A STUDY OF THE COMPARATIVE ACTIVITIES OF DESOXYCORTICOSTERONE AND REICHSTEIN'S COMPOUND S.*

BY

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Studies carried out in the past decade have clearly established the fact that overdosage with the steroid desoxycorticosterone acetate (DCA) produces in the rat a syndrome of malignant hypertension (Selye & Hall, 1944; Friedman & Friedman, 1949; Green & Glover, 1948; Masson, Corcoran & Page, 1949). This is characterized by polydipsia, polyuria, nephrosclerosis, myocarditis and a widespread panarteritis.

These results have been cited in support of the thesis that the elaboration of desoxycorticosterone or a mineralocorticoid of like action from the adrenal cortex might be responsible for many types of human hypertension. Such a relationship is easily envisaged in the case of hypertension associated with hyperfunctional states of the adrenal cortex and the pituitary gland, such as in patients with adrenal tumor or hyperplasia, and in Cushing's syndrome; but the genetic relationship of the adrenal cortex to essential hypertension is not quite as obvious. One of the chief drawbacks to the theory is that des-

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oxycorticosterone has been isolated from the adrenal cortex in only very minute quantities, indeed many investigators have cast doubt upon its presence in, or production by, the cortex.

The importance of Reichstein's compound S, 17-hydroxy-11-desoxycorticosterone (desoxocortisone), to the theory of adrenal participation in hypertension is obvious. Like DCA it possesses mineralocorticoid, but no glucocorticoid properties, differs from DCA structurally only in having an hydroxyl group at C17, and its extraction from the cortex in reasonable yields suggests its importance in normal adrenal physiology.

One group of investigators has compared the activity of compound S with that of DCA under several different experimental conditions and has reported that, in the rat, the former fails to produce hypertension, polydipsia, polyuria, the associated cardiovascular lesions and adrenal cortical atrophy, actions which are so characteristic of DCA (Masson et al., 1950; Masson et al., 1951; Deane & Masson, 1951). From these experiments it has been suggested that »— it is unlikely that compound S as such is of major importance in the field of hypertension«, although it was admitted that further study on the compound was warranted (Corcoran, 1950). On the other hand Selye (1950) found compound S to be so effective in producing hypertensive cardiovascular disease in the rat, with polydipsia as an associated phenomenon, that he stated »— under no condition has it been possible to produce such widespread hyalinosis with desoxycorticosterone within 24 days«. Further differences emerge from the data presented by the two different laboratories, for while the former found compound S to cause thymic involution while not effecting the size of the heart, the latter reported that the steroid produced insignificant thymic involution, and great cardiac hypertrophy. Neither group found the steroid to produce either the adrenal atrophy or renal enlargement so typical of DCA overdosage.

Obviously the results of the two groups of investigators are at variance and not readily compatible. In view of the fundamental importance of the actions of this steroid, with respect
to certain of the currently held concepts on the etiology and pathogenesis of hypertensive disease, and because of the divergence of opinion on its actions in the rat, it was felt that a reinvestigation of the hormone was warranted.

In seeking for differences in experimental procedure which might have accounted for the divergent results obtained by the two laboratories, it occurred to us that while hypertension had been achieved by injections of a suspension in fairly high dosage, it had not been observed either when pellet implantations were utilized or when injections had been employed for only a short period. We therefore chose to employ injections for a prolonged period, and under these circumstances to compare compound S with identical amounts of DCA. Even under these circumstances variations in response to the two steroids may well be due to dissimilar absorption rates, due to inherent structural differences and to the smaller particle size of the compound S suspension as compared to the DCA suspension.

MATERIAL AND METHODS

Thirty female albino rats derived from litters produced in our colony, the parent stock of which was obtained from the Holtzman-Rolfsmeyer Co., were divided into three equal groups. Each had an initial average body weight of 56 ± 2 gm. All of the rats were subjected to the usual sensitization procedures for the production of mineralocorticoid hypertension, namely unilateral nephrectomy and the replacement of tap water by a 1 per cent NaCl solution as the drinking fluid. Purina Laboratory Chow was fed ad libitum. The animals of group 1 served as untreated controls; those in group 2 received 2.5 mg. of compound S acetate in 0.1 ml. of a commercially prepared suspension daily, by subcutaneous injection; and the animals of group 3 received a like quantity of DCA in a similar suspension medium.

Blood pressures were taken at periodic intervals using unanaesthetized animals, employing the Sobin (1946) modificat-
tion of the method originally devised by Williams, Harrison & Grollman (1939). The arithmetic mean of four consecutive measurements, agreeing to within 5 mm. Hg, was taken as representative of the blood pressure in each rat. Although we never encounter pressures in excess of 135 mm. Hg in normal rats, 150 mm. Hg has arbitrarily been chosen as the lower limit of hypertension. Pressures between 140 and 150 are regarded as prehypertensive. The fluid intake of the three groups was measured daily beginning on the 9th day of the experiment and the animals were sacrificed on the 33rd day. At autopsy various tissues and organs were taken for weight and histology, the weights being determined after fixation in Bouin's fluid.

RESULTS

Growth and survival. The animals treated with compound S were indistinguishable from controls throughout the period of treatment. They grew as well and were sleek and healthy looking. The animals treated with DCA on the other hand soon showed evidence of edema and ascites, the abdomens being detectably distended. This change became manifest at about the third week of treatment, and coincidentally the fur of the rats became coarse and disheveled. Small subcutaneous nodules developed at the site of injection, suggesting a reaction to crystal deposits, as proved to be the case at autopsy. The rats treated with DCA failed to grow as well as those of the other groups, and seven of them died between the 24th and 29th day of treatment. With one exception, all of these had developed a marked degree of cardiovascular damage by this time, although the precipitating cause of death was pneumonia. It has been our experience that pneumonia is more prone to occur in DCA treated rats than is the case with other steroids. The data are summarized in Table 1.

Fluid intake. By the 9th day of the experiment, which marked the beginning of fluid intake measurement, the rats on
Table I.
A comparison of the anatomical changes produced by desoxy-corticosterone and Reichstein's compound S in the rat.

<table>
<thead>
<tr>
<th>Data</th>
<th>Controls</th>
<th>Compound S</th>
<th>DCA</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of rats</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>—</td>
</tr>
<tr>
<td>Final</td>
<td>10</td>
<td>10</td>
<td>3</td>
<td>—</td>
</tr>
<tr>
<td>Body wt. (gm.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>56±2*</td>
<td>56±2</td>
<td>56±2</td>
<td>—</td>
</tr>
<tr>
<td>Final</td>
<td>131±4</td>
<td>133±6</td>
<td>111±5</td>
<td>&gt;0.7</td>
</tr>
<tr>
<td>Thymus (mg.)</td>
<td>223±24</td>
<td>263±31</td>
<td>188±7</td>
<td>&gt;0.3</td>
</tr>
<tr>
<td>Adrenals (mg.)</td>
<td>40.4±2.0</td>
<td>31.5±1.5</td>
<td>20.8±0.9</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>mg/100 cm² B.S.†</td>
<td>17.2±0.7</td>
<td>13.3±0.8</td>
<td>11.0±1.2</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Heart (mg.)§</td>
<td>376±15</td>
<td>484±37</td>
<td>569±29</td>
<td>&lt;.02</td>
</tr>
<tr>
<td>mg/100 cm² B.S.</td>
<td>160±5</td>
<td>203±13</td>
<td>270±9</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Kidney (mg.)</td>
<td>975±50</td>
<td>1123±57</td>
<td>1429±115</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>mg/100 cm² B.S.</td>
<td>414±13</td>
<td>470±10</td>
<td>678±42</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Myocarditis %</td>
<td>0</td>
<td>10</td>
<td>100</td>
<td>—</td>
</tr>
<tr>
<td>Severity</td>
<td>0</td>
<td>+</td>
<td>++</td>
<td>—</td>
</tr>
<tr>
<td>Nephrosclerosis %</td>
<td>0</td>
<td>10</td>
<td>100</td>
<td>—</td>
</tr>
<tr>
<td>Severity</td>
<td>0</td>
<td>+</td>
<td>+++</td>
<td>—</td>
</tr>
<tr>
<td>Periarteritis %</td>
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<td>10</td>
<td>33</td>
<td>—</td>
</tr>
<tr>
<td>Severity</td>
<td>0</td>
<td>++</td>
<td>++</td>
<td>—</td>
</tr>
</tbody>
</table>

P* value for controls versus compound S. Values below 0.02 are considered statistically significant.

* Mean ± standard error of mean.
† Mg/100 square centimeters of body surface.
§ Ventricles only.
DCA were already consuming more than twice as much fluid as those in groups 1 and 2. At no time during the course of experiment was there any disproportion between the amount of fluid consumed by the control rats and those receiving compound S. Those treated with DCA on the other hand always exhibited a marked polydipsia, accompanied by polyuria. The development of polydipsia in group 3 is presented graphically in Fig. 1. A flattening out of the fluid intake curve between the 22nd and 29th day of the experiment, when many of the DCA animals were ill with pneumonia, is apparent. Following the death of these animals by the 29th day there was a sudden increase in the daily fluid consumption of the group, now consisting of three healthier animals.

**Blood pressure.** Fig. 2 summarizes the fluctuations in blood pressure. No evidence of hypertension was observed in the control animals. On the occasion of the first blood pressure measurement on the tenth day of the experiment, none of the animals in group 2 and two animals in group 3 were hypertensive. By the fourteenth day three animals in group 2 had blood pressure between 140—150 mm. Hg, which we consider to be prehypertensive, although none were frankly hypertensive; in group 3 on the other hand seven animals were hypertensive and one was prehypertensive. On the 21st day of the
Blood pressures in normal and hormone treated rats. The mean pressure for each group is indicated by the curve. Maximal and minimal readings at each determination are indicated by vertical bars through the median points.
experiment three animals in group 2 were hypertensive and one prehypertensive; whereas nine of the rats in group 3 were hypertensive. Following this until the experiment was terminated all of the rats surviving in group 3 were hypertensive, and there was a gradually increasing incidence of hypertension in group 2. On the 33rd day six of the animals in group 2 were hypertensive and two were prehypertensive. The pressures in the animals of group 1 ranged from 114—134 mm. Hg at the terminal reading; whereas those of group 2 ranged from 129—208 mm. Hg. and those of group 3 from 199—221 mm. Hg.

Organ weights. Table 1 contains all of the data pertaining to organ weights and pathological changes. Since the primary purpose of this study was to determine the effect of compound S, the changes effected by DCA, which are now so well documented, will not be discussed in detail, but merely tabulated in Table 1. It will be apparent from a perusal of the table that in the organs examined the changes resulting from compound S overdosage are identical to those resulting from DCA excess, but of lesser magnitude.

There was no evidence of thymic involution as a result of compound S treatment, in fact the glands of such rats were slightly larger than controls, but the difference was not statistically significant. The thymuses of DCA treated rats tended to be smaller than those of controls, but this difference similarly was not statistically significant.

The adrenal glands of rats treated with compound S were significantly smaller than those from controls when expressed either as absolute weights or when the weight was expressed as a function of the body surface.

The cardiac ventricles were increased in weight by treatment with compound S. That this was a purely mechanical response to the increase in blood pressure is indicated by the fact that there was good correlation between the magnitude of hypertension and the cardiac size. Normotensive animals treated with compound S exhibited no cardiac hypertrophy. As in the case of the adrenal glands, the difference in size was statistically significant when expressed either in absolute units
or as a function of the body surface. DCA produced an even greater degree of cardiac hypertrophy.

As in the case of the hearts, the remaining kidneys in the rats treated with compound S were larger than those of control rats both in absolute units and in those expressed as a function of body surface. An even greater enlargement was apparent in the DCA treated group. Here too the correlation with the degree of hypertension was obvious; since the kidneys of the most hypertensive animals were markedly hypertrophied while those from normotensive rats, treated with the same quantity of the hormone, were not larger than those from control rats.

Cardiovascular pathological changes. No evidence of structural deviation from normal was observed in any of the control rats. As synopsized in Table 1, however, all of the DCA treated animals exhibited varying degrees of nephrosclerosis and myocarditis; while periarteritis nodosa, especially prominent in the pancreatic and mesenteric arterioles, was also observed. This was also true of those animals which died during the course of the experiment although these are not included in the table. In the case of compound S, on the other hand, only one animal — that which had the highest blood pressure — exhibited such changes.

DISCUSSION

The results of this experiment are compatible with the view that the activity of compound S is qualitatively the same although quantitatively less than that of DCA. Using life maintenance, body growth and maintenance of normal blood chemistry in adrenalectomized rats as a measure of activity, Masson et al. (1950) estimate compound S to be about one-thirteenth as active as DCA. It is possible that its hypertensive potency is of this same order of magnitude, although enough of the hormone to test this experimentally has not been available. However, even a dosage sufficient to produce
hypertension fails to induce the polydipsia and polyuria characteristic of DCA overdosage.

In the dosage used, and the time interval studied, it would appear that the onset of hypertension is not as rapid with compound S as with DCA, nor does the elevation of blood pressure progress as rapidly. However, it is interesting to note that the elevation of blood pressure is accomplished without a preceding or concomitant augmented NaCl intake and with no evidence of either polydipsia or polyuria. This is in contrast to the behaviour of DCA treated animals and may account for the greater effectiveness of the latter steroid. Compound S has been shown to exhibit a weak sodium and chloride retaining potency (Clinton & Thorn, 1942), and it is probable that its hypertensive effect depends upon this action, but it quite obviously does not produce the profound disturbances in salt and water metabolism which accompany the injection of a similar amount of DCA. It may be that this accounts for the lesser incidence of cardiovascular lesions in compound S treated rats as compared to DCA treated rats. The augmented fluid intake and sodium retention produced by the latter may both aggravate the hypertension by increasing the blood volume and sensitize the vascular bed to the damaging effects of the increased lateral pressure exerted upon their walls.

Although the previous studies on the activities of compound S did not, in contrast to the present experiment, yield evidence of adrenal atrophy; a possible explanation is readily available. It is probable that the pellet implantations did not lead to a sufficient overdosage, as is indicated by their failure to produce hypertension (Masson et al., 1950). On the other hand the degrees of cardiovascular damage produced by injected compound S (Selye, 1950) may have caused a stress sufficient to elicit increased ACTH from the pituitary and thus offset the mildly inhibitory action of compound S. The present studies indicate that if a quantity sufficient to cause hypertension without severe vascular damage is employed, adrenal cortical atrophy is readily induced.
SUMMARY

Reichstein's compound S acetate, when given in a dosage of 2.5 mg./day to rats maintained on a 1 per cent NaCl drinking fluid, displays an activity which is qualitatively similar to but quantitatively less than of a like quantity of desoxycorticosterone acetate; excepting that it produces no evidence of either polydipsia or polyuria. Hypertension results from its administration but develops more slowly than that resulting from DCA overdosage.

The morphologic changes observed were compensatory adrenal atrophy in all compound S treated rats, and renal cardiac enlargement in those in which hypertension was induced. Cardiovascular lesions of myocarditis, nephrosclerosis and periarteritis nodosa may occur in the latter if the elevation of blood pressure is sufficiently severe, although such lesions are much more prominent in DCA treated rats.

Neither compound S nor DCA produce evidence of thymic atrophy, in which respect they differ from adrenal glucocorticoids.

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REFERENCES