OBSERVATIONS ON THE RÔLE OF THE RAT KIDNEY IN HYPERTENSION CAUSED BY DESOXYCORTICOSTERONE ACETATE*

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It is well established that desoxycorticosterone acetate (DCA) is capable of producing a rise in the blood pressure of a wide variety of species including man (1, 2). In the earlier animal experiments, large doses of the steroid or intensifying measures appeared necessary to elicit the reaction (3). Recently, however, we have been able to demonstrate that the pressor response can be elicited in the intact rat by small amounts of DCA without any additional sensitization and that the use of saline as drinking water intensifies the process but does not alter it qualitatively (4). Like others, we have concluded that the intensifying effect of saline resides in the sodium ion, although we have suggested that other ions may likewise be capable of increasing the effects (5).

Following on this, it appeared that the next phase of the problem was to elucidate the mechanism of action of DCA. We had noted that the rise in blood pressure in the rat appeared to be independent of alterations in renal function as determined by the clearance of inulin and sodium para-aminohippurate. Indeed, as in essential hypertension in man, the rise in blood pressure occurred before any change in renal function was demonstrable, and considerably preceded the onset of renal ischemia. Further, the intensification of renal functional derangement was not paralleled by any increase in the degree of pressor response. The suggestion that the pressor effects of DCA were independent of the kidney, and that the renal changes occurred secondarily, perhaps as a result of the hypertensive process itself, seemed reasonable, particularly since Goldman and Schroeder had recently demonstrated an immediate pressor response in man to administered DCA (6). In all our experiments, both published and unpublished however, we had noted that even where renal function as measured was undisturbed by DCA, kidney weight was increased. In other words, normal renal function was maintained only by hypertrophy. It thus became necessary to establish firmly whether kidney changes were or were not correlated with the pressor response.

Experiment 1

The experiments here reported were planned with the basic idea that the relation between blood pressure rise and kidney involvement could most easily

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be demonstrated by studying these two factors not only as they progressed following DCA administration, but also as they regressed following withdrawal of DCA.

One hundred and ten albino rats of the Sherman strain, averaging approximately 90 gm. in weight were divided into two groups. Forty-one animals served as untreated controls while the remaining 69 received DCA with 1 per cent saline as drinking water *ad libitum*. DCA was administered as one-third of a 75 mg. pellet (Schering cortate) subcutaneously implanted on the 1st day of the experiment and every 2 weeks thereafter. During the 4th week of the experiment renal function (7) and blood pressure (8) were determined in a group of 9 of the control and 18 of the DCA-saline–treated animals. Immediately following these procedures the control group and half of the treated group were sacrificed, their kidneys fixed, and then weighed. Pellets were removed from the remaining half of the DCA-saline–treated group which were then returned to tap water.

During the 6th week of the experiment renal function and blood pressure were again determined in a group of 8 control and 20 of the DCA-saline–treated animals, as well as in the group whose DCA-saline treatment had stopped 2 weeks earlier. Again, all animals were sacrificed except for half of the DCA-saline–treated group in which treatment was stopped at this time.

During the 8th week, the usual procedures were carried out in a control group, in a DCA-saline–treated group, and in the group whose treatment had been stopped in the previous period. Insufficient DCA-saline–treated animals remained to permit the experiment to be carried out beyond this period. Consequently, a subsidiary experiment was set up to determine the effects of stopping treatment in the 8th week. The base line data for control and DCA-saline–treated groups in the 8th week were again determined. Treatment was stopped in half of the treated group and their renal function and blood pressure determined again 1 week later.

Briefly, the clearance technique was as follows: PAH solution (12.5 mg./cc. in 2 per cent sodium sulfate) is injected into the lumbar region in accordance with a dose–body weight scale calculated to yield the desired 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 drained by suprapubic pressure, although micturition is usually free and spontaneous. Immediately following urine collection, 0.75 cc. of blood is obtained by heart puncture. Plasma and urine are then analyzed for inulin and PAH.

Blood pressure determinations are made with the tail plethysmograph using ether as anesthetic. While this method may fail to record a raised pressure in the occasional animal which is actually hypertensive, experience in many hundreds of animals indicates that it gives reliable and reproducible results. The actual pressure recorded is somewhat below the systolic level.

The results obtained in the two separate parts of the experiment (up to the 8th week, and then beyond this period) are presented in Table I. The number of animals indicated in each case refers to those sacrificed in that particular period. For simplicity, and in order to clarify the significant findings, the data have been rearranged graphically in Fig. 1. In this figure, the findings are presented as percentage deviation of the test group values from those obtained in a group of intact controls studied on the same day. Changes which are statistically significant ($p < 0.02$) are denoted.

TABLE I

Blood Pressure, Renal Function, and Kidney Weight Determined in Groups of Sherman Rats at Various Stages of Treatment with DCA and Saline, and Subsequent to the Cessation of Treatment

		Control	DCA-saline	DCA-saline treatment stopped in previous period
	No. of animals	9	9	
	Blood pressure	106 ±12	131 ±16	
	C_{IN}, *cc./100 cm.²*	0.34 ±0.08	0.34 ±0.08	
4th wk.	C_{PAH}, *cc./100 cm.²*	2.48 ±0.33	2.70 ±0.45	
	Tm_{PAH}, *mg./100 cm.²*	0.125 ±0.014	0.121 ±0.021	
	FF as per cent	13.7	12.9	
	C_{PAH}/Tm_{PAH}	19.8	22.2	
	Kidney weight, *mg./100 cm.²*	397 ±42	554 ±44	
	No. of animals	8	10	9
	Blood pressure	111 ±16	134 ±16	106 ±10
	C_{IN}, *cc./100 cm.²*	0.31 ±0.07	0.32 ±0.09	0.34 ±0.08
6th wk.	C_{PAH}, *cc./100 cm.²*	2.47 ±0.27	2.81 ±0.76	2.73 ±0.37
	Tm_{PAH}, *mg./100 cm.²*	0.127 ±0.010	0.134 ±0.033	0.129 ±0.011
	FF as per cent	12.5	11.4	12.4
	C_{PAH}/Tm_{PAH}	19.4	20.9	21.2
	Kidney weight, *mg./100 cm.²*	439 ±49	580 ±93	507 ±30
	No. of animals	8	10	10
	Blood pressure	107 ±13	151	97 ±17
	C_{IN}, *cc./100 cm.²*	0.33 ±0.08	Not graphed	0.39 ±0.08
8th wk.	C_{PAH}, *cc./100 cm.²*	2.59 ±0.27		2.87 ±0.39
	Tm_{PAH}, *mg./100 cm.²*	0.120 ±0.017		0.131 ±0.015
	FF as per cent	12.7		13.7
	C_{PAH}/Tm_{PAH}	21.7		21.9
	Kidney weight, *mg./100 cm.²*	459 ±45	602	501 ±30

TABLE I—*Concluded*

	Control	DCA-saline	DCA-saline treatment stopped in previous period
No. of animals	8	13	
Blood pressure	106 ±12	146 ±28	
C_{IN}, *cc./100 cm.²*	0.29 ±0.05	0.46 ±0.14	
8th wk. C_{PAH}, *cc./100 cm.²*	2.61 ±0.71	2.67 ±0.31	
Tm_{PAH}, *mg./100 cm.²*	0.140 ±0.022	0.126 ±0.014	
FF as per cent	11.1	17.2	
C_{PAH}/Tm_{PAH}	18.7	21.2	
Kidney weight, *mg./100 cm.²*	388 ±32	562 ±30	
No. of animals	8		8
Blood pressure	108 ±13		117 ±15
C_{IN}, *cc./100 cm.²*	0.27 ±0.04		0.29 ±0.05
9th wk. C_{PAH}, *cc./100 cm.²*	2.51 ±0.17		2.60 ±0.19
Tm_{PAH}, *mg./100 cm.²*	0.150 ±0.013		0.144 ±0.011
FF, as per cent	10.7		11.2
C_{PAH}/Tm_{PAH}	16.7		18.0
Kidney weight, *mg./100 cm.²*	388 ±32		507 ±78

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. Tm_{PAH} represents the minute tubular excretion of PAH 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.

The black arrows in this table connect the functional data of groups as determined before and after cessation of treatment. At the same time the table is arranged so that horizontal reading allows comparison between groups assessed at the same time.

Blood Pressure.—Blood pressure was significantly elevated as early as 25 days after the start of the experiment in the DCA-saline–treated animals and remained so throughout the 51 days of the treatment. Upon cessation of treat-

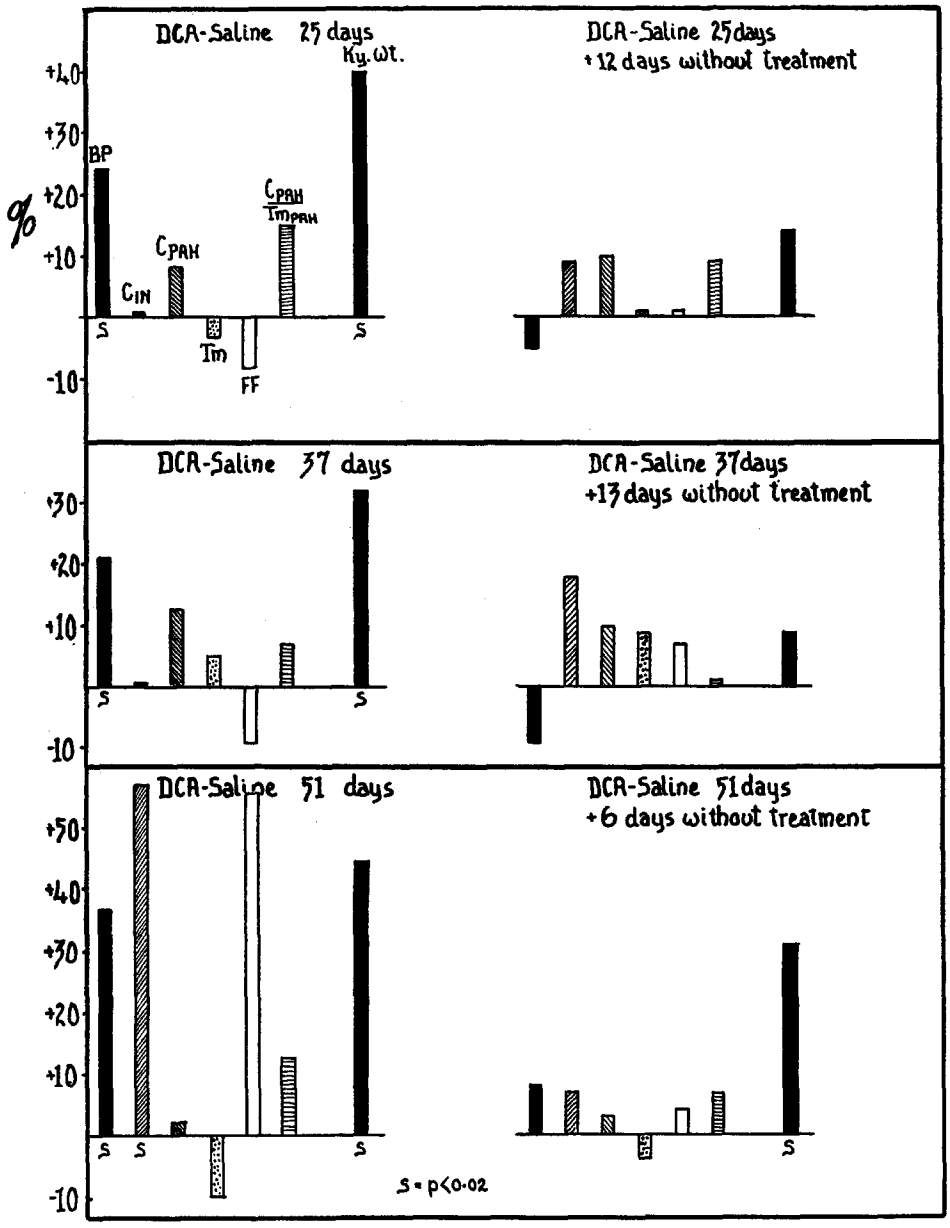

FIG. 1. Blood pressure, renal function, and kidney weight determined in groups of Sherman rats at various stages of treatment with DCA and saline, and subsequent to the cessation of treatment. The actual data on which this figure is based are presented in Table I.

ment either at 25, 37, or 51 days, blood pressure fell promptly to normal levels. Indeed, 6 days after cessation of DCA treatment which had been carried on for 51 days, a significant elevation of pressure was no longer demonstrable. Since blood pressure determinations were not carried out at times other than those specified in the table, the return of the elevated pressure to normotensive levels may have occurred even more rapidly than is here indicated.

Renal Function.—Renal function appeared undisturbed after 25 days and after 37 days of treatment with DCA-saline. This is in accord with our previous finding that Sherman animals do not develop renal functional changes as readily as do Wistar (9). Fifty-one days of treatment resulted in a significant increase in the glomerular filtration rate, and consequently, in the filtration fraction. Since in the two earlier periods renal function as here determined was unaffected by the DCA-saline treatment, it is not surprising that no real change was observed 12 days after cessation of treatment in either case. The marked deviation in filtration rate observed after 51 days of treatment disappeared 6 days after treatment was stopped.

The data concerning renal function fall in the same direction as others which we have reported and would, alone, suggest that the elevation in blood pressure is independent of renal functional derangement. Further, since change in renal function may only be observed relatively late in the course of treatment, the idea that it results from the elevated pressure might well be entertained. This suggestion is, however, contradicted by the observations concerning renal weight.

Kidney Weight.—Bearing in mind the obvious fact that changes in renal mass can probably not occur as rapidly as alterations in blood pressure, a remarkable parallel between these functions was observed. Elevation of the blood pressure even at the earliest date studied was accompanied by an increase in kidney weight, while restoration of the blood pressure was accompanied by a return towards normal of kidney weight. Since renal function was maintained only at the normal level despite this increase in size, it seems reasonable to assume that this process is a compensatory hypertrophy.

It is of some interest in relation to the theory of renal function tests that this renal involvement becomes apparent at once when renal function is related to actual renal mass.

The apparent correlation between renal mass and blood pressure was subjected to statistical analysis. In Fig. 2, renal mass in the DCA-saline–treated animals is plotted as a frequency distribution against blood pressure, the data being taken from part one of the experiment. The regression line for the data in which b, the coefficient of regression is 0.124, is statistically significant. Since this graph represents all treated animals, whether or not pellets were removed, the significant regression assumes even greater meaning. The

conclusion that the kidneys are in some way involved in DCA hypertension from the beginning of the process appears unavoidable.

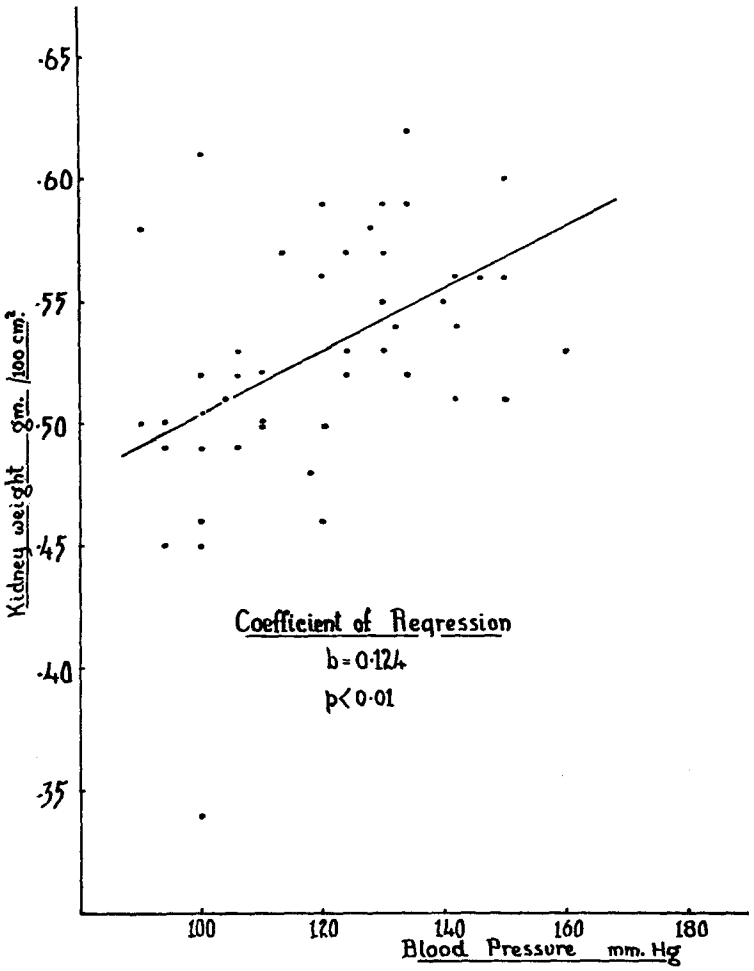

FIG. 2. Renal mass in DCA-saline–treated animals plotted as a frequency distribution against blood pressure.

Experiment 2

Since the kidneys are thus apparently immediately involved following DCA administration, two major possibilities concerning the mechanism suggest themselves—(*a*) the kidneys liberate a pressor substance upon stimulation by DCA or (*b*) the kidneys are actively concerned in the excretion and possible inactivation of DCA.

It seemed to us that the first step in distinguishing between these possibilities would be to examine the effects of DCA on blood pressure in the absence of the kidneys. The basic principle followed was to administer DCA until the blood pressure had attained a predetermined level, and then to nephrectomize the animals. A fall in pressure following nephrectomy would offer strong support to the idea that the pressor effect of DCA had been mediated by the kidney, perhaps through stimulation of a renal pressor mechanism. On the other hand, a rise in the blood pressure following nephrectomy would indicate that the pressor action of DCA was a more direct phenomenon.

Four separate experiments were carried out. In two of these, the blood pressure was elevated to a significant but low degree before nephrectomy so that either a fall or a rise might be easily discerned. Two were performed at an earlier stage in DCA treatment when the blood pressure was not yet significantly elevated. Since all four experiments yielded the same fundamental result only one experiment of each group is here reported.

Experiment 2a.—Twenty-eight male albino rats of an inbred Wistar strain and approximately 150 gm. in weight were maintained for 29 days. Eight animals served as untreated controls while the remaining 20 received a DCA pellet (one-third of a 75 mg. cortate pellet) as a subcutaneous implant on the 1st and 14th days of the experiment. On the 20th day, the left kidney was removed from each of 12 of the DCA-treated animals and on the 27th day of the experiment the remaining kidneys were removed. Blood pressure was determined at 1 or 2 day intervals beginning on the 18th day. The findings for this experiment are presented in Fig. 3. Seven of the 12 nephrectomized animals survived for the blood pressure determination 24 hours after complete nephrectomy, 2 for the 48 hour period.

Blood pressure was significantly elevated in the DCA-treated groups at the time of the first blood pressure determination on the 18th day. A fall in blood pressure occurred immediately following removal of one kidney but this was only temporary. In contrast, 24 hours after removal of the second kidney, a significant elevation above both untreated and DCA-treated controls was observed in the blood pressure of the nephrectomized animals. This result is the more remarkable since it occurred despite the undoubted operative shock, a factor not present in the control groups, and was observed not only as a group average but also in 6 of the 7 survivors, while the pressure of the seventh animal did not fall. The further elevation observed at 48 hours cannot be considered since it is based on only two survivors.

Experiment 2b.—Thirty male albino rats of an inbred Wistar strain, approximately 150 gm. in weight, were maintained for 14 days. Fifteen of these animals received 2 pellets (one-third of a cortate pellet) on the 1st day of the experiment and a third pellet on the 6th day. On the 12th day, 9 control and 9 DCA-treated animals were subjected to a one stage bilateral nephrectomy. Blood pressure was determined daily beginning on the 11th day. The results are presented in Fig. 4. All animals survived in good shape for 24 hours, but only 4 of the DCA-treated and 1 of the untreated nephrectomized animals were available for the 48 hour determination.

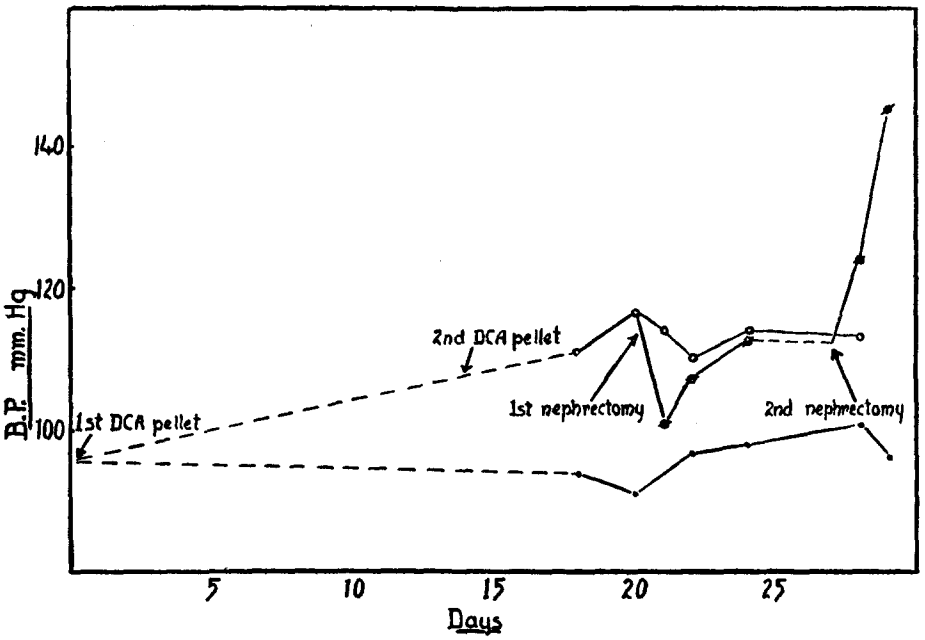

FIG. 3. The blood pressure in DCA-treated rats before and after removal of the kidneys.

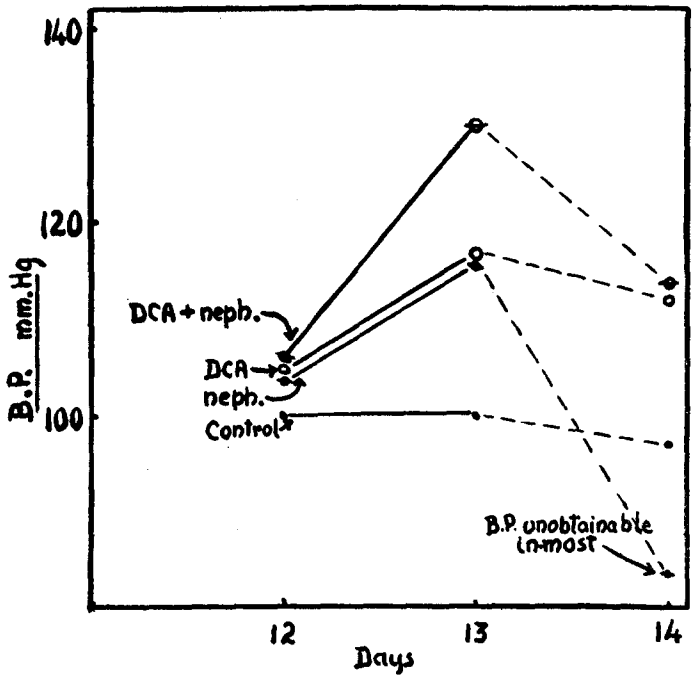

FIG. 4. The blood pressure in DCA-treated rats before and after removal of the kidneys.

Prior to nephrectomy no elevation in blood pressure was observed in any group although 2 days later the DCA-treated animals were beginning to show a rise. Twenty-four hours after removal of both kidneys blood pressure was elevated in both DCA-treated and untreated nephrectomized animals. In the case of the untreated group, this finding was significant but resulted from an elevation of pressure in only 3 of the 9 animals. On the other hand, all 9 DCA-treated nephrectomized animals participated in the observed elevation in this group, an elevation statistically significant not only in reference to the untreated control level but also in reference to the moderate elevation in the two other groups. The 48 hour findings are based on too few data to permit discussion.

DISCUSSION

Earlier workers had observed that when DCA was administered to suitably sensitized animals, hypertension and renal damage occurred. They assumed that the kidneys were primarily involved in DCA hypertension, although such a conclusion was not necessarily warranted by the data. Reinvestigating the problem from a functional approach we drew attention to the absence of renal functional change at a time when hypertension following DCA administration was well established, and pointed to the similarity of this observation to the findings in essential hypertension. Further exploration aimed at both the progression of DCA hypertension and its regression upon cessation of treatment showed, however, that the kidneys are involved from the start in the process. Attention is drawn particularly to those data which show clearly how compensatory hypertrophy may completely mask the presence of interference with renal function.

After removal of both kidneys, an aggravation of the hypertension was observed in DCA-treated animals. It is thus unlikely that the kidney enlargement reflects a stimulated production of renal pressor material, but it seems reasonable to suggest that the kidney is actively concerned with the excretion and possible inactivation of the steroid.

It is also possible to explain these findings according to Grollman's view that the kidney normally liberates an antihypertensive factor (10). In the present state of information, however, it is not possible to distinguish between the idea of a renal antihypertensive factor on the one hand and the renal destruction of a pressor agent on the other.

SUMMARY

Desoxycorticosterone acetate in pellet form was administered for 51 days to albino rats of the Sherman strain which also received 1 per cent saline as drinking water. Treatment was stopped in representative groups at 25, 37, and 51 days so that the regression of blood pressure and renal changes could be ob-

served. It was noted that both the elevation in blood pressure during treatment and its reversal when treatment was stopped were closely correlated with corresponding changes in renal mass. In the time for which the process was studied it did not become irreversible.

Removal of both kidneys from DCA-treated animals aggravated the hypertension, suggesting that the kidneys are actively concerned with the excretion and possible inactivation of the steroid.

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