to homografts(8,9). Those cells which survive the lymphoid invasion and retain a blood supply survive for prolonged periods. Were a toxic agent involved in this reaction, one would expect destruction of all the cells within the first-set transplant.

The present experiments also indicate that generalized cytotoxicity is probably not involved in the destruction of parenchymal cells during kidney transplant rejection in second-set kidneys. Interference with nutritional exchanges here also may be the primary cause of cellular damage. The abrupt destruction of second-set kidneys, occurring before massive invasion by white cells, favors the view that interruption of the blood supply is the primary cause of second-set rejection. Were the blood supply to remain intact, the *in vitro* studies suggest that kidney function would be prolonged even in second-set kidneys.

Summary. Oxygen consumption and para-aminohippurate accumulation have been studied in slices of renal cortex from second-set kidney transplants in dogs. The organs were removed for evaluation 24 hours after transplantation, at a time when they show gross hemorrhagic reactions and reduced rates of urine secretion. In spite of advanced rejection, these second-set transplants still accumulated PAH *in vitro* to the same high level as autotransplant controls. Oxygen uptake also remained within normal limits. Further, the turnover of phosphorus in the acid and lipid soluble fractions remained unaffected by the acute rejection process. A small increase in the specific activity of the nucleic acid phosphorus was observed. We interpret these observations to indicate that cytotoxic reactions are not a major factor in transplant rejection. An interruption of the vascular supply, or a mechanical interference with the nutritional exchange of the tissue, are suggested as more likely causes of transplant destruction.

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Effect of Angiotensin II on Adrenal Ascorbic Acid. (27747)

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Hog renin and synthetic asparaginyl 1, valine 5 angiotensin II stimulate secretion of adrenal cortical steroids in the dog(1,2). Saline extracts of dog kidneys stimulate dog adrenal steroid secretion, and it has been postulated that it is the renin present in the renal extract that stimulates steroidogenesis(3). There is indirect evidence in the rat, as shown by widening of the adrenal zona glomerulosa, that hog renin and saline extracts of rat kidneys stimulate steroidogenesis(4). As ACTH is also bound in significant amounts to kidney tissue(5), the role of renin in saline extracts of kidney cannot be assessed unless ACTH can be measured at the same time. Accordingly, we have compared the effects of ACTH with those of the renin-angiotensin system on adrenal ascorbic acid in the rat. The results

show that the two agents can be clearly distinguished by this procedure.

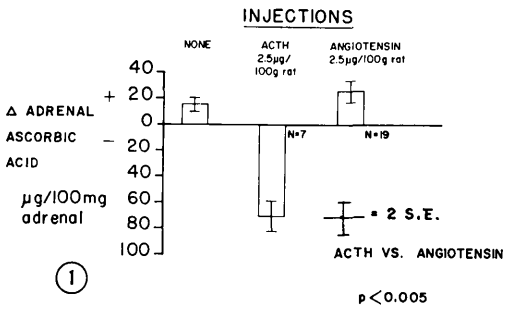
Methods. Male Sprague-Dawley rats (80-250 g) were hypophysectomized by the parapharyngeal route. Forty-eight hours later the left adrenal was removed for control. Immediately, ACTH or angiotensin II was given either into the left femoral vein as a single injection or into the right jugular vein as a one-hour infusion. One hour after the single injection or one hour after start of the infusion, the right adrenal was removed. All rats for which results are included here had complete hypophysectomy, as determined by inspection of the sella turcica. The ascorbic acid concentration of the right adrenal was compared to that of the left from the same animal(6). In the determination of ascorbic acid, ascorbic acid is oxidized to dehydroascorbic acid, which spontaneously undergoes partial conversion to diketogluconic acid. Both dehydroascorbic acid and diketogluconic acid react with dinitrophenylhydrazine to form a dinitrophenylhydrazone. The dinitrophenylhydrazone in sulfuric acid gives a characteristic color which is measured photometrically(7).

Both adrenals were removed simultaneously from 6 rats for comparison of adrenal ascorbic acid concentrations when no treatment was given.

ACTH (National Drug Co.) was given in normal saline as a single injection to 7 rats, and as a one-hour infusion in normal saline to 3 rats. All received a total dose of 2.5 μg per 100 g of body weight.

Asparaginyl 1, valine 5 angiotensin II (Ciba) was given in normal saline as a single injection to 19 rats, and as a one-hour infusion in normal saline to 9 rats. The total dose by single injection was 2.5, that by infusion, 3.1 μg/100 g of body weight.

Results. The results are shown graphically in Fig. 1 and 2. In the rats receiving no treatment, the difference in adrenal ascorbic acid concentrations was 14.7 $\pm$ 5.3 μg/100 mg of adrenal tissue (mean $\pm$ S.E.), expressed as concentration in the left adrenal minus that in the right. With ACTH given as single injections, there was a marked fall in adrenal ascorbic acid, and the mean difference was *minus* 70.6 $\pm$ 11.3 μg/100 mg of adrenal tissue. With angiotensin II given as single injections there was no fall in adrenal ascorbic acid, and the mean difference was 25.0 $\pm$ 7.3 μg/100 mg of adrenal tissue. The difference between the effect of ACTH and that of angiotensin II was significant at a level of $p<.005$ (Fig. 1).



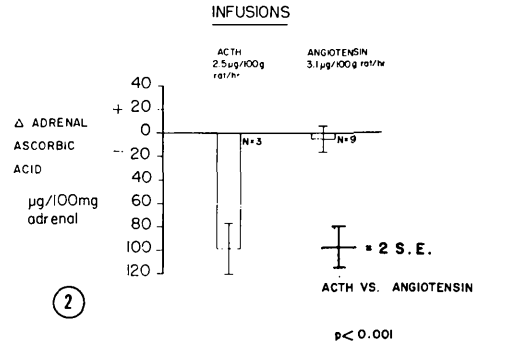


FIG. 1. Effect of single intrav. injections of ACTH and of angiotensin II on rat adrenal ascorbic acid: mean change plus and minus one stand. error of mean.

FIG. 2. Effect of 1-hr intrav. infusions of ACTH and of angiotensin II on rat adrenal ascorbic acid: mean change plus and minus one stand. error of mean.

With the infusions, ACTH induced a marked fall in adrenal ascorbic acid (mean difference *minus* 99.0 $\pm$ 23 μg/100 mg adrenal tissue), whereas angiotensin II induced no appreciable fall in ascorbic acid (mean difference *minus* 5 $\pm$ 11.6 μg/100 mg adrenal tissue). The difference between the effect of ACTH and that of angiotensin II was significant at the level of $p<.001$ (Fig. 2).

Discussion. There is evidence that renin will not, but angiotensin II will stimulate secretion of aldosterone, corticosterone and cortisol in outer slices of beef adrenal cortex(8).

It has been shown that the pressor effect of renin in rats depends upon the release of angiotensin I, which is converted to angiotensin II by circulating converting enzyme(9). This suggests that the effect of renin on adrenal steroidogenesis is mediated through angiotensin II. Thus, angiotensin II was taken in this experiment as representative of the renin-angiotensin system. Rapid inactivation of angiotensin II occurs in blood and tissues(10), and this might explain its failure to deplete adrenal ascorbic acid when given as a single injection. However, infusions of angiotensin II did not deplete ascorbic acid, so that rapid inactivation alone will not explain the lack of effect of angiotensin II. Transient bradycardia and apnea in all rats receiving injections of angiotensin served as evidence for its biologic activity. Thus, it is clear that ACTH and angiotensin II differ in their effect on rat adrenal ascorbic acid.

Although sensitivity of the assay has decreased somewhat by 48 hours after hypophysectomy, depletion of rat adrenal ascorbic acid by 0.37 mU of ACTH is still clearly demonstrable by this procedure(11). On the other hand, there is no appreciable increase in secretion of corticosterone into the adrenal vein of the dog with 0.5 mU of ACTH(12). Therefore, a dose of crude kidney extract which could stimulate corticosteroid secretion in the dog would, in our assay, deplete rat adrenal ascorbic acid if the active principle were ACTH alone. The assay can thus be used to distinguish renin from bound ACTH (5) in such extracts.

Summary. 1. ACTH and angiotensin II clearly differ in their effects on adrenal ascorbic acid in hypophysectomized rats. ACTH depletes adrenal ascorbic acid, whereas angiotensin II does not. 2. This difference can be used to determine the presence of ACTH in biological fluids or extracts which stimulate steroidogenesis by the adrenal cortex.

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Detection of Circumoval Precipitins by the Fluorescent Antibody Technic. (27748)

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Fluorescent antibody technics have been applied to the serological diagnosis of infections with *Schistosoma mansoni*(1). Cercariae preserved in 10% formalin were incubated with serum from known cases and then treated with fluorescent labeled anti-human globulin. Brilliant fluorescence was observed when cercariae were exposed to serum from infected cases and none with control sera.

The circumoval precipitin reaction observed when eggs of *Schistosome mansoni* are incubated in serum from individuals infected