Effects of Desoxycorticosterone Acetate on Cholesterolemia, Blood Pressure and Atherogenesis in Chicks

By J. Stamler, M.D., R. Pick, M.D., and L. N. Katz, M.D.

with the technical assistance of E. Levinson, P. Johnson, M. Dudley and C. Bolene

Chronic exhibition of chicks to desoxycorticosterone acetate produces a slight but definite rise in blood pressure. This effect is increased by addition of salt to the diet. This regimen does not affect cholesterol levels and atherogenesis in the chick on ordinary mash. However, in the presence of a mildly atherogenic diet (0.25 per cent cholesterol) concomitant DCA exhibition increases the incidence and severity of atherosclerosis.

Severe atherosclerosis is a significant "complication" in clinical hyperadrenocortical states, for example in Cushing's syndrome. These diseases are rare. However, many of their features are readily produced by overdosage with adrenocorticotropic hormone (ACTH) or corticosteroids (cortisone, desoxycorticosterone, and others). These endocrine preparations are now being extensively used in clinical therapeutics. The problem arises: will atherosclerosis supervene as a complication in patients treated with these drugs?

The possibility of this untoward development is emphasized by recent clinical observations indicating that therapy with cortisone effects a rise in plasma cholesterol concentration. It is further highlighted by laboratory data demonstrating that desoxycorticosterone and other steroids may induce hypertension and arteriolonephrosclerosis* in experimental animals.

These facts emphasize the need for investigations clarifying the relationships between adrenal corticoids and atherogenesis. Towards this end, we studied the effects of the mineralocorticoid, desoxycorticosterone acetate (DCA),† on cholesterolemia, blood pressure and atherogenesis (spontaneous and cholesterol-induced) in cockerels.

METHODS

Two series of experiments were completed. In both, 1 day old Hy-line cockerels were obtained from a certified hatchery and reared in a battery brooder. A total of 223 birds were used in these studies (table 1). Daily intramuscular administration of DCA was begun when the chicks were 1 week old. The initial daily dosage of DCA was 1 mg.; this was progressively increased during the experiment to a maximum of 4 mg. The experimental dietary regimens are indicated in table 1. Essentially, with these regimens the effects of DCA were studied in two sets of paired groups (groups 1 and 2, and groups 3 and 4), fed mashes respectively with and without a cholesterol-oil supplement. In series I, these experimental diets were initiated when the birds were 4 weeks old. Until then, all groups subsisted on a commercial chick starter mash (plain mash) of known composition. In series II, the diets indicated in table 1 were fed beginning with the first day of life. In series II, the effects of DCA were analyzed in chicks fed a cholesterol-oil mash containing a dosage of sterol (0.25 per cent) known to induce a minimal hypercholesterolemia and organ lipidosis, in contrast to series I wherein a relatively high dosage of sterol (2 per cent) was given. Further, in series II a supplement of 1 per cent sodium chloride was added to the mash of all four experimental groups in order to enhance the hypertensive effect of DCA in the two groups receiving

† We wish to express our appreciation to Dr. W. Alan Wright of the Schering Corp., Bloomfield, N. J., who supplied us with generous amounts of desoxycorticosterone acetate in oil (Cortate, Schering).
the steroid. In both series, birds received mash and tap water freely.

In both series, chicks were weighed weekly throughout the experiment and a record of feed intake was maintained. At intervals, data on water intake were also collected. In order to assay the role of dietary sodium in the pathogenesis of DCA-induced hypertension, and polydypsia, two additional groups of 5 chicks each were fed a low-sodium diet (Lonalac) during the fifth to twentieth weeks of life. One of these groups was given DCA in doses similar to those used in series I and II. This aspect of the study was carried out concurrently with the series II experiment.

At intervals throughout both series of experiments, blood was drawn from an alar vein or by direct cardiac puncture. Aliquots of heparinized plasma from individual birds were analyzed for total cholesterol by the method of Schoenheimer and Sperry. In both series, blood pressures were also determined at five-week intervals in unanesthetized quiescent birds by direct puncture of a sciatic artery, isolated by cut-down. Mean blood pressures were read on a mercury manometer. In series I, comb size indexes were estimated at five-week intervals, utilizing established methods of measurement and calculation. In series I, chicks were sacrificed by decapitation and exsanguination when 15 weeks of age, after 14 weeks on DCA and 11 weeks on the experimental diets. In series II, birds were sacrificed when 5, 10 and 15 weeks of age. At autopsy all the viscera were examined and the gross findings recorded. The hearts and great vessels were carefully inspected for gross atherosclerotic plaques. Established methods and precautions were utilized in grading these lesions. Adrenals of both series were pooled by group and weighed post mortem; total body weight and heart weight of individual birds were also obtained. Blocks of tissue were then fixed in 10 per cent aqueous formalin and subsequently stained with hematoxylin-eosin (paraffin sections), Sudan IV (frozen sections), and Van Giesson or Masson connective tissue stain (paraffin sections).

**Results**

*Feed Intake and Weight Gain.* In both series, DCA-treated birds exhibited feed intakes and rates of weight gain similar to their paired control groups. All groups on experimental regimens corresponded to the plain mash-fed controls (group 1, series I and group 5, series II—table 1) in these respects. Hence any observed group differences in atherogenesis cannot be attributed to differences in development or in ingestion of experimental regimens.

**Water Intake.** In both series, respectively with (series II) and without (series I) a supple-

<table>
<thead>
<tr>
<th>Table 1.—Experimental Regimens</th>
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<tbody>
<tr>
<td><strong>Series</strong></td>
</tr>
<tr>
<td>I. Group 1—PM</td>
</tr>
<tr>
<td>I. Group 2—PM + DCA</td>
</tr>
<tr>
<td>I. Group 3—C-O</td>
</tr>
<tr>
<td>I. Group 4—C-O + DCA</td>
</tr>
<tr>
<td>II. Group 1—SM</td>
</tr>
<tr>
<td>II. Group 2—SM + DCA</td>
</tr>
<tr>
<td>II. Group 3—C-O—S</td>
</tr>
<tr>
<td>II. Group 4—C-O—S + DCA</td>
</tr>
<tr>
<td>II. Group 5—PM</td>
</tr>
</tbody>
</table>

PM = Plain mash.
C-O = 2% Cholesterol-5% Oil mash.
SM = Plain mash supplemented with 1% NaCl.
C-O-S = 0.25% Cholesterol-5% Oil mash supplemented with 1% NaCl.

In series 2, the dosage of salt was 0.5% during the first 5 weeks of the experiment; thereafter it was 1%, as indicated above.

<table>
<thead>
<tr>
<th>Table 2.—Effect of DCA on Water Intake (cc. per chick per day)</th>
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</thead>
<tbody>
<tr>
<td><strong>Series I</strong></td>
</tr>
<tr>
<td>*</td>
</tr>
<tr>
<td><strong>Series II</strong></td>
</tr>
<tr>
<td>*</td>
</tr>
</tbody>
</table>

For symbols see table 1.

* Data collected during eighth week on DCA (chicks 9 weeks old).
† Mash of all groups supplemented with 1% NaCl; data collected during eighth week on DCA (chicks 9 weeks old).

ment of 1 per cent sodium chloride in the mash, chicks given DCA exhibited polydypsia (table 2). In contrast, DCA-treated birds subsisting on a low sodium diet (Lonalac) exhibited no polydypsia, compared with their paired controls.

* We gratefully acknowledge the assistance of Dr. Warren M. Cox, Jr. of Mead Johnson and Co., Evansville, Indiana, who contributed generous supplies of Lonalac for this and other projects.
receiving no corticoid. Thus during their seventh week on DCA, Lonalac-fed cockerels (12 weeks of age) ingested 300 cc. of water per bird per day; Lonalac controls not given DCA ingested 292 cc.

**Cholesterolemia.** Data on plasma cholesterol levels are summarized in table 3. In both series, DCA-treated chicks subsisting on mash without a cholesterol supplement (group 2) exhibited normal plasma cholesterol levels throughout. DCA-treated birds fed mash containing cholesterol and oil (group 4) had a hypercholesterolemia similar in degree to their paired controls receiving no steroid. This was true in both series, with 2 per cent and 0.25 per cent cholesterol supplement respectively. In series II, birds subsisting on the 0.25 per cent cholesterol mash beginning with the first day of life, did not develop a definitive minimal hypercholesterolemia until the latter weeks of the experiment (table 3). This hypercholesterolemia was slightly, but not significantly greater in the DCA-treated chicks (series II–group 4), compared with their paired controls (series II–group 3), not receiving steroid. Apparently DCA was without definitive, significant effect on cholesterolemia in cockerels fed a diet with or without a cholesterol supplement.

**Blood Pressure.** Data on mean blood pressures are summarized in table 4. In series I, DCA chicks on plain mash (group 2) had slightly higher mean blood pressures than their paired controls (group 1). DCA birds fed 2 per cent cholesterol–5 per cent oil mash in this series (group 4) exhibited blood pressures almost identical with those of their paired controls (group 3).

In series II, with addition of 1 per cent sodium chloride to the experimental diets of all groups, the DCA-treated cockerels consistently exhibited mean blood pressures greater than those of their paired controls. The DCA-induced increments in blood pressure became progressively greater with prolongation of the experiment (table 4). Addition of salt to the diet effected a slight blood pressure elevation in birds not receiving DCA (compare groups 1 and 3 with group 5, table 4).

In contrast to this rise in mean blood pressure in DCA chicks having access to salt in their diets, DCA-treated cockerels on a low sodium diet (Lonalac) failed to develop any increment in arterial pressure. Thus after seven weeks on DCA plus Lonalac, birds had a mean pressure of 133 mm. Hg (cf. group 5, table 4), compared with a value of 147 mm. Hg for their paired controls subsisting on Lonalac without DCA.

Our data fail to indicate any consistent tendency for cholesterol feeding to elevate the blood pressure of chicks. In series I, cockerels fed 2 per cent cholesterol–5 per cent oil mash (group 3) did have slightly higher mean blood pressures than birds subsisting on plain mash (group 1). However, in series II, chicks with and without a cholesterol supplement in their
diets (groups 3 and 1) had similar blood pressures (table 4).

Atherosclerotic Lesions. The gross findings in the great vessels are summarized in table 5. In both series, no chicks subsisting on mash without a cholesterol supplement exhibited lesions in the thoracic aorta (groups 1 and 2). In such cockerels, DCA failed to induce lesions in this area, the site of predilection for atherosclerosis of the cholesterol-induced type.1

At 14 to 15 weeks of age in both series, birds eating feed devoid of cholesterol supplement had atherosclerotic lesions of the spontaneous type1 in the abdominal aorta (groups 1 and 2). In both series, DCA-treated birds on this diet had slightly, but not significantly, greater incidence and severity of such abdominal aorta spontaneous lesions.

In series I, a diet of 2 per cent cholesterol–5 per cent cottonseed oil mash for 11 weeks induced severe atherogenesis in both groups 3 and 4, with and without DCA (table 5). The incidence and severity of cholesterol-induced atherosclerosis in both the thoracic and abdominal aorta were similar in these two groups.

The possibility presented itself that the atherogenesis occurring with this cholesterol dosage was of such intensity as to mask any aggravating effect of DCA. Hence in series II, we utilized the lower cholesterol dosage (0.25 per cent), with which minimal hypercholesterolemia and mild atherogenesis develop. With this second dietary regimen, chicks with and without DCA exhibited no significant differences in atherogenesis when sacrificed after 5 and 10 weeks of experimental feeding (4 and 9 weeks of DCA treatment for group 4, table 5). However, after

Table 5.—Effects of DCA on Spontaneous and Cholesterol-Induced Atherogenesis

<table>
<thead>
<tr>
<th>Duration of experimental feeding</th>
<th>% with lesions in thoracic aorta</th>
<th>% with lesions in abdominal aorta</th>
<th>% with lesions in thoracic aorta grade I or &gt;</th>
<th>% with lesions in abdominal aorta grade I or &gt;</th>
<th>Mean gross grading of lesions in birds with lesions</th>
<th>Mean gross grading of lesions—whole aorta— all birds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>weeks</td>
<td></td>
<td></td>
<td></td>
<td>Thoracic aorta</td>
<td>Abdominal aorta</td>
</tr>
<tr>
<td>I. Group 1</td>
<td>10</td>
<td>0</td>
<td>35</td>
<td>0</td>
<td>0</td>
<td>0.4</td>
</tr>
<tr>
<td>I. Group 2</td>
<td>10</td>
<td>0</td>
<td>50</td>
<td>0</td>
<td>0</td>
<td>0.6</td>
</tr>
<tr>
<td>I. Group 3</td>
<td>10</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>88</td>
<td>2.4</td>
</tr>
<tr>
<td>I. Group 4</td>
<td>10</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>75</td>
<td>2.9</td>
</tr>
<tr>
<td>II. Group 1</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.3</td>
</tr>
<tr>
<td>II. Group 2</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.3</td>
</tr>
<tr>
<td>II. Group 3</td>
<td>5</td>
<td>13</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.3</td>
</tr>
<tr>
<td>II. Group 4</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.3</td>
</tr>
<tr>
<td>II. Group 1</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.3</td>
</tr>
<tr>
<td>II. Group 2</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.3</td>
</tr>
<tr>
<td>II. Group 3</td>
<td>10</td>
<td>25</td>
<td>13</td>
<td>13</td>
<td>0</td>
<td>0.6</td>
</tr>
<tr>
<td>II. Group 4</td>
<td>10</td>
<td>25</td>
<td>25</td>
<td>13</td>
<td>0</td>
<td>1.0</td>
</tr>
<tr>
<td>II. Group 1</td>
<td>15</td>
<td>0</td>
<td>33</td>
<td>0</td>
<td>0</td>
<td>0.3</td>
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<tr>
<td>II. Group 2</td>
<td>15</td>
<td>0</td>
<td>57</td>
<td>0</td>
<td>14</td>
<td>0.5</td>
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<tr>
<td>II. Group 3</td>
<td>15</td>
<td>17</td>
<td>50</td>
<td>0</td>
<td>0</td>
<td>0.1</td>
</tr>
<tr>
<td>II. Group 4</td>
<td>15</td>
<td>45</td>
<td>91</td>
<td>9</td>
<td>18</td>
<td>0.3</td>
</tr>
</tbody>
</table>
cockers than in their paired controls (series II—groups 3 and 4, table 3).

Heart and Adrenal Weights, and Comb Size Indexes. In accordance with the elevations in blood pressure they tended to exhibit, all DCA-treated groups in both series had increased heart weights and wet heart weight: wet body weight ratios (table 6). Determinations of dry heart weight revealed that the foregoing alterations were attributable to a true increase in cardiac muscle mass (hypertrophy).

Corresponding to previous work in other species, DCA-treated cockers, with and without cholesterol in the diet, exhibited hypoplasia of the adrenals. Thus in series I, the adrenals of groups 1 and 2 weighed 95 and 65 mg. respectively; of groups 3 and 4, 98 and 72 mg. (groups 2 and 4 received DCA). From studies in other laboratories, it would appear that this adrenal hypoplasia may be a result of pituitary hypoadrenocorticotropicism, occurring secondary to administration of exogenous steroid.29 *

DCA-treated chicks had low comb size indexes compared with their paired controls. Thus in series I cockers, 12 weeks of age, groups 1 and 2 had comb size indexes of 30 and 16 units respectively; groups 3 and 4, 30 and 12. These data indicate that DCA administration was associated with decreased androgenic activity.15

Other Morphologic Findings. Microscopic sections of the kidneys of DCA-treated chicks, groups 2 and 4 in both series, revealed moderate arteriolar thickening. This was due to increased deposition of fibrous tissue in the outer layers of the media and in the adventitia. Intimal thickening was not observed. Moderate focal interstitial fibrosis was demonstrable by connective tissue stains. Amorphous basophilic material was deposited between the glomerular capillaries. Glomerular enlargement, hypertrophy of the parietal cells of Bowman’s capsule, and changes in the epithelial lining cells of the proximal convoluted tubules4, 5 were not prominent findings. Hyalinized glomeruli, hyaline tubular casts and hyalinized necrosis of arteriolar walls were not seen.4, 5

**Table 6—Effects of DCA on Heart Weight**

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean wet body weight</th>
<th>Mean wet heart weight</th>
<th>Mean dry heart weight</th>
<th>Wet heart: Wet body ratio</th>
<th>Dry heart: Wet heart ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Group 1</td>
<td>1560</td>
<td>7.68</td>
<td>1.66</td>
<td>.49</td>
<td>.22</td>
</tr>
<tr>
<td>I. Group 2</td>
<td>1543</td>
<td>9.35</td>
<td>2.72</td>
<td>.61</td>
<td>.29</td>
</tr>
<tr>
<td>I. Group 3</td>
<td>1551</td>
<td>7.94</td>
<td>1.80</td>
<td>.50</td>
<td>.24</td>
</tr>
<tr>
<td>I. Group 4</td>
<td>1586</td>
<td>10.52</td>
<td>2.60</td>
<td>.62</td>
<td>.26</td>
</tr>
<tr>
<td>II. Group 1</td>
<td>1546</td>
<td>6.84</td>
<td>1.53</td>
<td>.44</td>
<td>.22</td>
</tr>
<tr>
<td>II. Group 2</td>
<td>1520</td>
<td>11.12</td>
<td>2.58</td>
<td>.73</td>
<td>.23</td>
</tr>
<tr>
<td>II. Group 3</td>
<td>1760</td>
<td>7.15</td>
<td>1.72</td>
<td>.41</td>
<td>.24</td>
</tr>
<tr>
<td>II. Group 4</td>
<td>1364</td>
<td>10.24</td>
<td>2.00</td>
<td>.75</td>
<td>.20</td>
</tr>
</tbody>
</table>

I = series 1, 15 week sacrifice.  
II = series 2, 15 week sacrifice.

* Operation of this mechanism in the chick remains to be demonstrated. In our laboratory Stamler and associates, have been unable to record changes in blood glucose levels or white blood cell counts (including eosinophils) in chicks treated acutely with 5 mg. doses of mammalian ACTH. Others obtained similar negative results, indicating that the avian adrenal cortex is not stimulated by mammalian ACTH. To our knowledge, no studies have been accomplished on avian anterior pituitary preparations and their corticotropic activity.

**Discussion**

The data from both series of experiments clearly indicate that chronic administration of deoxycorticosterone acetate has little or no intensifying effect on the spontaneous atherogenesis of chicks. Moreover, in birds subsisting on a mash devoid of a cholesterol supplement, DCA fails to induce atherosclerotic plaques of the cholesterol-induced type in the thoracic aorta. These cockers are uniformly free of such lesions. These negative results were obtained despite the definite increases in blood pressure occurring in DCA-treated chicks. These observations are in accord with our previous findings on the influence of salt hypertension on atherogenesis in birds fed mash lacking a cholesterol supplement.30 They constitute further evidence that hypertension, per se, does not influence atherogenesis, when it is not accompanied by an alteration in lipid and cholesterol metabolism.1, 17, 19, 30–32

Several factors may account for the apparently discrepant results between Series I and Series II with respect to the influence of DCA on cholesterol-induced atherosclerosis:
the failure of DCA to intensify atherogenesis in series I* may be due to the absence of a definitive blood pressure elevation in these birds. It may also be attributed to the lack of any consistent effect of DCA on cholesterolemia in cockerels fed a mash supplemented with 2 per cent cholesterol plus 5 per cent cottonseed oil. As already indicated, it is further possible that cholesterol-induced atherogenesis was so severe with this dietary regimen as to mask any intensifying effect of DCA.

With the lower cholesterol dosage of series II, a definite, significant influence of DCA on atherogenesis was demonstrated. This intensifying effect of DCA on cholesterol-induced atherogenesis was clearcut, but not gross or extreme. It may be a by-product of the slightly greater hypercholesterolemia of these DCA-treated birds, compared with their paired controls. It may also be related to the DCA-induced blood pressure elevation, which in the presence of increased plasma cholesterol concentration may intensify atherogenesis.

Further, this effect of DCA on atherogenesis in chicks fed 0.25 per cent cholesterol–oil mash may be due to possible specific effects of the hormone on vascular tissue17, or on plasma cholesterol-bearing colloidal lipoprotein micelles. Finally, it may be a by-product of hypotuitarism, hypoadrenocorticism, and/or hypothyroidism possibly supervening with chronic DCA administration. Further studies are needed to clarify this problem of the precise mechanism of the DCA-intensifying effect on cholesterol-induced atherosclerosis of chicks.

Obviously, our experiments by no means represent exhaustive studies on anterior pituitary-adrenal cortical factors and atherogenesis. Many other aspects of the relationship of this hormonal system to cholesterol metabolism and atherogenesis merit exploration. The need for such investigations is emphasized by data demonstrating the active role of the adrenal cortices in sterol metabolism: their high content of cholesterol, their ability readily to discharge cholesterol, to synthesize cholesterol, and to accumulate ingested cholesterol.9, 41, 42–44 Further, recent clinical studies18 highlight the need to explore the effects of adrenal androgens (e.g. cortisone) on lipid metabolism and atherogenesis. Such studies are currently in progress in this laboratory.

**Summary**

The exhibition of deoxycorticosterone acetate over periods up to 15 weeks induces cardiac hypertrophy, polydipsia, adrenal hypoplasia, decreased comb size indexes and moderate nephrosclerosis in cockerels. A definite, but not severe, rise in blood pressure occurs. The polydipsia and hyperpiesis induced by DCA are increased by addition of 1 per cent sodium chloride to the mash; neither supervene in DCA-treated chicks on a low sodium diet.

The exhibition of DCA fails to influence cholesterolemia grossly in chicks on mash with or without a cholesterol supplement. Correspondingly, gross atherogenesis is not significantly influenced by DCA in birds fed only plain mash. However, in cockerels given a mildly atherogenic supplement of cholesterol in their mash (0.25 per cent cholesterol), concomitant DCA exhibition increases the incidence and severity of atherosclerosis. Possible mechanisms of this DCA effect on cholesterol-induced atherogenesis are discussed.

**Acknowledgments**

We wish to express our appreciation to G. Crowley, laboratory technician, for his assistance in this experiment.

**REFERENCES**


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* Bruger and Lowenstein also recorded negative results in studies on the effects of DCA on cholesterol-induced atherosclerosis of rabbits.39
EFFECTS OF DEOXYCORTICOSTERONE ACETATE

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