lease hemolysin unless treated with an agent that allows it to escape.

The *in vitro* effect of CPZ on antibody producing cells is probably different from its action when it is injected into animals. Preliminary studies from this laboratory(9) indicate that CPZ in high doses delays formation of hemolytic plaques, cellular production of RNA and release of antibody after primary immunization. Further, treatment with CPZ results in fewer hemolytic plaques, acridine orange stained cells and lower antibody titers after secondary immunization. This disparity between *in vivo* and *in vitro* effect may be due to the many actions of CPZ after it has entered a viable cell. It is well known that CPZ has a wide variety of biological properties in membraneless systems. For example, it inhibits a number of enzymes including DNA polymerase and thymidylate kinase(10). Since antibody formation depends on many cell functions it is possible that the *in vivo* inhibition of antibody synthesis by CPZ and the *in vitro* enhancement of antibody release by CPZ are unrelated.

Summary. Stimulation of hemolytic plaque formation by rat spleen cells after primary immunization was produced by *in vitro* incubation of these cells with chlorpromazine, promethazine and thioridazine in 2×10^{-3} M concentration. This effect may be the result of increased cell membrane permeability produced by these compounds. The phenothiazine derivatives may be useful agents for increasing the sensitivity of the hemolytic paque procedure by enhancing the release of antibody from immunized cells.

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Received October 17, 1966. P.S.E.B.M., 1967, v124.

Electrolyte-Fluid Exchanges and Renal Tissue Composition in Vasopressin Treated Polyuric-Polydipsic Rabbits.* (31849)

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The male rabbit when deprived of food, but allowed water *ad libitum,* develops a polyuria and polydipsia associated with a significant loss of sodium(1,2). Since the suppression of this polyuric-polydipsic syndrome by estrogens occurs even when sodium loss is enhanced by a spironolactone(3), a change in either vasopressin release or the renal response to this hormone may be involved. In view of the demonstration(4) that the medullary osmotic gradient can be washed out by a water diuresis, the possibility must be considered that this syndrome may be associated with a loss of the medullary osmotic gradient; this could account for an alteration in response to vasopressin. As an attempt to determine the factors promoting the increased fluid exchange and the mechanism of the estrogen inhibition, the response of food deprived rabbits to vasopressin administration and the renal tissue electrolyte

* This investigation was supported by National Science Foundation Research Grants G-12459 and GB-780.

composition were determined.

Methods. Six male Dutch rabbits weighing approximately 2 kg and individually housed in metabolism cages were used. The environmental conditions and diet composition were as previously reported(2). Daily observations of body weights and food and water intakes were made for 1 week prior to the start of each experiment to confirm that accommodation to these conditions had occurred. All measurements of intakes and urine volume, and food withdrawal were performed between 9-11 a.m. Each experiment covered a period of 11 days, which consisted of 2 days *ad lib* feeding, 7 days of food deprivation and 2 days of food restoration. Water was available *ad lib* throughout the experiment. Each animal was subjected to a complete 11-day experiment in order to determine its response to food deprivation. After a rest period of 2 weeks, the experiment was repeated on each animal with vasopressin administered on the 5th and 6th days (3rd and 4th days of food deprivation) of the experiment. The vasopressin (Pitressin; Parke, Davis) in a volume of 0.1 ml was injected subcutaneously at 10 a.m. (1 unit) and at 2 p.m. (1 unit) on these days for a total daily dose of 2 units. Sham injections, consisting of 0.1 ml distilled water, were given at identical times in the control experiment.

Daily measurement of body weight, food and water intakes and urine volume, and determinations of urinary sodium, potassium, chloride and osmolarity were performed by methods previously described(2).

For the tissue studies, the kidneys were removed from animals after a 7-day period with food available or after a 5-day period of food deprivation. The following groups, consisting of 5 adult Dutch rabbits each, were used: 1) normal males, 2) food-deprived males, 3) food-deprived males receiving 100 μg per day of estradiol (Progynon, Schering) subcutaneously as an aqueous suspension on the 3rd and 4th days of deprivation, 4) normal females and 5) food-deprived females. Following an intracardiac injection of an overdose of Nembutal, the kidneys were quickly removed, stripped of their capsule and each sliced with 2 longitudinal cuts through the central mass. From this sagittal section of each kidney, a small sample was obtained from the cortical area (150 $\pm$ 8 mg) and from the apical papilla (91 $\pm$ 6 mg). The sample was placed in a weighed 10 ml volumetric flask, and the wet weight obtained. The samples were dried to constant weight at 100°C. The dried tissues were prepared for electrolyte analyses according to the method of Malvin and Wilde(5).

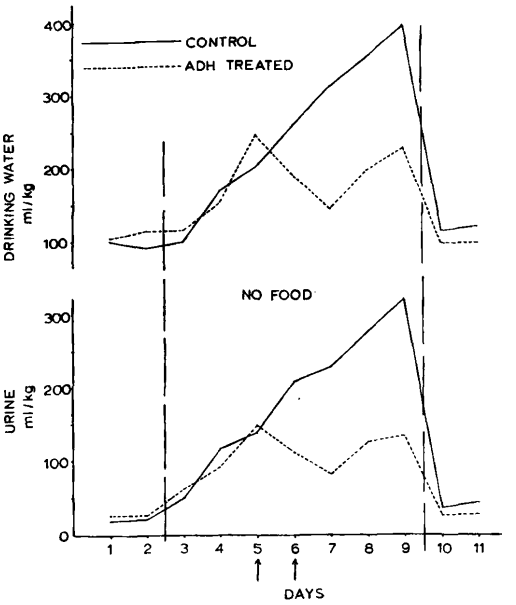

FIG. 1. Depressant effect of vasopressin (ADH—2 units/day) on fluid exchange in male rabbits. Each point is the average of 6 observations. Arrows indicate days of injection, *i.e.*, at the end of days 5 and 6. Food was withdrawn and restored at the end of days 2 and 9, respectively.

Results. As previously demonstrated, food withdrawal resulted in a significant increase in fluid exchange in adult male rabbits (Table I and Fig. 1). The polyuria and polydipsia were accompanied by the usual enhanced excretion of sodium, decreased excretion of potassium and chloride, and a decreased urinary osmolarity. The administration of 2 units of vasopressin per day for 2 days significantly ($p<.01$) lowered the urine volume and water intake and increased the urinary osmolarity (Table I and Fig. 1). These changes were present following the first day of treatment and became greater after the second day of injection (Fig. 1). Electrolyte

TABLE I. Comparison of Daily Water Intake and Urinary Volume, Osmolarity and Sodium Excretion in 6 Male Rabbits With and Without Vasopressin Treatment During Starvation Induced Polydipsia-Polyuria.

		Treatment	Water intake, ml/kg ± SEM*	Urine vol, ml/kg ± SEM*	Osmolarity, mOsm/l ± SEM	Urinary Na^+, mEq/kg ± SEM*
Day 2 (normal control)	A	none	91.5 ± 5.2	20.7 ± 2.8	1642 ± 164	.06 ± .02
	B	"	116.5 ± 12.3	26.9 ± 5.1	1698 ± 199	.05 ± .01
Day 6 and 7 (food deprivation for 5 days)	A	none	285.1 ± 26.0	219.0 ± 26.7	139 ± 24	.49 ± .09
	B	vasopressin	167.8 ± 14.0†	95.8 ± 15.2†	279 ± 39†	.48 ± .10
Day 10 (food restored)	A	none	113.0 ± 2.7	36.9 ± 7.6	1020 ± 217	.04 ± .01
	B	"	97.7 ± 4.6	26.0 ± 2.4	1100 ± 103	.02 ± .01

A = control study on 6 adult male rabbits. B = subsequent study on the same 6 rabbits but receiving vasopressin on days 5 and 6.
* ml or mEq kilogram body weight ± standard error of mean.
† $p < .01$ when B compared with A.

(Na^+, Cl^-, K^+) excretions during deprivation were not altered by vasopressin administration; daily total Na^+ values are given in Table I. All parameters approached their control values within 24 hours upon food restoration.

Typical(2) male and female responses to food deprivation and to exogenous estrogen administration were obtained in those animals used for the renal tissue analyses. Food deprivation in either sex, or estrogen administration to food-deprived males did not alter the potassium or water content of cortical or medullary renal tissue. Deprived males, with or without estrogen, displayed significantly lower medullary Na^+ concentrations than medullary tissue from normal males. In all cases the per cent water and the amount of sodium and potassium per gram of dry tissue were significantly greater in the medulla than cortex (Table II). When expressed as mEq per gram of tissue water, sodium, but not potassium, was increased in the medulla as compared with the cortex of the same group.

Discussion. The daily administration of 2 units of vasopressin in 2 divided doses for 2 days significantly diminished the polyuric-polydipsic syndrome in food-deprived adult male rabbits. A more marked effect might have been observed had measurements been made at a time interval less than 24 hours, or had a long-acting vasopressin preparation been used. In view of the well-known rapid clearance of vasopressin from the circulation, it is difficult to rationalize the consistently prolonged effect encountered in these studies.

TABLE II. Water and Electrolyte Content of Rabbit Renal Tissue Slices.*

Group	Water, %	Sodium		Potassium	
		mEq/g dry wt	mEq/g tissue water	mEq/g dry wt	mEq/g tissue water
Cortex					
Normal males	76.8 ± .5	.323 ± .016	.098 ± .005	.270 ± .016	.082 ± .003
Males (no food)	76.5 ± .4	.305 ± .014	.093 ± .003	.252 ± .012	.077 ± .004
Idem + estrogen†	77.1 ± .4	.317 ± .013	.095 ± .004	.235 ± .012	.071 ± .003
Normal females	77.7 ± .6	.361 ± .012	.102 ± .003	.278 ± .005	.080 ± .004
Females (no food)	76.5 ± .8	.361 ± .017	.101 ± .002	.252 ± .009	.078 ± .005
Medulla					
Normal males	84.1 ± .8‡	1.245 ± .073‡	.230 ± .011‡	.464 ± .033‡	.084 ± .004
Males (no food)	85.4 ± .5‡	1.092 ± .061‡	.185 ± .004‡	.390 ± .029‡	.066 ± .003
Idem + estrogen†	83.8 ± .1‡	.966 ± .043‡	.185 ± .008‡	.395 ± .029‡	.074 ± .007
Normal females	83.2 ± .9‡	1.105 ± .050‡	.223 ± .025‡	.413 ± .037‡	.076 ± .003
Females (no food)	83.1 ± .6‡	1.311 ± .062‡	.278 ± .020‡	.436 ± .049‡	.089 ± .011

* Each value is the average of 10 tissue samples ± standard error of mean.
† 100 μg estradiol/day on 3rd and 4th days of food deprivation.
‡ $p < .01$ when compared with cortex of corresponding group.

At the second collection period following the last vasopressin injection (48 hours), the fluid exchange had begun to increase at the same rate as in the pre-injection period. The response to vasopressin is in accord with that obtained during water diuresis in the rabbit using a comparable dosage(6), and indicates that the sensitivity to vasopressin in our rabbits was essentially normal.

The above response to vasopressin is indirect evidence against any major alteration in the medullary concentration mechanism in this syndrome. Under the conditions of these experiments, the medullary sodium concentration was significantly lowered only in food-deprived males, with and without estrogen. However, a significant cortico-medullary sodium gradient was maintained. Furthermore, the medullary sodium concentration was the same in both groups despite a markedly diminished fluid exchange in the estrogen treated animals. These data provide more direct evidence that a major change in the medullary concentration mechanism was not present. While the electrolytes do not represent the total osmolar components, they should serve as an index of any total osmolar change due to a washout or food deprivation, *per se*.

Aldactone administration to both sexes during food deprivation increased urinary Na^+ excretion but did not alter the inhibitory effect on fluid exchange of either exogenous or endogenous estrogen(3). The depressant action of estrogen thus appears to be independent of Na^+ excretion and adrenal mineralocorticoid activity. Since the estrogen inhibition of the polyuria-polydipsia is not related to an alteration in renal sodium handling, and since vasopressin effectively reduced the urine volume and water intake, an increase in vasopressin secretion may occur under the influence of estrogen. Although under quite different experimental conditions, Dance, Lloyd and Pickford(7) obtained sodium and water retention with stilbestrol administration in the ovariectomized and normal female dog during water diuresis, but were unable to obtain a consistent decrease in urine volume or sodium excretion when the pituitary stalk was sectioned. These data suggest that an altered vasopressin release may be involved in the development of this syndrome and in the inhibitory estrogen action. As suggested in a previous publication(2), the alternate possibility that estrogens may be acting on some central mechanism concerned with water intake exists and warrants further consideration.

Summary. Daily administration of 2 units of vasopressin for 2 days to adult male rabbits during the polyuric-polydipsic syndrome induced by food deprivation effectively reduced the urine volume and fluid intake and increased the urinary osmolarity. There was no alteration in renal cortical or medullary electrolyte and fluid content under the conditions of these experiments. These findings establish the effectiveness of vasopressin in food-deprived polyuric-polydipsic male rabbits and suggest a possible role of an altered vasopressin release in the development of this syndrome and in the inhibitory action of estrogen.

The authors express their gratitude to Mrs. A. Muda and Mr. J. Hegmann for their technical assistance.

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Received December 2, 1966. P.S.E.B.M., 1967, v124.