 52A).

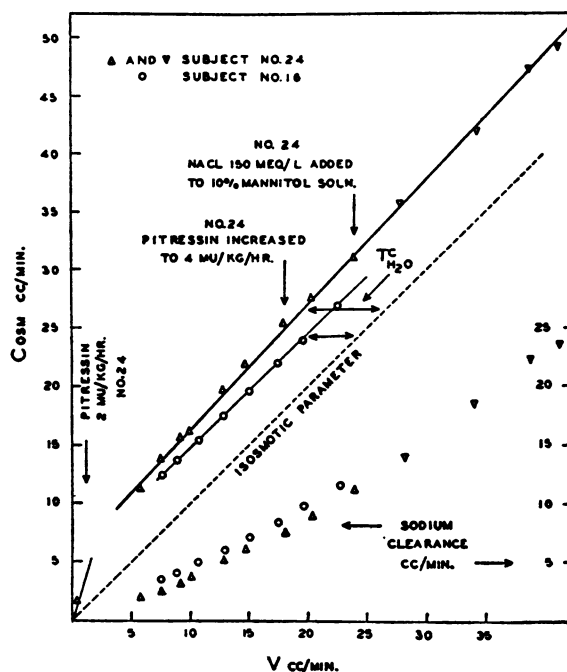


FIG. 1. EXAMINATION OF THE RENAL CONCENTRATING OPERATION IN TWO HYDROPENIC SUBJECTS RECEIVING 10 PER CENT MANNITOL INTRAVENOUSLY

The solute-free water abstracted from the urine is shown by the horizontal difference between the experimental curve and the isosmotic parameter. The slope in No. 24 is 1.063, and in No. 16 is 0.969. In so far as this slope deviates significantly from 1.0, $T^c_{H_2O}$ is not constant, and for the purposes of this paper the value interpolated when $V = 20$ ml. per min. has been reported in the tables. Possible reasons for inconstancy are discussed in the text.

TABLE I
Osmotic diuresis (10 per cent mannitol) in hydropenic subjects

1 Subject No.	2 Sex	3 Age	4 S.A.	5 Urine flow range	6 Correl. coeff.	7 Regres. coeff. (slope)	8 T_{H_2O} per 1.73 sq. m.	9 P_{osm} range	10 P_{Na} range	11 P_K range	12 Mean inulin clearance per 1.73 sq. m.
		yr.s.	sq. m.	ml./min.			ml./min.	mosm/L.	mEq./L.	mEq./L.	ml./min
1	M	22	1.70	3.6-12.3	.9990	1.168	7.4	287-288	146	5.1-4.9	117
2	M	15	1.60	7.0-20.8	.9992	1.104	4.6	280-289	144	4.4	104
3	M	18	1.73	10.1-21.3	.9998	1.012	5.4	286-295	146-141	4.3-4.8	139
4	F	43	1.72	8.0-23.4	.9997	1.054	4.9	294-300	137	4.7	75
5*	F	18	1.58	21.0-33.4	.9975	1.071	3.4	289-298	140	4.9	130
6	F	34	1.46	9.2-23.4	.9996	1.051	7.2	294-300	144	4.5	108
7	M	37	1.58	8.7-20.5	.9992	.987	4.2	286-295	136	4.9	95
10	M	21	1.49	9.2-23.6	.9998	1.046	6.2	292-293	139-139	4.5-4.9	91
11†	M	46	1.65	6.3-15.8	.9993	1.053	5.3	288-296	138-122	4.2-4.8	76
12	M	27	1.85	11.8-26.2	.9950	.916	4.1	286-291	139-136	4.5-4.4	108
13	F	30	1.57	8.1-26.7	.9999	.928	2.2	285-298	139-125	5.0-6.1	83
14	F	24	1.78	11.4-29.3	.9998	.863	1.5	291-302	135-128	4.6-4.8	85
15	F	27	1.62	9.5-24.2	.9983	1.016	6.4	278-280	134-123	4.1-4.3	137
16	M	18	1.56	7.7-22.7	.9999	.969	4.8	290-303	130-117	4.5-4.9	86
18	M	28	2.00	9.5-19.4	.9989	1.116	6.4	289-291	126-122	4.2-4.5	117
19	M	23	1.98	6.2-17.0	.9992	1.135	6.5	291-294	137-133	4.3-4.5	105
20	M	37	2.11	9.6-17.0	.9982	1.031	5.4	293-296	147-135	4.6-5.2	94
21	F	19	1.32	6.6-19.4	.9998	1.059	6.6	290-301	129-117	4.4-4.7	100
22	F	22	1.60	6.6-15.3	.9961	.976	4.9	286-296	132-118	4.9-5.3	107
23	M	28	1.95	5.5-16.8	.9997	1.02	4.9	287-293	135-132	4.4-4.6	92
27	F	28	1.51	9.3-20.0	.9997	1.029	5.2	291-302	122-116	4.7-6.1	85
Mean				8.8-21.4	.9989	1.033	5.1	288-295	135-127	4.5-4.9	102
Standard deviation						±0.075	±1.5				
Coefficient of variation						0.073	0.29				
Preinfusion mean ± S.D.								283±4.3	141±5.36	4.48±0.27	

* Rapid osmotic diuresis unexpectedly occurred early after the start of the mannitol infusion.

† Found at operation to have carcinoma of the stomach, and longstanding urethral stricture. Sodium excretion was low during the test.

Each series of observations on any one subject consisted of from 5 to 12 or more consecutive urine-collection periods obtained during increasing diuresis. The observations were generally continued until the urine flow had been increased by osmotic diuresis to 30 ml. per min. or more.

In all, 52 series of observations were made on 31 subjects; of these, 2 series on each of 3 subjects were made to check reproducibility.

RESULTS

Hydropenia without Pitressin

Twenty-one studies, on as many subjects (12 males and 9 females), were made postabsorptively and after abstinence from all fluids for 12 to 14 hours. Two to three liters or more of 10 per cent mannitol (20 ml. per min.) were generally required to establish a urine flow of 30 ml. per min. No Pitressin was administered, and no additional water was given during the test except in the form of occasional small pieces of ice to quench thirst. Smoking was not restricted.

Figure 1 shows a series of observations from one subject (No. 16) in this group. The osmolar clearance, C_{osm} , is here plotted against the simultaneous urine flow, V . In interpreting this figure it should be noted that if the glomerular filtrate underwent no osmotic concentration or dilution, the osmolar clearance would be identical with the urine flow over the whole range of observation, *i.e.*, all the data would fall on the bisector, which is here labeled the 'isosmotic parameter' (8). However, the osmolar clearance always exceeds the urine flow, the relations between the two being such that the data generate a linear regression line displaced to one side of the isosmotic parameter by a nearly constant amount, and having a slope of approximately 1.0. This regression line has been designated as the hypertonic parameter, an expression first used by Ladd (5, 8).

There are only two operations by which the kidney can transform the glomerular filtrate from the isosmotic to the hypertonic state: namely, by

the addition to the urine of solute without water, or by the removal of water without solute.⁷ During mannitol infusion, the urine flow is increasing primarily because of increasing quantities of mannitol claiming excretion, and the predominant urinary solutes are mannitol and sodium chloride.⁸ The concentration of potassium is negligible. The first explanation would require that mannitol or sodium be excreted by the tubules; since it is known that mannitol is excreted solely by filtration, and since there is no evidence for the tubular excretion of sodium, this explanation is unacceptable.

Alternatively, given a variable volume flow of isosmotic urine down the tubules, the rate of flow being determined *inter alia* by the amount of un-reabsorbed mannitol, passage from the isosmotic to the hypertonic state could be effected by the reabsorption of water, thus reducing the urine flow below the simultaneous osmolar clearance. This is the interpretation which we propose, and in Figure 1 this quantity of reabsorbed water is designated as $T^{\circ}\text{H}_2\text{O}$.

Table I summarizes the pertinent data on the 21 subjects in this group. Columns 1 to 4 of this table are self-explanatory; column 5 records the range of urine flow involved in the calculations shown in columns 6, 7, and 8; for reasons which will be discussed later, observations at urine flows below a critical value, which varies in different subjects, are excluded from these calculations. Column 6 records the coefficient of correlation calculated for linear regression. Column 7 records the slope of the regression line (regression coefficient). Column 8 records $T^{\circ}\text{H}_2\text{O}$ (corrected to 1.73 sq. m. S.A.) as determined arbitrarily at a urine flow of 20 ml. per min. Columns 9, 10, and 11 record the extremes of plasma osmolarity, sodium concentration and potassium concentration, respectively, at the minimal and maximal urine flows used in the calculations of columns 6 and 7. The average pre-infusion values of these terms are given at the bottom of the table. The last column records the average inulin clearance (cor-

rected to 1.73 sq. m. S.A.) during the entire duration of the mannitol infusion.⁹

The most striking feature of these data is the high coefficient of correlation for linear regression, which is invariably in excess of 0.995 (see column 6 of Table I), implying both considerable reliability in the individual observations and regularity in the underlying physiological processes.

The next most striking feature is the extent to which the slope of the regression lines in various subjects approaches unity; the mean slope in the 21 subjects in Table I is 1.033 ± 0.075 , yielding a coefficient of variation of only 7 per cent. In 15 out of 21 subjects, the deviation of slope from 1.00 is less than ± 10 per cent. The extent to which the slope approaches 1.00 over a wide range of urine flow is substantial evidence that the concentrating process operates basically by the extraction of an approximately constant quantity of water (identified in Figure 1 as $T^{\circ}\text{H}_2\text{O}$) from a variable volume of isosmotic urine. However, since the slope of the regression line in some subjects does in fact deviate significantly and consistently from 1.00, $T^{\circ}\text{H}_2\text{O}$ must tentatively be determined at an arbitrarily selected urine flow. For the purposes of this discussion only, we have chosen a urine flow of 20 ml. per min. At this urine flow, $T^{\circ}\text{H}_2\text{O}$ in these 21 subjects has an average value of 5.1 ml. per min. per 1.73 sq. m. body surface area. This average value has a high standard deviation (1.5) and coefficient of variation (29.4 per cent), but it must be emphasized that nothing is known concerning the possible effects of age, sex, diet, nutrition, or other factors on the concentrating operation, and also that the calculation at a urine flow of 20 ml. per min. is itself arbitrary.

Between the lowest and highest urine flow, the average plasma *osmolarity* increased (from 288 to 295 mosm per liter, as shown in column 9) in consequence of the infusion of hypertonic mannitol. This represents an increase of about 2.4 per cent and, added to pre-existing hydropenia, may

⁷ Presumably this operation is carried out low in the nephron, after the glomerular filtrate is substantially reduced in volume by isosmotic salt and water reabsorption.

⁸ As calculated from 10 subjects in Group I, mannitol supplies about two thirds of the osmotic pressure of the urine at a urine flow of 20 ml. per min.

⁹ Until near the conclusion of this work, commercial inulin was not routinely tested for hydrolysis, and it was subsequently discovered that in some of the ampouled material then in use a considerable fraction of the 'inulin' was fermentable by yeast and represents low molecular weight oligosaccharides, a circumstance that may have given erroneously low figures for the filtration rate. We therefore place little emphasis on this datum except as reflecting changes in filtration rate in any one subject.

have served to sustain endogenous antidiuretic hormone secretion.

The average plasma sodium concentration decreased from 135 to 127 mEq. per liter in consequence of dilution of body fluid by the infusion, withdrawal of water from the cells, and increased urinary excretion of sodium. No clinical evidences of hyponatremia were encountered.

The average plasma potassium concentration increased from 4.50 to 4.90 mEq. per liter.

The filtration rate underwent negligible changes in consequence of the mannitol infusion, some subjects showing a slight decrease toward the end of the experiment, others showing a slight increase.

Pitressin in variously hydrated subjects

In 13 of the 21 subjects reported above (7 male, 6 female) a second series of observations was made with mannitol plus Pitressin immediately after completion of the observations with 10 per cent mannitol alone. At the maximal urine flow shown in Table I, the mannitol infusion was stopped and the subject was given one liter of water to drink

to ameliorate the rather severe dehydration occasioned by the preceding osmotic diuresis. Pitressin (Parke, Davis & Co.) was given intravenously in a priming dose of 100 mU over a period of 3 to 4 minutes, followed by a sustaining infusion at a rate of 1 to 2 mU per Kg. per hr. up to the end of the test.¹⁰ One to one and one-half hours after the ingestion of water, at a time when the urine flow had fallen to some 8 to 10 ml. per min. in consequence of the elimination of the mannitol that had been administered previously, the second infusion of mannitol was started. Seven of the 13 subjects received 10 per cent mannitol at a rate of 20 ml. per min. (Subjects Nos. 1-6 and 15); the rest received a 5 per cent solution at rates of 30 ml. to 40 ml. per min., the intention being to attain as high a urine flow as possible within the practicable time of 1 to 1½ hours. The observations on these 13 subjects are recorded in Table II.

¹⁰ Possible inactivation of Pitressin in the infusion was examined in two subjects by doubling the sustaining dose in one (No. 24 see Figure 1) and by introducing a fresh solution midway in a test in the other one. There was no change in slope or $T^{\circ}\text{H}_2\text{O}$.

TABLE II
Re-examination of 13 of the subjects reported in Table I. Osmotic diuresis (5 and 10 per cent mannitol) in variously hydrated subjects with the addition of Pitressin

1 Subject	2 Sex	3 Age	4 S.A.	5 Urine flow range	6 Correl. coeff.	7 Regres. coeff. (slope)	8 $T^{\circ}\text{H}_2\text{O}$ per 1.73 sq. m.	9 P_{osm} range	10 P_{Na} range	11 P_{K} range
		yr.s.	sq. m.	ml./min.			ml./min.	mosm/L.	mEq./L.	mEq./L.
1*	M	22	1.70	9.2-16.4	.9998	1.153	8.5	283-286	141-133	4.90-4.4
2*	M	15	1.60	9.9-24.9	.9949	1.034	6.7	282-283	129-127	4.6-4.6
3*	M	18	1.73	9.1-21.9	.9970	1.006	6.6	287-288	-125	-4.7
4*	F	43	1.72	10.2-20.0	.9992	.999	5.7	296-301	123-120	4.9-4.6
5*	F	18	1.58	13.5-32.8	.9997	.948	7.8	294-298	130-120	5.0-4.8
6*	F	34	1.46	11.7-24.2	.9998	.999	5.9	305-303	128-121	4.7-4.0
10†	M	21	1.49	10.0-21.2	.9982	.928	6.2	277-280	128-122	4.5-4.3
11†	M	46	1.65	10.5-19.4	.9984	.940	5.3	292-293	125-118	-4.5
12†	M	27	1.85	11.2-24.8	.9931	.868	5.5	289-281	128-121	4.2-4.4
13†	F	30	1.57	11.8-24.5	.9996	.934	3.1	283-283	125-121	4.5-4.6
14†	F	24	1.78	16.8-42.5	.9997	.832	2.1	292-289	129-121	4.8-4.7
15†	F†	27	1.62	9.7-23.3	.9997	1.007	8.0	281-293	136-118	4.0-4.2
16†	M	18	1.56	12.0-18.3	.9962	1.036	2.8	287-296	133-117	4.5-4.5
Mean value				11.2-24.2	.9973	.976	5.7	288-290	129-122	4.6-4.5
Standard deviation						±.084	±2.0			
Coefficient of variation						0.086	0.35			

Corresponding mean values, standard deviation and coefficient of variation for the same 13 patients during hyponatremia alone, calculated for these subjects as reported in Table I:

9.5-23.3	.9992	1.020	4.9	288-303	138-129	4.6-4.8
		±0.070	±1.8			
		0.069	0.36			

* 10 per cent mannitol solution.

† 5 per cent mannitol solution.

‡ Not rehydrated.

TABLE III
Osmotic diuresis in hydrated subjects receiving Pitressin and 5 to 12.5 per cent mannitol solution

1 Subject No.	2 Sex	3 Age	4 S.A.	5 Urine flow range	6 Correl. coeff.	7 Regres. coeff. (slope)	8 T ² H ₂ O per 1.73 sq. m.	9 P _{osm} range	10 P _{Na} range	11 P _K range
		<i>yrs.</i>	<i>sq. m.</i>	<i>ml./min.</i>			<i>ml./min.</i>	<i>mosm/L.</i>	<i>mEq./L.</i>	<i>mEq./L.</i>
8	F	30	1.42	7.4-15.7	.9996	.966	5.0	276-272	125-	
9	M	31	1.79	7.4-12.2	.9941	.991	4.1	276-273	121-111	4.5-4.7
17	F	29	1.76	12.2-34.9	.9998	.874	3.7	275-275	130-121	4.2-4.0
28	M	25	1.83	8.3-21.3	.9986	.990	4.8	279-281	124-103	4.2-4.2
29	F	30	1.60	8.5-31.0	.9993	.863	2.2	277-284	134-121	4.8-
30	F	36	2.05	3.6-23.6	.9991	.855	3.8	285-290	144-134	4.1-4.0
31	F	25	1.65	6.5-23.8	.9990	.924	5.2	274-284	134-119	4.6
32	F	35	1.46	9.9-25.9	.9998	.874	3.6	279-281	132-120	4.3
Mean value				8.0-23.6	.9987	.917	4.1	278-280	131-116	4.3-4.2
S.D.						±.058	±1.0			
Coefficient of variation						0.064	0.24			
Two hydropenic subjects receiving Pitressin and hypertonic (10 per cent) mannitol:										
24	F	43	1.58	5.9-41.4	.9997	1.063	7.6	291-315	132-119	4.1-4.3
25	F	27	1.62	11.4-31.4	.9995	1.000	5.1	293-312	126-121	4.6-5.1

As between hydropenia alone and hydropenia combined with Pitressin, the mean slope changed from 1.033 to 1.020, a change to which no significance can be attached at this time. The slope decreased under Pitressin in 11 subjects, and increased in 2.

The mean value of T²H₂O increased from 4.9 ± 1.8 during hydropenia to 5.7 ± 2.0 during Pitressin. Nine subjects showed an increase in this value, 2 showed a decrease, and 2 did not change. Whether the change in mean value is significant is difficult to say because of the small number of subjects studied. Moreover, the conditions of the two tests with respect to the plasma sodium concentration and final osmolarity were not identical; also in the second series the subjects were initially in a more dehydrated state than in the first, despite the administration of water, a circumstance that may have influenced the concentrating mechanism itself. Therefore, we do not believe that the differences in the two series with respect to T²H₂O necessarily indicates that Pitressin plus hypertonic mannitol induces more effective anti-diuresis than hydropenia alone, an inference which is borne out by more critical experiments to be reported later.

Pitressin superimposed on hydration

Eight subjects (2 male, 6 female) in various degrees of hydration (breakfast with coffee or in some instances as much as a liter of water) were

examined with 5 to 12.5 per cent mannitol at infusion rates ranging from 20 to 40 ml. per min. and Pitressin in a priming dose of 50 to 100 mU followed by a sustaining infusion (Table III). This group shows the lowest average slope (0.917 ± 0.058) of any in our series. The mean value of T²H₂O (4.1 ± 1.0) in this series is lower than that recorded in Table I (5.1 ± 1.5). The group, however, is small and contains a preponderance of females (in whom all reported renal functions are less than in males), and again we cannot argue confidently that this difference has physiological significance.

Also recorded in Table III are observations on two hydropenic women (Nos. 24 and 25) infused with 10 per cent mannitol and Pitressin as described in the preceding paragraph. Since they received Pitressin they cannot be included with the patients listed in Table I, and since they were hydropenic they cannot be included with the subjects listed in Tables II and III. Both show a slope and correlation coefficient close to 1.0. Subject 24 is recorded in Figure 1.

Reproducibility in two consecutive tests made under identical conditions and on the same morning was examined in one hydropenic subject employing hypertonic mannitol alone (No. 7), and in two hydrated subjects employing isotonic mannitol and Pitressin in the priming and sustaining dosage used before. T²H₂O per 1.73 sq. m. increased from 4.2 in the first test to 5.3 in the sec-

ond test in the hydropenic subject, possibly because of the cellular dehydration occasioned by the first test. In the two hydrated subjects $T^{\circ}\text{H}_2\text{O}$ showed no change between the two tests.

Pitressin given in the course of hypertonic mannitol infusion

In order to eliminate complicating factors such as variable degrees of dehydration, etc., which are difficult to avoid in duplicate tests on the same subjects, Pitressin was added to an infusion of hypertonic mannitol midway in a continuous test in 6 hydropenic subjects. The first part of the test for these 6 subjects is reported in Table I (Nos. 18 to 23). Pitressin (priming and sustaining as described before) was added at the time when the maximal urine flow, as recorded in

Table I, had been attained, and without changing the rate or concentration of the mannitol infusion (10 per cent). Observations were continued for 3 to 10 urine collection periods, at which time the urine flow had reached rates of 25 to 30 ml. per min.

The results of these observations are illustrated in Figure 2, where the data on osmolar clearance and urine flow are plotted in such a manner as to bring into coincidence the time of Pitressin administration. These data show that the addition of Pitressin to a hypertonic mannitol infusion in hydropenic subjects has at most a slight effect on either the slope or $T^{\circ}\text{H}_2\text{O}$ (the actual values of the latter are not revealed in Figure 2 because of the use of elective coordinates). The observed deviations are fleeting in nature and small in magnitude, and appear to be referable to secondary, unidentified factors. We interpret these observations as indicating that hydropenia plus hypertonic mannitol produces maximal antidiuresis, and that the larger series of observations reported in Table I were obtained under maximal antidiuresis. This statement probably also applies to the subjects reported in Tables II and III with the qualification that the physiological status of these subjects was not the same as in those reported in Table I.

As a matter of interest, we have included in Figure 2 the sodium clearance, as related to urine flow, also plotted electively so as to bring into coincidence the administration of Pitressin. It is known that osmotic diuresis increases sodium excretion, a fact shown here by the progressive increase in sodium clearance as the urine flow increases. The administration of Pitressin does not appear to modify the uniformity of this relation.

It may also be recorded that Pitressin did not change the filtration rate, as shown by comparing the averages of two 10 min. periods immediately before and after Pitressin. The differences ranged from -1 to $+8$ ml. per min., which fall within the error of the method. In this observation we confirm Maxwell, Breed, and Smith (9).

The results presented in Figure 2 show that as between hydropenia plus hypertonic mannitol, on the one hand, and hydropenia plus mannitol and Pitressin on the other, there are no essential differences in slope, $T^{\circ}\text{H}_2\text{O}$, or sodium excretion, nor

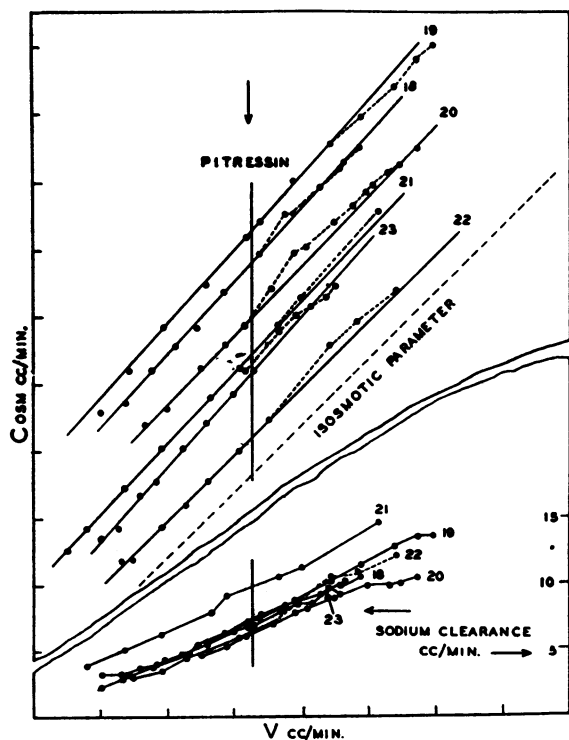


FIG. 2. THE EFFECTS OF PITRESSIN ON SLOPE AND $T^{\circ}\text{H}_2\text{O}$ WHEN ADDED IN THE COURSE OF A HYPERTONIC MANNITOL INFUSION IN HYDROPENIC SUBJECTS

The data on osmolar clearance and urine flow are plotted electively in order to bring into coincidence the time of Pitressin administration, and consequently the actual values of $T^{\circ}\text{H}_2\text{O}$ are not revealed by the graph. The slope, however, is not affected by this method of plotting. The data on the sodium clearance represent absolute values.

is there a perceptible change in the filtration rate, and it is therefore inferred that the action of Pitressin (in physiological doses) on the kidney is identical with that of the endogenous antidiuretic hormone. We believe that all our data are in agreement with this conclusion.

Effect of Mercurhydrin®

When 1 ml. of Mercurhydrin® was administered intravenously to 2 hydropenic subjects midway in time during 10 per cent mannitol plus Pitressin infusion, urine flows of 31 and 35 ml. per min. were reached, with no change in $T^{\circ}\text{H}_2\text{O}$ in one patient and possibly a slight decrease in the other. This observation confirms Ladd's (5) conclusion that Thiomerin® has no effect on the reabsorption of such water as is involved in the renal concentrating operation, a conclusion which may be inferred from the data of Welt, Goodyer, Darragh, Abele, and Meroney (10) though it is not so stated by these writers. Similarly, Page, Scott-Baker, Zak, Becker and Baxter (14) have found that Thiomerin® is without effect on the concentrating mechanism in the marine seal, *P. vitulina*.

DISCUSSION

Slope of the regression line relating C_{osm} to V

For $T^{\circ}\text{H}_2\text{O}$ to be constant, it is required that the slope of the curve relating C_{osm} to V must be 1.00 at all urine flows. The mean slope in the 31 subjects (derived from single tests or the first of duplicate tests) is 1.003 ± 0.083 (coefficient of variation 8.5 per cent), but the range is from 0.83 to 1.16.

If one allows an error of 5 per cent for methodological variations, $T^{\circ}\text{H}_2\text{O}$ has been found to be constant in only 12 out of 31 subjects (counting only single tests or the first one of duplicate tests on the same subject on the same day) and in only 23 out of 44 tests counting all tests separately. With respect to slope, the 31 subjects referred to above are nearly equally divided above and below 1.0.

In considering the possible reasons why the slope deviates from 1.0 in some subjects, it is important to note that any one subject, during consecutive observations extending over a period of two to three hours, reveals remarkable constancy in be-

havior as shown by the fact that the coefficient of correlation (relative to linear regression) of C_{osm} on V is invariably above 0.995, and frequently above 0.999.¹¹ This constancy in behavior is apparent even in subjects with a slope significantly greater or less than 1.0, as is shown by No. 24 in Figure 1, where the slope is 1.063 and the correlation coefficient is 0.9997, and by No. 14, who on two occasions showed the lowest slope of any subject studied (0.863 in Table I and 0.832 in Table II), and for whom the correlation coefficient was 0.9998 and 0.9997, respectively. This subject is illustrated in Figure 3, and it will be seen that in one test slightly hypotonic urine was excreted at urine flows above 33 ml. per min. This consistency suggests that we are dealing with factors which systematically affect the slope and which continue to operate in a regular manner in any one subject in observations extending over a period of several hours, and despite marked changes in urine flow.

Several factors which might contribute to variability in slope have been considered, but no one of them, or no two of them in combination, can be reconciled with the experimental data. A slope less than 1.0 could be explained during antidiuresis by the delivery of hypotonic urine to a distal concentrating mechanism which operated under the limitations of a fixed rate of reabsorption of water; but this explanation would require that the tubular urine/plasma osmotic ratio remain constant despite wide changes in urine flow induced by osmotic diuresis, a condition which renders the explanation implausible. Loss of Pitressin activity or too small a dose of Pitressin has been excluded as a cause of a slope less than 1.0 (Nos. 17 and 24).

¹¹ A similar high coefficient of correlation is shown between $U_{Na} V/kP_{Na} wC_T$ and V/wC_T in Ladd's (5) study of saline diuresis in prehydrated subjects who were receiving Pitressin.

In Ladd's studies, $T^{\circ}\text{H}_2\text{O}$ as calculated from sodium excretion alone had a value of only 2 ml. per min. per 100 ml. of glomerular filtrate. We cannot make an exact comparison with our figures because of some uncertainty in our data for the inulin clearance, but our average figure is 5.1 ml. per min. per 100 ml. of filtrate, a figure that is probably not very much in error. The remarkable difference in these figures may possibly be related to the fact that Ladd's subjects were prehydrated 8 to 13 hours before or to the fact that the loading substance was NaCl in Ladd's study.

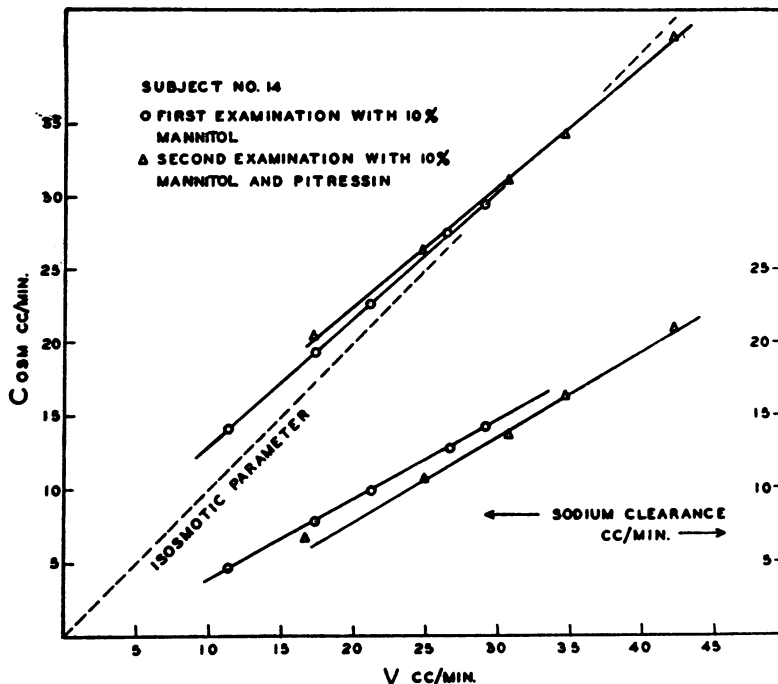


FIG. 3. DUPLICATE EXAMINATION OF ONE SUBJECT, FIRST IN THE HYDROPENIC STATE WITHOUT AND THEN WITH PITRESSIN AFTER PARTIAL REHYDRATION

Hypertonic mannitol was used in the first test, isotonic in the second. This subject showed the lowest slope of all subjects examined.

Conversely a slope greater than 1.0 could be explained by the delivery to the concentrating mechanism of urine which is consistently hypertonic, but this speculation is contrary to such information as is available on the composition of the urine in the proximal tubule.

A consistent over- or underestimation of the plasma osmolarity (method error) can be ruled out as causing the slopes to differ from 1.0, as both the plasma and the urine samples of each patient were measured consecutively and no longer than 2 to 3 hours after conclusion of the experiment.

Gradual attainment of the maximal antidiuretic state would increase the slope above 1.0, but our observations indicate that maximal antidiuresis is attained early in the tests and is not significantly enhanced by the administration of Pitressin. A slope greater than 1.0 would also be obtained if maximal activity in the concentrating mechanism were approached asymptotically with increasing urine flow, but the fact that the correlation coefficient is greater than 0.999, even in those subjects

in whom the slope of the regression line exceeds 1.0, argues against this assumption.

Dead space error of significant magnitude would tend to lower the slope during ascending urine flows, but in a non-linear manner and with negligible effects at high urine flows. In no case could it produce a slope above 1.0.¹² Nor does it seem possible to explain the deviations in slope by progressive changes in the volume of the dead space, or by the use of an incorrect delay time correction.

As matters stand, therefore, we are unable to find any satisfactory explanation for the fact that the slope of the regression line of C_{osc} on V shows the range it does.

Throughout the experimental part of this paper

¹² All observations reported in the body of the paper were made during increasing osmotic diuresis, but two subjects were also examined during descending diuresis. In both individually, the slope was slightly less during the descending than during the ascending phase, a result contrary to what would be anticipated if dead space error were significant.

we have used the designation $T^c_{H_2O}$ to indicate the water abstracted from the urine in raising it to the hyperosmotic state. We believe that our observations can be interpreted as indicating that during antidiuresis the concentrating mechanism operates essentially by the removal of a constant quantity (in ml. per min.) of solute-free water. The magnitude of this constant quantity we consider a fixed renal function under carefully controlled circumstances, a qualification which it is difficult to realize experimentally. This hypothesized function can be designated as $T^c_m H_2O$, the addition of the letter *m* indicating that the operation represented by $T^c_{H_2O}$ has a limiting maximal value. This limiting maximal rate, however, may be subject to secondary factors and if such arise in the course of the experiment (*e.g.*, progressive dehydration) the slope of the regression line will be affected.

Limiting osmotic U/P ratio

The relationship indicated by the regression line of C_{osm} on *V* obviously cannot hold when the urine flow has a value less than $T^c_{H_2O}$ and at low urine flows a new limitation, the maximal osmotic U/P ratio, is imposed on the process of urinary concentration (8).¹⁸ A constant osmotic U/P ratio requires direct proportionality between C_{osm} and *V*, and data conforming with a constant osmotic U/P ratio will fall on a regression line intersecting the ordinates and abscissae at zero, such as the short line shown at the extreme left of Figure 1. No special effort has been made in this study to examine the maximal osmotic U/P ratio at low urine flows, but it is apparent from unpublished experiments that a maximal osmotic U/P

¹⁸ In the majority of our subjects the osmotic U/P ratio in the control urine after 12 to 14 hours without fluids ranged from 2.5 to 3.87. Most of our figures are significantly lower than those reported by other investigators, for reasons unknown to us.

A possible explanation may be the following: All the patients were ambulatory. On reaching the 'unit' after a 3-minute walk they were put to bed and catheterized. The urine found in the bladder was discarded and the control urine was collected over a period of 20 to 30 minutes. We have noticed in several patients that the control urine elaborated in the supine position was markedly lighter in color than the discarded urine elaborated during the erect position; this was particularly striking in 2 rather anxious patients.

ratio can be reached only when the osmolar clearance is small, and that the transition from the one regression line to the other is gradual and characterized by significant splay. It is because of this gradual transition from one regression line to the other that we have necessarily excluded from our analysis data at lower urine flows where this transitional area has been entered. The lower limit of *V* at which linear regression ceases has ranged from 3 to 8 ml. per min. in different individuals.

Osmotic U/P ratio during osmotic diuresis

As the urine flow increases during antidiuresis in consequence of an increasing osmolar clearance, the osmotic relations between blood and urine pass gradually from the parameter determined by the limiting osmotic U/P ratio to the parameter determined by $T^c_{H_2O}$, so that the osmotic U/P ratio decreases asymptotically from the maximal osmotic U/P ratio to approach a value of 1.0 at large urine flows, giving rise to the approximately hyperbolic relationship originally described by Hervey, McCance, and Tayler (11) and subsequently by Rapoport, Brodsky, West, and Mackler (12).

Observation on the dog and seal

Since this work was initiated, a similar investigation in the dog has been completed and reported from this laboratory by Page and Reem (13), who conclude that at urine flows ranging from 11 to 28 per cent of the filtration rate, $T^c_{H_2O}$ had reached maximal values (7.6 and 6.2 ml. per min. per 100 ml. of filtrate), as judged by constancy and reproducibility, but the data do not rule out an asymptotic approach to these or slightly higher values. In the seal, Page, Scott-Baker, Zak, Becker, and Baxter (14), utilizing hydropenia, saline diuresis, and Pitressin, have found that at the highest values of C_{osm} attainable (16 to 18 per cent of the filtered load), an apparent maximal value of $T^c_{H_2O}$ was attained in only one animal—in all others this value continued to increase with increasing urine flow. This is perhaps related to the special adaptations of the seal as a mammal which lives on metabolic water, and to the fact that the maximal value of $T^c_{H_2O}$ must be as much as 10 ml. per min. per 100 Gm. kidney weight as

compared to 3.8 to 5.4 ml. in the dog, and some 1 to 2 ml. in man. It is possible that failure to attain maximal values of $T^{\circ}\text{H}_2\text{O}$ in the seal is related to the relatively large magnitude of this function.

SUMMARY

1. The urinary concentrating process has been examined in 54 tests on 31 normal subjects in the antidiuretic state by the induction of osmotic (mannitol) diuresis at urine flows ranging from 5 to 40 ml. per min. Antidiuresis was induced either by abstinence from fluids for 12 to 14 hours (hydropenia), by hydropenia supplemented by Pitressin, or by the administration of Pitressin to hydrated subjects.

2. The degree of osmotic concentration of the urine was determined by application of the cryoscopic method to plasma and urine, and the calculation of the quantity of water (in ml. per min. and here designated as $T^{\circ}\text{H}_2\text{O}$) required to restore the urine to an isosmotic state with respect to the glomerular filtrate.

3. The conclusion is reached that the concentrating mechanism operates essentially by the removal of a constant, maximal quantity of solute-free water irrespective of the urine flow, so long as the latter somewhat exceeds this maximal reabsorptive rate.

4. The presumed constancy of $T^{\circ}\text{H}_2\text{O}$ requires that the regression line generated by plotting the osmolar clearance (C_{osm}) against the urine flow (V) should have a slope of 1.00. In 31 subjects during a first examination on any one day, the slope averages 1.003 ± 0.083 . In 12 of these subjects it has a value between 0.95 and 1.05, which we do not consider to be significantly different from 1.0. In the remaining 19 subjects the slope deviates from 1.0 by more than ± 5 per cent, with extremes of 0.83 and 1.16. It is believed that unknown factors operate systematically in some individuals or under certain circumstances to cause slight, regular increases or decreases in $T^{\circ}\text{H}_2\text{O}$ with increasing diuresis.

5. As opposed to this apparent inconstancy of $T^{\circ}\text{H}_2\text{O}$ under our experimental conditions, the consistency of behavior of any one individual is remarkable, as shown by the fact that in all subjects, despite protracted observations and rather unphysiological circumstances, the coefficient of cor-

relation (relative to linear regression) of C_{osm} on V is invariably above 0.995, and frequently above 0.999.

6. This concentrating operation appears to be generally identical whether the antidiuretic state is induced by endogenous antidiuretic hormone, by Pitressin in physiological doses, or both.

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