THE EFFECT OF DESOXYCORTICOSTERONE ACETATE ON BLOOD PRESSURE, RENAL FUNCTION, AND ELECTROLYTE PATTERN IN THE INTACT RAT*

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The problem of the relation between experimental renal hypertension and essential hypertension in man, hinging on the primacy of the kidney, has assumed a critical importance in recent years. The alternate positions have been summarized by Smith, Goldring, and Chasis (1), Goldring and Chasis (2), and more recently by Goldblatt (3). Recent work by Selye and his colleagues implicating the adrenal cortex in the etiology of the hypertensive syndrome has, in a sense, offered an intermediate position (4, 5). These workers demonstrated, first in the chick (6), and later, in the rat, dog, and monkey (7), that overdosage with desoxycorticosterone acetate (DCA) could produce hypertension and renal damage in animals sensitized with saline. Later these workers demonstrated that an endogenous overproduction of cortical hormones resulting from stress could also cause this nephrosclerosis and hypertension in the rat (8). The rôle of the adrenal in experimental hypertension in animals has been confirmed by many workers, more recently by Anderson, Page, Li, and Ogden (9), and Page, Ogden, and Anderson (10), who showed that renal hypertension could be restored in hypophysectomized rats by administration of the adrenocorticotropic hormone alone, and to a lesser extent by desoxycorticosterone or adrenal cortical extract.

Clinical support for Selye's findings has come from several sources. Perera and his coworkers have demonstrated that patients with Addison's disease being treated with DCA may develop elevated blood pressure (11–13), while F. Selye has shown the presence of an elevated Na/Cl ratio—supposedly characteristic of DCA overdosage (5)—in some patients with hypertension (14). More recently Hoagland (15) has demonstrated strikingly the intimate correlation between cortical hormone production and stress in man.

The idea that the adrenal plays a primary rôle in the development of hypertension has, however, not gone uncriticized (3, 16). Thus Goldblatt (3) has stated, "it is doubtful that any of the known endocrines plays a primary part in either essential hypertension associated with vascular disease in man, or in experimental renal hypertension in animals." Further it must be admitted that even though the dosages of DCA usually employed are large, saline feeding, preferably coupled with uninephrectomy or other intensifying measures, seems necessary to elicit the full character of the lesions (17).

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It seemed to us that insufficient evidence had been presented either for discounting or affirming the rôle of the adrenal cortex in the genesis of hypertension. From the literature, it seemed possible that reasons for disagreement among authors might rest in part with the mode of administration and the quantity of DCA used as well as with the technique of blood pressure determinations. With this in mind, several preliminary experiments were carried out in which we were able to determine that small doses of DCA administered in pellet form were capable of raising blood pressure and causing kidney damage in the intact animal. Encapsulation of the pellets occurred within about 14 days and was followed by the disappearance of the effects. If, instead of a single large pellet, small pellets were implanted singly every 10 days into a new site, progressive changes were consistently observed. Pellets are recovered amazingly intact, indicating slow rates of absorption. In these experiments it was also noted that sustained increases of blood pressure were paralleled by increases in heart weight due largely to ventricular hypertrophy determined histologically. It became evident that the determination of heart weight was an objective means of confirming the presence of chronic hypertension in experiments involving treatment with DCA.

With this background, the progressive changes in renal function, electrolyte pattern, and blood pressure following the administration of DCA to intact rats were investigated.

EXPERIMENTAL

Four groups of twenty male albino rats were maintained for 42 days. The animals were 28 days old and approximately evenly matched in weight at the start. They were housed five to a cage and received Purina Fox Chow *ad libitum*. The first and third groups drank tap water, while the second and fourth received 1 per cent saline. In addition, the third and fourth groups carried subcutaneous implants of DCA throughout the experiment. There were thus: group 1, intact control; group 2, intact control drinking saline; group 3, animals with DCA pellet; and group 4, animals with DCA pellet also drinking saline.

As already mentioned, lead experiments had demonstrated that for this type of work the mode of administration of DCA is important. In this experiment, 75 mg. pellets of DCA[1] were broken into three roughly equal parts, and each third was separately implanted on the 1st, 11th, and 25th days of the experiment. On the 42nd day the animals were sacrificed. Tissues were fixed for subsequent histological examination, and the kidneys and heart were weighed after 6 hours' fixation.

During the course of the experiment blood electrolyte studies (18) were carried out on individual animals once every 2 weeks beginning on the 12th day. Similarly, clearance studies using inulin and sodium *p*-aminohippurate were performed every 2 weeks beginning on the 10th day. Blood for both procedures was obtained by heart puncture. Half of each group of twenty animals were thus used throughout for electrolyte studies, while the other half were used for renal function tests, so that no animal was subjected to heart puncture oftener than once in 14 days. This is an ample recovery period as shown by the rapid return of normal behaviour in the animals, as well as by hematocrit studies.

[1] Schering cortate.

Briefly the clearance technique is as follows: PAH solution (12.5 mg./cc. in 2 per cent sodium sulfate) is injected into the lumbar region in accordance with body weight. The total dose in normal animals weighing 110 gm. is 40 mg., for animals weighing 150 gm., 47 mg., and for animals weighing 200 gm., 55 mg., with intermediate doses for intermediate weights. Where renal function is impaired, smaller doses may suffice to yield the correct plasma level of 5 to 7 mg per cent at 50 minutes.

Immediately following this injection, 3 cc. of warm 2 per cent inulin solution is injected intraperitoneally. The completion of this second injection marks the start of the urine collection period, and the rat is immediately placed into a metabolism funnel.

Fifty minutes after injection, the rat is picked up over the funnel and the bladder is drained by suprapubic pressure, although micturition is usually quite free and spontaneous. Immediately following urine collection, 0.75 cc. of blood is obtained by heart puncture in the 51st minute, using a 24 or 25 gauge heparinized needle. The animal is returned to its cage unharmed and the blood and urine analysed for inulin and PAH.

The rationale for this procedure and the rigid criteria necessary for its execution and for calculation of valid clearance data have already been discussed in detail elsewhere (19). Thirty-two animals are handled per day.

Blood pressure determinations were carried out on all animals on the day before the electrolyte or clearance test, using the method of Byrom and Wilson (20) with ether anesthetic. While this method may fail to record a raised pressure in the occasional animal which is actually hypertensive, it has been our experience that it never falsely indicates a rise.

Table I presents the data for the 2nd week of the experiment.

Blood Pressure.—Blood pressure was significantly elevated in both groups 3 and 4; *i.e.*, DCA and DCA-saline. Four animals in group 3 and six animals in group 4 had pressures exceeding the control average by more than twice the standard deviation of the latter value.

Renal Function.—Group 2, receiving saline, but no DCA, showed an increase in glomerular filtration rate (GFR, C_{IN}) and in filtration fraction (FF). On the other hand, renal function appeared undisturbed in the two DCA groups. When group 4 is compared with its saline-fed control, however, the administration of DCA is seen to have negated the expected rise in GFR and FF. This difference is statistically significant.

Plasma Electrolytes.—The only significant electrolyte change at this period is the fall in plasma K observed in groups 3 and 4; *i. e.*, DCA and DCA-saline. Other trends, however, are interesting in the light of later observations. Thus the slight elevation in both Na and Cl seen in group 2 was maintained throughout the later experimental period. The small rise in Na occurring in both groups receiving DCA compared to their controls was increased later. At this time an increase in the Na/Cl ratio had occurred only in the DCA-saline group compared with its control, but this change became increasingly significant. The total ionic concentration indicated by Na plus K remains normal, the fall in K being insufficient to alter this total.

Table I presents the data obtained in the 4th week of the experiment.

Blood Pressure.—Blood pressure was significantly elevated in the two DCA groups and had progressed beyond that previously observed. Six animals in group 3 and eight animals in group 4 had pressures exceeding the control average by more than twice the standard deviation of the latter value.

Renal Function.—Renal function was definitely altered at this time. The aberrations observed at 2 weeks in the saline-fed group disappeared although the slight hyperemia indicated in the first test (C_{PAH}/Tm_{PAH}, renal plasma flow per unit of excretory tissue), still appears to

TABLE I

	2nd week				4th week				6th week				
Group No.	1	2	3	4	1	2	3	4	1	2	3	4	
Treatment	Control	Saline	DCA	DCA-saline	Control	Saline	DCA	DCA-saline	Control	Saline	DCA	DCA-saline	No. of animals per group
Blood pressure	101 ±11.7	97 ±9.5	110 ±13.9	113 ±13.3	100 ±13.8	96 ±13.0	115 ±13.0	120 ±13.6	108 ±14.8	103 ±10.4	117 ±18.8	129 ±19.6	16
C_{IN}, *cc./100 cm.²*	0.34 ±0.07	0.48 ±0.05	0.34 ±0.04	0.40 ±0.09	0.34 ±0.07	0.34 ±0.07	0.34 ±0.06	0.31 ±0.09	0.35 ±0.03	0.30 ±0.04	0.32 ±0.07	0.22 ±0.03	8
C_{PAH}, *cc./100 cm.²*	2.18 ±0.26	2.34 ±0.35	2.44 ±0.61	2.48 ±0.45	2.55 ±0.36	2.57 ±0.44	2.16 ±0.41	1.76 ±0.30	2.47 ±0.52	2.76 ±0.46	2.37 ±0.36	1.55 ±0.44	8
Tm_{PAH}, *mg./100 cm.²*	0.119 ±0.012	0.118 ±0.014	0.128 ±0.014	0.126 ±0.013	0.126 ±0.012	0.115 ±0.012	0.111 ±0.010	0.107 ±0.007	0.118 ±0.020	0.125 ±0.014	0.107 ±0.013	0.093 ±0.012	8
FF as per cent	14.5 ±4.6	20.7 ±3.6	14.7 ±2.4	15.3 ±5.0	13.3 ±2.0	13.8 ±1.9	16.4 ±2.0	17.6 ±3.6	16.2 ±4.0	11.2 ±2.1	13.5 ±3.6	15.6 ±6.0	8
C_{PAH}/Tm_{PAH}	18.6 ±2.3	20.0 ±2.6	19.8 ±5.1	19.6 ±3.3	20.3 ±2.7	22.4 ±2.9	18.7 ±2.4	17.7 ±3.2	20.9 ±1.4	20.8 ±2.5	22.2 ±3.0	16.7 ±2.8	8
Na, *m. eq.*	149.2 ±2.7	152.8 ±2.4	151.8 ±2.0	153.8 ±3.3	151.4 ±4.8	152.5 ±6.6	155.7 ±6.6	155.1 ±3.1	148.0 ±4.3	149.2 ±2.0	154.7 ±4.0	158.6 ±3.7	8
Cl, *m. eq.*	98.1 ±1.5	103.9 ±0.6	99.1 ±3.9	100.2 ±2.0	103.6 ±1.6	104.8 ±2.1	103.6 ±3.3	94.6 ±2.0	104.8 ±1.3	106.3 ±1.0	98.1 ±1.4	95.7 ±1.1	8
K, *m. eq.*	8.0 ±0.7	5.0 ±0.3	4.2 ±0.2	3.8 ±0.3	6.8 ±0.5	6.2 ±0.6	3.5 ±0.4	3.2 ±0.6	5.4 ±0.8	5.3 ±0.4	4.1 ±0.6	2.7 ±0.6	8
Ca, *m. eq.*	—	—	—	—	6.9 ±0.1	6.8 ±0.3	6.5 ±0.2	6.7 ±0.2	6.4 ±0.1	6.3 ±0.3	6.3 ±0.3	6.0 ±0.4	8
Na/Cl	1.52	1.47	1.53	1.53	1.46	1.45	1.50	1.64	1.41	1.40	1.57	1.65	8

Na + K	157.2	157.8	156.0	157.6	158.2	158.7	159.2	158.3	153.4	154.5	159.8	161.3	8
Hematocrit, *per cent*									48 ±1	47 ±2	47 ±1	48 ±3	6
Heart rate, *per min.*									390 ±50	420 ±40	380 ±40	360 ±50	16
Heart weight, *mg./100 cm.²*									188 ±12	191 ±11	209 ±24	228 ±22	16
Kidney weight, *mg./100 cm.²*									465 ±54	474 ±30	544 ±67	683 ±58	16
Final body weight, *gm.*									193 ±21	201 ±22	204 ±24	158 ±24	16

The inulin clearance, C_{IN} measures the glomerular filtration rate (GFR). The clearance of PAH, C_{PAH}, measures the renal plasma flow (RPF) at the plasma levels of PAH used here. Tm_{PAH} represents the minute tubular excretion of PAH (total excretion of PAH less the amount filtered) and hence measures the functioning tubular excretory mass. The ratio C_{IN}/C_{PAH} represents the fraction of plasma filtered at the glomerulus and is termed filtration fraction (FF). The ratio C_{PAH}/Tm_{PAH} expresses the plasma flow for each unit of functioning tubular excretory tissue.

be present. Contrasting the DCA groups with their respective controls, it is readily seen that GFR was maintained but the total renal plasma flow, (RPF, C_{PAH}), was reduced. Similarly, the mass of functioning tubular excretory tissue, (Tm_{PAH}), was reduced in these groups. Reflecting these changes was a rise in FF which probably indicates constriction of the efferent arteriole since there was also a relative ischemia of kidney tubules as shown by the decline in C_{PAH}/Tm_{PAH}, particularly in group 4.

Plasma Electrolytes.—Clear cut changes in electrolyte pattern were also well established at this time. The elevation in both Na and Cl in the saline-fed group 2 was maintained. Plasma Na was significantly increased in both groups receiving DCA, a change previously indicated, and the Na/Cl ratio was likewise significantly elevated in these groups. The increase in this ratio was aggravated in the DCA-saline group by the definite fall in plasma Cl, despite the fact that this group received extra Cl from the NaCl. The fall in plasma K in both DCA groups was still pronounced but had not progressed beyond the level previously observed. The total ionic concentration indicated by Na plus K remained normal, the fall in K being balanced by the rise in Na. Plasma calcium was normal in all groups.

Table I presents the data obtained in the 6th week of the experiment.

Blood Pressure.—Blood pressure was significantly elevated in the DCA-saline group and higher than previously observed. On the other hand, while the pressure in the DCA group was still elevated, it had not progressed and indeed, it is questionable whether any significant elevation actually existed at this time. (The advance of the average blood pressure value in the control animals during the 6 week period is our usual finding for growing animals.) Four animals in group 3 and ten animals in group 4 had pressures exceeding the control average by more than twice the standard deviation of the latter value.

Renal Function.—The changes in renal function at this time are interesting. The temporary aberrations in the saline-fed group 2 have disappeared and the FF is now rather reduced.

In the DCA-treated animals of group 3 some adaptation must have been effected, for renal function was now well within normal limits, except that Tm still appeared reduced. These findings are in obvious contrast to the observations of the previous period, and suggest that the kidney changes may be to some extent reversible.

In the DCA-saline group the changes observed at the end of the 4th week had intensified. The GFR was no longer maintained, indicating a considerable degree of interference with the filtering mechanism. Renal plasma flow was cut to only a little more than half the normal value, and the functioning excretory tubular mass was considerably reduced. These changes are even more striking in view of the considerable renal enlargement which occurred during the experiment. The FF was unchanged on the average but the broad spread of data indicates that as glomerular damage progressed the raised FF previously observed changed to a reduced FF with individual animals in various phases of the process. Renal ischemia was definitely present.

Plasma Electrolytes.—The electrolyte pattern differed from that previously observed only in degree. Thus in the saline-fed group 2 there was still the slight expected increase in both Na and Cl. In the two DCA groups the elevation of plasma Na was now more marked, while the depression of plasma Cl seen only in group 4, DCA-saline, at 4 weeks was now seen in both groups. The decline in plasma K was still present but in no greater degree. The elevation in the Na/Cl ratio was now more marked while the sum of Na plus K was also increased, the decreased K no longer being able to compensate for the increased Na. Plasma calcium remained undisturbed.

Organ Weights.—The hypertension observed during the course of the experiment was substantiated by the significant increase in heart weight which occurred in both DCA groups.

The increase in heart weight in the DCA group was less than that observed in the DCA-saline group paralleling the relative blood pressure increases noted. The kidney weights are interesting, for the significant renal enlargement observed in both DCA-treated groups indicates how poorly renal efficiency was actually maintained. It is similarly clear from the hematocrit values that the animals had adjusted to the previous withdrawals of blood.

Heart rate was determined under light nembutal anesthesia, a procedure which gives rates somewhat above the basal level (21). The absence of any significant change in heart rate makes it unlikely that the increased blood pressure was due to increased cardiac output.

Histological examination of the kidneys showed the presence of diffusely distributed early glomerular sclerosis in both DCA groups. No tubular damage or vascular lesions were seen and the glomerular lesions were actually minimal.

DISCUSSION

As a result of DCA treatment, renal structure and function were affected, the electrolyte pattern was upset, and the blood pressure rose. Each of these factors may be examined separately.

The Effect on the Kidney.—It is apparent that DCA alone in small doses is capable of interfering with renal function, while the addition of saline intensifies the process.

The first change is a decrease in renal plasma flow with the maintenance of normal filtration, findings suggesting efferent vascular constriction. At this time the flow of blood to each unit of functioning excretory tissue is normal so that no true ischemia is present. Later the mass of functioning tubular excretory tissue decreases while renal plasma flow decreases even more, so that renal ischemia occurs. In this later stage gross interference with filtration is present.

The pattern of renal functional change seems remarkably similar to that observed in essential hypertension in man. When the DCA administration was accompanied by saline feeding the process was progressive, while in those animals receiving DCA alone the vascular spasm and renal ischemia disappeared, suggesting the reversibility of the process, a finding reminiscent again of essential hypertension in man (22). Interestingly, the elevation of blood pressure in these latter animals became less apparent concurrently with the improvement in renal function.

Superficially the blood pressure elevation occurred well before the onset of renal interference, but it is obviously not possible to come to any definite conclusion as to the primacy of either the blood pressure increase or the renal damage from these data. Certainly, however, the rise in blood pressure may occur without evidence of renal ischemia.

The Effect on Electrolytes.—A progressive increase in plasma sodium occurs, together with a decrease in plasma chloride and potassium. These changes are in accord with reported observations following DCA administration both in animals and man. Selye, Hall, and Rowley (5) noted the elevation in Na/Cl ratio and the fall in potassium in rats receiving DCA with saline, but failed to

observe an actual rise in sodium with their methods. Similar findings were reported by F. Selye in some hypertensive patients (14). Knowlton, Loeb, Stoerk, and Seegal (17) found the same changes as here reported in rats, but did not mention the total increase in cations denoted by Na plus K, although it is indicated in their data. Similar results have been published by Ferrebee *et al.* (23). Thus, DCA alone in small doses is capable of interfering with the electrolyte balance, while the addition of saline intensifies the process. It seems to us of importance to establish whether or not the increase in the total of sodium and potassium represents an osmotic plasma to tissue differential which could be etiologically significant. The data seem to indicate that DCA causes sodium retention, that potassium is lost in a failing effort to maintain a normal total concentration of cations, and that chloride is lost passively, coupled with potassium. Further work is necessary on these points.

The Rise in Blood Pressure.—There does not seem to be any reasonable doubt that the blood pressure rose early in the experiment. It might be argued from a perusal of the absolute data that these elevations cannot be considered true hypertension. We would agree that an average blood pressure rise of 10 or 15 mm. Hg does not seem very much when compared with the magnitude of the figures usually associated with clinical and experimental renal hypertension. The actual figures here presented are, however, not absolute. The instrument, in our hands, records a pressure which probably is somewhat below the systolic level and the actual values obtained lie in a restricted range. Further, as pointed out earlier, in dealing with a process in which different animals are necessarily in different stages of the pathological disturbance, the average pressure rise is at most an arithmetic mean between hypertensive and normotensive animals. It is for this reason that in the description of results, the number of animals with blood pressure exceeding the average control level by more than twice its standard deviation is presented. Proof of the validity of the estimates rests on the demonstration of cardiac hypertrophy in the hypertensive animals.

Several workers have doubted that any of the known endocrine organs play a primary part in either essential hypertension in man or in experimental hypertension (3, 16). In the light of our present findings this thesis may be untenable. It has been demonstrated that a syndrome presenting certain marked similarities to essential hypertension can be produced in the intact rat by DCA. Further, it is clear that small amounts are sufficient to elicit the aberrations reported without resort to any intensifying measures, such as adding saline or reducing renal reserve by uninephrectomy. When these facts are added to the demonstrated responsiveness of the human adrenal cortex to stress, to recent reports on the blood pressure elevation observed in patients receiving DCA implants for Addison's disease, and when the renal functional alterations here reported are compared with those observed in man, it would appear that the rôle of the adrenal cortex cannot be lightly dismissed.

This is not to suggest that hypertension is an endocrine disease, but rather to indicate that there may be patients with hypertension of hormonal etiology to be distinguished from those in whom the disease is truly "essential."

SUMMARY

Small doses of DCA administered at intervals in pellet form are capable of raising the blood pressure, altering renal function, and changing the electrolyte pattern in the intact rat. The concomitant feeding of 1 per cent saline intensifies the process.

The elevation in blood pressure occurs prior to demonstrable changes in renal excretory function.

The alteration in renal function consists first of a reduction in C_{PAH} with the maintenance of a normal filtration rate. Filtration fraction is elevated while there is no reduction in renal plasma flow per unit of tubular excretory tissue. Later, filtration is interfered with and renal ischemia occurs.

The electrolyte change is characterized by a sustained fall in plasma K and Cl, a rise in plasma Na, an increase in the Na/Cl ratio, and finally an elevation of Na plus K. Plasma Ca is unaffected.

These observations suggest the possible etiological significance of the adrenal cortex in some types of hypertension.

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BIBLIOGRAPHY

1. Smith, H. W., Goldring, W., and Chasis, H., *Bull. New York Acad. Med.*, 1943, **19**, 449.
2. Goldring, W., and Chasis, H., Hypertension and Hypertensive Disease, New York, The Commonwealth Fund, 1944.
3. Goldblatt, H., *Physiol. Rev.*, 1947, **27**, 120.
4. Selye, H., Hall, C. E., and Rowley, E. M., *Canad. Med. Assn. J.*, 1943, **49**, 88.
5. Selye, H., Hall, O., and Rowley, E. M., *Lancet*, 1945, **248**, 301.
6. Selye, H., and Stone, H., *Proc. Soc. Exp. Biol. and Med.*, 1943, **52**, 190.
7. Selye, H., and Hall, C. E., *Arch. Path.*, 1943, **36**, 19.
8. Selye, H., *Rev. Canad. Biol.*, 1943, **2**, 501.
9. Anderson, E., Page, E. W., Li, C. H., and Ogden, E., *Am. J. Physiol.*, 1944, **141**, 393.
10. Page, E. W., Ogden, E., and Anderson, E., *Am. J. Physiol.*, 1946, **147**, 471.
11. Perera, G. A., Knowlton, A. I., Lowell, A., and Loeb, R. F., *J. Am. Med. Assn.* 1944, **125**, 1030.
12. Perera, G. A., *J. Am. Med. Assn.*, 1945, **129**, 537.
13. Perera, G. A., and Blood, D. W., *Ann. Int. Med.*, 1947, **27**, 401.
14. Selye, F. L., *Canad. Med. Assn. J.*, 1947, **57**, 325.
15. Hoagland, H., *J. Aviation Med.*, 1947, **18**, 450.

16. Braun-Menéndez, E., Fasciolo, J. C., Leloir, L. F., Muñoz, J. M., and Taquini, A. C., Renal Hypertension, Springfield, Illinois, Charles C. Thomas, 1946.
17. Knowlton, A. I., Loeb, E. N., Stoerk, H. C., and Seegal, B. C., *J. Exp. Med.*, 1947, **85,** 187.
18. Polley, J. R., in press.
19. Friedman, S. M., Polley, J. R., and Friedman, C. L., *Am. J. Physiol.*, 1947, **150,** 340.
20. Byrom, F. B., and Wilson, C. J., *J. Physiol.*, 1938, **93,** 301.
21. Friedman, S. M., and Martin, W. S., *J. Lab. and Clin. Med.*, 1947, **32,** 1284.
22. Goldring, W., Chasis, H., Ranges, H. A., and Smith, H. W., *J. Clin. Inv.*, 1941, **20,** 637.
23. Ferrebee, J. W., Parker, D., Carnes, W. C., Gerity, M. K., Atchley, D. W., and Loeb, R. F., *Am. J. Physiol.*, 1941, **135,** 230.