sex and pre-shock environment. Females were more sensitive to noxious stimulation than males, as reflected in a more rapid increase and subsequent fall in circulating levels. Post-weaning group living, compared to isolation, was associated with both greater sensitivity and a more pronounced total response. Infantile-manipulated rats had a numerically greater sensitivity and total response, in comparison with non-manipulated controls.

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Effect of Intravenous Angiotensin Infusion on Adrenal Medullary Function in Man. (32179)

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It has been shown in animal experiments that angiotensin can cause the release of catecholamines from the adrenal gland(1-5). In the cat, 0.1 μg of angiotensin intravenously (I.V.) will produce this effect; an even smaller dose injected into the celiac artery can release up to 100 times its weight in epinephrine(1). However, the effect of an I.V. infusion of angiotensin on adrenal medullary function has not been investigated in man.

The purpose of this work was, therefore, to study in man the effect of an I.V. infusion of a moderate pressor dose of angiotensin (a) on the urinary excretion rates of catecholamines and 3-methoxy-4-hydroxy mandelic acid (V.M.A.) and (b) on the fasting blood glucose level.

* Renal Research Fellow, supported by a grant from the Lions Clubs of Southern Queensland and Northern New South Wales.

Methods. (a) *Effect of angiotensin on catecholamine and V.M.A. excretion rates.* Studies were done on 2 male and 2 female volunteers, aged from 23 to 35 years. The subjects were normal or were patients who had recovered from minor illnesses and were in good health at time of the study. Subjects were kept at rest in bed in a hospital for 2 days, on the second of which the experiments were performed. Vanilla, vanilla-containing foods, bananas and coffee were excluded from the diet for the 2 days. With these restrictions, meals and fluids were permitted during the experiments. To compensate to some degree for depression in renal excretory capacity which angiotensin might induce, the inulin clearance was used throughout these experiments as a measure of glomerular filtration rate (G.F.R.). Excretion rates of catecholamines and V.M.A. were then expressed in μg/hr/100 ml G.F.R.

On the experimental day, a priming dose of inulin, sufficient to achieve a plasma level of 5-10 mg/100 ml, was given about 10:20. a.m. This level was then maintained by an I.V. infusion of inulin in N NaCl, with the aid of a constant infusion pump. After 40-50 minutes equilibration time, the bladder was emptied with the patient standing (male) or sitting on a bed-pan, on a chair (female). Collection periods were then begun.

The first collection (control) period was for 3 hours, at the end of which the bladder was again emptied and all urine measured and collected. Asparginyl valine-5 angiotensin (Hypertensin, CIBA) was then added to the infusion, so that a dose of 0.025 μg/kg/min was given. This was maintained for 5 hours, when the bladder was again emptied (angiotensin period). A further 3 hours collection was then carried out (recovery period), during which only inulin in N NaCl was infused. In 2 instances, the overnight urine was collected to a known time about 7 a.m. next morning; the infusion was not continued overnight in these cases. Urine samples were collected in bottles thoroughly cleaned with glass-distilled water. Ten ml of 15% HCl were previously added to each bottle as a preservative, and the samples were frozen. Some urine was measured and kept separately for inulin estimation.

At the mid-point of each of the 3 clearance periods, a heparinized blood sample was withdrawn from a vein in the arm opposite the infusion, for estimation of the plasma inulin level, and blood pressure was taken at hourly intervals throughout the experiments.

Total catecholamine excretion was measured in duplicate in the urine by the ethylenediamine method of Small(6). Acetic acid extracts were made within 24 hours on all samples and stored at 2°C; the remainder of the catecholamine estimation was completed within 2 days. A Shimadzu (QR50) spectrophotofluorometer was used, with a mercury lamp and a primary filter (VV1) of wavelength 360-390 mμ, and a secondary filter (Y3) with a gradual cut-off below 520 mμ. All glassware used was soaked in nitric acid and triple-washed with glass-distilled water. 750 mg of alumina were used for adsorption, instead of 500 mg, as this appeared to give improved recoveries. The alumina was first washed in hot N HCl, as recommended by Hingerty(7).

The relation between fluorescence and concentration of pure L-norepinephrine standards was linear up to 0.20 μg/ml. The S.D. of the variation of duplicate readings from the duplicate mean was ± 9.5% for 25 separate readings. Recoveries of L-norepinephrine (Koch Light, purified powder), added to urine, ranged from 66.2 to 84.4% with an average of 75.1%, which compares well with the 75% quoted by Small(6). Final figures for urine catecholamines were not corrected for recoveries, but were corrected for the volume of added preservative and for the amount of urine kept separately for inulin estimation.

3-Methoxy-4-hydroxy mandelic acid (V.M.A.) was measured by the method of Pisano, Crout and Abraham(8). Inulin was measured in duplicate in plasma and urine by the method of Walser, Davidson and Orloff(9).

(b) *Effects of angiotensin on the fasting blood glucose level.* The subjects were 2 male and 2 female volunteers, aged 22 to 59 years, who had regained normal health following minor illnesses. They were fasted overnight before the experiment. Next morning, with the aid of a constant infusion pump, N NaCl was infused I.V. for 1 hour, then 0.025 μg/kg/min of angiotensin in N NaCl for 1 hour, then N NaCl alone for a further half hour. Blood pressure was taken 8 times throughout, and following each of these measurements a heparinized blood sample was withdrawn from a vein in the arm opposite the infusion, for blood glucose estimation. In each case, the first urine passed after the experiment was tested for glucose with glucose-oxidase test-paper.

Blood glucose levels were estimated with a Technicon Auto-analyzer. Hoffman's ferricyanide method (Technicon method N 2 A) was used(10).

Results. (a) *Effect of angiotensin on catecholamine and V.M.A. excretion rates.* Suffi-

TABLE I. Effects of a 5-Hour Intravenous Infusion of 0.025 μg/kg/Min of Angiotensin in Man. Control and recovery periods are of 3 hours each. Results are the mean of 4 experiments.

Period	Diastolic b.p., mm Hg	G.F.R.,* ml/min	Urine flow, ml/min	Catecholamine excretion, μg/hr/100 ml of G.F.R. ± S.D.	V.M.A. excretion, μg/hr/100 ml of G.F.R. ± S.D.
Control	78	119	2.9	2.92 ± 1.34	201 ± 77.5
Angiotensin	105	93	.8	1.95 ± .89	173 ± 40.4
Recovery	71	113	2.6	2.98 ± .93	201 ± 62.2

* G.F.R. not corrected for body surface area.

cient angiotensin was given to produce vascular and renal effects. During the angiotensin infusion, the diastolic blood pressure was significantly elevated above control values (mean rise, 27 mm Hg, $p<.025$), and the G.F.R. was significantly reduced below control values (mean fall, 26 ml/min, $p<.05$). Although the urine flow rate fell during angiotensin infusion in 3 experiments, the mean change was not significant. During the recovery period, these parameters returned towards control levels (Fig. 1; Table I).

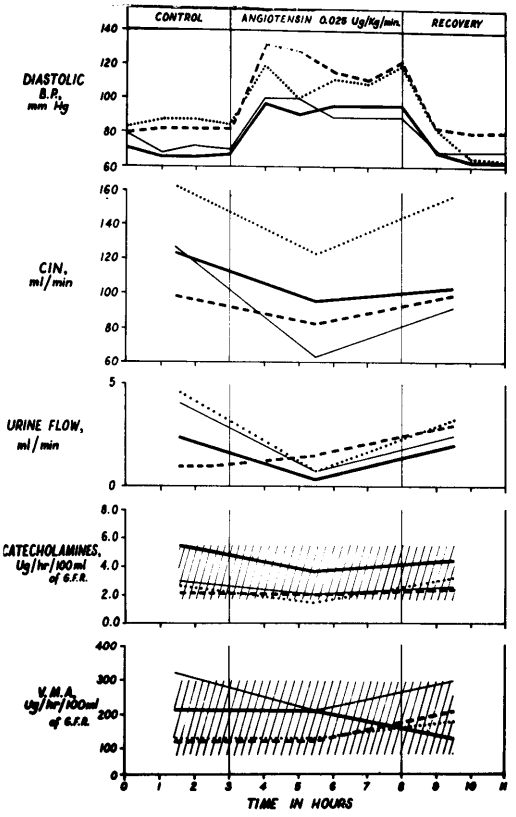

FIG. 1. Effect of a 5-hr intravenous infusion of 0.025 μg/kg/min of angiotensin on diastolic blood pressure, G.F.R. (C_{IN}), urine flow and catecholamine and V.M.A. excretion rates in 4 experiments in man. Shaded areas represent approximate normal excretion rates for catecholamines and V.A.M. G.F.R. not corrected for body surface area.

In spite of this evidence of angiotensin activity, catecholamine excretion rates remained within an approximate normal range (1.7-5.2 μg/hr/100 ml G.F.R.) throughout all experiments (Fig. 1). The mean catecholamine excretion rates during control, angiotensin and recovery periods were not significantly different (2.92, 1.95, and 2.98 μg/hr/100 ml G.F.A., respectively; Table I). Similarly, V.M.A. excretion rates remained within an approximate normal range (83-292 μg/hr/100 ml G.F.R.) throughout each study (Fig. 1), except that one control value just exceeded this range. The mean V.M.A. excretion rates in the control, angiotensin and recovery periods of the experiments (201, 173 and 201 μg/hr/100 ml G.F.R., respectively; Table I) did not differ significantly.

Catecholamine and V.M.A. excretion rates were both within the normal range in the 2 specimens of urine collected overnight.

(b) *Effect of angiotensin on fasting blood glucose level.* Although the angiotensin infusion caused a significant rise in diastolic blood pressure in relation to the control period (mean rise, 21 mm Hg, $p<.025$), blood glucose levels remained almost unchanged (Fig. 2). The mean blood glucose levels ± S.D. were: control, 74.5 ± 6.9 mg/100 ml; angiotensin period, 73.8 ± 8.9 mg/100 ml; recovery period, 75.5 ± 4.9 mg/100 ml. These were not significantly different. Glycosuria did not occur following any experiment.

Discussion. The results show that I.V. angiotensin in the dose used did not increase the urinary excretion of adrenal medullary hormones, nor did it increase the excretion

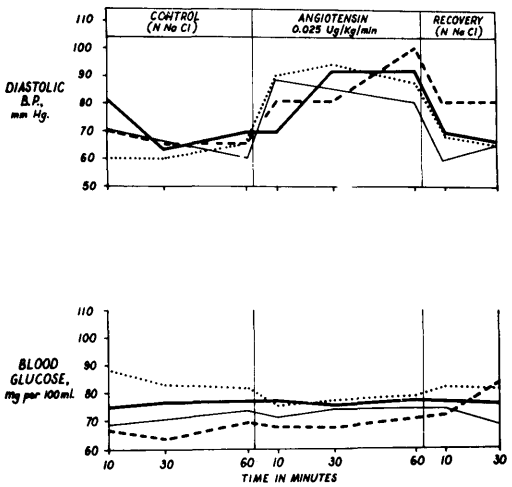

FIG. 2. Effect of 1 hr intravenous infusion of 0.025 μg/kg/min of angiotensin on diastolic blood pressure and fasting blood glucose level in 4 studies in man.

of V.M.A., a major catecholamine metabolite. Therefore it is unlikely that catecholamine release made a significant contribution to the observed pressor response. The constancy of the blood glucose level during angiotensin infusion can be regarded as further evidence of unchanging adrenal medullary activity.

Although the effect of angiotensin on the adrenal medullary hormones has not previously been studied in detail in man, in one report(11) the 24-hour V.M.A. excretion rate was not increased by a 6-hour angiotensin infusion in one normal and 3 hypertensive patients. This is in accord with the present findngs. Catecholamine excretion rate was not examined in that study.

In another study(12), it has been shown that epinephrine does not play an important role in the vasoconstriction induced by angiotensin in hand vessels in man, which supports the view that the vascular response to angiotensin is not mediated by catecholamine release from the adrenal medulla.

The dose of angiotensin used in the present study produced a marked pressor response. Prolonged infusions of larger doses were not felt to be entirely safe, but it is possible that angiotensin in greater quantities may stimulate the release of catecholamines. It is also possible that angiotensin injected into the adrenal artery in man may stimulate catecholamine release, since this route of administration is more effective than I.V. angiotensin in the cat(1,2). However, in most experimental studies in man in which the various physiological effects of angiotensin have been examined, a dose of the order of that used in the present study has been given and the intravenous route of administration used. Further, I.V. infusion more closely resembles the physiological situation, since active angiotensin is normally released into the systemic circulation *via* the renal vein.

The lack of an effect of angiotenin on the blood glucose level and the absence of glycosuria during angiotensin infusion in the present study is in agreement with findings in the dog(13). The occurrence of glycosuria or elevation of blood glucose levels described in the rabbit and chicken during angiotensin infusion(14,15) may be due to a species difference.

Summary. In studies in man, an intravenous infusion of 0.025 μg/kg/min of angiotensin for 5 hours significantly elevated the diastolic blood pressure, but had no effect on the urinary catecholamine or 3-methoxy-4-hydroxy mandelic acid (V.M.A.) excretion rates. The same dose of angiotensin infused intravenously for 1 hour had no effect on fasting blood glucose levels and did not cause glycosuria. It is concluded that catecholamine release does not play a significant role in the vascular responses observed with this dose of intravenous angiotensin in man.

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Human Febrile Response to Influenza Virus or Its Ether Isolated Hemagglutinins. (32180)

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An earlier study(1) has shown that monovalent influenza virus vaccines prepared from two strains of influenza A virus processed to yield either intact virus vaccines or vaccines composed of the ether isolated hemagglutinins induced equivalent hemagglutinating inhibiting (HI) and neutralizing antibodies in children and adults. Additionally, the isolated hemagglutinin vaccines failed to induce febrile responses in children, while the same or lower doses of intact virus were clearly pyrogenic. The earlier study did not include type B influenza virus, and the processing had included a lanthanum precipitation step designed to reduce or eliminate the soluble (S) influenza antigens. It was important therefore, to determine if ether treatment alone, without lanthanum precipitation, would render the antigens nonpyrogenic for children; and if treatment of type B influenza virus would reduce its reactivity in infants.

Only minimal antibody response could be anticipated from the single vaccine dose in the very young subjects who participated in this study. However, it was of interest to compare the immunogenicity of the standard and extracted antigen vaccines under these adverse conditions.

Methods and materials. Virus. The strains of influenza virus used for vaccine production were Asian/170, Type A-2 and Maryland/59, Type B obtained from the Division of Biologics Standards, National Institutes of Health.

Vaccines. Intact Virus Vaccine. The virus was concentrated approximately 20-fold and partially purified by differential centrifugation in the Sharples and International Centrifuges. A portion of the concentrate was inactivated by addition of formalin to a final concentration of 1 part in 2,000 and incubation at 37°C for 48 hours. The chick cell agglutinating (CCA) activity(2) of the inactive concentrate was determined and the maximum test strength vaccine prepared by dilution of an appropriate volume of the inactive concentrate with isotonic saline containing 1 part in 10,000 each of thimerosal and formalin and buffered to pH 7.2 with 0.01 molar phosphate.

Isolated hemagglutinin vaccine. To the remaining aliquot of the active monovalent concentrate from the Sharples and International Centrifuges was added 1 mg/ml of Tween 80, and the mixture was treated with 2 volumes of fresh anesthetic ether at 4°C for 5 hours. Constant agitation was maintained during the period of extraction. After separation, the ether phase was decanted and to remove residual ether the aqueous phase was aerated with nitrogen and held under vacuum at room temperature. Formalin and thimerosal were each added to a final concentration of 1 part in 10,000.

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