Antidiuretic Hormone Release in the Monkey During Hypo- and Hyper-Osmotic Dehydration.*† (32305)

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The decreased blood volume in hemorrhagic shock has been shown to stimulate antidiuretic hormone (ADH) release(1) presumably *via* volume receptors(2). On the other hand when small volume of hypertonic saline is injected into the carotid artery an antidiuresis results which has been attributed to ADH liberation(3,4). This paper reports studies on monkeys in which dehydration was produced using the techniques reported by Darrow and Yannet(5), and Gregersen and his colleagues(6) which resulted in a hypo- or hyper-osmotic plasma depending on the technique used. It will be shown that in the dehydration with hyperosmotic plasma there is a marked release of ADH. Further, in the dehydration with plasma osmolarity below normal there is also appreciable ADH release.

Materials and methods. Eight monkeys (*Macaca cyclopis*) of both sexes with body weight between 2.30 and 5.17 kg obtained from the mountain area of Taiwan were used. Food and water were withdrawn for at least 16 hours before actual experiment to render them somewhat dehydrated. They were anesthetized with sodium pentobarbital in doses of 25 mg/kg intravenously, with supplemental doses of 10 mg/kg every 4 hours by the same route. The left femoral artery was cannulated for collecting blood samples and the left femoral vein for administering fluid. Two types of dehydration were produced experimentally.

Dehydration with high plasma osmolarity was produced by the diuresis resulting from intravenous injection of freshly prepared 50% sucrose in doses of 22 ml/kg at the rate of 1 ml/min(6). Diuresis due to the sucrose was usually complete within 10 hours. This type of dehydration was corrected, after a lapse of 2 hours, by intravenous infusion of a hypotonic solution which contained d-glucose and NaCl in concentrations of 1.1% and 0.07%, respectively. The infused volume equalled the urine output in the period of diuresis.

Dehydration with low plasma osmolarity was produced by intraperitoneal injection of 5% glucose in a dose of 100 ml/kg(5). Marked dehydration was present 2 hours after intraperitoneal injection of the glucose solution. This type of dehydration was corrected, after obtaining an arterial blood sample, by intravenous infusion of 30 ml/kg of 2% NaCl at the rate of 2 ml/min. Hydration was maintained by intravenous infusion of 0.9% NaCl at the rate of 1 ml/min. This was necessary since water and electrolytes continued to be "lost" into the peritoneal cavity.

Four heparinized blood samples of 12 ml each were taken from each animal. Sample A was taken in the pre-dehydration or control period, about 2 hours after the animal was anesthetized. Sample B was taken at the end of dehydration period. Sample C was taken when dehydration had been corrected. Sample D was taken one hour after sample C for a further check.

Six determinations of microhematocrit (V_c)(7) were made on each blood sample and the results averaged. The plasma obtained by centrifugation was shaken with 2 volumes of absolute alcohol and the mixture centrifuged. Antidiuretic hormone was removed in the supernatant which was then concentrated by distillation *in vacuo*(8). Each extract was assayed against U.S.P. Standard Posterior Pituitary Powder by its antidiuretic action after intravenous injection into 3 water loaded rats anesthetized

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TABLE I. Data on Dehydration with High Plasma Osmolarity.

Sample*	Na†	K†	Cl†	Osm‡	G_p	V_c	ADH†
A	158 ± 1.7§	3.4 ± .2	120 ± 1.9	305 ± 6.9	1.026 ± .001	35.3 ± .8	10 ± .4
B	171 ± 2.6	4.6 ± .4	127 ± 2.2	355 ±11.1	1.034 ± .001	43.0 ±1.6	279 ±75.2
C	149 ± 2.7	3.2 ± .2	109 ± 2.7	308 ± 7.3	1.027 ± .001	36.9 ±1.3	13 ± 1.7
D	147 ± 4.5	3.2 ± .2	108 ± 2.9	301 ± 9.7	1.027 ± .001	36.8 ± .9	18 ± 9.3
	P values between A and B (I) and between B and C (II)						
I	<.01	<.05	<.05	<.01	<.01	<.01	<.05
II	<.01	>.05	<.01	<.05	<.01	<.01	<.05

* Sample A's were taken in control period, sample B's at the end of dehydration, sample C's and D's in the period when dehydration had been corrected.
† Expressed as mEq per liter plasma water or μU per ml plasma water.
‡ Expressed as mOsm per liter plasma.
§ Values are means ± standard error of mean (4 experiments).
G_p represents plasma specific gravity.
V_c represents hematocrit expressed in percentage.

TABLE II. Data on Dehydration with Low Plasma Osmolarity.

Sample*	Na†	K†	Cl†	Osm‡	G_p	V_c	ADH†
A	164 ± 2.5§	2.9 ± .3	123 ± 2.1	314 ± 6.0	1.025 ± .001	32.2 ± .6	13 ± 3.2
B	153 ± 1.8	2.7 ± .2	110 ± .9	299 ± 6.8	1.029 ± .001	41.9 ±1.3	44 ±11.1
C	160 ± 1.5	2.3 ± .3	124 ± 1.3	310 ± 4.3	1.024 ± .001	35.2 ±1.6	31 ± 5.6
D	159 ± .9	2.4 ± .4	125 ± .9	307 ± 4.3	1.022 ± .001	34.2 ± .6	20 ± 4.1
	P values between A and B (I) and between B and C (II)						
I	<.01	>.05	<.01	>.05	<.01	<.01	<.05
II	<.05	>.05	<.01	>.05	<.02	<.01	>.05

* † ‡ § G_p V_c—See footnotes to Table I.

with ethanol and sodium salt of ethyl-(1-methyl-propyl)-thiobarbituric acid (Inactin)(9). Plasma samples were also analyzed for specific gravity (G_p) by the copper sulfate method(10), sodium and potassium by flame photometry, chloride by the Cotlove titrator and osmolarity by the Fiske osmometer. The packed cells in each blood sample were resuspended with 0.9% NaCl and infused back into the animal's circulation. Sampling losses were thus confined to plasma protein and electrolytes other than sodium and chloride.

Results. The findings by these two types of dehydration are summarized in Table I and Table II, respectively. Data of electrolytes and ADH have been corrected for plasma protein concentration by conversion to mEq per liter of plasma water or μU per ml of plasma water(11). Dehydration was successfully produced as evidenced by significant increase of V_c and G_p in samples labelled B. There was marked and significant increase of plasma ADH level in dehydration with high plasma osmolarity in which electrolytes such as sodium and chloride were significantly elevated (Table I). When the alterations of blood volume and plasma osmolarity were corrected by rehydration, the plasma ADH returned to predehydration level.

There was moderate, but significant increase of plasma ADH level (Table II) when dehydration with low plasma osmolarity was produced in which both sodium and chloride concentration in plasma were significantly lowered. The antidiuretic activity in each plasma extract was completely inactivated by adding freshly prepared potassium thioglycollate to a final concentration of 0.05 M.

Discussion. It has been reported that antidiuretic hormone is liberated in response to an increase in osmotic pressure of body fluid (4,12) even in the absence of definite volume change, while in hemorrhage as reviewed by Moore(13) or in patients suffering from cholera, isotonic fluid deficiency has been postulated to be responsible for the striking increase in the rate of ADH liberation. Our experiments in dehydrated monkeys compared these variables in two different combinations, *i.e.*, reduction of blood volume with

high osmolarity and reduction of blood volume with low osmolarity. In the first case tremendous release of ADH was noted. This 27-fold increase of plasma ADH level (Table I) is apparently due to concomitant responses to these two potent stimuli. In dehydration with low plasma osmolarity there was also a significant elevation in plasma ADH level though less marked in degree. This 3-fold increase of plasma ADH level (Table II) is in agreement with the observation of Mirsky *et al*(14). They found that in adrenalectomized rats, a state of hypo-osmotic dehydration with salt loss, plasma ADH level increased 3 to 4 times that of control rats within 7 days after adrenalectomy. Our results imply that these two stimuli are basically independent. Elimination of osmolar stimulus did not abolish the response to reduction of blood volume.

Comparison of the hematocrit (V_c) in Tables I and II indicates that the dehydration produced by sucrose diuresis or by shifting fluid into peritoneal cavity is about the same in the two experimental procedures; sample B is 1.22 $\times$ sample A in Table I and sample B is 1.30 $\times$ sample A in Table II.

Summary. Release of antidiuretic hormone in two types of dehydration was studied in monkeys. It was found that dehydration with high plasma osmolarity led to a tremendous elevation of plasma ADH level, and dehydration with low plasma osmolarity also led to a significant increase of plasma ADH level though less marked in degree.

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Demonstration of Increased Catecholamine Excretion in the Nephrotic Syndrome.* (32306)

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Hypovolemia, secondary to reduced circulating albumin, is postulated to stimulate increased secretion of aldosterone in the nephrotic syndrome(1-3); however, measurements during diuresis, when aldosterone excretion is low, have not consistently shown an increase of plasma volume(4-5). Physiologic responses to hypovolemia also include increased activity of the autonomic nervous system (adrenergic)(6). This suggested that investigation of catecholamine excretion

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