pophysectomized rats, but not in adrenalectomized animals. Administration of DOC to hypophysectomized rats failed to lower alanine transaminase, nor did it alter the response of this enzyme to treatment with ACTH. The inhibitory effect of DOC on alanine transaminase activity appears to be due to suppression of ACTH release by the pituitary.

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Diminished Adrenal Corticoid Response to Burn and ACTH in the Nephrectomized Dog.* (26857)

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Recent reports have implicated the kidney in the control of aldosterone secretion(1,2,3), and have suggested that this organ may play some role in control of 17 hydroxycorticoid (17 OHCS) secretion as well(3,4). Since ACTH is known to affect both aldosterone and 17 OHCS secretion, the present studies were undertaken to determine whether nephrectomy alters the 17 OHCS response to burn, a known stimulus to ACTH release, and if so, by what means.

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Methods. Experiments were performed on

22 dogs anesthetized with sodium pentobarbital. The right lumboadrenal vein was cannulated by the method of Hume and Nelson(5). Simultaneous bilateral nephrectomy was carried out in 10 dogs. One liter of normal saline was given intravenously through a femoral venous cannula to prevent hypovolemia during preparation. Arterial pressure was monitored by means of a cannula in the femoral artery. Following completion of the preparation, the animal was heparinized and left undisturbed for one hour. The level of anesthesia was maintained. Blood lost through adrenal venous collections or incidentally was replaced with dextran in saline.

Burn experiments were carried out in 6 normal and 7 nephrectomized dogs. Of these, 4 normal and 3 nephrectomized dogs were burned twice; all other dogs were burned once. In all cases of 2 burns, the magnitude of response to the second burn was equal to that from the first. A control timed sample of adrenal venous blood was first collected. The animal was then burned on the thigh with cautery for 2 minutes. This procedure resulted in a uniform third degree burn measuring more than 35 sq. cm. The edges of the burned area were sharp and there appeared to be virtually no region of submaximal burn. Additional timed adrenal venous samples were collected 10, 30 and 60 minutes after the start of the burn. In those dogs which were burned twice, the second burn (contralateral thigh) was begun after collection of the 60-minute post-burn sample. The same protocol was then observed following the second burn.

Nine ACTH experiments were carried out in 5 normal and 4 nephrectomized dogs. A control sample of adrenal venous blood was collected and 10 mμ of ACTH (Armour, crystalline, dissolved in saline) was injected into the adrenal vein over a 15-second period and washed in with 10 ml of saline. A second sample of adrenal venous blood was collected beginning exactly 5 minutes after ACTH injection. This collection extended over the period of maximal response described by Nelson and Hume(6). After a recovery period of 30 to 45 minutes, the procedure was repeated with a dose of 100 mμ; then, after a similar period, with a dose of 1 μ; then, after another period, with a dose of 22 μ. Hypophysectomized dogs were not used for these experiments because the intent was to determine the effect of the absence of kidneys alone. A series of increasing doses was used in the same animal in order to demonstrate the cumulative, as well as the acute, effects of ACTH.

Adrenal venous samples were centrifuged immediately, the plasma was withdrawn and analyzed for 17 OHCS by Peterson's modification(7) of the method of Silber and Porter (8). Secretion rates of 17 OHCS were calculated from concentration, rate of adrenal blood flow and hematocrit.

Results. Average initial control levels of 17 OHCS secretion were 2.67 $\pm$ 0.61 (SEM) μg/min for all normal dogs and 3.99 $\pm$ 0.61 μg/min in all nephrectomized dogs. These values are not significantly different. Adrenal blood flows were equal in the 2 groups.

The effects of burns upon adrenal 17 OHCS secretion in normal and nephrectomized dogs are shown in Fig. 1 and tabulated and analyzed in Table I. The difference between the 10-minute post-burn and control secretion rates is a measure of the response to burn, while the differences between the 30- and 60-minute post-burn and control secretion rates are measures of rate of return to control levels following stimulation. It may be seen that burn elicited a significantly greater rise in 17 OHCS secretion rates in normal than in nephrectomized dogs. Further, 17 OHCS secretion had returned to control levels after 30 minutes in nephrectomized dogs but were still elevated in normal dogs. 17 OHCS secretion rates 60 minutes after burn were not significantly different from control levels in either group. The high mean and large standard error at 60 minutes in the normal group are the results of a single experiment in which 17 OHCS secretion remained elevated. This was in an animal which was burned twice, and this result followed the second burn.

The effects of ACTH administration on adrenal 17 OHCS secretion rates are shown in Fig. 2 and tabulated and analyzed in Table II. It may be seen that ACTH led to a

TABLE I. a. Effect of Third Degree Burns on 17 OHCS Secretion (μg/min.) in Normal and Nephrectomized Dogs.

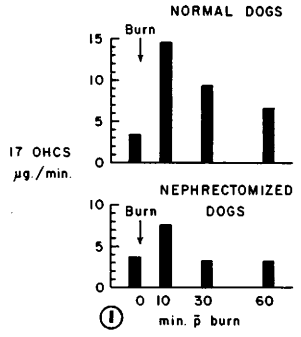
	No. of experiments	Control	10 min. after burn	30 min. after burn	60 min. after burn
Normal	10	3.29 ± .67*	14.47 ± 1.31	9.40 ± 2.20	6.68 ± 2.44
Nephrectomized	10	3.56 ± .55	7.52 ± .69	3.18 ± .74	3.15 ± .46

b. Analysis of differences in individual experiments

	Normal		Nephrectomized		Comparison, normal *vs* nephrectomized	
10 min. after burn minus control	11.18 ± 1.27	$p < .001$	3.96 ± .64	$p < .001$	$t = 5.79$	$p < .001$
30 min. after burn minus control	5.78 ± 1.87	$p < .02$	−.65 ± .54	$p > .2$	$t = 3.31$	$p < .005$
60 min. after burn	3.06 ± 2.43	$p > .2$	−.71 ± .50	$p > .2$	$t = 1.51$	$p > .1$

* Mean ± stand. error.

higher maximal secretion rate in normal than in nephrectomized dogs. The acute rise obtained with ACTH was, in addition, greater in normal dogs than in nephrectomized dogs in response to 10, 100 and 1000 mμ. This difference was obscured by the high control secretion rates in the group given 22 μ. The cumulative effect of multiple doses of ACTH, evidence by the rising control levels with repeated doses was also greater in normal than in nephrectomized dogs.



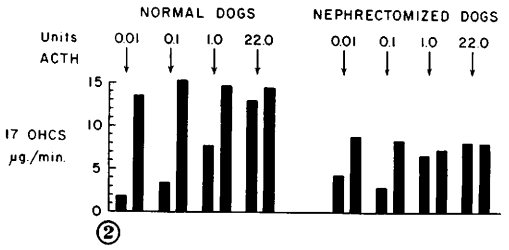


FIG. 1. Effect of burn on 17 OHCS secretion (μg/min.) in normal and nephrectomized dogs. Each bar is mean value (10 experiments in each group).

FIG. 2. Effect of ACTH in sequentially increasing doses on 17 OHCS secretion (μg/min.) in normal and nephrectomized dogs. Each bar is mean value (5 normal, 4 nephrectomized dogs).

Discussion. In general, dogs with chronic adrenal cannulae have been used for experiments such as those reported here. Though mean control secretion rates in the present experiments were slightly higher than those reported for dogs with chronic adrenal cannulae(5), there were many individual values below 2 μg/min, and the responses to stimuli were not prevented by the elevated control levels. Before starting this study, it was decided arbitrarily to exclude any dog with "resting" 17 OHCS secretion rate greater than 8 μg/min since a control level near to or at maximum secretion rate would obscure any response to a stimulus. Of 25 dogs prepared, only 3 were excluded because of this criterion. This proportion of animals lost does not seem to us excessive, compared to probable losses in chronic preparations from death, clotting, inadvertent removal of the cannula, and occasional very high control values, despite a 48-hour wait(5).

Third degree burns of the magnitude employed in these studies lead to rates of secretion of 17 OHCS equal to those achieved with large doses of ACTH in similar animals. The 17 OHCS response to burn is attenuated markedly in previously nephrectomized animals. This effect is not the result of different control secretion rates (Table I). It cannot be ascribed to physical damage to the adrenal in the course of nephrectomy, since adrenal blood flows were equal in the 2 groups, and

TABLE II. Effect of Sequentially Increasing Doses of ACTH on 17 OHCS Secretion (μg/min.) in Normal and Nephrectomized Dogs.

	Normal (5 dogs)			Nephrectomized (4 dogs)			Comparison, Δ normal *vs* Δ nephrectomized	
ACTH	Control	5 min. after ACTH	Δ*	Control	5 min. after ACTH	Δ	t	p
10 mμ	1.9	13.5	11.6 $\pm$ 1.8†	4.2	8.7	4.5 $\pm$ 1.3	3.8	<.01
100 mμ	3.4	15.2	11.8 $\pm$ 1.9	2.9	8.3	5.4 $\pm$ .5	3.3	<.02
1 μ	7.7	14.7	7.0 $\pm$ 1.6	6.6	7.3	.7 $\pm$ 1.0	3.2	<.02
22 μ	13.0	14.5	1.5 $\pm$ 1.1	8.2	8.1	-.1 $\pm$ .7	1.2	>.2

Mean secretion rate after ACTH: Normal, 14.47 $\pm$ .94; nephrectomized, 8.11 $\pm$ .48.
$t = 6.01$ $p < .001$

* Analysis of differences in individual experiments.
† Mean $\pm$ stand. error.

there was no impairment of resting secretion rates of 17 OHCS. This limitation of response could be explained either by decreased ACTH release by the pituitary or by decreased adrenal sensitivity to ACTH.

The ACTH experiments described were designed to distinguish between these possibilities. It is evident from these experiments that the maximum secretion rate in response to ACTH is reduced markedly in nephrectomized dogs. Both immediate response to injected ACTH and cumulative effect on 17 OHCS secretion rates of repeated doses of the drug are less in nephrectomized than in normal dogs. It seems reasonable to conclude that the adrenal glands of nephrectomized dogs are less sensitive to ACTH than are those of normal dogs, so far as 17 OHCS secretion is concerned. The different responses to burn of normal and nephrectomized dogs can be ascribed to diminished sensitivity to ACTH in the nephrectomized group. Indeed, the observed difference between the 2 burned groups (Table I) is almost quantitatively accounted for by the differences in maximal secretion rates (Table II), since burns led to apparently maximum secretion rates in both groups.

The diminished sensitivity to ACTH of adrenal 17 OHCS secretion in nephrectomized dogs may be explained in 2 principal ways, or in combinations of these. First, substances which inhibit adrenal corticoid secretion or synthesis might accumulate in the blood in the absence of the kidneys. The fact that the nephrectomized dogs reported here did not have lower control 17 OHCS secretion rates than did the normal dogs would seem to militate against this hypothesis, particularly since the control secretion rates observed in these studies were not absolutely basal, and are significantly different from zero (See, *e.g.* Table I).

Second, the kidney might secrete a substance which stimulates adrenal 17 OHCS secretion. Evidence suggests that the renin-angiotensin system plays such a role. Deane and Masson(9) reported that injection of renin into rats may cause hypertrophy of the zona fasciculata of the adrenal as well as of the zona glomerulosa. There are recent reports that both renin and angiotensin infused into hypophysectomized nephrectomized dogs may stimulate 17 OHCS secretion(2,3,10,11). ACTH may play a role in regulation of kidney renin content(11,12). Further, injected ACTH is rapidly bound to kidney tissue in large proportion(13). It is possible that part of its adrenotropic action is mediated through the renin-angiotensin system. However, it is also possible that angiotensin, released by mechanisms other than ACTH, may alter adrenal sensitivity to ACTH. It has been reported that renin is released in hemorrhagic shock(14,15). Hume and Nelson(5) studied 17 OHCS secretion during hemorrhagic shock in hypophysectomized dogs maintained on constant ACTH, and found an increase in 17 OHCS secretion with hemorrhage. They proposed an extra-pituitary mechanism regulating adrenal sensitivity to ACTH. The present data support such a possibility and suggest that the kidney plays an important role in this regulation.

It has been proposed recently that angiotensin may be a specific aldosterone stimulating hormone(1,2). The evidence presented here suggests, however, that the action of the kidney in regulation of adrenal cortical secretion is not limited to aldosterone. Slater, Casper, Delea and Bartter(3) were unable to separate aldosterone stimulating and 17 OHCS stimulating effects of angiotensin, even at low doses, though Mulrow and Ganong(2) were able to separate these effects. It seems there must be some reservations concerning the specificity of the role of the kidney in control of aldosterone secretion.

In the burn experiments, 17 OHCS secretion fell to levels not distinguishable from normal after 30 minutes in nephrectomized dogs and 60 minutes in normal dogs. The more rapid return to control levels in nephrectomized dogs may be simply a reflection of the smaller rise after burn rather than a specific effect.

It has been suggested(16) on the basis of experiments on dogs with isolated pituitaries that there may be a humoral factor arising in the hind-brain mediating ACTH release. It is possible that a part of the increase in 17 OHCS secretion observed in those experiments results from extra-pituitary stimulation of the adrenal rather than from extra-hypothalamic stimulation of the pituitary. The present experiments suggest that the kidney may mediate such extra-pituitary stimulation.

Summary. Nephrectomized dogs respond to third degree burns with much less increase in 17 OHCS secretion than do normal dogs. ACTH injection also produces much less increase in 17 OHCS secretion in nephrectomized than in normal dogs. These diminished responses appear to result from decreased adrenal sensitivity to ACTH. It is suggested that the kidney plays an important role in the control of 17 OHCS secretion.

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Acridine Orange Staining of Purified Polyoma Virus.* (26858)

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Recent experimental evidence indicates that polyoma is a DNA virus. Di Mayorca *et al.*(1) extracted an infectious nucleic acid from the virus and demonstrated that infectivity was abolished by treatment with DNAase while RNAase had no effect. The studies

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