that they stimulated hormone synthesis. Mammary gland growth data suggest that less lactogenic hormone is released from the pituitary by 1.0 μg ED than by either 2.0 or 4.0 μg ED, which presumably are stimulating maximal secretion and release since only 1.0 μg ED could be potentiated with additional pituitary tissue. These results also indicate that the anterior pituitary is responsive to estrogen prior to weaning, in this case 21 days of age, and that the hypothalamic controlling mechanism(s) responsible for the synthesis and release of lactogen is either mature at this time or is matured by exogenous estrogen in suckling rats.

Summary. Anterior pituitary tissue alone and with estradiol dipropionate increased mammary area in suckling, ovariectomized, hooded Norway rats. Anterior pituitary tissue injected with 1.0 μg estrogen increased mammary area to a greater extent than did 1.0 μg estrogen alone and similar to that stimulated by 2.0 μg estrogen. When injected alone, anterior pituitaries from rats previously injected with estrogen increased mammary area to a greater extent than did anterior pituitaries from non-injected rats. Estrogen injections, either 1.0 or 2.0 μg, increased pituitary lactogen content.

1. Silver, M., *J. Endocrinol.*, 1953, v10, 35.
2. Macdonald, G. J., Reece, R. P., PROC. SOC. EXP. BIOL. AND MED., 1962, v110, 647.
3. Reece, R. P., Turner, C. W., *Mo. Agr. Exp. Sta. Res. Bull.*, 1937, 266.

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Renal Tubular Effect of Val$_5$-Angiotensin II Amide in Rats. (28166)

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The angiotensins are vasoconstrictor drugs. Their most evident effect on renal function in the dog(1,2,3), in man(4-6), and in the rat (7) is an increase in vascular resistance in the kidney which is apparently more pronounced than in other parts of the circulation(8). This vascular action of angiotensin appears to affect the afferent arterioles more than the efferent vessels and, therefore, causes a decrease in glomerular filtration rate(1,3,5,6). Since changes in glomerular filtration rate usually induce large changes in tubular function, any direct effect of angiotensin on renal tubules tends to be obscured by the effect on renal hemodynamics and glomerular filtration. In contrast to normal humans, patients with severe chronic hypertension do not respond to infusions of angiotensin by a major drop in effective renal blood flow: the clearance of p-aminohippurate (PAH) either remains unchanged or decreases only slightly(6,9-11). Glomerular filtration rate (GFR) remains unchanged. In such patients angiotensin induces an increase in urine flow and in sodium excretion which must be attributed to a tubular effect of the drug. A similar tubular effect in normal rats will be described here.

Methods. Male rats weighing 200-300 g, of mixed stock, were used. Renal clearance and water-electrolyte excretion was measured as described by Cotlove, with modifications suggested by the writer(14). The restraining cages used have been described(15). A 0.15 M NaCl solution, containing suitable amounts of inulin and Na-PAH, was infused into a tail vein at a rate of approximately 0.85 ml/kg • min. After an equilibration period of approximately one hour, renal functions were measured for 2 control periods of 15-30 minutes each. Blood samples were taken after each period, and were replaced by i.v. injections of normal donor blood, containing inulin and PAH. At the conclusion of the second control period, angiotensin II amide ("Hypertensin CIBA") was added to the infusion fluid in amounts calculated to result in a drug administration rate of 0.008-0.2 μg/kg • min. In control animals, the isotonic saline infu-

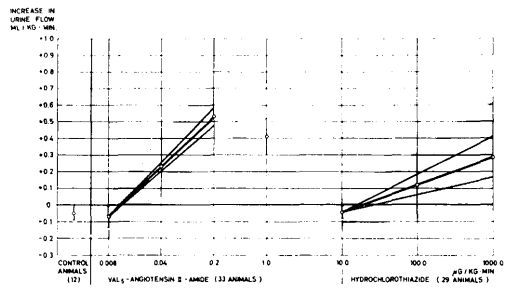

FIG. 1. Diuretic effects of val$_5$-angiotensin II amide and of hydrochlorothiazide in rats infused with 0.8-0.9 ml/kg • min 0.15 M saline. Diuretic effects are expressed as differences between urine flow 5 to 50 min after adding the drugs to infusion fluid and during the 2 preceding control periods. The non-significant change of urine flow in control animals is indicated as mean ± S.E. Standard error, of a, in the regression equation $Y = a + b x$, is similarly given with the values for urine flow for lowest doses used. Standard error of the regression coefficient b is shown by the pair of thin lines above and below regression line. Shaded area shows 0.95 fiducial limits for the increase in diuresis.

sion was continued as before. Five minutes after adding the drug the first "treatment" clearance period was started, followed by another period beginning 90 minutes after starting administration of angiotensin.

Urine was collected, practically without evaporation, under light mineral oil. Concentrations of sodium and potassium in urine and plasma were estimated by flame photometry (flame photometer attachment to Beckman DU spectrophotometer). Methods for measuring concentrations of inulin and PAH in plasma and urine have been discussed(16). Pure hydrochlorothiazide was dissolved in 0.15 M NaCl solution by adding 0.1 ml of N NaOH solution for each 10 mg of drug. Like angiotensin hydrochlorothiazide was added to the infusion solution.

Arterial blood pressure was recorded in parallel experiments in unanesthetized rats treated precisely as those used in the clearance experiments. A polyethylene tube was ligated in the common carotid artery 24 hours before the actual experiment and was led out through the skin of the neck through a stab wound. The protruding part of the polyethylene tube was closed by a polyethylene stopper and protected against scratching and biting by a short external piece of glass tubing. Blood pressure was recorded, by strain gauge pressure transducer, continuously over periods of 4-6 hours.

Results. While urine flow in untreated control animals remained stable or even declined slightly after 2 control periods, infusion of angiotensin II had a most pronounced diuretic and natriuretic effect (Fig. 1). When the diuretic response was expressed as the absolute difference in rates of urine flow during the first "treatment" period and the control periods, the response was a linear function of log dose, for doses from 0.008 to 0.2 μg/kg • min; 1.0 μg/kg • min apparently did not produce a larger diuretic effect than 0.2 μg/kg • min.

When hydrochlorothiazide was infused under the same conditions, which are not particularly favorable for demonstration of the diuretic effects of this type of drug, the slope of the log dose response regression line was clearly different from that for angiotensin II. This deviation from parallelism precludes a precise comparison of the potencies of the 2 drugs. Within the range of doses studied the relation of equally effective doses was approximately 5000-10,000 weight units of hydrochlorothiazide to one weight unit of angiotensin II amide, *i.e.*, 17,000-35,000/1 mole.

When angiotensin was infused together with isotonic saline under the same conditions, for more than one hour, the diuretic and natriuretic effect declined or disappeared after one hour (Table I). When hydrochlorothiazide was infused for similar periods the diuretic effect also disappeared within 1½ hours.

The increase in blood pressure in rats infused with 0.2 μg/kg • min angiotensin II remained quite stable for 1 to 1½ hours, and declined progressively thereafter. The pattern of the decrease differed markedly from that of "angiotensin tachyphylaxis" as observed in the unanesthetized dog infused with large doses of angiotensin II(17).

There was no consistent relationship between the pressor and the diuretic response to angiotensin. Thus, in a group of 7 rats, an infusion of 0.04 μg/kg • min of angiotensin produced an increase in urine flow from 0.66 ± 0.09 to 0.93 ± 0.10 ml/kg • min, but had practically no pressor effect (mean increase

TABLE I. Influence of Angiotensin II Amide (.2 μg/kg·min) on Renal Excretion of Water, Sodium and Potassium in Rats.

	Urine flow, ml/kg · min	$[Na^+]_U \cdot V$, μeq/kg · min	$[K^+]_U \cdot V$, μeq/kg · min	$\frac{V}{GFR}$, %	$\frac{[Na^+]_U \cdot V}{[Na^+]_P \cdot GFR}$, %
Controls	.39 ± .06	47 ± 10	7.6 ± 1.3	4.0 ± .6	4.4 ± .8
Angiotensin II: .2 μg/kg·min					
5- 50 min	.96‡ ± .13	111† ± 16	7.9 ± .6	10.0‡ ± 1.3	7.8† ± 1.3
90-130 "	.62‡ ± .09	90† ± 10	6.2 ± .6	6.1* ± .9	5.5 ± 1.2

Values are means from groups of 6-7 rats each, ± S.E. Significance of "paired" differences between treatment and control periods is shown as * for $P < 0.05$, † for $P < 0.01$, ‡ for $P < 0.001$. All animals were infused with 0.15 M NaCl solution at 0.85 ml/kg·min (i.e. 128 μeq Na^+/kg·min).

in blood pressure + 5 ± 8 mm Hg). The diuretic effect of angiotensin was neither mediated nor accompanied by an increase in glomerular filtration rate (Table II). There was no significant change in sodium concentration of the urine, or in sodium-U/P. The tubular rejection fraction for sodium and for water increased to a comparable extent under the influence of angiotensin (Table I). The infusion of angiotensin had no demonstrable effect on renal excretion of potassium.

Renal clearance of PAH decreased very sharply in angiotensin diuresis. This decrease resulted in a steep increase in the "filtration fraction," calculated as C_{IN}/C_{PAH}.

Discussion. Angiotensin II in unanesthetized rats infused with isotonic NaCl solution at a fairly high rate has diuretic and natriuretic effects comparable to the well-known effect of exogenous renin in the rat(18). Analagous to most other effects of exogenous renin the diuretic effect of renin may, therefore, be assumed to be mediated by angiotensin. Angiotensin was found by previous investigators to have no diuretic effect when injected intraperitoneally(18) and a small very brief diuretic effect when injected intravenously into rats in isotonic saline diuresis (19). These negative results are readily explained by the very rapid degradation of angiotensin in the blood and other tissues, particularly in the kidneys(20).

Since glomerular filtration rate is definitely not increased by infusion of angiotensin II, the observed diuretic effect in rats in isotonic saline diuresis must be ascribed to a change in tubular function, *i.e.*, a depression of overall tubular reabsorption of sodium ions followed by a decrease in back diffusion of water to the peritubular blood. The same statement applies to the diuretic and natriuretic effect of angiotensin in human patients with severe hypertension(6,9-13). This change could either be due to a direct tubular effect of angiotensin, or could be a corollary to some other action of angiotensin as, for instance, its pressor action. In dogs, increases in the perfusion pressure of the kidneys have been shown to accelerate urine flow(21), sodium excretion(22), and total solute excretion(23). Within the limits of "autoregulation" of renal blood flow, *i.e.*, at blood pressures below 180 mm Hg, "pressure diuresis" occurred without an increase in glomerular filtration rate(22,

TABLE II. Influence of Angiotensin II Amide (.2 μg/kg·min) on GFR, C_{PAH} and Mean Arterial Blood Pressure in Rats Receiving Infusions of 0.85 ml/kg·min of 0.15 M NaCl Solution.

	GFR (C_{IN}), ml/kg·min	C_{PAH}, ml/kg·min	"Filtration fraction"	Mean arterial blood pressure, mm Hg
Controls	9.8 ± 1.1	29.0 ± 8.0	.34 ± .08	114 ± 7
Angiotensin II: .2 μg/kg·min				Change:
5 – 50 minutes	9.9 ± 1.5	18.0† ± 3.5	.59† ± .06	+28‡ ± 6
90 – 130 "	11.0 ± 2.0	23.6 ± 5.0	.50 ± .05	+14‡ ± 4

†, ‡ See Table I.

Values are means ± S.E. for groups of 6-7 rats each. "Paired" differences in C_{PAH} and F.F. are significant in spite of large inter-individual variation. Blood pressure was recorded in parallel experiments under comparable conditions on a group of 5 rats.

23). It is thought to be due to a change in renal medullary blood flow(23). "Pressure diuresis" has not, to our knowledge, been shown to occur in the rat. If it occurs, it could account, partly or wholly, for the diuretic effect of angiotensin infusion. The increases in sodium and water excretion with increasing arterial pressure observed in dogs within the range of blood pressures encountered in rats infused with angiotensin was, however, much smaller than the diuretic responses found in the present experiments. Furthermore, in our normal rats as well as in human hypertensive patients(13), sub-pressor doses of angiotensin enhanced renal sodium and water excretion. A direct inhibition of tubular sodium reabsorption by angiotensin would, therefore, appear as a more reasonable explanation of the observed diuretic effects. An inhibitory effect on tubular sodium transport would also be in keeping with the inhibition of sodium transport in isolated rat or rabbit kidney slices *in vitro* which could be induced by very small concentrations of angiotensin(24). The antidiuretic (and antinatriuretic) effect of angiotensin in dogs and humans in moderate water diuresis(1-7, 9-12) has been explained(10) as a direct consequence of a decrease in renal blood flow and in glomerular filtration rate. A decrease in renal blood flow has also been observed in dogs(8) and postulated to occur in rats(7). In normal human subjects, in moderate water diuresis, there is a marked fall in the clearance of PAH which is also interpreted as an expression of decreased renal blood flow, though the influence of angiotensin on renal PAH extraction in man apparently has not been measured. In hypertensive patients in whom angiotensin entails a diuretic response, the clearance of PAH either remains unchanged or decreases very slightly(11,13). In normal rats, on the other hand, there is a marked decrease in clearance of PAH. This could also be due to a decrease in renal blood flow: if renal vasoconstriction were to affect the afferent and the efferent segments of the renal circulation to the same degree, glomerular filtration rate would not be expected to decrease very much when blood pressure rises at the same time. On the other hand, the 40% decrease in C_{PAH} which occurred in rats under the influence of an angiotensin infusion could also be due to a decrease in tubular PAH secretion, possibly consequent upon the inhibition of tubular sodium transport. In kidney slices from rabbits, inhibition of the sodium-potassium exchange mechanism has been shown to interfere with transport of PAH(25). In normal rabbits angiotensin apparently also exerts a diuretic effect(26), accompanied by a decrease in clearance of diodone.

There is ample evidence that angiotensin stimulates the secretion of aldosterone from the adrenals of humans and dogs(13,27-31) as well as of rats(32). In humans with arterial hypertension, an increased urinary excretion of aldosterone has been shown to occur at the same time as decreased renal tubular reabsorption of sodium under the influence of angiotensin(13). In normal rats in isotonic saline diuresis aldosterone would, however, not be expected to have a marked influence on sodium excretion(33). On the other hand, in human patients with glomerular nephritis an infusion of aldosterone, preceding an infusion of angiotensin, has been shown to increase the natriuretic activity of angiotensin(34). This finding raises the possibility that endogenous aldosterone, secreted under the influence of angiotensin, may enhance rather than oppose the natriuretic effect of angiotensin by an unknown mechanism.

Summary. In unanesthetized restrained male rats infused with 0.15 M NaCl solution at 0.8-1.0 ml/kg · min, infusions of angiotensin had a strong diuretic and natriuretic effect. The log dose-response relation was linear for doses of angiotensin from 0.008-0.2 μg/kg · min. The diuretic potency of angiotensin per Mol was approximately 17,000-35,000 times greater than that of hydrochlorothiazide. GFR (C_{IN}) did not increase with angiotensin diuresis. Tubular rejection fractions for sodium ions and for water were increased to the same extent by angiotensin. Subpressor doses of angiotensin II also were diuretic. Both diuretic and pressor effects tended to decrease after 1-1½ hours though the infusion was continued. There was a large decrease in C_{PAH} during angiotensin diuresis which may reflect

a depression of PAH secretion and/or a decrease in renal blood flow.

1. Barac, G., *C. R. Soc. Biol.,* 1959, v153, 704.
2. Gross, F., Turrian, H., in M. Schachter, Ed., *Polypeptides Which Affect Smooth Muscles and Blood Vessels,* Oxford, Pergamon Press, 1960, p137.
3. Del Greco, F., Page, I. H., *Circulation,* 1961, v24, 917.
4. Finnerty, F. A., Jr., *ibid.,* 1962, v25, 255.
5. Bock, K. D., Dengler, H., Krecke, H. J., Reichel, G., *Klin. Wochenschr.,* 1958, v36, 808.
6. Peart, W. S., *Brit. Med. J.,* 1959, vII, 1421.
7. Mandel, M. J., Sapirstein, L. A., *Circulation Res.,* 1962, v10, 807.
8. McGiff, J. C., Aviado, D. M., *ibid.,* 1961, v9, 1327.
9. Peart, W. S., Brown, J. J., *Lancet,* 1961, vI, 28.
10. Barbour, B. H., Gill, J. R., Jr., Slater, J. D. H., Bartter, F. C., *Clin. Res.,* 1962, v10, 245.
11. Brown, J. J., Peart, W. S., *Clin. Sci.,* 1962, v22, 1.
12. Del Greco, F., PROC. SOC. EXP. BIOL. AND MED., 1961, v107, 943.
13. Biron, P., Chrétien, M., Kolw, E., Genest, J., *Brit. Med. J.,* 1962, vI, 1568.
14. Cotlove, E., *J. Appl. Physiol.,* 1961, v16, 764.
15. Peters, G., Hedwall, P. R., *Arch. int. pharmacodyn. et thér.,* in press, 1962.
16. Peters, G., *Arch. exp. Path. u. Pharmakol.,* 1958, v235, 113.
17. Gross, F., Bock, K. D., *Circulation,* 1962, v25, 193.
18. Croxatto, H., Barnafi, L., Passi, J., *Acta physiol. latino-amer.,* 1952, v2, 159.
19. Schroeder, R., Meyer-Burgdorff, C., Rott, D., Brahms, O., *Arch. exp. Path. u. Pharmakol.,* 1962, v240, 285.
20. Brunner, H., Regoli, D., *Experientia,* 1962, v18, 504.
21. Goll, F., *Z. f. rat. Med.,* 1854, vIV, 86.
22. Selkurt, E. E., *Circulation,* 1951, v4, 541.
23. Thurau, K., Deetjen, P., *Pflüger's Arch.,* 1962, v274, 567.
24. Leyssac, P., Lassen, U. V., Thaysen, J. H., *Biochim. biophys. acta,* 1961, v48, 602.
25. Burg, M. B., Orloff, J., *Am. J. Physiol.,* 1962, v202, 565.
26. Hughes-Jones, N. C., Pickering, G. W., Sanderson, P. H., Scarborough, H., Van Den Broucke, J., *J. Physiol.,* 1949, v109, 288.
27. Carpenter, C. C. J., Davis, J. O., Ayers, C. R., *J. Clin. Invest.,* 1961, v40, 2026.
28. Davis, J. O., Ayers, C. R., Carpenter, C. C. J., *ibid.,* 1961, v40, 1466.
29. Ganong, W. F., Mulrow, P. J., Boryczka, A., Cera, G., PROC. SOC. EXP. BIOL. AND MED., 1962, v109, 381.
30. Mulrow, P. J., Ganong, W. F., *Circulation,* 1962, v25, 213.
31. Mulrow, P. J., Ganong, W. F., Cera, G., Kuljian, A., *J. Clin. Invest.,* 1962, v41, 505.
32. Gláz, E., Sugar, K., *J. Endocrinol.,* 1962, v24, 299.
33. Peters, G., *Arch. exp. Path. u. Pharmakol.,* 1958, v235, 155.
34. Del Greco, F., PROC. SOC. EXP. BIOL. AND MED., 1961, v107, 943.

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Colchicine Teratogenesis in Hamster Embryos.* (28167)

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The natural resistance of the adult golden hamster, *Cricetus auratus,* to the mitotic inhibitory action of colchicine has been described(1) and confirmed(2,3). It has also been reported that hamster embryonic cells grown in tissue culture have at least a 100-fold greater resistance to colchicine than do human cells in tissue culture(3). However, Vincaleukoblastine, another mitotic inhibitor, has as marked an effect on hamsters as on other animals(4). During a study on mitotic inhibition in hamster embryos it was noted that colchicine had an unexpected effect on the developing embryos. This report is based on a study of the effects of colchicine in pregnant hamsters, using fetal mortality and congenital malformations as criteria.

Materials and methods. Virgin female golden hamsters were bred under direct observation in the early evening hours and left with the male overnight. The day following

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