Characteristics of Adrenal-Regeneration and Aldosterone Hypertension.* (32280)

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The etiology of adrenal-regeneration hypertension remains obscure. Although it initially appeared that both adrenal enucleation and a high salt consumption were essential to development(1-3), it now seems that increased salt consumption is not a prerequisite (4,5) although there is no question that it will greatly accelerate onset and enhance severity. The notion that some type of hypercorticism might supervene during glandular regeneration is contraindicated by the fact that measured hormone output is slightly subnormal(6-11). The supposed predominance of mineralocorticoid secretion or the appearance of an unusual mineralocorticoid (2) has been challenged by data from *in vitro* studies indicating that there may be, in fact, an excess of glucocorticoid(12): a finding itself difficult to reconcile with the observation that glucocorticoid hypertension is not, and adrenal-regeneration hypertension is, enhanced by salt excess.

Of the known steroids secreted by the rat adrenal cortex, corticosterone and aldosterone would seem to be the most culpable progenitors of adrenal-regeneration hypertension, perhaps because of an altered ratio. Masson (13) examined this possibility, and found that aldosterone failed to fully sustain hypertension when regenerated adrenals were removed, and that whereas corticosterone did so, it also caused complete adrenal atrophy, a change not characteristic of early adrenal-regeneration hypertension. Neither ideally fitted the requirements as *causa proxima* of the disease, although the author did suggest that larger doses of aldosterone might have proved effective.

The ability of aldosterone to induce hypertensive vascular disease in rats has been erratic, as pointed out in a recent compendium of relevant data(14), yet it is clearly evident that it does so under appropriate circumstances(14-16). Various characteristics mitigating against exhibition of its hypertensive properties in experimental studies have been reviewed(14). Chief among them is the short biologic half-life, which can be overcome to a degree by employing, in divided doses, the acetate ester of d-aldosterone. Desoxycorticosterone acetate (DCA) has 3 times the potency of the free alcohol, attributable to its slower absorption and greater resistance to biologic inactivation(17). Aldosterone acetate is also more slowly absorbed than the alcohol (Ciba Pharmaceutical Co., personal communication) and, presumably, more slowly inactivated. Employment of the acetate in divided doses has been more successful in causing hypertensive vascular disease in the rat(14,15) than has the alcohol, perhaps in part because prolonged activity of the ester compensates for the short biologic half-life of the parent steroid(18).

The present experiment was undertaken to determine how closely aldosterone-induced hypertension resembled the disease induced by adrenal enucleation.

Materials and methods. Sixty female rats of the Houston-Cheek strain, a Sprague-Dawley derivative, weighing 55-75 g were right nephrectomized and divided into 6 groups. Groups 1 and 2 were bilaterally adrenalectomized. Groups 3 and 4 were right adrenalectomized and left adrenal enucleated. These groups each contained 8 rats. Groups 5 and 6, each containing 6 rats, were subjected to right adrenalectomy. Rats of groups 1, 3 and 5 each received two 50 μg injections of d-aldosterone acetate in sesame oil by subcutaneous injection daily: those of 2, 4 and 6 received oil only in the same amount and manner. Animals were individually caged in temperature-controlled quarters and received both 1% NaCl drinking solution and Purina laboratory chow *ad lib.* A 24-hour fluid intake was measured

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on each rat on days 22, 25 and 26.

Blood pressures were measured periodically in unanesthetized animals by tail plethysmography and systolic pressures over 150 mm Hg were considered to reflect hypertension.

Survivors were killed with ether on the 26th day and various tissues and organ were excised and placed in neutral 10% formalin for weight and subsequent histology. Organs to be weighed were removed after fixation, trimmed, blotted and weighed on an analytical balance. Vascular lesions were arbitrarily graded on a 0 to 4+ scale, and the severity scored by being expressed as a percentage of the maximum possible, *e.g.*, 24 for a six-membered group.

Results. Blood pressure estimation on the 16th experimental day revealed that all untreated unilaterally adrenalectomized controls were normotensive, systolic pressure averaging 126 ± 1 mm Hg: 6 bilaterally adrenalectomized rats were hypotensive at 107 ± 1 mm Hg ($P<.001$) and 6 of the 8 enucleated controls were hypertensive, the group average pressure being 168 ± 8 mm Hg. Six of the seven adrenalectomized animals receiving aldosterone were hypertensive 154 ± 3 mm Hg as were all 6 hormone-treated unilaterally adrenalectomized rats at 183 ± 6 mm Hg and all 7 of the aldosterone-treated enucleated rats, 198 ± 8 mm Hg. Aldosterone caused hypertension in both adrenalectomized groups and appeared to enhance the severity of early adrenal-regeneration hypertension, the pressures averaging 168 mm Hg in untreated enucleated rats and 198 mm Hg in those treated with the steroid. The difference was barely significant at the 5% level of confidence, but this was not so in subsequent pressure measurements.

Untreated adrenalectomized rats continued to exhibit a statistical hypotension on the 20th and 25th days. On the 20th day hypertension in untreated enucleated rats (187 ± 10 mm Hg) was as severe as it was in aldosterone-treated unilaterally adrenalectomized (194 ± 4 mm Hg) or adrenal enucleated (198 ± 12 mm Hg) rats. On the 25th day the blood pressure of unilaterally adrenalectomized rats had reached prehypertensive levels (143 ± 2 mm Hg) the highest individual pressure being 146 mm Hg. The only difference between the various hypertensive groups was that blood pressure averaged lower in adrenalectomized rats receiving aldosterone (186 ± 9 mm Hg) than in untreated enucleated rats, which had the highest average pressure 219 ± 10 mm Hg. The results are shown in Fig. 1.

The fluid intake on the 22nd, 25th and 26th day, showed considerable uniformity within individual groups. When averaged over the period, untreated enucleated rats evidently drank significantly larger quantities than either bilaterally adrenalectomized ($P<.001$) or unilaterally adrenalectomized controls ($P<.05$). Aldosterone significantly increased fluid consumption of adrenalectomized or adrenal enucleated rats ($P<.001$) to about

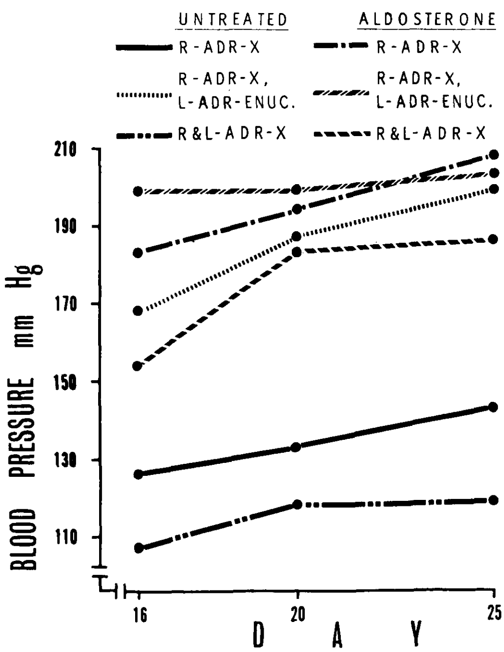

FIG. 1. Arterial pressure changes in unilaterally adrenalectomized, bilaterally adrenalectomized and unilaterally adrenalectomized-contralaterally adrenal enucleated rats with and without concomitant aldosterone treatment. All rats were unilaterally nephrectomized. Note that bilaterally adrenalectomized, untreated rats display hypotension, and that those given aldosterone have somewhat lower pressures than other hormone-treated groups. Hypertension at first recording was greatest in aldosterone-treated, enucleated rats, less in untreated enucleated rats and intermediate in unilaterally adrenalectomized, aldosterone-treated rats. Thereafter the differences diminished.

TABLE I. Principal Effects of Aldosterone in Unilaterally Nephrectomized Rats Subjected to Adrenalectomy or Adrenal Enucleation.

Data		Bilaterally adrenalectomized		Unilaterally adrenalectomized		Adrenal enucleated	
		Aldosterone	Control	Aldosterone	Control	Aldosterone	Control
Body wt, g	Initial	64 ± 3	63 ± 2	59 ± 2	60 ± 2	63 ± 3	63 ± 2
	Final	124 ± 10*	141 ± 9	119 ± 12	161 ± 1	118 ± 5	146 ± 4
No. rats	Initial	8	8	6	6	8	8
	Final	4	5	5	6	7	8
Fluid intake day 22, 25 & 26, ml/rat/day		108 ± 6	39 ± 2	117 ± 10	50 ± 3	116 ± 9	70 ± 7 [a]
Organ wt, mg/100 g body wt							
Heart		479 ± 11	366 ± 13	580 ± 29	382 ± 7	582 ±33	509 ±23 [a]
Kidney		1494 ± 37	887 ± 40	1521 ± 53	943 ±30	1604 ±63	1233 ±41 [a]
Thymus		345 ± 19	409 ± 13	145 ± 30	249 ±13	115 ±20	216 ±16
Adrenal		—	—	26.4± 1.8	26.9± 0.8	18.4± 0.9	18.9± 1.0[a]
Vascular lesions							
Heart	% incidence	25	0	100	0	71	63
	% severity	6	0	45	0	50	34
Kidney	% incidence	100	0	100	0	100	88
	% severity	38	0	80	0	61	34
Polyarteritis	% incidence	25	0	40	0	29	25
	% severity	19	0	15	0	22	6

* Mean ± standard error.
Underlined figures differ from similarly operated untreated controls ($P < .05$ or better).
[a] Indicates that untreated enucleated rats differ from either of the other untreated groups ($P < .05$ or better).

the same degree. The data are given in Table I.

Organ weights revealed considerable cardiac hypertrophy in each of the hypertensive groups as compared with either control group ($P < .001$ or better), the response to aldosterone being as great as to adrenal-regeneration.

Untreated adrenalenucleated rats had enlarged kidneys as compared with either control group ($P < .001$), but significantly smaller than in aldosterone-treated bilaterally adrenalectomized ($P < .001$), unilaterally adrenalectomized ($P < .001$), or adrenal enucleated ($P < .001$) rats.

The thymus glands of untreated adrenalectomized rats were significantly larger than in unilaterally adrenalectomized or adrenal enucleated animals ($P < .001$), which themselves did not differ from each other. In each case aldosterone caused significant thymus involution as compared with similarly operated but untreated controls ($P < .001$). Regenerated adrenals were smaller than hypertrophied glands, but in neither instance did aldosterone exert a demonstrable effect.

Hypertensive vascular lesions were generally fewer and least advanced, except in the kidneys, in adrenalectomized rats given aldosterone. This group had averaged the lowest pressure on the first and last blood pressure determinations and had significantly smaller hearts than did the other hypertensive groups. The other two aldosterone-treated groups had equally severe and advanced lesions, probably slightly more prominent (particularly in the kidneys which in each case were significantly larger) than in rats with uncomplicated adrenal-regeneration hypertension. The lesions are tabulated in Table I.

Discussion. Growth rate was impaired equivalently by either total adrenalectomy ($P < .05$) or adrenal enucleation ($P < .01$), but to an even greater degree by aldosterone which had substantially the same effect upon animals in each of the 3 treated groups. Hormone and adrenal surgery were neither summative nor synergistic. Thymus glands of untreated bilaterally adrenalectomized rats were enlarged in comparison with those in either of the comparable untreated groups (P<.001). In contrast with adrenal enucleation, which did not cause thymic atrophy, aldosterone caused significant glandular involution in the three groups to which it was given; nevertheless, the glands of hormone treated adrenalectomized animals were larger than in untreated enucleated (P<.001) or unilaterally adrenalectomized (P<.01) rats.

Cardiac size correlated well with blood pressure findings. Aldosterone caused significantly increased heart weight (P<.001) in both unilaterally adrenalectomized and bilaterally adrenalectomized rats, but did not further augment the enlargement in adrenal enucleated rats. The hearts of aldosterone-treated adrenalectomized rats, which had shown the mildest hypertension, were less hypertrophied than in either hormone-treated unilaterally adrenalectomized rats (P<.01) or hormone-treated adrenal enucleated rats. The kidney enlargement evident in untreated adrenal enucleated rats as compared with either of the other untreated groups (P<.001), was nevertheless less pronounced than that caused by aldosterone in either unilaterally or bilaterally adrenalectomized animals (P<.001). The largest kidneys were found in aldosterone-treated adrenal enucleated rats, although the difference in weight between these and the two other hormone-treated groups was not statistically significant. Aldosterone had no significant effect upon adrenal weight of either unilaterally adrenalectomized or adrenal enucleated rats.

Although there were no deaths among adrenal enucleated or unilaterally adrenalectomized rats during the experiment, many totally adrenalectomized animals died regardless of whether or not hormone was given. Survival time averaged 9.7 days in untreated rats and 20.2 days in those receiving aldosterone. Longer survival, together with the fact that 3 of the 4 in the latter category had developed severe hypertension prior to death, suggests that hypertensive vascular disease was poorly tolerated by adrenalectomized rats, and that these died from the effects of treatment rather than from simple acute adrenal insufficiency.

There was little difference between hypertension caused by adrenal regeneration or by aldosterone excess as regards time of onset, rate of development, incidence, or ultimate severity. The similarity in overall response was striking considering that the dosage of aldosterone was arbitrarily chosen, making it quite unlikely that the quantity of steroid administered closely approximated what was being endogenously secreted by the regenerating adrenals.

The vascular lesions in general accorded well with the severity of hypertension within a given group. Although it might have been anticipated that adrenalectomized rats would have been more severely affected by hypertensive disease, such was not the case. Tobian and Perry(19), similarly, did not find adrenalectomized rats to be more susceptible to desoxycorticosterone hypertension than intact animals.

The dosage of aldosterone used in this experiment appears to be the lowest yet reported to cause severe hypertensive vascular disease in rats, although, as recently reported from this laboratory(14), the literature is ambiguous regarding the cardiovascular effects of this steroid in experimental animals. The response to 100 μg daily was much the same as previously obtained with two and a half times as much(14). Thus it appears that the lower quantity, when given in divided doses, still exceeds the quantity required to elicit hypertensive disease in salt-loaded rats. This accords well with the known sodium retaining potency of the hormone if it be accepted that it is the sodium retention which causes hypertension. It is quite conceivable that lesser quantities of aldosterone could induce hypertensive disease in rats, without provoking the greater saline polydipsia, growth impairment and more marked kidney enlargement which, in the present experiment, clearly demarcated the hypertensive response to hormone from that due to adrenal enucleation. If so, a slight aldosterone hypersecretion, perhaps only relative to glucocorticoid output, would be a possible protagonist of adrenal-regeneration hypertension.

Summary. Unilaterally nephrectomized rats were subjected to either unilateral adre-

nalectomy, bilateral adrenalectomy or unilateral adrenalectomy and contralateral adrenal enucleation and given 1% sodium chloride solution to drink. Their response to the daily administration of 100 μg of d-aldosterone acetate was compared. Hormone-treated or adrenal enucleated rats both developed hypertension at about the same rate and with a comparable incidence and severity, and in this respect the two influences were not additive. In contrast to adrenal enucleation, aldosterone treatment caused impairment of growth and thymus involution. Thymus glands of aldosterone-treated bilaterally adrenalectomized rats were nonetheless larger than those of untreated enucleated rats. Although adrenal enucleation induced saline polydipsia and caused kidney enlargement, both responses were significantly greater in hormone-treated rats. The implication of these findings with respect to the role which endogenous aldosterone hypersecretion has been suspected of playing in the genesis of adrenal-regeneration hypertension is considered.

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Effect of Hydrochlorothiazide on Preference Threshold of Rats for NaCl Solutions.* (32281)

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During an earlier study in which the diuretic agent, hydrochlorothiazide (HCZ), was administered chronically to rats with post-desoxycorticosterone acetate (DCA)-induced hypertension, the impression was gained that HCZ increased the NaCl intake of these rats above that of controls given DCA alone. The experiments reported here were carried out to verify this observation and to study it in more detail. Both the time course for development of the NaCl appetite after HCZ administration and the effect of this diuretic agent on preference (taste) threshold for NaCl solution were studied and are described below.

Methods. Experiment 1. Effect of chronic administration of HCZ on spontaneous NaCl intake by rats. Eighteen male rats of the Carworth CFN strain weighing 420 to 460 g were kept in individual cages in a thermoregulated

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