doxal phosphate. Bergeret *et al.*(6) found that brain cysteinesulfinic acid apodecarboxylase was not saturated with pyridoxal phosphate in rats fed 15 μg of pyridoxine hydrochloride daily although the liver apoenzyme appeared saturated.

With the other adult rats previously fed 50 μg of pyridoxine daily, however, taurine excretion after feeding cysteine and pyridoxine was somewhat less than after feeding cysteine alone. This suggests that their previous intake of pyridoxine may have been sufficient to "saturate" the enzymes concerned in taurine formation although it is possible that other factors involved in taurine formation may have altered during this collection period.

Summary. Feeding cysteine alone or with pyridoxine greatly increased urinary excretion of taurine in young or adult rats fed adequate amounts of pyridoxine. In Vit. B_6-deficient young rats, these treatments caused only a slight increase in taurine excretion. The results indicated that the relative increase in urinary excretion of taurine after an oral dose of cysteine or cysteine and pyridoxine might be used as an index of Vit. B_6 nutrition.

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Comparative Effects of Valine-5 Angiotensin II Amide and Pitressin on Renal Excretory Function in Diabetes Insipidus.* (27117)

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Angiotensin causes antidiuresis, sodium retention and renal vasoconstriction in normal human subjects(1). These effects are associated with an increased secretion and excretion of aldosterone(2,3). Whether the antidiuretic effect of angiotensin is attributable wholly or in part to its vascular action on the kidney is not clear, nor is it known what role the concomitant stimulation of aldosterone secretion plays. The suggestion has been made(4) that the antidiuresis by angiotensin may be due to a mechanism similar to that of the antidiuretic hormone (ADH).

The present experiments were undertaken with the aim of comparing the effects of angiotensin to those of pitressin on excretion of water, sodium and potassium. To eliminate the influence of endogenous ADH the studies were performed in subjects suffering from diabetes insipidus.

Material and methods. The experiments were performed on 3 subjects. One patient had diabetes insipidus of unknown etiology, and 2 patients developed diabetes insipidus after complete hypophysectomy for removal of a chromophobe adenoma and for treatment of metastatic carcinoma of the breast, respectively. In all subjects the absence of antidiuretic hormone (ADH) production was confirmed by a negative response to nicotine administered intravenously according to Dingman *et al.*(5), and by their inability to form concentrated urine after fluid deprivation for 12 to 14 hours. Two subjects (B.M. and P.R.) were placed on a low salt diet (2 g NaCl daily); the third subject (G.C.), however, was given a regular hospital diet (approximately 10 g NaCl daily).

The effects of valine-5 angiotensin II

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amide† (angiotensin II) were determined in 7 experiments. The effects of pitressin‡ were determined in the course of 2 of these and in another 2 experiments. The therapeutic administration of pitressin was discontinued from 3 to 5 days before the experiments were performed. All subjects were studied recumbent in the fasting state.

Diuresis was induced in 4 experiments by intravenous infusion of 20 to 25% mannitol in water for 20 to 40 minutes at rates of 10 to 12 ml per minute, followed by a sustaining infusion of 10 to 12.5% mannitol at rates of 5 to 6 ml per minute. In 5 experiments diuresis was induced by administration of an oral water load (20 ml per kg body weight) imbibed over 30 minutes, followed by supplemental oral water or by infusion of 5% dextrose in water at rates equal to urine flow.

After a steady flow of urine had been achieved, 3 to 5 control urine collection periods of 10 to 15 minutes each were obtained. Upon completion of the control periods an intravenous infusion of angiotensin II (0.0025 to 0.0050 μg per kg per minute) or pitressin (3 to 18 μU per kg per minute) in 5% dextrose in water was started and continued for 30 to 45 minutes. The urine collected during the first 5 to 10 minutes was discarded and two to five 10 to 15 minute clearance periods were then obtained. Upon completion of the infusions 2 to 3 additional 10 to 15 minute periods were collected in most of the experiments.

The urine was collected by indwelling catheter. When the urine flow was below 2 ml per minute, air "wash outs" and suprapubic pressure were utilized. Blood samples were taken at regular intervals from an indwelling Cournand needle inserted into an antecubital vein. Blood pressure was measured sphygmomanometrically at frequent intervals throughout the experiments.

Plasma and urine samples were analyzed for endogenous creatinine chromogen according to Bossnes and Tausky(6), and for sodium and potassium by flame photometry. Urine and plasma total solute concentration was measured with a Fiske osmometer. The free water clearance (C_{H_2O}) was calculated according to Smith(7), and Wesson and Anslow(8), by the formula $C_{H_2O} = V\text{-}U_{Osm}V/P_{Osm}$, where V is urine flow in milliliters per minute and U_{Osm} and P_{Osm} are the solute concentrations of urine and plasma in milliosmols per kg of water. C_{H_2O} is positive when urine flow is greater, and negative when urine flow is less than the osmolar clearance.

Results. The results of all experiments are summarized in Tables I, II and III.

1. *Blood pressure.* Angiotensin II administered at rates of 0.0025 to 0.0050 μg per kg of body weight per minute caused a modest but definite increase in blood pressure ranging from 5 to 20 mm Hg systolic and 5 to 10 mm Hg diastolic in 5 out of 7 experiments. In general, the blood pressure increased immediately after infusion of angiotensin II began and remained sustained throughout the infusion. Upon discontinuing the angiotensin II infusion blood pressure promptly returned to control values. None of the subjects studied offered any complaints throughout the angiotensin II infusion. Blood pressure did not change significantly during infusion of pitressin.

2. *Endogenous creatinine clearance.* During both mannitol and water diuresis angiotensin II infusion caused an average 11% decrease of endogenous creatinine clearance (C_{Cr}). The infusion of pitressin produced in 2 experiments a decrease in C_{Cr} of approximately 40% (P.R. and B.M., Table II), but in the other 2 experiments (P.R., Table I; G.C., Table II) C_{Cr} remained unchanged.

During the recovery periods C_{Cr} returned to or near control levels in all experiments but one (P.R., Table I).

3. *Urine flow (V), total solute concentration (U_{Osm}), Osmolar clearance (C_{Osm}) and free water clearance (C_{H_2O}).* Angiotensin II promoted a moderate antidiuresis in all experiments. Urine total solute concentration (U_{Osm}) increased above control values by 33 to 119 mOsm per kg of water, osmolar clearance (C_{Osm}) diminished by about 25%, while urine flow (V) and free water clearance

† Lot E 6286 A, supplied by Ciba Pharmaceuticals, Summit, N. J.

‡ Lot 3-161-10, supplied by Parke, Davis and Co., Detroit, Mich.

TABLE I. Effects of Angiotensin II and Pitressin during Mannitol Diuresis on Blood Pressure (BP), Urine Flow (V), Urine Osmolality (U_{Osm}), Osmolar Clearance (C_{Osm}), Free Water Clearance (C_{H_2O}), Sodium ($U_{Na}V$) and Potassium (U_KV) Excretion Rates.

(Data corrected to 1.73 m^2 B.S.A.)

Subject, date. NaCl intake (g/day)	Procedure	Rate, kg/min. I.V.	B.P., mm Hg	V, ml/min.	U_{Osm}, mOsm per kg H_2O	C_{Osm}, ml/min.	C_{H_2O}, ml/min.	$U_{Na}V$, μeq/min.	U_KV, μeq/min.	C_{Cr}, ml/min.	V per 100 ml C_{Cr}	C_{Osm} (ml/min.)	C_{H_2O}
B.M., 9/16/60, 2	Control*		98/70	6.6	297	6.7	- .1	45	71	72	9.1	9.3	- .2
	Angiotensin†	.0050 μg	120/80	5.0	358	6.2	- 1.2	18	52	66	7.5	9.3	- 1.8
	Recovery‡		114/72	5.8	343	6.9	- 1.1	33	60	73	8.0	9.4	- 1.4
G.C., 12/21/60, 10	Control*		125/86	12.4	265	10.6	+1.7	456	54	66	18.7	16.1	+2.6
	Angiotensin†	.0050 μg	144/92	7.4	326	7.7	- .3	193	95	59	12.5	13.1	- .6
	Recovery‡		125/84	9.2	309	8.8	+ .4	309	102	64	14.8	14.2	+ .6
P.R., 3/30/61, 2	Control*		106/64	12.8	203	8.3	+4.5	127	134	96	13.4	8.6	+4.8
	Angiotensin†	.0038 μg	122/74	9.6	236	7.3	+2.5	72	96	82	11.6	8.6	+3.0
	Recovery‡		108/64	10.3	209	6.7	+3.6	83	111	76	13.5	8.8	+4.7
P.R., 4/3/61, 2	Control*		104/62	9.9	201	6.4	+3.5	86	27	75	13.2	8.5	+4.7
	Pitressin†	18 μU	106/60	7.7	410	8.5	- 1.8	111	84	80	8.4	10.6	- 2.2

* Avg of 3 to 5 periods. † Avg of 2 periods at peak of effect. ‡ Avg of 2 to 5 periods.

(C_{H_2O}) decreased by nearly 40%. The effects of pitressin were qualitatively similar to the above, except that the changes in U_{Osm} were greater. Upon cessation of angiotensin II infusion V and C_{H_2O} increased but failed to return to control levels.

In Table III are assembled the net averaged changes in V, C_{Osm} and C_{H_2O} found during the infusions of angiotensin II and pitressin. Angiotensin II, like pitressin, produced a decrease of C_{H_2O} proportionate to that of V but consistently greater than C_{Osm} changes.

4. *Electrolyte excretion.* Angiotensin II caused a slight to moderate decrease of urine sodium concentration averaging 30% in all experiments. However, during infusion of pitressin, urine sodium concentration rose markedly in 3 experiments and remained unchanged in one. Rate of sodium excretion per minute ($U_{Na}V$) followed the pattern of urine flow, but decreased more during angiotensin II than during pitressin infusion.

During infusion of angiotensin II urine potassium concentration increased slightly in 2 experiments, but did not change significantly in the other. During the infusion of pitressin urine potassium concentration increased in all experiments. Potassium excretion rates (U_KV) decreased in all the experiments but one (G.C., Table I) during infusion of angiotensin II, but increased during the infusion of pitressin in 2 of 4 experiments. After cessation of angiotensin II $U_{Na}V$ rose but failed to reach control levels.

Discussion. The results show that in normotensive subjects with diabetes insipidus angiotensin II administered intravenously causes antidiuresis, increased tonicity of the urine and mild salt retention. These effects are manifest during high and low rates of solute excretion. The findings confirm and extend those reported in normal subjects having received natural and synthetic angiotensin II(1,9). They also show that in a dosage range in which angiotensin II produces no or only slight increases in blood pressure, there occurs definite antidiuresis and sodium retention.

In view of the known action of angiotensin (10) on renal circulation, the water and electrolyte changes found in the present study

TABLE II. Effects of Angiotensin II and Pitressin during Water Diuresis on Blood Pressure (BP), Urine Flow (V), Urine Osmolality (U_{Osm}), Osmolar Clearance (C_{Osm}), Free Water Clearance (C_{H_2O}), Sodium ($U_{Na}V$) and Potassium (U_KV) Excretion Rates.

(Data corrected to 1.73 m² B.S.A.)

Subject, date, NaCl intake (g/day)	Procedure	Rate, kg/min. I.V.	B.P., mm Hg	V, ml/min.	U_{Osm}, mOsm per kg H_2O	C_{Osm}, ml/min.	C_{H_2O}, ml/min.	$U_{Na}V$, μeq/min.	U_KV, μeq/min.	C_{Cr}, ml/min.	V	C_{Osm} (ml/min.) per 100 ml C_{Cr}	C_{H_2O}
B.M., 9/20/60, 2	Control*		124/78	5.8	78	1.6	+4.2	37.5	34.5	91	6.4	1.7	+4.7
	Angiotensin†	.0050 μg	138/88	3.9	138	1.9	+2.0	23.0	15.5	77	5.0	2.5	+2.5
	Control*		112/78	8.9	56	1.8	+7.1	68.0	19.5	97	9.1	1.8	+7.3
	Pitressin†	14 μU	120/78	.4	587	1.0	− .6	24.5	46.0	78	.6	1.3	− .7
G.C., 12/19/60, 10	Control*		130/90	2.9	204	1.9	+1.0	76	48	104	2.7	1.8	+ .9
	Pitressin†	8 μU	132/96	1.1	386	1.5	− .4	73	40	100	1.1	1.5	− .4
P.R., 4/7/61, 2	Control*		106/60	4.5	130	1.8	+2.7	2.5	45	111	4.0	1.6	+2.4
	Angiotensin†	.0030 μg	104/62	1.4	231	1.0	+ .4	.2	24	91	1.5	1.1	+ .4
	Recovery‡		104/56	1.9	200	1.2	+ .7	.2	26	113	1.7	1.0	+ .7
P.R., 4/11/61, 2	Control*		108/64	5.3	142	2.3	+3.0	9.2	114	131	4.0	1.7	+2.3
	Angiotensin†	.0025 μg	106/72	4.2	172	2.3	+1.9	9.0	71	129	3.2	1.8	+1.4
	Recovery‡		102/68	5.0	134	2.1	+2.9	10.0	51	127	3.9	1.6	+2.3
	Angiotensin†	.0025 μg	104/78	2.6	190	1.6	+1.0	2.0	39	114	2.3	1.4	+ .9
	Recovery‡		102/68	4.4	140	2.0	+2.4	10.0	59	123	3.5	1.6	+1.9
P.R., 4/14/61, 2	Control*		104/66	2.7	179	1.6	+1.1	2	24	121	2.3	1.3	+1.0
	Pitressin†	3 μU	104/62	.3	535	.5	− .2	.1	11	51	.6	1.0	− .4
	Control*		110/68	1.9	226	1.5	+ .4	3.0	28.7	118	1.6	1.3	+ .3
	Angiotensin†	.0038 μg	116/66	1.0	328	1.1	+ .1	1.3	23.0	111	1.0	1.1	− .1

* Avg of 3 to 5 periods. † Avg of 2 periods at peak of effect. ‡ Avg of 2 to 5 periods.

TABLE III. Summary of Changes Produced by Angiotensin II and Pitressin.

Subject	ΔV, ml/min.			ΔC_{Osm}, ml/min. per 100 ml C_{Cr}			ΔC_{H_2O}, ml/min. per 100 ml C_{Cr}		
	B.M.	G.C.	P.R.	B.M.	G.C.	P.R.	B.M.	G.C.	P.R.
Angiotensin II									
Mannitol diuresis	−1.6	−6.2	−1.8	0	−3.0	0	−1.6	−3.2	−1.8
Water "	−1.4		−1.3	+.9		− .2	−2.2		− .7
Pitressin									
Mannitol diuresis			−4.8			+2.1			−6.9
Water "	−8.5	−1.6	−1.7	−.5	− .3	− .3	− .8	−1.4	−1.3

The data presented were obtained from all experiments listed in Tables I and II. Values for urine flow (V), osmolar clearance (C_{Osm}) and free water clearance (C_{H_2O})/100 ml of endogenous creatinine clearance (C_{Cr}) during infusions of angiotensin II and pitressin have been averaged and are expressed here as "net" changes from control figures.

could be ascribed to decreased renal blood flow and glomerular filtration. However, although endogenous creatinine clearance decreased in most of the experiments, the decrease was small and proportionately less than that of water and salt excretion. Further, upon cessation of infusion of angiotensin II, endogenous creatinine clearance returned to control levels while antidiuresis and sodium retention tended to persist. On the basis of the present findings, the inhibition of diuresis caused by angiotensin II cannot be attributed with assurance to the decrease of glomerular filtration alone. On the contrary, the data suggest that the antidiuresis may be attributable to increased tubular reabsorption of water and salt.

Whether the observed effects of angiotensin II are due to direct or indirect action upon the renal tubules is not clear. The concept that the renal pressor system is functionally related to the metabolism of corticosteroids (11) has received support from human and animal experimentation(2,3,12). The secondary release of aldosterone during infusion of angiotensin II might have caused the decreased sodium excretion found in the present experiments. This interpretation, however, is not convincing because it does not give account for the decreased antidiuresis, nor for the lack of changes in potassium excretion. The present findings rather show a similarity between the effects of angiotensin II and those of pitressin on reabsorption of water. Thus, it appears that angiotensin II might inhibit water excretion by direct tubular action. A similar mechanism has been suggested for explaining the renal effects of the catecholamines(13).

The water and electrolyte changes found in the present study contrast with those caused by angiotensin II in hypertensive subjects (10). The discrepancy is difficult to explain on the basis of the available data. Further studies conducted under standardized conditions are needed to ascertain whether angiotensin plays a physiological role, if any, in the regulation of kidney function.

Summary. Angiotensin II (valine-5 angiotensin II amide) and pitressin were administered intravenously during mannitol and water diuresis to subjects with diabetes insipidus. Angiotensin II in doses of 0.0025 to 0.0050 μg per kg per minute caused a small but definite increase in blood pressure in 5 of 7 experiments, a decrease in endogenous creatinine clearance of about 11%, and a decrease in urine flow and water clearance of about 40%. The rate of sodium excretion was depressed by nearly 50%, while that of potassium decreased much less. Pitressin produced changes of water excretion qualitatively similar to those caused by angiotensin II. However, this effect was achieved without alteration in blood pressure, although filtration rate decreased. Further, pitressin did not consistently affect excretion of sodium and potassium. The results suggest that angiotensin II at the dose-ranges examined inhibits water and salt excretion by direct tubular action.

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Substrate Utilization in Bovine Kidney Culture Cells Infected with Foot-and-Mouth Disease Virus. (27118)

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Infection of bovine calf kidney culture cells with foot-and-mouth disease virus (FMDV) results in an increased use of glycolytic pathways, the metabolic sequences previously found necessary for virus production(1,2,3). A further study of the acids produced during the metabolism of glucose revealed only pyruvic, lactic, and acetic acids,* the quantities of which were influenced by the presence of lactalbumin hydrolyzate in the maintenance medium.

The present investigation was undertaken to study in more detail the metabolic interactions of normal and infected cells, partially depleted of endogenous nutrients, in contact with a defined medium(4) containing varying quantities of glucose, pyruvate, acetate, and lactalbumin hydrolyzate.

Materials and methods. Tissue culture. Primary monolayer cultures from bovine kidney tissue were prepared in 4-oz. prescription bottles as previously described(5). The growth medium consisted of 0.5% lactalbumin hydrolyzate and 6% bovine serum in Hanks' balanced salt solution. Resting cells were obtained by storage of 6-day-old confluent cultures, each containing from 11 to 13 million cells, for 5-9 days at 30°C without change of culture fluid.

Virus. High-titer stocks of FMDV-A119 were produced from infected tissue culture fluids by precipitation with methanol, and the alcohol removed by subsequent dialysis (1).

Virus assay. The plaque method using the monolayer cultures in prescription bottles was used(5).

Starvation. Cells were depleted of endogenous nutrients by washing 3 times with 5-ml portions of 1% tris(hydroxymethyl) amino methane (Tris) buffer, pH 7.5. They were then incubated at 37°C for 2 hours in presence of 5 ml of Hanks' balanced salt solution without glucose made up in 1% Tris buffer in which the sodium chloride concentration was reduced to 2.1 g per liter to maintain isotonicity (TB-H).

Respiration. Cell suspensions were prepared by scraping cells from ½-hour starved

* Unpublished data.