SUSTAINED HYPERTENSION FOLLOWING THE ADMINISTRATION OF DESOXYCORTICOSTERONE ACETATE*

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PLATE 28

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It is well established that the administration of desoxycorticosterone acetate (DCA) to a wide variety of species including man may lead to the development of hypertension (1, 2). Because of the obvious possible implications of this finding in essential hypertension in man, a considerable body of literature has accumulated concerning both the mechanism of action of DCA and the conditions under which the steroid exerts its characteristic effects. Despite a considerable parallel between the effects of DCA and the findings in the human disease, only indirect evidence links essential hypertension to the DCA-induced condition (3). A distinct barrier to a direct linkage between the two conditions still remains in the fact that there is little proof of adrenal cortical hypersecretion in most instances of essential hypertension.

In studying this problem we noted that there was little information concerning the course of DCA hypertension once treatment with the steroid was discontinued. It seemed to us that, under certain circumstances, exposure to DCA might well be followed by irreversible changes and a sustained hypertension. If this were so, then the necessity for finding evidence of adrenal cortical hyperfunction in the human disease would fall away. Of course, if such a sustained post-DCA hypertension resulted, but was traceable to an anatomical renal lesion, then any attempt to draw a parallel would be difficult, since in man, the bulk of the evidence suggests that most instances of hypertension are not traceable to any distinct anatomical change in the kidneys.

Following out this line of thought, we studied the course in animals subjected to DCA treatment and observed that, following a short period of intensive treatment, hypertension sustained long beyond the period of initial administration resulted in some animals (4). As far as we could determine, the anatomical state of the kidney bore no relation to the hypertension, for any lesion (in

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any case a minimal one) which we could find in the kidneys of the hypertensive animals could be found with equal facility in the kidneys of those animals which became normotensive after discontinuation of the DCA. Shortly after publication of these initial findings in a small group of rats, Prado (5) also reported a "residual hypertension" with a tendency to become permanent in about 40 per cent of animals after treatment with DCA.

It seemed to us of importance to establish on a firm basis the features of this sustained post-DCA hypertension over a longer period and in greater detail. The present report is concerned with these experiments. Rats have been maintained for over a year after cessation of DCA treatment, and it is clear that approximately one-third of the animals so treated develop a fully sustained hypertension. This hypertension could be considered by definition "essential" for no cause has as yet been found to explain it and it clearly does not reside in any renal anatomical fault. It would seem that this disease is closely parallel at least to certain forms of the disease in man and consequently raises fundamental issues.

EXPERIMENTAL

Experiment 1.—

Procedure.—75 male albino rats of an inbred Wistar strain, weighing approximately 75 gm., were divided into two groups. The first group, consisting of 22 animals, served as untreated controls, while the remaining 53 animals were uninephrectomized and then, beginning 6 days after operation, were subjected to the intermittent administration of DCA combined with 1 per cent saline as drinking water for three short courses, each separated by a 2 week rest interval. At the conclusion of treatment the animals were observed without further interference for an additional period of 54 weeks.

In detail the treatment courses were as follows: 1st course. One DCA pellet (one-third of a 75 mg. cortate pellet) was implanted subcutaneously, followed by a second pellet 2 days later. 1 week after the start of treatment 1 per cent saline was substituted for the drinking water. 2 weeks after the start of treatment the pellets were recovered and the animals returned to tap water for 2 weeks. The 2nd course was the the same as the first. The 3rd course was also the same but was carried for a 3rd week during which time a third pellet was implanted.

The present report is concerned with our observations during the 54 weeks following DCA treatment. In this period, routine blood pressure determinations were carried out frequently. Blood pressure was measured by a modification of the method of Byrom and Wilson (6) using a 1 cm. cuff and ether anesthesia (7).

In addition, blood urea nitrogen was determined during the post-DCA period using the method of Barker (8) on a 0.2 cc. sample obtained from the tail. Small groups of animals were sacrificed during the course of the experiment in order to obtain organ weight and histological data. Similar data were obtained at the conclusion of the experiment.

(It should be recorded that in the 43rd week after DCA treatment had been stopped the animals were transported in a pressurized cabin of a commercial aircraft from Montreal to Vancouver.) For histological work prior to this period Susa was used as fixative and tissues were stained with hematoxylin and eosin and Weigert's elastic tissue stain; after this period formalin fixation was used and sections were stained with hematoxylin and eosin, Weigert's elastic tissue stain, and by McManus' periodic acid method.

Observations.—

Blood Pressure.—At the conclusion of the active treatment period, 21 control and 47 treated animals were available for comparison. At this time, 2 control and 4 treated animals were killed.

The intermittent, moderate treatment with DCA and saline was highly effective in elevating the blood pressure in this relatively susceptible strain of rats (Table I) the real nature of the blood pressure elevation being confirmed by the marked increase in ventricular mass found in the 4 animals autopsied at this time. Calculating the absorption as 100 μg. per day, per pellet of this size—an assumption warranted by our previous work and that of others (9)—it would appear that this marked hypertension required only about 11 mg. of DCA when administered intermittently.

Table I presents a simplification of the data obtained in this prolonged experiment. It was abundantly evident as the experiment progressed that, as we had previously reported, some animals remained hypertensive. Since the object of the experiment was to determine (*a*) whether a real hypertension persists following cessation of DCA treatment and (*b*) whether this hypertension is renal in origin, the data were analyzed accordingly. Three periods were arbitrarily set up as check points, 14 weeks post-DCA, 24 weeks post-DCA, and 54 weeks post-DCA, *i.e.*, at the conclusion of the experiment. The blood pressure values obtained from each animal during the period preceding the check point were then averaged, as were those of the available controls. To avoid biasing the data, the first blood pressure value accepted for inclusion was that obtained 2 weeks after cessation of DCA treatment. It seemed that these average blood pressure values could be considered to indicate hypertension if they exceeded twice the standard deviation of the control average. Application of this rigid standard was in fact hardly necessary as can be seen by inspection of the sample graphs of blood pressure values over the weeks (Text-fig. 1).

For purposes of comparison, the treated animals during the post-treatment phase were readily regrouped into those which maintained a continual hypertension over the year on the one hand, and those which never showed a sustained elevation of blood pressure. In between these two contrasting groups were those animals which showed a hypertension at one or more of the check points but not throughout the experiment. 14 of the 47 treated animals succumbed before reaching the 14 week check point.

Of the treated animals available for comparison at the end of the experiment, 7 were never hypertensive, 4 had occasionally been hypertensive, and 4 were still definitely hypertensive. This conclusion, based on the blood pressure findings, was confirmed by the heart weights. To these figures should be added the 6 definitely hypertensive and 8 definitely normotensive animals which were either killed at 24 weeks for histological data or died during the course of the experiment.

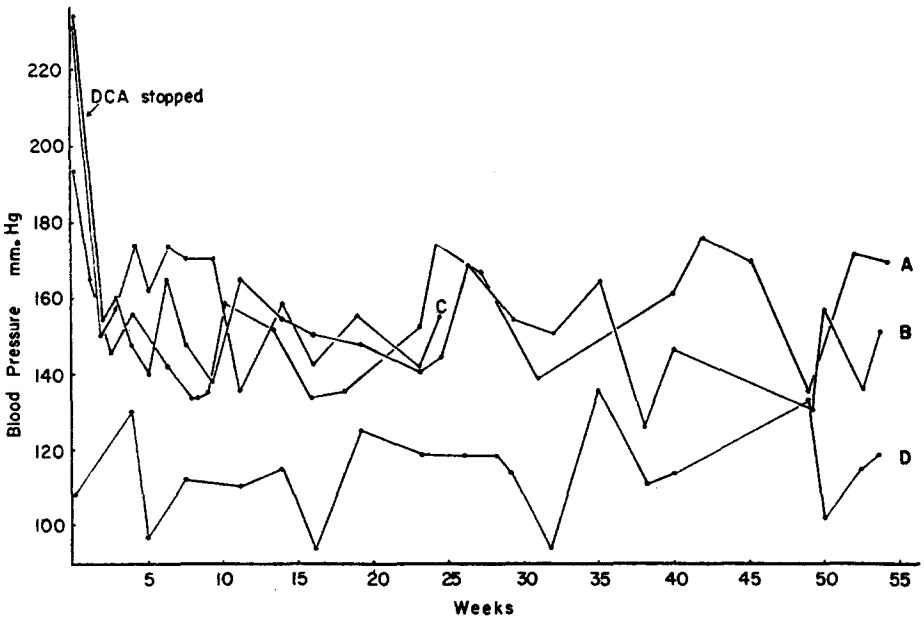
TABLE I

Group	Animal No.	End of DCA		+14 wks.	+24 wks.		+54 wks.	
		Blood pressure	Heart weight	Blood pressure	Blood pressure	Heart weight	Blood pressure	Heart weight
		mm. Hg	*gm.*	*mm. Hg*	*mm. Hg*	*gm.*	*mm. Hg*	*gm.*
Control	—	114 ±13 (21)	0.73 (2)	116 ±8 (15)	117 ±8 (12)	1.09 (3)	120 ±8 (7)	1.24 ±0.04 (6)
Treated	—	180 ±27 (47)	1.19 ±0.1 (4)	Considered individually below				
Post-DCA normotensive	779-3			126	132		128	1.22
	779-1			109	125		111	1.16
	780-2			125	131		106	1.13
	782-1			110	124		111	1.11
	792-1			122	110		124	1.37
	793-2			118	121		123	1.20
	795-2			132	119		104	
	785-3			119	111	0.86		
	785-4			129	121	0.76		
	794-2			132	116	1.09		
	787-3			118	132			
	784-2			132	123			
	790-1			115				
	792-3			109				
	794-1			128				
Post-DCA occasionally hypertensive	789-4			148	129		109	
	792-2			139	123		131	
	787-4			129	138		133	
	797-1			133	141		129	
	788-3			141	144			
	784-1			149	125			
	780-3			120	145			
	791-4			121	136			
Post-DCA continually hypertensive	791-2			152	147		147	1.48
	795-1			137	145		143	1.40
	795-4			135	139		135	1.41
	783-2			123	134		137	1.40
	782-3			160	150	1.29		
	783-3			149	217	1.71		
	789-3			152	148	1.36		
	786-2			141	148			
	778-3			159				
	782-2			141				

Subscripts indicate the number of animals available for the datum.

Text-fig. 1 illustrates the pattern of the blood pressure throughout the experiment as seen in several representative animals.

Urea.—As a first attempt to determine whether gross renal insufficiency was present in the hypertensive animals, blood urea nitrogen was determined at several points during the experiment and again at the end. As indicated in Table II there was only a slight increase in blood urea nitrogen in the post-DCA animals with no detectable difference between the hypertensive and non-hypertensive members of this group.



TEXT-FIG. 1. The pattern of the blood pressure in representative animals after cessation of DCA treatment. A, hypertensive rat 812-1 (Experiment 2). B, hypertensive rat 791-2 (Experiment 1). C, hypertensive rat 782-3 (Experiment 1). D, control rat 774-3 (Experiment 1).

Kidney Histology.—Histological examination of the kidneys at the end of active DCA treatment yielded surprising results in view of the reported findings of ourselves and others in rats treated with DCA. Selye (10), using large doses of the steroid, observed marked tubular damage in addition to glomerular sclerosis and vascular changes. Using minimal doses of DCA we (11) had observed little tubular damage and moderate glomerular sclerosis with arteriolar change. Here, using minimal doses intermittently administered, almost no tubular damage was seen and little glomerular sclerosis. The predominant change occurred in the small arteries and arterioles and consisted mainly of medial hypertrophy progressing to the point of near obliteration of the lumen.

The territories supplied by the most affected vessels seemed to be relatively avascular and in process of disintegration and replacement. The over-all picture, consequently, was one of focal infarction in numerous areas. The parenchyma between involved areas appeared nearly normal. No relation was observed, however, between the number and extent of these scar areas and the height of the blood pressure.

Histological examination of the kidneys in the post-treatment phase was in contrast to the earlier observations at the time of cessation of treatment. In general, the kidneys obtained both at 24 weeks and at the end of the experiment were close to normal (Figs. 1–7). All showed some small parenchymal scars which were obviously the healed remnants of the original more acute process; these were no more frequent in the hypertensive animals than in the

TABLE II

Group	Blood urea nitrogen	
	Random sampling during experiment	At termination
	mg. per cent	*mg. per cent*
Control	45.4 ± 3.5 (10)	39.5 ± 4.5 (7)
Post-DCA normotensive	55.4 ± 7.7 (7)	50.9 ± 6.9 (8)
Post-DCA hypertensive	50.9 ± 7.0 (10)	47.8 ± 9.2 (5)

Subscripts indicate the number of animals available for the datum.

non-hypertensive. All showed some increase in the prominence of arterioles and small arteries due mainly to medial hypertrophy; they also showed some glomerular sclerosis, but again this was no different in the two subgroups of post-DCA animals.

The histological picture of the adrenal and pituitary and of blood vessels in the pancreas, spleen, liver, and gut was entirely normal.

Experiment 2.—

Procedure.—Fifty-five male albino rats of an inbred Wistar strain, weighing approximately 70 gm., were divided into 2 groups. The first group, consisting of 15 animals, served as untreated controls, while the remaining 40 animals were uninephrectomized and then, beginning 6 days after operation, were subjected to the cumulative effects of DCA combined with 1 per cent saline as drinking water. Following the conclusion of treatment, the animals were observed for an additional 54 weeks.

The detail of treatment with DCA and saline was as follows: One-third of a 75 mg. cortate pellet was implanted subcutaneously on the 1st, 5th, 11th, 15th, 25th, and 29th days of

treatment. 1 per cent saline was substituted for the drinking water from the 4th to the 19th day. On the 40th day of exposure to DCA, treatment was stopped by the surgical recovery of all implanted pellets.

The present report is concerned with our observations preceding cessation of DCA treatment and over the 54 following weeks. In addition to frequent determinations of blood pressure, blood urea nitrogen and plasma volume (12) were studied. Plasma potassium according to the method of Polley (13) was also measured.

(Again, these animals were transported by air from Montreal to Vancouver during the latter part of the experiment.)

Animals surviving the various procedures were killed at the end of the experiment. Organs were fixed in Zenker's solution, weighed, and studied histologically as in the preceding experiment.

Observations.—

Blood Pressure.—At the conclusion of the treatment period, 15 controls and 31 treated animals remained for comparison. The treatment was very effective in elevating the blood pressure in most instances. Calculating the absorption on the basis of 100 μg. per pellet per day, each rat must have received approximately 15 mgm. of DCA. Despite the larger amount of the steroid used in this experiment, the blood pressure elevation was not so great as that in the previous experiment.

Table III presents a simplification of the basic data obtained in this experiment. It is clear that, again, following the cessation of DCA treatment, some animals remain hypertensive. The data were analyzed as before, the check periods in this case being 11, 17, and 25 weeks after treatment was stopped, and at termination, 54 weeks. Of the animals surviving at the end of the experiment, 4 remained consistently hypertensive throughout (confirmed by heart weight), 5 were occasionally hypertensive, and 6 remained normotensive. Of the original 31 animals at the start of the post-treatment period it is to be noted that 7 died shortly after cessation of treatment and hence are not of interest to the prolonged study. For the remaining 24, sufficient data are available to place 11 in the non-hypertensive group, 6 in the occasionally hypertensive group, and 7 in the hypertensive group, although some of these died before the end of the experiment. This distribution closely approximates the observations in the previous experiment.

Blood Urea Nitrogen, Plasma Volume, and Plasma Potassium.—Table IV presents the findings for blood urea nitrogen. While there was an elevation in average blood urea levels in animals still under treatment with DCA this reverted to normal in the post-treatment period. Again, the urea levels were not significantly greater in the hypertensive than in the non-hypertensive animals.

Similarly, no real difference was observed between the hypertensive and non-hypertensive animals in regard to their plasma volume, although we do not place too much value on this determination in the rat.

TABLE III

Group	Animal No.	Blood Pressure					Average heart weight at end
		End of DCA	+11 wks.	+17 wks.	+25 wks.	+54 wks.	
		mm. Hg	*mm. Hg*	*mm. Hg*	*mm. Hg*	*mm. Hg*	*gm.*
Control	—	104 ± 12 (15)	113 ± 9 (14)	117 ± 11 (14)	119 ± 10 (13)	122 ± 5 (9)	
Treated	—	164 ± 22 (31)	Considered individually below				
Post-DCA normotensive	812-2		126	110	122	116	1.11 ± 0.10
	813-3		120	120	131	124	
	814-3		105	100	114	118	
	817-1		117	115	107	106	
	816-2		124	124	132	117	
	815-4		115	110	135	129	
	814-1		109	110	116		
	816-4		130				
	815-3		107				
	815-2		116				
	813-2		126				
Post-DCA occasionally hypertensive	809-2		147	129	128	150	
	809-4		137	144	127	115	
	813-1		117	115	136	127	
	812-4		116	126	127	137	
	813-4		124	117	116	134	
	817-3		135	123	128		
Post-DCA continually hypertensive	812-1		148	143	159	158	1.37 ± 0.19
	812-3		147	138	137	139	
	817-4		132	138	144	144	
	814-2		126 (?)	138	142	140	
	809-3		143	128	149		
	808-4		165				
	814-4		138				

Subscripts indicate the number of animals available for the datum.

TABLE IV

Group	Blood urea nitrogen			Plasma volume		Plasma K		
	End of DCA	+11 wks.	+17 wks.	End of DCA	+11 wks.	End of DCA	+11 wks.	+25 wks.
	mg. per cent	*mg. per cent*	*mg. per cent*	*cc.*	*cc.*	*m. eq.*	*m. eq.*	*m. eq.*
Control	44.3 ± 6.0 (14)	51.0 ± 7.0 (7)	46.6 ± 3.4 (7)	5.1 ± 0.6 (8)	5.1 ± 0.6 (6)	4.5 ± 0.3 (8)	5.1 ± 0.6 (8)	4.5 ± 0.5 (7)
Post-DCA normotensive		45.5 ± 2.1 (6)	46.9 ± 2.5 (5)		5.6 ± 0.4 (3)		5.4 ± 0.4 (4)	4.6 ± 0.7 (3)
	53.9 ± 10.9 (12)			4.9 ± 0.7 (12)		3.7 ± 0.6 (13)		
Post-DCA hypertensive		49.9 ± 3.5 (7)	52.7 ± 5.9 (5)		5.1 ± 0.3 (5)		4.8 ± 0.5 (6)	4.9 ± 0.4 (4)

Subscripts indicate the number of animals available for the datum.

Plasma potassium, significantly depressed during acute treatment with DCA, became normal in both groups after cessation of treatment.

Kidney Histology.—Some pathological changes were observed in the kidneys of the treated animals. These consisted of scattered parenchymal scars indicative of old DCA activity, some focal tubular damage with dilatation and casts, and, finally, some glomerular sclerosis. Careful comparison was made between the kidneys of those animals which had remained consistently hypertensive throughout and those which had been equally consistently normotensive, but no difference in either the severity or diffuseness of the lesions could be found. In short, no anatomical basis for the hypertension was revealed by our examination of the kidney.

DISCUSSION

The two experiments here reported confirm our previous observation that DCA treatment can result in a permanent self-sustained hypertension in the rat (4). Such a hypertension apparently may result from different doses of DCA given for different lengths of time, provided that a definite and marked pressor response to the steroid is induced in the first instance. It is of more than passing interest that intermittent treatment with the steroid seems highly effective, a point which should receive further study.

As far as we are able to determine, the anatomical change present in the kidney does not account for the sustained elevation in blood pressure. Consequently, this form of hypertensive disease in the rat bears considerable resemblance to the benign phase of essential hypertension in man. Certainly, in both instances, while a renal relation is apparent there is no real basis for considering the disease primarily renal in origin. The lack of renal change in the present experiments is in exact accord with our previous experiment in which measurement of vessels failed to reveal any differences in the hypertensive animals (4).

To reconcile these and other findings, we have already suggested an hypothesis that the adrenal cortex normally secretes a pressor agent which is eliminated (converted or excreted) by the kidney. Hypertension could result from any condition tending to increase the concentration of this pressor agent, either by increasing its secretion or impairing its renal handling. Not all adrenal cortical tumors would thus be expected to result in hypertension, rather only those involving the secretion of the specific pressor agent. Not all renal diseases, on the other hand, would result in hypertension, rather only those which involved an interference with the specific renal mechanisms for handling the pressor agent.

By this view, if the synthetic steroid DCA be considered only as representative of the natural pressor agent, then its administration would result in hypertension only when the amounts supplied are sufficient to overcome the natural

ability of the kidney to handle it. This would explain the fact that this steroid is most effective in animals sensitized by reduction in renal parenchyma, or by the administration of saline or nephritic serum. The agent would be still more effective in the completely nephrectomized animal, a fact which has been demonstrated by ourselves (14) and more fully by Hall and Hall (15).

The present hypothesis does not require that extreme amounts of some DCA-like material be present, since even a small excess could suffice to "set" the homeostatic mechanism at a higher level. We would call attention to the recent report by Forbes *et al.* (16) which indicates the marked capacity of the kidney for the conversion of progesterone; to the well known fact that DCA can give rise to urinary pregnanediol (17); and to the observations supporting the idea that DCA may be converted to progesterone (18). In view of the similarity of these steroids, the role of metabolic conversion which we would assign to the kidney seems not unreasonable.

The present finding of sustained hypertension following the administration of DCA is tentatively to be explained as the result of a permanent impairment of a specific renal process normally concerned in the elimination of a structurally similar and naturally occurring pressor agent. Further experiments are in progress to test this hypothesis.

SUMMARY

DCA was administered in two separate experiments to rats in amounts sufficient to cause marked elevation of the blood pressure. It was then abruptly withdrawn. Following this treatment, observations of blood pressure, blood urea nitrogen, plasma potassium, and plasma volume were carried out for over a year.

Approximately one-third of the animals so treated developed a sustained, permanent hypertension after treatment had been stopped.

This sustained hypertension is not due to any anatomical fault in the kidney and in that sense is not primarily renal in origin. An hypothesis to explain the findings is presented.

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BIBLIOGRAPHY

1. Selye, H., and Hall, C. E., *Arch. Path.*, 1943, **36,** 19.
2. Perera, G. A., Knowlton, A. I., Lowell, A., and Loeb, R. F., *J. Am. Med. Assn.*, 1944, **125,** 1030.
3. Davies, D. F., and Clark, H. E., *Circulation*, 1950, **2,** 494.
4. Friedman, S. M., and Friedman, C. L., *Canad. Med. Assn. J.*, 1949, **61,** 596.
5. Prado, J. L., Thesis, Escola Paulista de Medicina, Sao Paulo, Brazil, 1950.
6. Byrom, F. B., and Wilson C. J., *J. Physiol.*, 1938, **93,** 301.

7. Friedman, S. M., and Friedman, C. L., *Rev. Canad. Biol.*, 1949, **8,** 482.
8. Barker, S. B., *J. Biol. Chem.*, 1944, **152,** 453.
9. Soffer, L. J., Diseases of the Adrenals, Philadelphia, Lea and Febiger, 1948.
10. Selye, H., Hall, C. E., and Rowley, E. M., *Canad. Med. Assn. J.*, 1943, **49,** 88.
11. Friedman, S. M., Polley, J. R., and Friedman, C. L., *J. Exp. Med.*, 1948, **87,** 329.
12. Braun-Menendez, E., and Covian, M. R., *Rev. Soc. Argent. Biol.*, 1948, **24,** 44.
13. Polley, J. R., *Canad. J. Research*, 1948, **E26,** 188.
14. Friedman, S. M., and Friedman, C. L., *J. Exp. Med.*, 1949, **89,** 631.
15. Hall, C. E., and Hall, O., *Proc. Soc. Exp. Biol. and Med.*, 1949, **71,** 690.
16. Forbes, T. R., Hooker, C. W., and Pfeiffer, C. A., *Endocrinology*, 1950, **47,** 83.
17. Kochakian, C. D., A Symposium on Steroid Hormones, Madison, University of Wisconsin Press, 1950, 91.
18. Zarrow, M. X., Hisaw, F. L., and Bryans, F., *Endocrinology*, 1950, **46,** 403.

EXPLANATION OF PLATE 28

Low power photomicrographs of kidney sections taken from rats at the termination of Experiment 1 (54 weeks after cessation of DCA treatment). ×100.

FIG. 1. Control. Periodic acid stain.

FIG. 2. Relatively normal glomeruli and tubules in a uninephrectomized rat with permanent hypertension. Periodic acid stain.

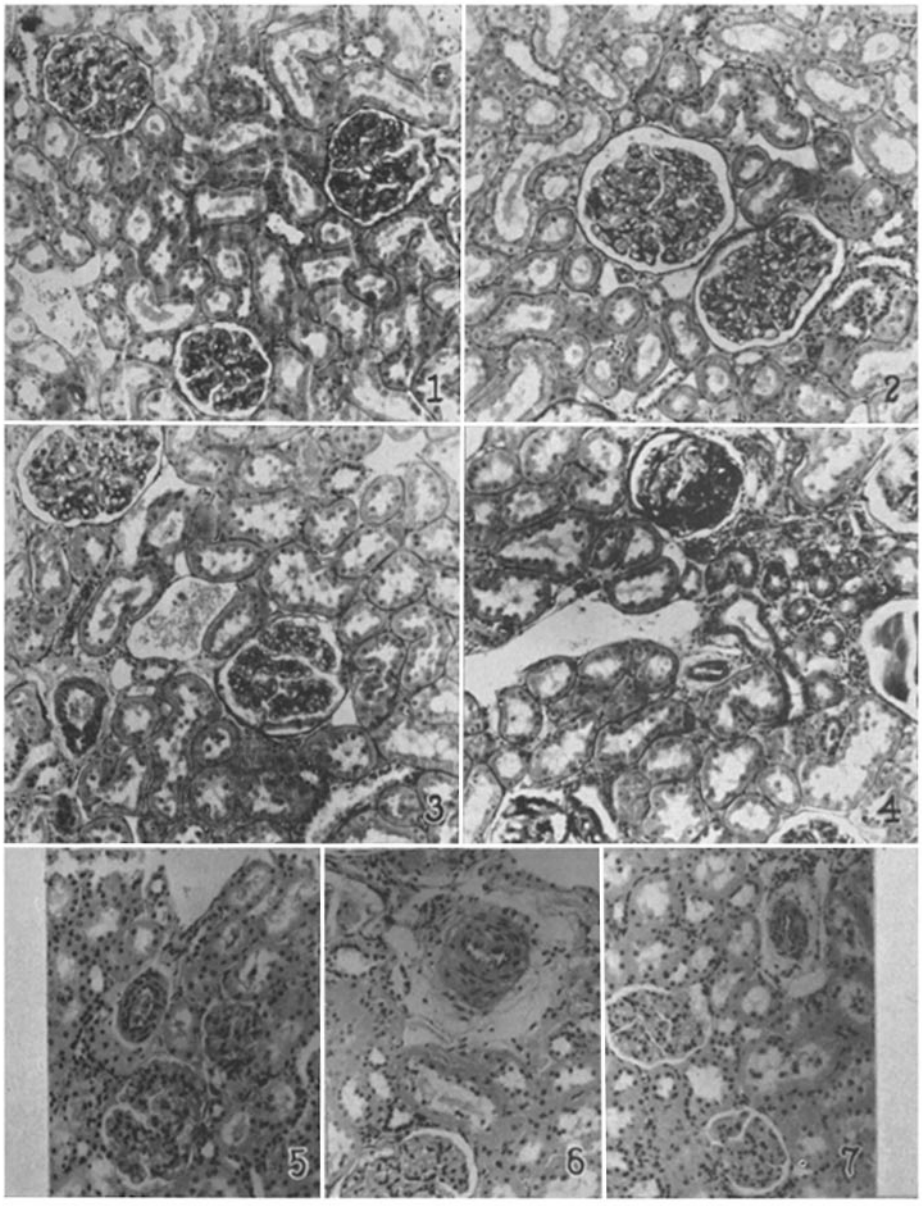
FIG. 3. Relatively normal glomeruli and tubules in a uninephrectomized rat with normal blood pressure. Periodic acid stain.

FIG. 4. Residual glomerular sclerosis in a uninephrectomized rat with normal blood pressure. Periodic acid stain.

FIG. 5. Small artery in a control rat. Hematoxylin and eosin.

FIG. 6. Small artery showing marked medial hypertrophy and narrowed lumen in a rat with permanent hypertension. Hematoxylin and eosin.

FIG. 7. Small artery showing marked medial hypertrophy and narrowed lumen in a treated rat with normal blood pressure. Hematoxylin and eosin.



(Friedman *et al.*: Desoxycorticosterone acetate and hypertension)