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## The Effect of Various States of Hydration and the Plasma Concentration on the Turnover of Antidiuretic Hormone in Mammals \*

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To evaluate the rate of production and turnover of antidiuretic hormone (ADH), it is essential to know its volume of distribution. Our previous paper (1), and those in the literature (2-4), have suggested that this volume approximates the plasma volume, and most authors have explained this as the result of binding of ADH to plasma proteins. In the accompanying study (1) on the physiology of ADH in man, we were unable to demonstrate the binding of the hormone to plasma proteins using *in vitro* ultrafiltration and dialysis techniques. Despite this, the volume of distribution of arginine-vasopressin, calculated from the injected dose and the extrapolated zero time concentration in the plasma, was only 2.5% of the body weight, a volume significantly less than plasma volume. We concluded, however, that under the circumstances of these experiments the volume calculated from the extrapolation technique was erroneously low. In the same study we found that the turnover rate of both endogenous and exogenous ADH (arginine-vasopressin) in humans was related to the state of hydration. Three days of dehydration significantly accelerated while a comparable period of sustained over hydration slowed the observed normal turnover rate.

The present study was undertaken in an effort to determine the true volume of distribution in animals, using a constant infusion technique, and to determine whether the fractional turnover rate

was truly influenced by the state of hydration per se or by the different levels of circulating ADH resulting from the various states of hydration.

### Methods

*The fractional turnover rate and volume of distribution of endogenous and exogenous ADH*

*Dog experiments.* 1) *The disappearance curve of endogenous ADH and injected arginine-vasopressin in the water-loaded animal.* Four trained female dogs were deprived of fluids for 12 hours. At the start of the experiment a sample of blood was drawn from an indwelling needle in the foreleg vein, and the animals were hydrated by stomach tube with 800 to 1,000 ml of tap water (5% of body weight). This state of hydration was maintained by a continuous iv infusion of 2.5% glucose in 0.45% sodium chloride at a rate equivalent to urine output. At suitable intervals blood samples were obtained for ADH determination. Timed urine specimens were collected by an indwelling catheter. The osmolality of the urine samples was measured by freezing point depression, using a Fiske osmometer.<sup>1</sup> ADH was assayed by a modification of the bioassay of Heller and Stulc (1, 5). After maximal water diuresis was attained, two of the dogs were injected intravenously with 2.5 mU of synthetic arginine-vasopressin,<sup>2</sup> and its disappearance from the circulation was followed. From these data the fractional turnover rates and apparent volume of distribution were calculated.

2) *Steady-state levels of ADH during a constant infusion of arginine-vasopressin into hydrated animals and after stopping the infusion.* Two trained dogs were hydrated by stomach tube and prepared similar to the above experiments. At the point of maximal water diuresis, when the level of ADH was zero ( $< 0.25 \mu\text{U}$  per ml), vasopressin was added to the constant intravenous infusion.

One dog received 230  $\mu\text{U}$  per minute for 1 hour, then 470  $\mu\text{U}$  per minute for 1 additional hour and, finally, 1,700  $\mu\text{U}$  per minute for 50 minutes. The second dog received

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<sup>1</sup> Fiske Associates, Inc., Bethel, Conn.

<sup>2</sup> Mr. Harry Althouse of the Sandoz Co., Hanover, N. J., donated the supplies of arginine-vasopressin.

4,000  $\mu\text{U}$  per minute under similar conditions. The volume of distribution of the infused vasopressin was calculated from the following relationship:  $I/V = Kc$  or  $V = I/Kc$ , where  $c$  = the constant concentration of ADH in plasma attained during the infusion,  $K$  = fractional turnover rate per minute calculated from the slope of the semilog disappearance after the infusion of vasopressin is stopped,  $I$  = rate of infusion of ADH in microunits per minute, and  $V$  = volume of distribution. After the highest constant levels were attained in each animal (19 and 40  $\mu\text{U}$  per ml, respectively), the vasopressin infusion was stopped, and the disappearance of the hormone from the circulation was followed.

An additional experiment was designed to examine the concentration of ADH in the interstitial space while the plasma level was elevated by a constant infusion of vasopressin in a pentobarbital-anesthetized dog. Lymph was considered a reasonable approximation of interstitial fluid. The lymph samples were collected from a large vessel entering the cisterna chyli. To avoid contamination with liver lymph, the hepatic arteries were ligated. A portacaval shunt operation was performed to prevent venous stasis in the gastrointestinal tract subsequent to the arterial ligation. The ADH content of simultaneously collected samples of blood and lymph were compared.

*Rat experiments.* 1) *The disappearance curve of ADH after a single intravenous injection of arginine-vasopressin into hydrated rats.* Female rats of the Sprague-Dawley strain weighing 100 to 130 g were divided into groups of three to five animals. The average weight in each group was 115 g. The rats were hydrated and anesthetized by the intragastric administration in divided doses of 12% ethanol in water. A total volume of 8% of their body weight was administered. Immediately after the hydration, 25 animals (lot A) were injected intravenously with 5  $\mu\text{U}$  and 43 animals (lot B) with 150  $\mu\text{U}$  of arginine-vasopressin contained in 0.5 ml of saline. All injections were given into a tail as rapidly as possible. One group (3 to 5 animals) in each lot was killed immediately after the injection by decapitation and their blood pooled for ADH assay. The bulk of the blood from each animal was collected in about 5 seconds. This time was consistent from animal to animal. The other groups were killed similarly in intervals of 2 minutes. From the concentration of ADH in the plasma of the pooled blood of each group a disappearance curve was plotted.

2) *"Steady-state" levels of ADH after constant infusion of arginine-vasopressin.* Four rats weighing 120 g were hydrated as in the former experiment; 10 to 15 minutes after the hydration they were infused intravenously with 2.0  $\mu\text{U}$  per minute of arginine-vasopressin for 1 hour. The infusion was continued up to the moment of decapitation, and their blood was collected individually into heparinized tubes. From the ADH concentration of their plasma the volume of distribution was calculated using the formula described above in the dog experiments. The  $K$  value used for these 4 rats was

that derived from the experiments in the above paragraph (1).

3) *Turnover rate of circulating endogenous ADH in a patient with vasopressin-resistant and three patients with vasopressin-sensitive diabetes insipidus.* Patient D.K., a 53-year-old male with congenital nephrogenic diabetes insipidus, was described in detail in a previous publication (6). Thiazide diuretics were discontinued 2 weeks before the experiment. On the day before the experiment a random sample of blood was drawn and assayed for its ADH activity. On the day of the experiment the patient was taken off fluids for slightly longer than 2 hours. At the end of this period he complained of severe headache, muscle weakness, and extreme thirst. He described these symptoms as typical of water deprivation of this duration. After procaine infiltration an indwelling needle was painlessly inserted into a forearm vein, and a blood sample was taken for zero time concentration of ADH in his plasma. Immediately after this a rapid infusion (20 ml per minute) of 0.45% sodium chloride was started and continued for 52 minutes. He then drank 1,000 ml of water and continued drinking in amounts equal to his urine output. After 50 minutes the patient was taken off fluids and the experiment continued for another hour. During the whole test he reclined in bed, standing only to void. At suitable intervals samples of blood were drawn for ADH assay, timed urine samples were collected, and their osmolality was determined. Similar experiments were carried out on three patients with vasopressin-sensitive diabetes insipidus. The only difference was that these patients were hydrated orally. They initially drank 1,000 to 1,500 ml of water and subsequently orally replaced a volume equivalent to urinary output. Patient S.P., a 30-year-old female, had congenital diabetes insipidus and anterior pituitary insufficiency secondary to removal of a craniopharyngioma at the age of 9. He had been maintained on glucocorticoid, thyroid, and androgen replacement therapy that was not interrupted for the present experiments. Both of these patients stopped their Pitressin tannate therapy 72 hours before the experiments. The third patient, S.K., a 29-year-old male, developed diabetes insipidus of undetermined etiology in early childhood. The diagnosis was confirmed, and the present experiment was carried out during the same hospital admission. Before this admission he had never received therapy for his disease. Patients S.P., B.G., and S.K. were injected with 10, 10, and 25 mU of arginine-vasopressin, respectively.

*Ultrafiltration and dialysis of endogenous ADH.* Forty ml of blood was drawn from each of four dogs after 15 hours of water deprivation, and 40 ml was pooled from ten decapitated rats after a similar period of water restriction. Immediately after clotting, the serum was separated and part of it placed in Lavietes (7) anaerobic ultrafiltration units at both 20° and 37° C. One unit, set up in such a way that no ultrafiltration occurred, served as a control. Two 10-ml samples of dog and one 10-ml pooled sample of rat serum were each dialyzed against 50 ml

TABLE I  
The disappearance of endogenous and exogenous vasopressin in water-loaded dogs\*

Elapsed time	Blood sample no.	Urine sample no.	Urine flow				Plasma ADH†			
			Dog				Dog			
			1	3	5	6	1	3	5	6
<i>min</i>			<i>ml/min</i>				<i>μU/ml</i>			
-10	B <sub>0</sub>						6.0	4.0	4.8	6.0
-5 to 0‡			Water load 5% of body weight by stomach tube +4.4 ml/min iv infusion of 2.5% glucose in 0.45% saline infusion started at zero time							
2		U <sub>0</sub>								
15	B <sub>1</sub>						1.5	1.0	1.2	1.5
30	B <sub>2</sub>						0.4	0.25	0.25	0.4
40		U <sub>1</sub>	3.8	2.66	3.2	5.6				
45	B <sub>3</sub>						0	0	0	0
55		U <sub>2</sub>	6.5	8.24	8.0	8.2				
60			2.5 mU arginine-vasopressin iv							
70	B <sub>4</sub>							2.0	2.5	
71		U <sub>3</sub>		3.0	4.0					
80	B <sub>5</sub>							0.8	1.0	
85	B <sub>6</sub>							0.5	0.6	
95		U <sub>4</sub>		2.5	5.0					

\* Dog weights (kg): 1 = 15.5, 3 = 18.4, 5 = 15.0, and 6 = 20.9.

† ADH = antidiuretic hormone.

‡ Completion of the oral water load represents zero time. The zero-time plasma concentration of ADH is considered equal to the basal steady-state plasma level. This assumes that the ADH concentration in the plasma has not changed appreciably during the 5 minutes of gavage.

Ringer's solution for 6 hours at 37° C with continuous stirring, by the technique described by Craig and King (8). The dialysis bag was filled completely so that no visible air space was left. In both ultrafiltration and dialysis Visking membrane<sup>3</sup> was used. The ADH content of the ultrafiltrates, the control serums, and the fluid on both sides of the dialyzing membrane were determined.

Results

The fractional turnover rate and volume of distribution of endogenous and exogenous ADH

*Dog experiments.* Table I shows the results of four dog experiments, and Figure 1 demonstrates the disappearance curves of endogenous and exogenous ADH. The basal concentrations of ADH in microunits per milliliter in the plasma of these animals were 6.0, 4.0, 4.8, and 6.0, respectively. The disappearance curve of endogenous and exogenous ADH approximated a straight line when plotted on semilogarithmic paper. The slope of these lines is extremely constant, representing a fractional turnover of 0.0924 per minute and a t<sub>1/2</sub> of 7½ minutes. Figure 2 compared the above curves with those of two hydrated dogs whose

plasma level of ADH was raised to 19 and 40 μU per ml, respectively, by continuous intravenous infusion of arginine-vasopressin. The slopes from the higher values down to 10 μU per ml are steeper, whereas below 10 μU per ml they are similar (Figure 2). The fractional turnover rate for the steeper part of the curve is 0.139 per minute, and the t<sub>1/2</sub> is 5 minutes.

*Rat experiments.* Table II gives the results in

TABLE II  
Disappearance curve of exogenous ADH from hydrated rats after iv injection of 5 and 150 μU arginine-vasopressin

Elapsed time	Lot A		Lot B	
	Normal rats in group	Plasma ADH content of pooled blood	No. of rats in group	Plasma ADH content of pooled blood
<i>min</i>		<i>μU/ml</i>		<i>μU/ml</i>
-1	3	0	3	0
0		5 μU arginine-vasopressin iv		150 μU arginine-vasopressin iv
0.1	5	1.6	5	50
2	5	1.1	4	20
4	4	0.7	5	8
6	4	>0.25 <0.5	3	4
8	4	0	5	2
10			4	1.3
12			4	0.9
14			4	0.6
16			3	>0.25 <0.5
18			3	0

<sup>3</sup> Dialyzing tubing by Division of American Hospital Supply Corp., Evanston, Ill.

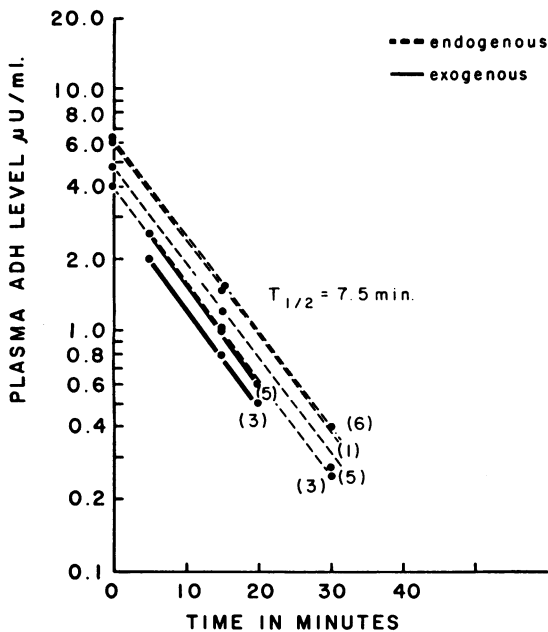


FIG. 1. DISAPPEARANCE OF ENDOGENOUS AND EXOGENOUS ANTIDIURETIC HORMONE (ADH) (ARGININE-VASOPRESSIN) FROM THE PLASMA OF HYDRATED DOGS. The lines represent visual approximation to the points. In the curves representing the disappearance of endogenous ADH, the basal steady-state concentration obtained 5 minutes before the gavage (see Table I) is considered the zero-time plasma concentration. To avoid further overlap of the points, the curves for exogenous ADH have been drawn through points at 5, 15, and 20 minutes rather than (see Table I) 10, 20, and 25 minutes, the times the samples were collected.

the two rat experiments. After hydration the rat plasma contained no ADH activity, and immediately after the injection of vasopressin, plasma levels of 1.6 and 50  $\mu$ U per ml were obtained. The pooled values fell in an exponential way (Figure 3). In the first experiment (curve 1), starting with a plasma level of 1.6  $\mu$ U per ml, one straight semilogarithmic line was obtained. The fractional turnover rate was 0.203 per minute, the  $t_{1/2}$ , 3.4 minutes. In the second experiment (curve 2), starting with a plasma level of 50  $\mu$ U per ml, three different slopes were obtained, the steepest being associated with the highest ADH levels. The disappearance curve from 50 to 8  $\mu$ U per ml corresponds to a fractional turnover of 0.462 per minute and a  $t_{1/2}$  of 1.5 minutes; from 8 to 2  $\mu$ U per ml, to a fractional turnover of 0.362 and a  $t_{1/2}$  of 2 minutes; below 2  $\mu$ U per ml the fractional turnover and  $t_{1/2}$  were identical with the former

experiment (curve 1) (0.203 per minute and 3.4 minutes). These results, demonstrating the relationship of fractional turnover to plasma concentration of ADH, are similar to those of the dog experiments (Figure 2).

#### Volume of distribution

After intravenous injection of vasopressin into hydrated animals (rats and dogs) the apparent volume of distribution was calculated with the injected amount of ADH and the zero time concentration arrived at by extrapolating the disappearance curve to the ordinate. The results were tabulated in Table III, and the apparent volume of distribution was expressed as a percentage of the body weight. This value averages 2.6% of the body weight.

Table IV gives the relevant data for the calcu-

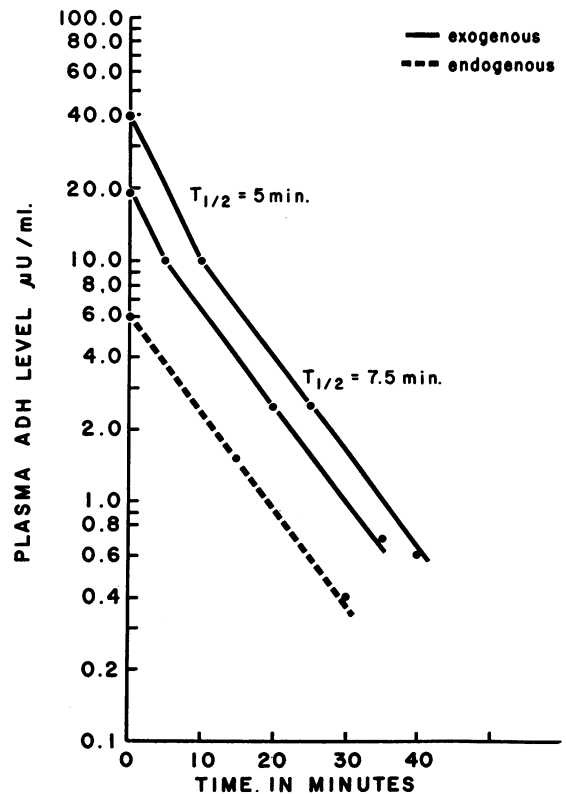


FIG. 2. DISAPPEARANCE OF ARGININE-VASOPRESSIN FROM THE PLASMA OF TWO HYDRATED DOGS AFTER THE CONCENTRATIONS HAD BEEN ELEVATED BY CONTINUOUS INTRAVENOUS INFUSION. The curve to the left is derived from the values in Figure 1. The lines represent visual approximation to the points.

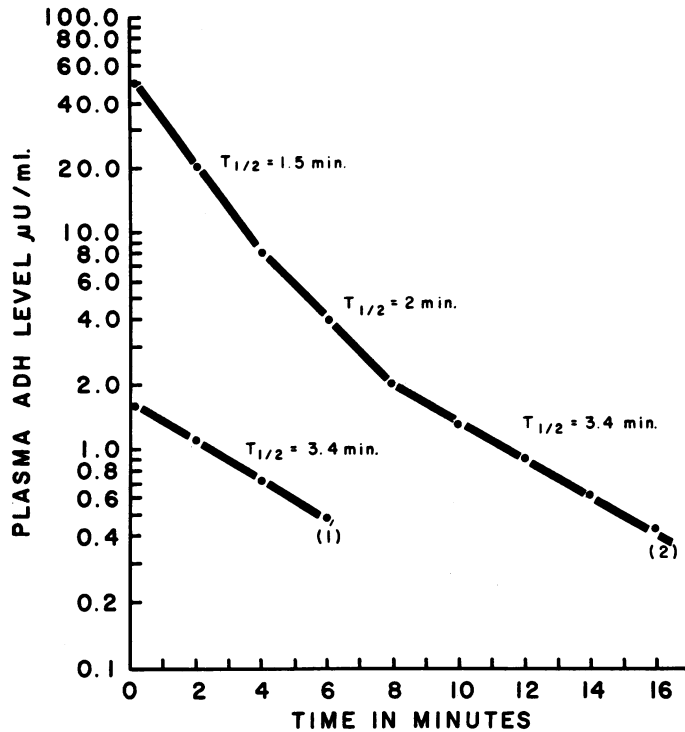


FIG. 3. DISAPPEARANCE OF ARGININE-VASOPRESSIN FROM THE PLASMA OF HYDRATED RATS AFTER INTRAVENOUS INJECTION. The lines represent a visual approximation to the points. The lowest points on both curves represent concentrations between 0.25 and 0.5  $\mu$ U per ml (see Table II).

lation of the volume of distribution derived from steady-state levels of plasma ADH after continuous infusion of known amounts of arginine-vasopressin into dogs and rats (see Methods). The volume of distribution is expressed again as a percentage of body weight, and this value is averaged (4% in the dogs and 4.2% in the rats). Table V gives the ADH content of simultaneously collected lymph and plasma in the surgically prepared dog (see Methods). With plasma levels of ADH maintained over 200  $\mu$ U per ml, the con-

centration of ADH in the lymph remained below 1.0  $\mu$ U per ml, thus supporting the assumption that the plasma compartment represents the true volume of distribution.

*Vasopressin-resistant diabetes insipidus* (patient D.K.). In Table VI are the tabulated results of the experiment. A random sample of blood drawn on the day before the experiment while the patient was allowed free access to water contained 4.3  $\mu$ U per ml. On the day of the experiment, despite a weight loss of 1 kg after 2 hours of wa-

TABLE III  
Apparent volume of distribution of injected ADH (arginine-vasopressin) in dogs and rats

Animal	Extrapolated zero value	Injected amount of ADH	Wt of animal	Vol of distribution	Vol of distribution as % of body wt
	$\mu$ U/ml	mU	kg	ml	%
Dog 3	5.0	2.5	18.4	500	2.7
Dog 5	6.0	2.5	15.0	420	2.8
Rats					
Lot A	1.7	0.005	0.115	2.93	2.5
Lot B	52	0.150	0.115	2.99	2.5

TABLE IV  
*Volume of distribution of ADH (arginine-vasopressin) calculated from steady states attained after constant infusion of the hormone into dogs and rats*

Animal	Infused amount of ADH (I)	Steady-state concentration of ADH (c)	K*	Volume of distribution (V)	Body wt	Volume as % of body wt
	$\mu\text{U}/\text{min}$	$\mu\text{U}/\text{ml}$		$\text{ml}$	$\text{kg}$	
Dog 1	4,000	40.00	0.139	721	19.0	3.8
Dog 2	230	4.05	0.0924†	617	15.5	4.0
	470	8.15	0.0924†	625	15.5	4.05
	1,700	19.00	0.139	645	15.5	4.15
Rat 1	2.0	1.8	0.203‡	5.47	0.120	4.56
Rat 2	2.0	2.0	0.203‡	4.92	0.120	4.10
Rat 3	2.0	2.1	0.203‡	4.80	0.120	4.00
Rat 4	2.0	1.9	0.203‡	5.2	0.120	4.35

\* Fractional turnover rate per minute; see figures.  $V = I/Kc$ .

† These values for K were obtained from the semilog disappearance curve in this dog after a single intravenous injection of arginine-vasopressin. See Figure 1.

‡ The value of K for the rats was derived from the lot A experiments shown in Table II and Figure 3.

ter deprivation, the concentration of ADH in the initial sample ( $B_0$  in Table VI) was no higher than in the random sample. Under the influence of rehydration the ADH levels fell in an exponential manner (Figure 4, curve 1). Despite the fact that this patient was hydrated by a rapid continuous intravenous infusion, while the previously described (1) normal subjects were hydrated orally, the slopes of the first part of their curves were very similar. The fractional turnover rate calculated from the second part of curve 1, Figure 4, was 0.0693 per minute, and the  $t_{\frac{1}{2}}$  was 10 minutes. This value was very similar to that observed in normal subjects after 3 days of dehydration (1). As shown in Table VI, after all water ingestion stopped the plasma level returned to  $3.0 \mu\text{U}$  per ml in 1 hour. In contrast,

a previously tested normal subject required, in a similar experiment, 90 minutes to reach a plasma level of less than  $2.0 \mu\text{U}$  per ml (1). The rapid return in this patient occurred despite the fact that his fractional turnover rate of ADH was significantly greater than normal.

*Vasopressin-sensitive diabetes insipidus* (patients S.P., B.G., and S.K.). Figure 4 (curves 2, 3, and 4) demonstrates the disappearance of the injected arginine-vasopressin from the circulation of these three patients. The slopes are very similar to the second part of the curve in the patient with Pitressin-resistant diabetes insipidus. The fractional turnover rates and  $t_{\frac{1}{2}}$  for patients S.P., B.G., and S.K. were 0.057, 0.057, and 0.053; 12, 12, and 13 minutes, respectively.

*Ultrafiltration and dialysis.* Examination of Table VII indicates that in the *in vitro* systems utilized, plasma ADH was completely ultrafilterable as well as dialyzable.

TABLE V  
*Protocol of simultaneous blood and lymph collection in a dog after portacaval shunt operation and ligation of the hepatic artery*

Time	Blood sample no.	Plasma ADH	Lymph sample no.	Lymph ADH
		$\mu\text{U}/\text{ml}$		$\mu\text{U}/\text{ml}$
11:00	Start of experiment; dog anesthetized and intubated			
12:40	Portacaval shunt operation completed			
12:50	Ligation of hepatic artery			
12:55	Cannulation of lymph vessel			
1:00	$B_0$	58.0	$L_0$	$> 0.25 < 0.5$
1:05	Infusion of arginine-vasopressin, 5 mU/minute			
1:35	$B_1$	220	$L_1$	0.9
2:15	$B_2$	220	$L_2$	0.5
3:00	$B_3$	250	$L_3$	0.9

## Discussion

In a previous study (1) on the metabolism of ADH, the state of hydration significantly altered the turnover rate of the hormone. Three days of dehydration increased and three days of overhydration decreased the turnover rate. The above states of hydration were associated with a high and zero ( $< 0.25 \mu\text{U}$  per ml) concentration of ADH, respectively, and it was not possible in these earlier experiments to determine whether the

varying turnover rates were primarily due to the altered ADH level or to the state of hydration per se. In the dog and rat experiments of the present study it was possible to observe the effect of various levels of ADH on the turnover rate while a constant state of hydration was maintained. In dogs a  $t_{1/2}$  of  $7\frac{1}{2}$  minutes was observed when the plasma level of ADH was below  $10 \mu\text{U}$  per ml, while between 40 and  $10 \mu\text{U}$  per ml the  $t_{1/2}$  was shortened to 5 minutes. This shorter  $t_{1/2}$  was similar to that calculated by Lauson and Bocanegra (9) in experiments on pentobarbital-anesthetized dogs receiving constant infusions of bovine Pitressin. When they elevated the plasma level of ADH to between 80 and 2,000  $\mu\text{U}$  per ml they found that after stopping the infusion the concentration fell within 15 minutes to about 14% of the steady-state level. Assuming a semilogarithmic disappearance, they calculated a mean  $t_{1/2}$  of 5.4 minutes and a fractional turnover rate of 12.9% per minute. In the rats a  $t_{1/2}$  of 3.4 minutes was observed at a concentration below  $2 \mu\text{U}$  per ml; between 8 and  $2 \mu\text{U}$  per ml the  $t_{1/2}$  was 2.0 minutes, whereas be-

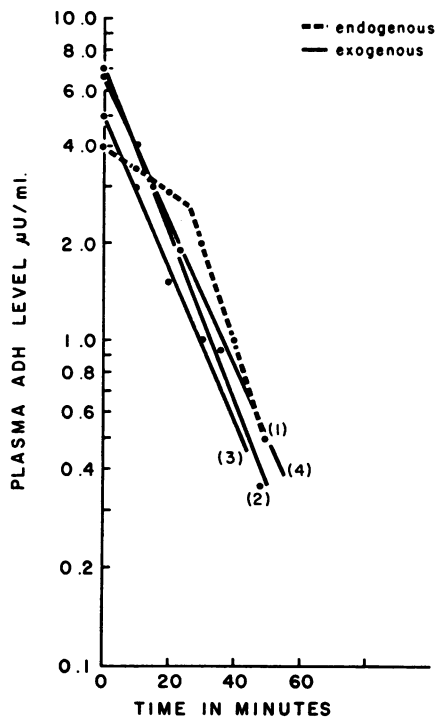


FIG. 4. DISAPPEARANCE OF ENDOGENOUS ADH FROM THE PLASMA OF A PATIENT WITH VASOPRESSIN-RESISTANT DIABETES INSIPIDUS (1) AFTER AN INTRAVENOUS AND ORAL WATER LOAD AND THE DISAPPEARANCE OF ARGININE-VASOPRESSIN FROM THE PLASMA OF THREE PATIENTS WITH VASOPRESSIN-SENSITIVE DIABETES INSIPIDUS (2, 3, AND 4) AFTER INTRAVENOUS INJECTION. The zero-time value represents the concentration in the plasma 10 minutes after the injection. The lines represent visual approximation to the points.

TABLE VI

The effect of water restriction followed by intravenous and oral hydration on circulating ADH and water diuresis in patient D. K. (vasopressin-resistant diabetes insipidus)

Elapsed time	Blood sample no.	Urine sample no.	Urine flow	Urine osmolality	Plasma ADH
min			ml/min	mOsm/L	$\mu\text{U/ml}$
-130					
-78		U <sub>0</sub>		103	
-35		U <sub>1</sub>	8.6	105	
-5	B <sub>0</sub>				4.0
0					
4		U <sub>2</sub>	9.2	105	
10	B <sub>1</sub>				3.4
20		U <sub>3</sub>	9.4	93	
20	B <sub>2</sub>				2.9
30	B <sub>3</sub>				2.0
35		U <sub>4</sub>	10.0	93	
40	B <sub>4</sub>				4.0
50	B <sub>5</sub>				0.5
52					
57		U <sub>5</sub>	10.0	93	
72		U <sub>6</sub>	10.5	86	
80	B <sub>6</sub>				0
96		U <sub>7</sub>	10.0	90	
110	B <sub>7</sub>				0
116		U <sub>8</sub>	9.0	86	
136		U <sub>9</sub>	9.3	87	
140	B <sub>8</sub>				1.6
156		U <sub>10</sub>	10.0	88	
170	B <sub>9</sub>				3.0

tween 50 and  $8 \mu\text{U}$  per ml the  $t_{1/2}$  was 1.5 minutes. Ginsburg (10) and Ginsburg and Heller (11), utilizing the antidiuretic activity of blood after the intravenous injection of Pitressin,<sup>4</sup> estimated its half-life in the circulation to be about 1 minute with a plasma clearance and fractional turnover of 100% per minute. Recently Sawyer, Chan, and Van Dyke (12) demonstrated that the mean half-life of the vasopressor response to the injection of 4 mU of arginine-vasopressin was 2.05 minutes. Ginsburg's results (10, 11) compare favorably with the  $t_{1/2}$  observed in the present study for ADH levels between 50 and  $8 \mu\text{U}$  per ml. Although there may be some question about the validity of comparing the duration of a pressor response to the level of antidiuretic activity as

<sup>4</sup> Parke, Davis & Co., Detroit, Mich.



TABLE VII  
*Concentration of endogenous ADH in anaerobic ultrafiltrate  
 and in dialyzate compared with control plasma*

Material	Procedure	Temp.	ADH in	ADH in
			filtrate or dialyzate	control
		°C	$\mu\text{U/ml}$	$\mu\text{U/ml}$
Dog serum	Ultrafiltration	20	5.9	6.0
	Ultrafiltration	20	6.0	5.8
	Ultrafiltration	37	4.2	4.3
Rat serum	Ultrafiltration	37	4.8	4.7
	Ultrafiltration	20	8.4	8.0
Dog serum	Ultrafiltration	37	6.8	6.6
	Dialysis	37	3.2	3.0
Rat serum	Dialysis	37	3.1	3.1
	Dialysis	37	5.4	5.2

measured in our study, the half-life of 2.05 minutes is very similar to the 2.0 minutes observed between 8 and 2  $\mu\text{U}$  per ml. However, when the concentration of ADH in plasma approaches the more physiologic range (< 2 to 3  $\mu\text{U}$  per ml), the  $t_{1/2}$  was significantly longer. The results of the present study seem to indicate that the level of circulating ADH per se does influence the turnover rate.

On the other hand, all four patients with diabetes insipidus demonstrated turnover rates that were significantly greater than normal. This was despite the fact that the tissues of untreated patient S.K. were not exposed to ADH; those of patient D.K. (vasopressin-resistant diabetes insipidus) were exposed continuously to higher concentrations (4  $\mu\text{U}$  per ml) than observed in normally hydrated subjects (2  $\mu\text{U}$  per ml) (1), and those of the other two patients (S.P. and B.G.) intermittently exposed to high and low concentrations of ADH as a result of treatment. The only evident common denominator in these four patients was the presence of varying periods of mild to moderate dehydration extending over many years.

If the assumption is correct that the factors controlling the turnover of ADH are similar in all mammals, we may conclude that both the concentration of ADH in plasma and the state of hydration influence the peripheral disposal of ADH. Under physiological conditions of enhanced secretion, both factors would tend to lower plasma levels of the hormone and therefore protect the organism from any adverse cardiovascular effects of vasopressin.

The enhanced disposal of ADH at higher plasma levels might reasonably be explained by the effect of increased amounts of substrate on the enzyme (s) systems involved in inactivation of the hormone (13). However, the mechanisms by which the state of hydration could influence hormonal turnover remain unclear.

*Volume of distribution.* In a previous study in man the apparent volume of distribution determined by the extrapolation technique after injection of arginine-vasopressin into hydrated subjects was found to be 2.6% of the body weight (1). It was assumed that the true volume of distribution was probably equivalent to plasma volume and that the calculated volume, for unknown reasons, was erroneously low. In the present study volume of distribution in dogs and rats was also 2.6% of the body weight when calculated by extrapolation (Table III). We are again at a complete loss to explain this low volume of distribution particularly when at least in the dog, at low plasma concentrations, the  $K_d$ , or fraction of the volume of distribution cleared per minute, is the same for endogenous and exogenous ADH (Figure 1).

Lauson and Bocanegra (9) in the dog experiments described above calculated the apparent volume of distribution of ADH from the semilog disappearance curve after stopping the constant infusion. The apparent volume ranged from 0.98 to 1.34 times the true plasma volume (T-1824). To overcome the error introduced in our studies by the extrapolation technique, arginine-vasopressin was constantly infused until a prolonged constant plasma level was achieved. Under these conditions we can reasonably assume that equilibration in the true volume of distribution occurred. From the rate of infusion, the fractional turnover rate, and the steady-state concentration of vasopressin in plasma, it was possible to calculate this volume (Table IV). The mean value of 4% of body weight reasonably approximates the known plasma volume in these species (14). The detection of less than 1  $\mu\text{U}$  per ml of ADH in the lymph while plasma levels were maintained above 200  $\mu\text{U}$  per ml (Table V) affords additional evidence that ADH is distributed in a space equivalent to plasma volume.

TABLE VIII

*Neurohypophyseal secretion rate of ADH necessary to maintain steady-state plasma levels of 4.0  $\mu$ U per ml in human, dog, and rat*

Species	Estimated plasma vol	K*	Steady-state secretion	Surface area†	Steady-state secretion
	ml		$\mu$ U/min	m <sup>2</sup>	$\mu$ U/min/m <sup>2</sup>
Man (normal) 70 kg	3,200	0.0433	555	1.73	320
Dog (normal) 20 kg	900	0.0924	330	0.86	383
Rat (normal) 100 g	4	0.362	5.8	0.016	362

\* Fractional turnover rate per minute.

† Surface area of dog and rat was calculated according to the formula  $S.A. (cm^2) = W^{1.7} \cdot K$ , where  $W$  = weight in grams.  $K$  values were taken from Table 146 of the Handbook of Biological Data, National Academy of Science, National Research Council. Philadelphia, W. B. Saunders Co., 1961.

As previously reported for human plasma (1), the authors were unable to detect binding of ADH to plasma proteins of the dog and rat *in vitro* (Table VII). The results in the dog resemble those of Bocanegra and Lauson (15). However, our failure to demonstrate protein binding of the hormone in the rat conflicts with the observations of Thorn and Silver (4). These authors were measuring the protein binding of very high concentrations of endogenous ADH released under the powerful stimulus of severe hemorrhage in contrast to the relatively low concentrations studied in the present investigation (Table VII). We must assume from our results that either the hormone is bound *in vivo*, but not *in vitro*, to the plasma proteins, or that the capillary membrane is a significant barrier to the free diffusion of arginine-vasopressin.

Knowing the volume of distribution and the fractional turnover rates of the hormone in man, dog, and rat, it was possible to calculate the approximate rate of secretion from the neural lobe for any given steady-state concentration of ADH in the plasma. In Table VIII the calculated secretion rates for a plasma level of 4  $\mu$ U per ml are tabulated for these three species. Per square meter of surface area the secretion rates were very similar (300 to 380  $\mu$ U per m<sup>2</sup> per minute).

The conclusions of Lauson and Bocanegra (9) and Ginsburg and Smith (16) that there is a definite relationship between body size and the fractional turnover rate and half-life of ADH are confirmed. The smaller the animal and the smaller the plasma volume, the greater the  $K$ . This results in the relationship shown in Table VIII.

### Summary

1) The turnover rates of endogenous and exogenous antidiuretic hormone (ADH) (arginine-vasopressin) were determined in dogs, rats, and patients with vasopressin-sensitive and vasopressin-resistant diabetes insipidus by utilizing a sensitive bioassay technique.

2) In the rat and dog we found that at higher plasma levels the turnover of the hormone was more rapid.

3) In all four patients the hormonal turnover was significantly faster than observed in normal subjects during comparable states of hydration.

4) We concluded that both the plasma level of ADH and the state of hydration influenced the peripheral disposal of the hormone.

5) The volume of distribution of ADH, determined by a constant infusion technique, and the rate of disappearance of the hormone after stopping the infusion, approximated the assumed plasma volume. This was substantiated by finding less than 1  $\mu$ U per ml in lymph at a time when the plasma level was above 200  $\mu$ U per ml.

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