after radiation, but the increase of acid phosphatase activity in the thymus and spleen 2 hours after 1000 r remained unexplained. The percentage of free activities of these 2 enzymes did not seem to be affected by radiation.

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## Evidence for a Direct Effect of Angiotensin-II on Adrenal Cortex of the Dog.*† (27210)

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Angiotensin-II stimulates aldosterone secretion in normal humans(1,2) and nephrectomized hypophysectomized dogs(3,4). This could be due to a direct action on the adrenal, or it could be an indirect effect. It has been claimed that some polypeptide hormones and other substances release ACTH from binding sites in the body(5,6) and angiotensin-II might have this action. Its hemodynamic effects might also cause liberation of an aldosterone stimulating substance. To investigate the possibility of an indirect effect, we have compared the changes in adrenocortical secretion produced by infusing angiotensin-II into the arterial supply of the adrenal gland with the changes produced by its infusion into a peripheral vein in nephrectomized hypophysectomized dogs.

*Methods.* Six male mongrel dogs weighing 9.4 to 16.2 kg were used. The right kidney and the right adrenal were removed and the right lumbo-adrenal artery and the 2 pairs of lumbar arteries leaving the aorta at the level of the adrenals and the kidney tied. Six to 36 days later, each dog was anesthetized with pentobarbital and the left lumbo-adrenal vein cannulated. The left kidney was removed and the aorta and vena cava tied just below the renal vessels (Fig. 1). The left lumbo-adrenal artery was tied 1 cm lateral to the left adrenal. All branches to the diaphragm were ligated. A PE-50 polyethylene cannula was then inserted into the lumbo-adrenal artery, adjusted so that its tip was just at the aortic orifice of the lumbo-adrenal artery, and tied in place. The left carotid artery was cannulated for continuous monitoring of blood pressure with a Statham strain gauge and Grass Model 4 polygraph. A PE-50 cannula for infusing angiotensin-II and a larger cannula for administering transfusions were inserted into the left jugular vein. The dog was then hypophysectomized via the transbuccal route. Two control adrenal venous blood specimens were collected 60 and 80 minutes after hypophysectomy. Synthetic angiotensin-II in saline was then infused into the jugular vein (in 2 dogs) or the lumbo-adrenal artery (in 4 dogs) for 14 minutes.

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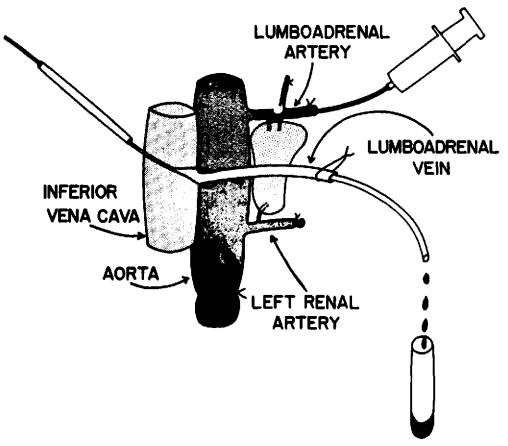


FIG. 1. Technic for injecting into adrenal arterial blood supply. For explanation, see text.

Adrenal venous blood was collected from the fourth to the fourteenth minute of infusion, and 2 additional samples were obtained 30 and 60 minutes after start of the infusion. After the 60-minute collection, angiotensin-II was infused into the other catheter (lumboadrenal artery or jugular vein) at the same rate and collections made at the same time intervals. The angiotensin-II was dissolved in saline just before use, and the solution infused at a rate of either 0.12 or 0.25 ml/min. Care was taken to fill the catheter with the angiotensin solution before the infusion started. From the beginning of the infusion into the adrenal artery until one minute after it was over, the choker around the lumboadrenal vein was tightened and all adrenal venous blood flowed out of the body, so that no angiotensin-II was permitted to escape into the systemic circulation through the adrenal vein. The one dog given norepinephrine received 12.5 $\mu$g/min in 0.25 ml/min of 5% glucose. All blood loss from the adrenal vein was replaced by transfusion at frequent intervals of an equal volume of bank blood from nephrectomized hypophysectomized donor dogs. All adrenal venous samples were centrifuged promptly and the plasma stored in the frozen state for subsequent analysis of Porter-Silber chromogens by the method of Silber and Porter(7) and aldosterone by the double isotope derivative technic(8). The adrenal output of these hormones was calculated by multiplying their concentration in adrenal venous plasma by the plasma flow per minute.

Just before sacrificing each animal, India ink in saline was infused into the lumboadrenal artery cannula. At autopsy, the position of the cannula was checked and the area stained by the India ink noted. Completeness of hypophysectomy was checked by gross examination.

*Results.* The effects of angiotensin-II on adrenal 17-hydroxycorticoid and aldosterone secretion are summariezd in Table I. In all instances, aldosterone output was greater when the peptide was injected into the adrenal arterial supply than when it was infused into the jugular vein. The differences were especially marked 30 minutes after the start of the 14-minute infusion. The increase in 17-hydroxycorticoid output was also greater, after intra-arterial infusion. Results were similar whether the angiotensin-II was first infused locally or systemically. The adrenal blood flow was slightly decreased during infusion in 5 of the 6 dogs, but the decline was not marked. The ratio of the volume infused into the adrenal artery to the adrenal plasma flow was 0.34 in one dog and less than 0.18 in all other instances. Norepinephrine had no effect on adrenocortical secretion and no greater effect than angiotensin on adrenal blood flow. Systemic pressure did not change more than 5 mm Hg when angiotensin-II was infused intraarterially (Table I). No change in systemic blood pressure was observed when norepinephrine was infused into the adrenal arterial supply. The animals withstood the experiment well, and blood pressure was maintained throughout.

At autopsy, all hypophysectomies were found to be complete by gross examination. In all the dogs, the adrenal gland was blackened by the injected India ink. In most dogs, there was a small amount of India ink in a 1-3 mm band of tissue around the adrenal. Other tissues were not blackened.

*Discussion.* When angiotensin-II was infused into the adrenal arterial supply, the effect on adrenocortical secretion was always greater than when it was infused into the jugular vein, and there was little or no change in systemic blood pressure. These observations indicate that angiotensin can stimulate

TABLE I. Effect of Angiotensin-II Infusions on Adrenocortical Secretion.

| Dog | Angiotensin dose (μg/min.) | Route | Aldosterone secretion rate (mμg/min.) Before | 4 min.* | 30 min.* | 17-Hydroxycorticoid secretion rate (μg/min.) Before | 4 min.* | 30 min.* | Blood pressure rise, systolic/diastolic (mm Hg) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | .42 | Jugular | 0 | 32 | 1 | 1.8 | 5.0 | 4.3 | 15/30 |
| | | Arterial | 0 | 33 | 30 | 4.3 | 8.9 | 11.2 | 5/5 |
| 2 | .083 | Jugular | 7 | 11 | 6 | 3.6 | 4.0 | 2.8 | 20/15 |
| | | Arterial | 3 | 27 | 20 | 1.4 | 7.1 | 2.7 | 0/5 |
| 3 | .083 | Jugular | 15 | 80 | 34 | .4 | .5 | 0 | 15/25 |
| | | Arterial | 17 | 96 | 64 | 0 | 2.3 | .2 | 0/0 |
| 4 | .083 | Jugular | 5 | 6 | 7 | .7 | .5 | 0 | 15/15 |
| | | Arterial | 1 | 11 | 15 | .6 | 4.1 | 2.0 | 5/0 |
| 5 | .042 | Jugular | 0 | 8 | 4 | .1 | .1 | 0 | 10/10 |
| | | Arterial | 9 | 18 | 13 | .2 | .2 | 0 | −5/5 |
| 6 | .042 | Jugular | 0 | 15 | 3 | .9 | 0 | 0 | 10/10 |
| | | Arterial | 8 | 63 | 71 | 0 | 2.3 | 0 | 5/0 |

* Min. after start of infusion.

the adrenal gland directly. How it exerts this effect is unknown. Our results do not rule out increased secretion secondary to an effect on the blood vessels in the gland, but this seems unlikely in view of the fact that norepinephrine had no effect. The dose of norepinephrine used causes a systolic blood pressure rise of 105-135 mm Hg when infused intravenously into nephrectomized hypophysectomized dogs(9). Recently, it has been reported that increased amounts of adrenocortical hormones appear in the medium when angiotensin is added to incubating beef adrenal tissue *in vitro*(10).

Our studies do not rule out the possibility that angiotensin-II acts only by releasing preformed hormones in the gland, since no analysis of gland content was made. However, this seems unlikely since aldosterone secretion was still elevated 16 minutes, and in some cases 46 minutes after the local infusion of angiotensin was stopped. In other studies, we have found that aldosterone secretion remains elevated during intravenous infusion of angiotensin-II for 4 hours(9). Secretion of this much hormone strongly suggests that synthesis as well as release of aldosterone was stimulated.

Although the sensitivity of the various dogs to intravenous angiotensin-II varied, it was generally greater than that of the dogs with 2 adrenal reported previously from this laboratory(3,4,9). Intravenous doses of 0.042 μg/min stimulated aldosterone secretion in both dogs receiving this dose in the present studies, but generally was ineffective in dogs with 2 adrenals. This increased sensitivity is probably due to compensatory hypertrophy of the adrenal, since the magnitude of the 17-hydroxycorticoid response to a given dose of ACTH is known to increase after unilateral adrenalectomy in the dog(11).

*Summary.* In 6 nephrectomized hypophysectomized dogs, the increase in aldosterone and 17-hydroxycorticoid secretion produced by infusion of angiotensin-II into the arterial blood supplying the adrenal gland was greater than the increase produced by infusion of the same dose into the jugular vein. It is concluded that angiotensin-II can stimulate adrenocortical secretion by a direct action on the adrenal gland.

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# A "Heat-Activated" Diphosphopyridine Nucleotide Pyrophosphatase Activity of *Staphylococcus aureus.** (27211)

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The presence of normally inhibited diphosphopyridine nucleotide (DPN) pyrophosphatases and diphosphopyridine nucleotidases (DPN'ases) in *Proteus vulgaris* and *Mycobacterium butyricum* has been pointed out by Swartz *et al.*(1) and by Kern and Natale(2). Because of the fortuitous association of a relatively heat-stable enzyme and a heat-labile protein inhibitor in extracts of these organisms, it was possible to demonstrate the particular activity only after heating. Other less vigorous manipulations, such as alteration in pH, also accomplished activation of these enzymes. Unpublished studies by Osler and Rodriguez(3) suggested the presence of a similar enzyme in a strain of *Staphylococcus aureus* isolated from an infected wound. Because the possible intraleucocytic activation of a normally inhibited pyridine nucleotide cleaving enzyme of staphylococci might have some influence on leucocyte pyridine nucleotide levels and thus on phagocytosis, some of the properties of this enzyme in that organism have been investigated.

*Material and methods.* Cultures of staphylococci were obtained from the diagnostic Bacteriology Lab. Sensitivity to antibiotics was tested by the disc method and coagulase production by the tube method(4). Hemolysis was observed during growth of the organisms on sheep blood agar plates. The strain of *Staphylococcus aureus* which had been converted to the "L form"† was grown in standing culture in a medium consisting of 4% trypticase, 3% NaCl, and 1000 units/ml of penicillin, with 10% horse serum added. For enzyme demonstration, cultures were grown in 250 ml of 2% trypticase broth (Baltimore Biol. Labs.) overnight in 1000 ml Erlenmeyer flasks on a rotary shaker. The cultures were centrifuged and the cells were thoroughly washed twice in demineralized water, then suspended in approximately 5 times their volume of water and disrupted by sonic vibration for 15 min. in a 10 K.C. Raytheon Magneto-stricktor oscillator at 0°C. Assays of DPN were carried out spectrophotometrically at 340 mμ by the alcohol dehydrogenase method(5). Triphosphopyridine nucleotide (TPN) was also measured spectrophotometrically, utilizing the isocitric dehydrogenase method(6). Activation of the extract was carried out by placing a 0.38 to 2.0 ml quantity of the preparation in a boiling water bath for one minute and then chilling it in ice. Incubation mixtures for demonstration of the DPN-cleaving activity usually consisted of 0.38 ml of enzyme solution, 0.9 μmoles of DPN, and 0.2 ml of 0.2 M Tris (trishydroxymethyl aminomethane) buffer, pH 9.5 in a final volume of .75 ml. 0.2 ml aliquots were taken at varying time intervals and DPN content was measured in the model DU Beckman spectrophotometer. Inorganic phosphate was measured by a modification of the Fiske-SubbaRow method(7). For determination of the products of DPN cleavage, a large incubation mixture was employed, consisting of 44.5 μmoles of DPN, 5 ml of boiled sonic extract of *Staph. aureus* and 6 ml of 0.2 M tris buffer (pH 9.5) in a final volume of 20 ml. After 7 hours' incubation, the mixture was centrifuged in the cold at 9,000 × g to remove cell remnants. The supernatant was

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