TABLE I. Series 1—Effect of Aureomycin Supplementation.

| Lot No. | Avg initial wt in lb. | Supplement/kg basal diet | Avg daily gain in lb. first 37 days | Avg daily gain in lb. total 54 days | Hb.§ |
|---|---|---|---|---|---|
| 1 | 35.6 | 44 γ vit. $B_{12}$ (as concentrate) † | 0.77 | 0.75 | 10.7 |
| 2 | 35.8 | 44 γ vit. $B_{12}$ + 50 mg aureomycin | 1.30 | * | — |
| 3 | 35.6 | 44 γ vit. $B_{12}$ + 200 mg aureomycin | 1.61 | 1.69 | 13.1 |
| 4 | 35.6 | 10 g APF‡ | 1.36 | 1.46 | 12.0 |

* Lot 2 was stopped after being on experiment 37 days.

† Vit. $B_{12}$ concentrate contained approximately equal parts of vit. $B_{12}$ and $B_{12b}$ and had at least 10 mg of vit. $B_{12}$ activity per g of solids.

‡ Supplement 1 was a commercial APF supplement (8108-109) prepared from the aureomycin fermentation at Lederle Laboratories. By microbiological assay, Supplement 1 was found to contain about 3 mg of aureomycin per g. Its $B_{12}$ content by chick assay appears to be about 4 μg per g. The APF, aureomycin and $B_{12}$ were obtained from Dr. T. H. Jukes, Lederle Laboratories, Pearl River, N. Y.

§ Average of hemoglobin at end of 54 days.

TABLE II. Series 2—Effect of Aureomycin and Supplement 2.

| Lot No. | Avg initial wt in lb. | Supplement/kg basal diet | Avg daily gain in lb., 67 days |
|---|---|---|---|
| 1 | 42.5 | 1.5 g methionine* + 27.5 γ vit. $B_{12}$† | 0.76 |
| 2 | 41.7 | 1.5 g methionine + 10 g APF‡ (1%) + 100 mg aureomycin‡ | 1.26 |
| 3 | 44.5 | 1.5 g methionine + 10 g APF (1%) | 1.26 |
| 4 | 44.8 | 1.5 g methionine + 30 g APF (3%) | 1.56 |

* DL-methionine supplied through the courtesy of Dr. H. J. Prebluda, U. S. Industrial Chemicals, New York City.

† Vit. $B_{12}$ concentrate (fuller's earth) from Dr. D. F. Green, Merck, Rahway, N. J.

‡ Supplement 2 (lot 24) was similar to Supplement 1 except that microbiological assay indicated its aureomycin content was 6 mg per g.

*Summary.* Aureomycin supplementation produced considerable growth response with the pig when added to a diet containing vitamin $B_{12}$ and $B_{12b}$. APF Supplement 2 contains some factor(s) other than aureomycin and $B_{12}$ which accelerates growth in the young pig with the basal ration used. One per cent of APF Supplement 2 in the ration supplied enough aureomycin for the pig under the conditions of this experiment.

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## Desoxycorticosterone Hypertension and Ammonium Chloride.* (18227)

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Prolonged administration of desoxycorticosterone acetate (DCA) results in the development of hypertension in a variety of animal species including man(1-4). In the rat, this is accompanied by a condition of

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3. Selye, H., Hall, C. E., and Rowley, E. M., *Canad. M. A. J.*, 1943, v49, 88.

4. Perera, G. A., Knowlton, A. I., Lowell, A., and Loeb, R. F., *J. A. M. A.*, 1944, v125, 1030.

widespread arteriolosclerosis, nephrosclerosis, periarteritis nodosa and cardiac lesions(1,3,5). Increased sodium chloride intake may augment both the hypertension and the severity of the lesions(5,6), but does not apparently do so when high doses of DCA are employed (6). Although a sodium free diet prevents these effects of DCA in rats(7), clinical evidence suggests that sodium retention is not a factor in the increased blood pressure which develops in either Addisonian(4) or hypertensive patients(8) receiving DCA. The relationship which exists between DCA-induced hypertension and sodium therefore is not entirely clear. Ammonium chloride, administered to rats receiving DCA, inhibits the widespread cardiovascular lesions(9,10). This has been ascribed to the prevention of the hypertensive effect of the hormone(9,11), although the blood pressure was not measured; the deduction being made from the inhibition of cardiovascular lesions. Further, although the beneficial effect of ammonium chloride has been ascribed to its sodium diuretic effect, there is some doubt as to whether blood sodium may be effectively lowered by ammonium chloride(12,13). Evidence indicates that hypertension resulting from DCA treatment precedes the development of cardiovascular lesions(14), and may persist even when DCA is withdrawn(15). The foregoing considerations suggested to us that it might be well to re-evaluate the influence of ammonium chloride on DCA hypertension.

*Methods.* Sixty albino rats of the Holtzman strain, half females and half castrate males, weighing between 40 and 65 g were unilaterally nephrectomized and divided into 6 equal groups. Groups 1 and 2 received a solution containing 0.5% sodium chloride to drink: groups 3 and 4 were given a solution of ammonium chloride containing an equivalent number of chloride ions, *i.e.* 0.45% $NH_4Cl$: groups 5 and 6 received a solution which contained 0.5% NaCl and 0.45% $NH_4Cl$. A pellet of crystalline desoxycorticosterone acetate† weighing 50 mg was implanted subcutaneously into each of the rats in the odd numbered Groups 1, 3 and 5; whereas those of Groups 2, 4 and 6 served as controls. All of the solutions were made with distilled water. The food consisted entirely of Purina Laboratory Chow. On the first day of the experiment all animals were allowed free access to drinking fluids, and it was found that those of Group 4 consumed the least. Thereafter the animals of this group were allowed fluid *ad lib.*, and the other groups were restricted to the amount which Group 4 voluntarily consumed. The blood pressures were taken, beginning on the 15th day of treatment, twice weekly for the duration of the experiment by the tail plethysmograph method of Williams, Harrison and Grollman(16), as modified by Sobin(17), on unanesthetized animals. In order to avoid bias one of us (L.A.P.) measured the blood pressure once weekly while another (O.H.) recorded the pressures at the second weekly determination. On each of these occasions the operator measured the pressures while being unaware of the group to which the animal in question belonged or of the previous pressures determined in that week. An average of 4 successive readings on each rat was taken to be an accurate representation of the

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† We are indebted to the Schering Corp., Bloomfield, N. J. for supplying the desoxycorticosterone acetate used in these studies.

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17. Sobin, S. S., *Am. J. Physiol.*, 1947, v146, 179.

blood pressure in that animal on each occasion. On the 30th day the animals were sacrificed and the kidneys, hearts, adrenals and portions of the pancreas and mesentery were placed in Bouin's fixative. The auricles were removed from the hearts at the time of autopsy since we have found ventricular weights to be a more sensitive reflection of cardiac hypertrophy than total heart weights, and organs were weighed after fixation.

*Results.* In our experience normal rats do not exhibit blood pressures in excess of 135 mm Hg, which therefore represents the upper limit of normal. Pressures of 150 mm Hg or higher have arbitrarily been designated as reflecting a hypertensive state. The blood pressure of the rats in Groups 2, 4 and 6 did not deviate from the normal at any time during the course of the experiment. Those receiving DCA however began to manifest hypertensive pressures about the 19th day of treatment in Groups 1 and 3, and those of Group 5 about the 23rd day. Actually those of Group 3, receiving ammonium chloride and DCA, had blood pressures slightly higher than those of Groups 1 and 5. Although this difference between Groups 1 and 3 was not statistically significant at the final reading, the blood pressure of the animals in Group 5 was significantly lower ($P<0.02$) than that of Groups 1 and 3. The rats of Group 5 lagged behind those of the other DCA treated groups, both in the time at which the elevation of blood pressure became manifest and the magnitude of the elevation (Fig. 1). The reason for this is not readily apparent although the rats of Groups 5 and 6, while they appeared to be in good condition, did not gain as rapidly in weight as the other rats in this experiment, perhaps because they received a hypertonic solution to drink.

Since the rats in Group 3 did not consume their fluid at a much greater rate than those of Group 4, these groups, for all practical purposes, had fluid available at all times. Those of Group 2 consumed their daily allotment within 4 or 5 hours; those of Group 1 within an hour; and those of Groups 5 and 6 in about 8 and 12 hours respectively. At the beginning of the experiment the average fluid

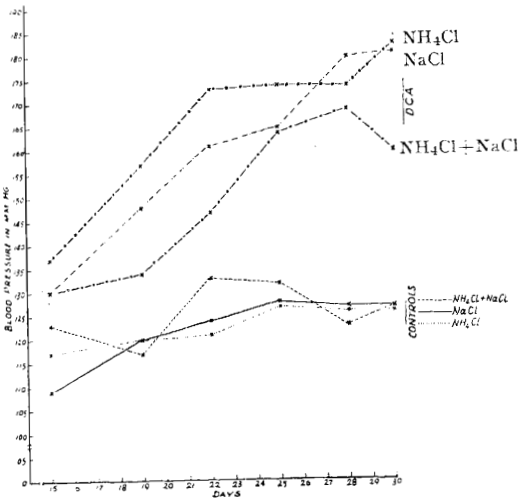


FIG. 1.

Blood pressure of DCA-treated and control rats. The slope of the curve for rats receiving the hormone and ammonium chloride is paralleled, at a somewhat lower level for the greater part of the experiment by that of the rats receiving DCA and sodium chloride. Hypertension in rats receiving DCA and both electrolytes is delayed in development and of lesser magnitude. Days on which blood pressure determinations were made are indicated on the chart by the letter X.

intake of each rat was 14 cc; whereas terminally it reached 25 cc daily.

At the end of the experimental period every rat in Groups 1, 3 and 5 was hypertensive. The organ weights, together with their standard errors calculated according to the formula

$$\text{S. E. M.} = \sqrt{\frac{d^2}{n\,(n-1)}},$$

are given in Table I. The effect of DCA in producing cardiac and renal enlargement is apparent in each of the groups receiving the steroid. Similarly the weight of the adrenals was depressed in those animals receiving DCA, below that of the appropriate controls, this difference not being marked between Groups 1 and 2. This may perhaps be due to the fact that being deprived of drinking fluid for about 23 hours of the day proved a sufficient stress to permit the pituitary to elaborate normal amounts of ACTH despite the mild inhibitory influence of DCA on this function of the hypophysis.

Histologic study of the tissues examined failed to reveal pathologic lesions in any of

TABLE I. Effect of Desoxycorticosterone Acetate on the Body and Organ Weights of Rats Drinking Various Electrolyte Solutions.

| Drinking solution | NaCl | | $NH_4Cl$ | | NaCl and $NH_4Cl$ | |
|---|---|---|---|---|---|---|
| Group | I DCA | II Control | III DCA | IV Control | V DCA | VI Control |
| No. of rats (final) | 9 | 7 | 8 | 10 | 7 | 8 |
| Body wt (g) | | | | | | |
| Initial | 51 ± 2 | 46 ± 1 | 54 ± 2 | 51 ± 2 | 43 ± 1 | 48 ± 5 |
| Final | 120 ± 4 | 132 ± 4 | 127 ± 1 | 140 ± 3 | 111 ± 1 | 112 ± 4 |
| Kidney wt | | | | | | |
| mg | 1100 ± 30 | 953 ± 13 | 1127 ± 67 | 899 ± 38 | 919 ± 64 | 807 ± 48 |
| % body wt | .921 ± .028 | .688 ± .011 | .862 ± .041 | .639 ± .084 | .812 ± .031 | .715 ± .011 |
| Heart wt | | | | | | |
| mg | 444 ± 17 | 420 ± 19 | 493 ± 10 | 354 ± 21 | 393 ± 9 | 354 ± 21 |
| % body wt | .368 ± .009 | .317 ± .017 | .398 ± .023 | .282 ± .007 | .349 ± .011 | .282 ± .007 |
| Adrenal wt | | | | | | |
| mg | 24 ± 1 | 25 ± 1 | 24 ± 1 | 27 ± 1 | 19 ± 0 | 22 ± 0 |

the rats. This may be due to the low dosage of DCA and short duration of the experiment. Also the rather rigorous restriction of fluid, and hence electrolytes, perhaps militated against their development.

*Discussion.* Rats receiving desoxycorticosterone acetate develop a marked and persistent hypertension against which ammonium chloride exerts no protective effect. The elevation of blood pressure to hypertensive levels occurs as promptly and as markedly in rats receiving ammonium chloride to drink as in those given comparable quantities of sodium chloride; indeed, as may be observed from a perusal of Fig. 1, the blood pressure of the former group was at all times slightly above that of the latter. Hypertension produced by the steroid in rats which received a hypertonic solution of sodium and ammonium chloride together was slightly delayed in time of onset and was of lesser magnitude. It has been shown that ammonium chloride does not interfere with the life maintaining action of DCA in the adrenalectomized rat(18). The present study would indicate that this electrolyte similarly does not interfere with the hypertensive effect of the steroid. Perhaps rats allowed free access to unlimited amounts of sodium chloride and treated with DCA might have developed a more severe hypertension than those on restricted quantities, but it is evident that when sodium chloride intake is limited it does not potentiate the pressor effect of DCA to a greater extent than equivalent amounts of ammonium chloride.

The inhibition of cardiovascular lesions in DCA treated animals by ammonium chloride would therefore seem to be due to a local effect on the tissues involved rather than to any interference with the production of hypertension *per se*. This would appear to be of considerable therapeutic interest since although cardiovascular pathology is an almost invariable consequence of sustained hypertension, any form of therapy which fails to reduce the blood pressure is usually discarded. Ammonium chloride, while failing to reduce the blood pressure of DCA treated animals, may nonetheless be effective in moderating the severity of the widespread vascular damage which follows sustained hypertension.

*Summary.* Ammonium chloride does not inhibit the hypertensive effect of desoxycorticosterone acetate in the rat. When both received the steroid, no difference can be detected between the blood pressure of rats given excess sodium chloride or an equivalent amount of ammonium chloride. The protection against cardiovascular lesions by $NH_4Cl$ in DCA treated animals must therefore be ascribed to a lack of response of the vascular territories to hypertension.

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