A COMPARISON OF THE RELATIVE EFFECTIVENESS OF HYDROPENIA AND OF PITRESSIN® IN PRODUCING A CONCENTRATED URINE [1, 2]

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(Submitted for publication October 7, 1954; accepted February 16, 1955)

It is a common assumption that exogenous anti-diuretic hormone (Pitressin®) is equal to the endogenous anti-diuretic hormone (ADH) in its ability to stimulate the production of a concentrated urine. However, clinical studies of urine formation after the administration of Pitressin® or Pituitrin® leave some doubt as to the ability of the exogenous hormone to produce a maximally concentrated urine. Sodeman and Engelhardt (1) found that if urinary specific gravity was low at the time of Pituitrin® administration, the value attained was not so high as could be produced in the same subject by water deprivation. Similar observations were made by Little, Wallace, Whatley, and Anderson (2). Taylor, Peirce, and Page (3) gave Pituitrin® at frequent intervals for 12 hours and found the urine to be often more dilute and of larger volume than after a 12-hour period of water deprivation. In every case the amounts of anti-diuretic hormone administered were much greater than are assumed to be normally produced.

In the present study the ability of exogenous anti-diuretic hormone, administered as Pitressin®, to concentrate the urine has been reinvestigated in humans and in the dog. Because it is now recognized that the concentration of urine depends not only on the degree of hydropenia, but also on the prevailing rate of solute excretion (4–6), it seemed pertinent to investigate urinary concentration after Pitressin® administration during mild solute diuresis as well as under normal conditions of urine formation. To insure absence of circulating endogenous anti-diuretic hormone at the time Pitressin® was tested, the subjects were hydrated and having a water diuresis at the start of hormone infusion. In evaluating the results, the urinary concentrations after Pitressin®, expressed in milliosmols, have been compared with the urine osmolarity of hydropenic subjects, taking into account the fall in osmolarity normally occurring during solute diuresis.

It was found that once water diuresis was inhibited by the administration of Pitressin®, urine osmolarity slowly increased to approach, but rarely equal, values observed during hydropenia. Differences in plasma osmolarity in the hydrated-Pitressin® and hydropenic groups accounted to some extent for the discrepancy. When urine osmolarity was expressed as a function of plasma osmolarity, either as the osmotic U/P ratio or the urine-plasma osmotic pressure difference, values after a sufficient period of Pitressin® infusion were often comparable to those of hydropenic subjects providing solute diuresis was present. In the absence of solute diuresis, the results were not as consistent. Prolonged Pitressin® administration in several instances failed to elevate osmotic pressure differentials to values observed in hydropenic subjects.

METHODS

For the study 32 experiments were performed, 17 on eight female mongrel dogs and 15 on five male subjects. At least two experiments were usually performed on each subject, one to determine urinary concentration during hydropenia and a second to test the effectiveness of Pitressin® in concentrating the urine during hydration. All experiments were performed in the morning. The general plans of experimentation are described below.

[1] This investigation was supported in part by a research grant (H-1638) from the National Heart Institute of the National Institutes of Health, United States Public Health Service.

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Experiments on dogs

Previous experimental work (7) has indicated that dogs produce a more concentrated urine during hydropenia when the previous diet has been high in protein. Accordingly, in the present study, the animals were fed on a diet rich in protein (horsemeat) for two days preceding each experiment to insure conditions optimal for maximal urine concentration. If a portion of the diet was refused on either day, the experiment was not performed. All experiments were preceded by 18 hours of fasting. Urine was collected by indwelling catheter and blood either by venipuncture or from an indwelling polyethylene venous catheter. The blood was either mixed with a small amount of dry heparin or allowed to clot.

A. *Experiments on hydropenic animals:* Hydropenia was induced by 18 hours of water deprivation. In four of the experiments, the animals were anesthetized with Nembutal® (27 mg. per Kg.) administered intravenously, and in three, no anesthetic was used. Anesthesia appeared to have no influence on the results obtained. In experiments in which no solute load was administered, the procedure consisted simply in the collection of several urine specimens over intervals of 20 to 30 minutes. For experiments in which solute diuresis was induced, a priming dose of mannitol was given as a 20 per cent solution after one or two preliminary urine collections. The amount of priming dose varied; the largest dose was calculated to raise the plasma level to 32 milliosmols per L. and the smallest to 2.5 milliosmols per L. The priming dose was given over a 10 to 20-minute interval and was followed by a sustaining infusion amounting to 1.5 per cent of the priming dose per min. Following a period of time allowing for equilibration of the priming dose throughout its distribution volume, urine was collected every 10 to 20 minutes and blood specimens at appropriate intervals.

B. *Hydrated-Pitressin® experiments:* Preceding the experiments employing Pitressin®, the animals were hydrated by two loads of tap water given by stomach tube, each amounting to 5 per cent of the body weight. The first was given 16 hours and the second 45 to 60 minutes before experimentation. In 5 of the 10 experiments the animals were anesthetized with chloralose (90 mg. per Kg.) administered intravenously as a 1 per cent solution in 5 per cent glucose. Sufficient time was allowed for the absorption of the ingested water before the anesthetic was given. Experiments on animals anesthetized with chloralose did not differ in their results from the others. In experiments employing solute diuresis, hydration was maintained by the infusion of mannitol loads in large volumes of water. The volume of the priming injection amounted to 2 per cent of the body weight and of the sustaining infusion, 0.03 per cent of the body weight per minute. The concentration of mannitol in the infusions was never greater than 300 milliosmols per L. and, if less than 180 milliosmols per L., glucose was added in amounts sufficient to bring the total tonicity to this value. For experiments in which a load of mannitol was not given, a quantity of 3 per cent glucose or 1.5 per cent glucose—1.5 per cent fructose equal to 1 per cent of the body weight was infused rapidly before urine collection was started. This was followed by a continuous infusion of the same solution at the rate of 0.015 per cent of the body weight per minute. When urine collections indicated that water diuresis was approximately at its maximum, a priming injection of Pitressin®, amounting to 6.4 milliunits per kilogram contained in 4 cc. of 5 per cent glucose, was given by rapid intravenous injection and was followed by a continuous infusion of this agent at the rate of 48 mUnits per Kg. per hour contained in 30 cc. of 5 per cent glucose. Urine and blood collections were continued at appropriate intervals. Dilutions of Pitressin® were freshly prepared the morning of the experiment.

Experiments on human subjects

Preceding the experiments, the subjects were on regular diets. Breakfast was omitted the morning of experimentation. During the experiments, the subjects were recumbent, standing only to void.

A. *Experiments in the hydropenic state:* Hydropenia was induced by 15 hours of water deprivation. In experiments employing solute diuresis, the mannitol levels attained varied from 2.5 to 10 milliosmols per L. The experimental procedure did not differ essentially from that described for dogs.

B. *Hydrated-Pitressin® experiments:* The subjects were allowed water *ad libitum* the night before the experiment. In the morning just before or after arrival in the laboratory, 1,500 cc. of tap water was drunk over a 30 to 45-minute period. In the experiments in which the effect of solute diuresis was studied, a priming infusion of mannitol in 5 per cent solution was started when urine collections indicated that water diuresis was increasing. After equilibration of the priming dose and the collection of the two urine specimens during combined water and solute diuresis, a continuous intravenous infusion of Pitressin® was started. The rate of administration was 4 or 16 mUnits per kilogram per hour contained in 30 cc. of 5 per cent glucose. The collection of urine and blood specimens at regular intervals was continued. During the entire experiment, mannitol was infused as a 5 per cent solution at a rate of 1.5 per cent of the priming dose per minute. Hydration was maintained by ingestion of water in amounts approximately equal to the volumes of the urine.

In five of the experiments on hydrated subjects, inulin was included in the priming and maintenance solutions in amounts sufficient for the measurement of inulin clearance. The ampouled inulin was not subjected to alkali hydrolysis prior to use.

The freezing point of urine was determined with a Hortvet cryoscope and that for serum by means of a thermistor. Both instruments were calibrated by determining the freezing points of standard solutions of sodium chloride. The thermistor was calibrated several times during each series of determinations; the Hortvet cryoscope less frequently. The osmotic pressure of plasma

TABLE I

Effect of Pitressin® on urine flow and urine and plasma osmolarity when administered to the hydrated human subject

Period	Concurrent time min.	Urine flow cc./min./1.73 m²	Urine osmolarity mOsmols./L.	Plasma osmolarity mOsmols./L.
Experiment No. 56				
	−108 to −81	1,500 cc. tap water drunk*		
	−54 to −29	5% mannitol, 620 cc. i.v.		
	−29 to 83	5% mannitol, 9.3 cc./min. i.v.		
P-1	−26 to −13	20.1	153	284
P-2	−13 to −1	16.6	176	285
	0 to 83	Pitressin®, 4 mUnits/Kg./hr. i.v.		
1	−1 to 12	10.2	245	283
2	12 to 26	5.69	454	283
3	26 to 40	6.04	477	278
4	40 to 52	5.86	480	279
5	52 to 66	5.94	477	278
6	66 to 83	5.86	484	277
Experiment No. 121				
	−103 to −88	1,500 cc. tap water drunk*		
P-1	−58 to −33	9.24	90	
P-2	−33 to −19	11.3	59	
P-3	−19 to 3	14.5	49	
	0 to 198	Pitressin®, 16 mUnits/Kg./hr.		
1	3 to 25	1.88	242	
2	25 to 42	.846	605	281
3	42 to 70	.623	626	
4	70 to 91	.505	672	277
5	91 to 112	.468	693	
6	112 to 136	.459	739	277
7	136 to 154	.501	753	
8	154 to 178	.438	777	272
9	178 to 198	.406	787	

* Additional water was ingested during the experiments approximately equal in volume to the rate of urine flow.

and urine, expressed in milliosmols per liter, was calculated as:

$$\text{Osmolarity} = \frac{\Delta^\circ}{1.86^\circ} \times 1{,}000$$

Inulin was determined by a modification (8) of the method of Hubbard and Loomis (9).

RESULTS

Inhibition of water diuresis

Following administration of Pitressin® to the hydrated subjects, water diuresis was inhibited. Urine osmolarity promptly rose and urine flow diminished to values dependent on the amount of solute presenting for excretion. Data for typical experiments on human subjects are presented in Table I. In Experiment 56, mannitol as well as water diuresis was present prior to Pitressin® administration. Because of the magnitude of the excreted solute load, diuresis was still present after Pitressin® infusion and the urine was not highly concentrated. In Experiment 121, only a water diuresis was present prior to Pitressin® infusion. In contrast to conditions of solute loading, Pitressin® caused a marked fall in urine flow and rise in urine osmolarity. In these experiments, as in most of the others, urine became hypertonic to plasma during the second collection period after Pitressin® administration. Data for all collection periods in which the urine was hypertonic to plasma are included in subsequent figures.

Urine and plasma osmolarity in hydropenic subjects

In both hydropenic man and dog, urine osmolarity diminished with increasing solute diuresis as indicated in Figures 1 and 2. This relationship has been described previously (4–7) and has been shown to be independent of the composition of the urine or the nature of the solutes excreted. The present data indicate that at flows of less than 2 cc. per min. per m² in the dog, urine osmolarity varies widely. At higher flows in the dog and at all flows in man, the range is comparatively small and the values reproducible.

Prior to loading, the osmotic activity of plasma in hydropenic man was relatively constant and

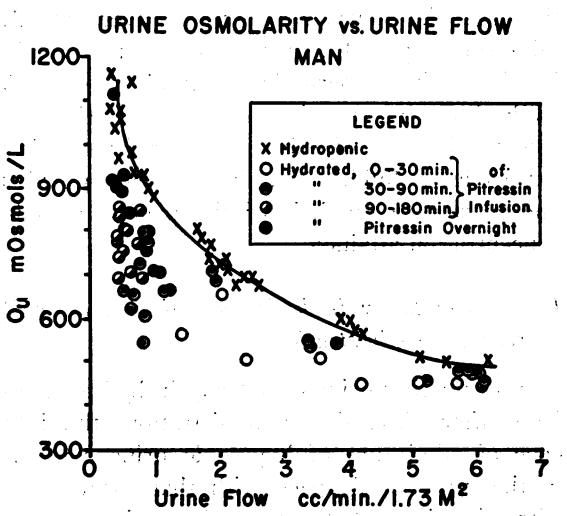

FIG. 1. URINE OSMOLARITY *versus* THE RATE OF URINE FLOW DURING HYDROPENIC AND HYDRATED-PITRESSIN® EXPERIMENTS (DATA FOR MAN)

The relation for hydropenic subjects is indicated by the hand-drawn line. Times after Pitressin® are for midpoints of urine periods.

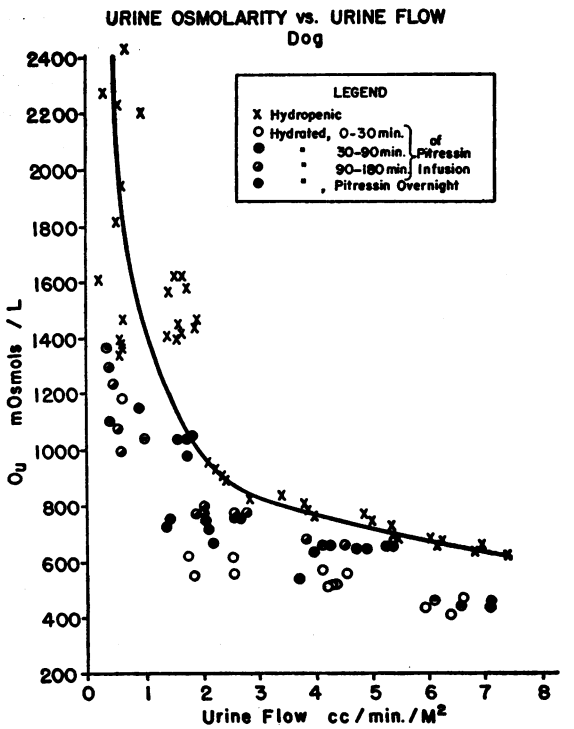

FIG. 2. URINE OSMOLARITY *versus* URINE FLOW DURING HYDROPENIC AND HYDRATED-PITRESSIN® EXPERIMENTS (DATA FOR THE DOG)

See legend Figure 1.

averaged 292 milliosmols per L. (Table II). In the dogs, the values were greater, averaging 324 milliosmols per L., and were more variable. Loading with hypertonic mannitol caused relatively little increase in plasma osmolarity, presumably because of shifts of water from cells to extracellular fluid. The increase amounted to one-quarter to one-half of the plasma mannitol concentration attained.

Urine osmolarity in hydrated subjects after Pitressin® administration

In evaluating urine osmolarity in hydrated subjects after Pitressin® administration, the influence of admixture of hypotonic urine contained in the renal dead space with that formed after Pitressin® is started must be borne in mind. A number of workers (10, 11) have given evidence that equilibrium of inflow and outflow in the renal system is not reached until thirty minutes after a change in experimental conditions. For this reason, urine collections with midpoints within thirty minutes after the start of Pitressin® infusions (Figures 1 and 2) are of doubtful physiological significance. After this interval, it would appear from the data of the experiments as given in Table I that a relatively steady state is reached.

In general, the urine osmolarities achieved after Pitressin® were, to a variable extent, less than during hydropenia. In the dog, urine osmolarity increased gradually after Pitressin® infusion but never attained values observed at comparable flows in hydropenia (Figure 1). In human subjects undergoing solute diuresis, urine osmolarity rapidly increased with Pitressin® infusion (Figure 2); values 30 to 90 minutes after start of Pitressin® were only slightly, albeit consistently, less than those observed at corresponding flows during hydropenia. On the other hand, in the absence of mannitol diuresis in man (flows usually less than 1 cc. per minute) Pitressin® was considerably less effective in producing a maximally concentrated urine. In four experiments, the highest urine osmolarity obtained after 90 to 180 minutes of Pitressin® infusion was 847 and the average peak value was 797 milliosmols per L. These values are in contrast with osmotic activities averaging 1,000 milliosmols per L. at comparable flows in hydropenic subjects.

The slow response and the failure to reach peak values with Pitressin® in the absence of solute diuresis might conceivably be explained as an artifact attributable to admixture of dilute urine contained in the dead space with the concentrated urine formed after hormone administration. This dilution effect would be of greater moment at low rates of urine flow than at the high rates of flow obtaining during solute diuresis. However, the experimental procedure and the pattern of response observed in these experiments make this

TABLE II

Average values and range of plasma osmolarity in man and dog during hydropenic and hydrated-Pitressin® experiments *

Condition	Human		Dog	
	Av.	Range	Av.	Range
Hydropenic (preloading)	292	290–294	324	314–332
Hydrated (during Pitressin®)	281	272–288	282	268–300

* Values are in mOsmols. per liter.

TABLE III

Urine flow and urine and plasma osmolarity in a hydrated subject given five pressor units of Pitressin® Tannate in Oil 15 hours previously

Period No.	Concurrent time *Min.*	Urine flow *cc./min./ 1.73 m²*	Urine osmolarity		Plasma osmolarity *mOsm./L.*	Osmotic U/P	
			Observed *mOsm./L.*	Expected* *mOsm./L.*		Observed	Expected*
Experiment 146							
Disc	— to 0	—	790			2.83	
1	0 to 25	.54	809	1,017	279	2.90	3.45
2	25 to 50	.90	801	905	275	2.91	3.10
	54 Aqueous Pitressin®, 35 mUnits i.v.						
	54 to 117 Aqueous Pitressin®, 4 mUnits/Kg./hr. i.v.						
3	50 to 77	1.09	716	875	275	2.60	3.00
4	77 to 98	1.25	669	847	274	2.44	2.91
5	98 to 117	1.00	723	892	274	2.64	3.05

* Values for corresponding rates of urine flow in hydropenic subjects (from relationship expressed by lines in Figures 1 and 3).

explanation unlikely. In Experiment 121 (Table I), for example, urine was voided at approximately 20-minute intervals, thus reducing the effect of bladder dead space. Equilibrium conditions appear to be established 40 to 60 minutes after the start of Pitressin® infusion. Thereafter urine flow showed relatively little change and urine osmolarity gradually increased at a rate of about 1 milliosmol per L. per minute. In this experiment, specimens obtained after three hours of Pitressin® infusion, during which time the subject had voided eight times, had osmolarities considerably less than observed in the hydropenic subject.[5] The procedure and data of the other three experiments of this type were essentially similar.

It would appear from the gradual increase in urine osmolarity with time during infusion of Pitressin® that eventually hydropenic values would be achieved. To test this possibility several experiments were performed on hydrated subjects in which Pitressin® was present in the circulating fluids for 15 hours before experimentation. The human subjects were given 3 to 5 units of Pitressin® Tannate in Oil intramuscularly at a time when normally hydrated the afternoon preceding the experiment. During and after the evening meal they drank 2,500 cc. of water. The values for urines collected the next morning in one such experiment are given in Table III and data for both experiments are shown in Figure 1 (solid circles). With the exception of one period (Figure 1), urine was of somewhat lower osmolarity than would be predicted from the relation between osmolarity and flow for hydropenic subjects. In both experiments the infusion of aqueous Pitressin® was without effect in further concentrating the urine. Similar experiments were performed on dogs. Pitressin® in oil as well as aqueous Pitressin® was given the afternoon before experi-

[5] It is obvious that under conditions of Experiment 121, the urine formed during a given period will be of greater concentration than that voided. The osmolarity of the urine formed may be calculated by a simple mixing equation, assuming the new formed urine to mix completely with dead space urine and that it is formed at a rate equal to the observed rate of urine flow. The equation is based on the identity

Number of solutes in urinary tract before mixing = Number of solutes in urinary tract after mixing

or

$$(V_{DS}O_{u_1}) + (VO_{u_e}) = O_{u_2}(V + V_{DS})$$

in which V_{DS} represents the dead space volume, O_{u_1}, the osmolarity of urine last voided (assumed osmolarity of dead space urine at start of period), O_{u_e}, the osmolarity of urine formed during period, V, the volume of urine formed during period (volume voided), and O_{u_2}, the osmolarity of urine voided at end of period.

Applying this equation to the last three periods of Experiment 121 and assuming a dead space volume of 30 cc. the osmolarity of the urine formed (O_{u_e}) was found to average 45 milliosmols per L. greater than that voided. Thus urine formed during period 9 would have, by these assumptions, an osmolarity of 822 rather than 777 milliosmols per L.

Solving the equation for V_{DS}, assuming that urine formed during the last three periods was concentrated to 1,000 milliosmols per L. (as would be predicted from hydropenic values), the calculation indicates that the urine formed would have to mix with dead space volumes of 110 to 180 cc. to account for the osmolarities observed.

mentation together with water to the amount of 5 per cent of the body weight by stomach tube. The next morning a second load of water equal in amount to the first was given and the experiment performed with aqueous Pitressin® infusion in the usual manner. In both of these experiments (Figure 2, solid circles), urine osmolarity was less than at comparable flows during hydropenia.

The two rates of Pitressin® infusion employed in the human subjects, 4 and 16 mUnits per Kg. per hr., did not differ significantly in their effects on urine osmolarity.

Interrelationships between urine and plasma osmolarity

Plasma osmolarity was in all cases lower in the hydrated than in the hydropenic subjects (Table II). The average difference in dogs amounted to 42 milliosmols per L. and in man to 11 milliosmols per L. It seemed possible that the lower urine osmolarity after Pitressin® might be in part related to the dilution of body fluids in the hydrated subjects. Accordingly, several expressions relating urine and plasma osmolarity were investigated as a means of further assessing concentrating ability after Pitressin®. These included the osmotic U/P ratios (O_u/O_p), the osmotic pressure differences of urine and plasma (O_u–O_p), and the water economy.

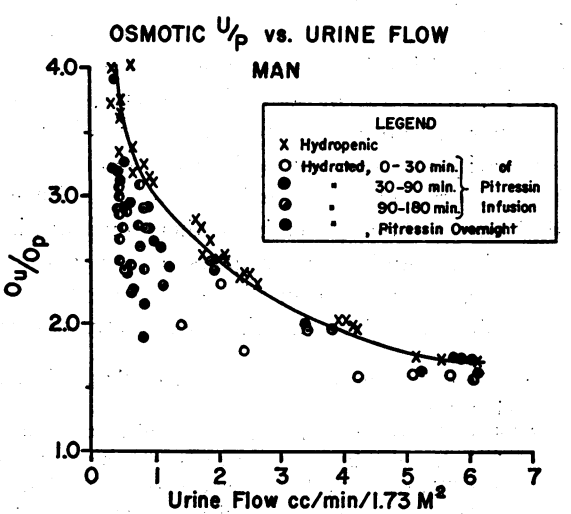

FIG. 3. OSMOTIC U/P RATIO *versus* URINE FLOW (DATA FOR MAN)

See legend Figure 1.

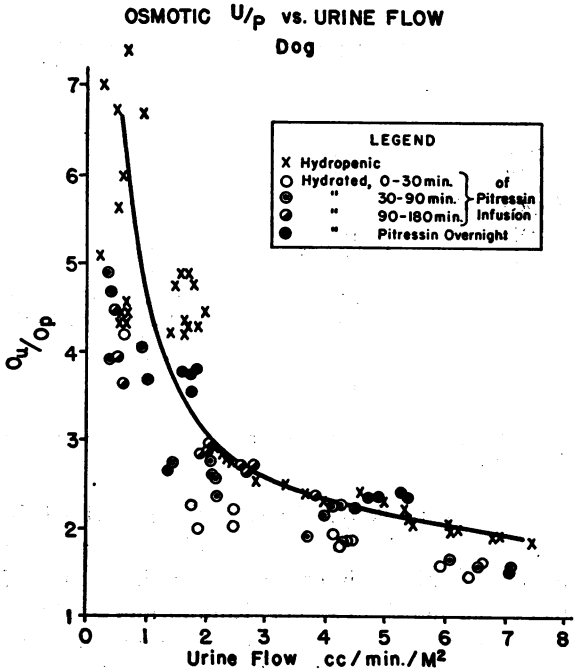

FIG. 4. OSMOTIC U/P RATIO *versus* URINE FLOW (DATA FOR THE DOG)

See legend Figure 1.

The relation between osmotic U/P ratios and urine flow for man and dog are shown in Figures 3 and 4, respectively. In man, the relative positions of hydropenic and hydrated-Pitressin® points were not significantly altered by expressing the data as the osmotic U/P ratio as would be predicted from the small differences in plasma osmolarity of hydrated and hydropenic subjects. Under conditions of solute diuresis, urines collected within 30 to 90 minutes of Pitressin® infusion gave osmotic U/P ratios differing little from hydropenic values. In the absence of solute diuresis, the agreement between hydropenic and hydrated Pitressin® values was also improved. Several specimens collected after Pitressin® had been infused for 90 to 180 minutes or had been present in circulating fluids overnight had osmotic U/P ratios comparable to hydropenic values. On other occasions, after Pitressin® had been present for similar periods, the ratios were significantly less than would be predicted from hydropenic values (Table I, Experiment 121, and Table III).

In the dog, expressing urine osmolarity as the osmotic U/P ratio (Figure 4) resulted in much better agreement of hydropenic and hydrated-

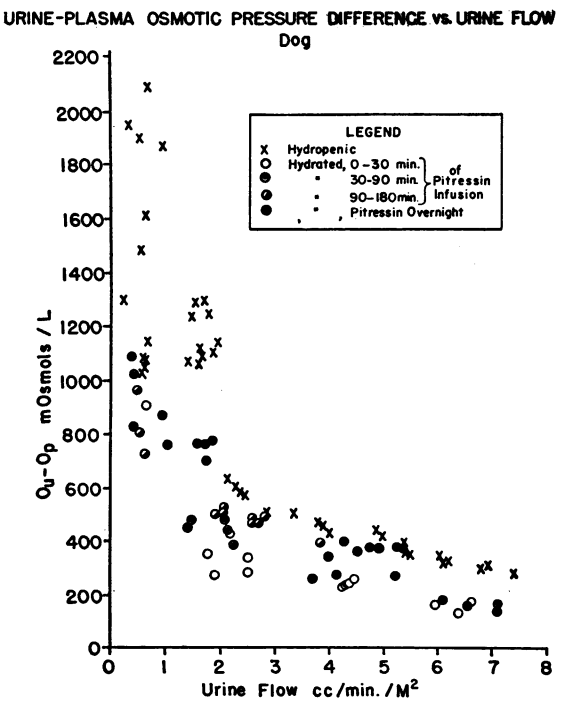

FIG. 5. THE URINE-PLASMA OSMOTIC PRESSURE DIFFERENCE PLOTTED *versus* THE RATE OF URINE FLOW (DATA FOR THE DOG)

Pitressin® values than was the case for the plot of urine osmolarity *versus* urine flow (Figure 2). After 90 minutes of Pitressin® infusion, and in some instances after a shorter interval, the osmotic U/P ratios were clearly comparable to those observed in hydropenia. As for the experiments in man, in the absence of solute diuresis, osmotic U/P ratios after Pitressin® tended to fall short of the high values observed in hydropenic animals but the scatter in this region makes assessment of the data difficult.

The osmotic activity differences between urine and plasma (O_u–O_p) in hydrated dogs (Figure 5), were in agreement with the hydropenic values only after overnight Pitressin® administration. With shorter periods of Pitressin® infusion, values of O_u–O_p tended to be lower than in the hydropenic animals. For man, the plot of O_u–O_p *vs.* urine flow showed agreement between hydrated and hydropenic values similar to that for osmotic U/P ratios (Figure 3) and is not shown.

The rate of water economy (7), termed the negative free water clearance or T^cH_2O by other workers (12), was also investigated in comparing data for hydrated-Pitressin® and hydropenic experiments. Water economy was calculated as the difference between the solute, or osmolar clearance and rate of urine flow [6] by the expression—

$$H_2O_{Ec} = \frac{O_u}{O_p} V - V$$

in which O_u and O_p are the osmolarities of urine and plasma respectively and V, the rate of urine flow. Because water economy is a function of the osmotic U/P ratio and urine flow, plots comparing water economy and urine flow show the same relation for hydrated-Pitressin® and hydropenic experiments as observed in plots of osmotic U/P ratio *vs.* urine flow. Data pertaining to water economy in man are shown in Figure 6. The graph is similar to that employed by others (13, 14) to assess the renal concentrating function. The solute (osmolar) clearance $\frac{O_u V}{O_p}$, has been plotted on the ordinate *vs.* urine flow on the abscissa. The diagonal line, termed the isosmotic parameter, indicates the relation between these variables which would have been observed had tubular fluid been unaffected by the concentrating mechanism and remained isosmotic with plasma. The horizontal distance from any point to the isosmotic parameter thus indicates the rate of economy of water resulting from the concentrating operation. It may be seen that at urine flows greater than 1.0 cc. per min., 30 to 90 minutes of Pitressin® infusion resulted in rates of water economy about equal to those observed in hydropenic subjects. On the other hand, at low rates of urine flow, shown in detail in the inset, hydropenic rates of water economy were reproduced by Pitressin® in some experiments; in others performed in an identical manner, the values were less than would be expected.

The effectiveness of Pitressin® in maintaining maximal urine osmolarity after hydration of the hydropenic subject

In view of the failure of Pitressin® to concentrate the urine maximally in the absence of solute diuresis in several experiments, it was of

[6] The water economy is the rate at which water must be reabsorbed to produce a concentrated urine, assuming reabsorption to occur from a tubular fluid isosmotic with plasma containing only the solutes to be excreted.

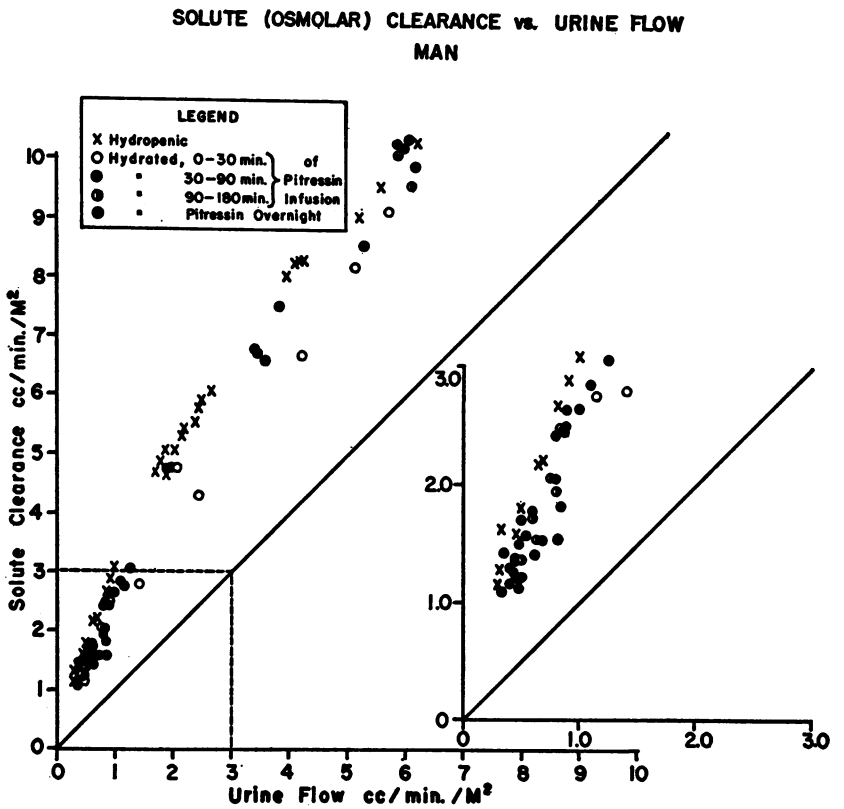

FIG. 6. THE SOLUTE CLEARANCE *versus* URINE FLOW (DATA FOR MAN)
The region within the dotted lines is shown in detail in the inset at right.

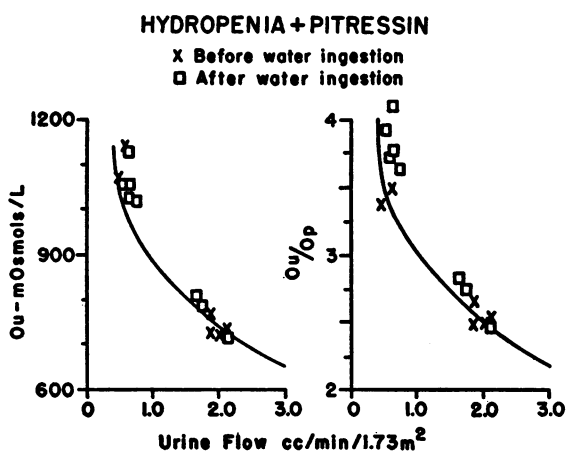

FIG. 7. URINE OSMOLARITY *versus* URINE FLOW (LEFT) AND OSMOTIC U/P RATIO *versus* URINE FLOW (RIGHT) BEFORE AND AFTER WATER INGESTION BY TWO HYDROPENIC SUBJECTS RECEIVING PITRESSIN®

In the experiment in which points group about urine flows of 2 cc. per minute, mild mannitol diuresis was present. In this experiment plasma osmolarity fell from 293 to 285 mOsmols. per L. with water ingestion; in the other, the fall was from 286 to 272.

interest to determine whether in the hydropenic subject Pitressin® would keep urine osmolarity at a high level after water ingestion. Two experiments of this type were performed. In one, the subject was undergoing mild solute diuresis with mannitol throughout; in the other, there was no solute diuresis. After several urines were collected while the subject was hydropenic and receiving Pitressin® at rates of 16 mUnits per Kg. per hr., 1500 cc. of water were ingested and the Pitressin® infusion continued. In one experiment, urines were collected for one hour after hydration and in the other for two hours. The results are indicated in Figure 7. In neither experiment was there any significant deviation from hydropenic values after hydration, either in urine osmolarity or in osmotic U/P ratio. Thus, it appears that Pitressin® will keep the urine at maximal osmolarity when the hydropenic subject becomes hydrated but may fail to produce a maximally concentrated urine when administered to the hydrated subject undergoing water diuresis.

Glomerular filtration rate

In four hydrated subjects, inulin clearances were measured during Pitressin® infusion. Average values [7] in three experiments in which there was concomitant solute diuresis with mannitol were 101, 111 and 78 cc. per min. per 1.73 m^2. In the fourth experiment in which no solute diuresis was present, the average was 140 cc. per min. per 1.73 m^2. In this experiment, Pitressin® was infused for 100 minutes and the maximum osmolarity achieved was 847 milliosmols per L. Unfortunately, clearances were not determined in hydropenic subjects. However, in similar loading experiments on 15 normal hydropenic subjects previously reported (15), the clearance of mannitol averaged 83.6 cc. per min. per 1.73 m^2 with a standard deviation of 15.2. The data suggest some enhancement of filtration rate in the hydrated subject.

DISCUSSION

Assessment of the present data as regards the ability of Pitressin® to concentrate urine rests in part on the nature of the concentrating mechanism and the means of measuring its activity. Page and Reem (16) from studies on the dog, and Zak, Brun, and Smith (14) from studies on man, have shown that at high rates of urine flow, attained by solute loading in hydropenic subjects, the rate of abstraction of solute free water from tubular fluid, or the water economy, tends to reach a constant maximal volume. Although in about one-half of the human subjects examined, this quantity demonstrated some systematic variation with changes in urine flow, its magnitude was nevertheless regarded as a fixed renal function. For its demonstration, the solute clearance (measured approximately by the rate of solute excretion) must be high so that a large quantity of water reaches the concentrating mechanism. At lower rates of solute clearance, approximating those of the present study, the rate of water abstraction by the concentrating mechanism diminishes. Under these conditions, the authors state that maximal urinary concentration is dependent on a limiting osmotic U/P ratio.

[7] Averages are for three or four urine collection periods. Values obtained within 30 minutes of the start of Pitressin® infusion are not included in the averages.

Assuming from these interpretations that under the conditions of the present study, the osmotic U/P ratio is a direct measure of the activity of the concentrating mechanism, it may be said that with solute diuresis in man and in the dog, Pitressin® acts as a maximal stimulus to the concentrating mechanism, provided it is infused over an interval of approximately thirty minutes or longer in man and ninety minutes in the dog. This interpretation is in accord with the studies of human subjects reported by Zak, Brun, and Smith (14).

A similar conclusion would be reached if the urine-plasma osmotic pressure difference were taken as a measure of the activity of the concentrating mechanism. Using this standard of reference, the sole difference in the interpretation would be that in the dog, Pitressin® must be present in circulating fluids for twelve hours or more before the concentrating mechanism is maximally stimulated. As a fundamental measure of the renal concentrating operation, the present study cannot discriminate among the osmotic U/P ratio, the urine-plasma osmotic pressure difference, and the water economy.

In the absence of solute diuresis, the results with Pitressin® are less consistent and more difficult to interpret. When administered to the hydrated subject undergoing water diuresis, urine osmolarity increased, at first rapidly and then more slowly. In only one experiment did the osmotic differentials attained come within the range of hydropenic subjects. Similar results were obtained after Pitressin® had been present in circulating fluids overnight. On the other hand, once maximal urine osmolarity is achieved in the hydropenic subject, it appears to be maintained by Pitressin® despite water ingestion. The latter result confirms the observations of Sodeman and Engelhardt (1) and agrees with data given in a recent study by Leaf, Bartter, Santos, and Wrong (17).

An artifact in these observations seems unlikely. It is, of course, possible, as mentioned previously, that in the experiments in which water diuresis preceded Pitressin® infusion, hypertonic urine formed after Pitressin® was given was contaminated with residual hypotonic urine present in the renal dead space. However, the conduct of the experiments with frequent voidings and the data

indicating the attainment of relatively steady state makes such an artifact seem unlikely. One conclusion which seems obvious is that with Pitressin®, ceiling values for urine-plasma osmotic differentials are achieved much more slowly at normal rates of solute excretion than under conditions of solute diuresis. The reason for this is not clear. It is conceivable that the high urine-plasma osmotic differentials characteristic of low rates of urine flow require greater activity of the concentrating mechanism for their production than the lower differentials (or the tubular maximum for water abstraction) present during solute diuresis.[8] If such is the case, the ability to attain high values at low rates of flow might then constitute a test of the concentrating mechanism more sensitive than that correlating urine osmolarity with urine flow during solute diuresis (15). The results give no evidence for and, in fact, make doubtful the assumption that a greater blood concentration of ADH is needed to induce maximum anti-diuresis when the rate of solute excretion is high than when it is in the usual range (19).

The experiments in which Pitressin® was present in circulating fluid overnight give only meager support to a conclusion that might be drawn from the data of the acute experiments, namely, that with prolonged Pitressin® infusion maximal osmotic differentials would always be achieved. After Pitressin® had been present for 15 hours, maximal values were observed for a few periods in one experiment. In the other, the values were less than predicted. In these experiments there was no possibility of contamination with hypotonic dead space urine. The possibility that the blood concentrations of Pitressin® were too low to produce maximum osmotic differentials seems unlikely from the fact that infusion of the aqueous material produced no increase in concentration. The results in general confirm the more extensive studies by Taylor, Peirce, and Page (3) in which specific gravities were employed to measure urinary concentrations. As was pointed out by these authors, not only may the urinary concentrations be less after Pitressin® than during hydropenia, but also the results are not consistent even when the agent is given over prolonged periods. Because of the inconsistencies, use of Pitressin® as a substitute for water deprivation in clinically testing the concentrating function of the kidney would appear to be of limited value.

A factor which should not be overlooked as contributing to the generally lower and inconsistent osmotic differentials in the absence of solute diuresis is the hydration of the subjects receiving Pitressin®. There are a number of ways by which hydration could conceivably influence the concentrating mechanism. Dilution of body fluids might diminish the concentrations of certain intra- and/or extracellular solutes, critical for maximal activity of the concentrating mechanism. The possibility that hydration might variably stimulate secretion of adrenal cortical hormones antagonistic to Pitressin®, evidence for which has recently been reviewed (20), should also be considered. Similarly, substances with anti-diuretic activity other than ADH might be present in the hydropenic subject, supplementing the action of the pituitary hormone and disappearing with hydration. Finally, differences in filtration rate in the hydrated and hydropenic subjects might be responsible for the differences in urinary concentration.

Data in the literature, however, appear to indicate that hydration is not a significant factor responsible for the lower and inconsistent urinary concentrations after Pitressin®. In the experiments by Taylor, Peirce, and Page (3), no attempt was made to hydrate the patients during Pitressin® administration, yet the urinary concentrations measured by specific gravities were often lower than after water deprivation. Perhaps more germane are observations made on patients with diabetes insipidus. Studies by Brodsky and Rapoport (21) suggest that even when hydropenic, patients with this disease while receiving Pitressin® fail to produce a maximally concentrated urine at low rates of urine flow. Subsequent examination, in this laboratory, of two additional patients with diabetes insipidus have confirmed these observations. On the other hand,

[8] It should be noted that this assumption is not in accord with thermodynamic calculations of the theoretical, minimal energy required for the production of a concentrated urine. When "distal osmotic" work is calculated as described in a previous paper (18), by substituting in the equation of von Rohrer values for urine and plasma osmolarity, the minimal energy requirement is found to be relatively small for highly concentrated urine at low rates of flow as compared to conditions of mild solute diuresis.

data given by Leaf, Mamby, Rasmussen, and Marasco (22) would suggest that maximal urine osmolarity may occasionally be achieved by these subjects. It is of interest that under conditions of solute diuresis, subjects with diabetes insipidus given Pitressin® and rendered hydropenic are able to produce urine comparable in concentration to that of normal individuals (21). Their behavior under both loading and non-loading conditions thus appears to correspond closely to that of the hydrated normal subject given Pitressin®

Data on glomerular filtration rate are too scanty in the present study to establish a relationship between this function and urinary concentration. The observations do suggest that filtration rate was greater in the hydrated than in hydropenic subjects, a trend which has been frequently reported. Reports by other workers on the influence of filtration on urinary concentration allow some prediction as to the effect of this function in the present study. Platt (23) has pointed out the importance of considering the osmolarity-flow relationship in terms of nephron flow. When a large fraction of the renal tissue is removed in the experimental animal, renal concentrating ability is reduced although the nephrons remaining are presumably not injured. Since the partially nephrectomized animal has daily rates of solute excretion differing little from the normal, it is apparent that in terms of the nephrons remaining, a solute diuresis is occurring, a condition in which a reduced urine osmolarity would be predicted. This observation points out that the osmolarity-flow relationship would be more precisely expressed in terms of flow per nephron rather than total urine flow. The relationship observed with total flow is fairly well defined because a significant change in the number of functioning nephrons probably does not occur with solute diuresis (24) so that average flow per nephron is a relatively fixed fraction of total flow. Applying this concept to the hydrated-Pitressin® experiments, if activation of previously inactive nephrons accounted for the enhancement of filtration rate, average flow per nephron would be a smaller fraction of a given total flow and the osmolarity-flow relationship would be shifted to the right, *i.e.*, urine osmolarity would be greater at a given flow. Alternatively, if the increase in filtration rate occurs, as commonly believed, without change in the number of functioning nephrons, flow per nephron as a fraction of total flow would be the same as in hydropenic subjects and the osmolarity-flow relationship would be unchanged. This prediction has experimental verification in the studies of Thompson and Barrett (25). Moderate reduction in filtration rate in dogs undergoing mannitol diuresis by inflation of a balloon catheter in the aorta had no effect on the osmolarity-flow relationship. Marked reduction, presumably accompanied by a diminished number of functioning nephrons, resulted in a lower osmolarity than in the normal animal. The observation suggests that under relatively normal conditions, alterations in filtration rate unaccompanied by a change in the number of functioning nephrons will not affect the osmolarity-flow relationship.

SUMMARY

Administration of Pitressin® in supramaximal amounts to hydrated man and dog resulted in urine osmolarities somewhat less than in hydropenic subjects at comparable rates of urine flow. The discrepancy was reduced when the lower plasma osmolarity of the hydrated subject was taken into account. When the data obtained under conditions of solute diuresis were expressed in terms of the osmotic U/P ratio or the urine-plasma osmotic pressure difference, values for hydropenic and hydrated-Pitressin® conditions were comparable.

In the absence of solute diuresis, particularly in man, less consistent results were obtained. The urine-plasma osmotic differentials approached hydropenic values much more slowly than when Pitressin® was administered during solute diuresis and in several experiments in which Pitressin® was present in circulating fluids for prolonged periods, maximal values were never attained. On the other hand, water ingestion by the hydropenic subject receiving Pitressin® did not lower the osmotic differential. The reason for these inconsistencies is not known.

ACKNOWLEDGMENT

The technical assistance of Mrs. Ruth Bayless, Mrs. Ethel Barber, and Miss Anne Hobson is gratefully appreciated.

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