ARTERIOLAR NECROSIS IN ADRENAL-REGENERATION HYPERTENSION: INFLUENCE OF PREVENTIVE TREATMENT WITH HYDRALAZINE ON TISSUE ELECTROLYTES

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AFTER enucleation of an adrenal, hypertension develops quickly in rats given salt to drink (Skelton, 1955). Such animals sustain widespread visceral arteriolar muscle necrosis. Evidence derived from experiments with an antihypertensive agent has shown that muscle cell death may be prevented by intermittent reduction of the blood pressure (Gardner and Brooks, 1962). These observations are in accord with studies made in steroid hypertension with the drugs hydralazine (Gardner, 1960) and bretylium tosylate (Gardner, 1962).

Although raised blood pressure and vasospasm play essential parts in the genesis of hypertensive arteriolar necrosis (Byrom, 1954), it appears possible that an associated initial alteration in arteriolar metabolism may increase the susceptibility of muscle cells to the injurious effects of sustained high blood pressure (Gardner, 1960). Disturbance in tissue electrolyte concentrations may accompany the hypertensive state in the rat (Tobian and Redleaf, 1958) and these changes may be related to the destructive effect of raised pressure. During a recent experiment with adrenal-regeneration hypertension (Gardner and Brooks, 1962) the opportunity was therefore taken to examine the concentrations of electrolytes in selected relevant tissues. These figures were compared with those obtained from tissues of rats in which arteriolar necrosis had been prevented by appropriate treatment. The results of this comparison are described in the present paper which represents therefore a continuation of the previous report (Gardner and Brooks, 1962).

MATERIAL AND METHODS

Fifty-five male albino Wistar rats, mean weight 145 g., were maintained on a diet of known adequacy. Adrenal-regeneration hypertension was induced in 43 animals. Twenty of these animals were treated with hydralazine once daily, as described previously (Gardner and Brooks, 1962); 11 survived 56 days of treatment. Twenty-three animals received no hydralazine; 12 survived 56 days. Twelve rats were maintained as untreated controls. The body weights, salt intakes and blood pressures were recorded. At the conclusion of the experiment, samples of kidney, aorta, remaining adrenal, heart, skeletal muscle and mesentery were taken and analysed for sodium, potassium and chloride, using methods to be described elsewhere (Gardner, unpublished observations). Serum sodium, potassium and chloride levels were measured on samples of blood collected at the time of death and have been reported previously (Gardner and Brooks, 1962). The results of the analysis on tissues and sera of the 12 surviving untreated rats and 11 surviving treated rats were compared with those obtained from 12 normal animals.

From these figures were calculated the concentration of sodium, potassium and chloride

per 100 g. of fat-free, dry tissue. Chloride results were also calculated in terms of wet tissue weight. Chloride space was obtained from the ratio

$$\frac{\text{tissue chloride in m.Eq./kg. wet weight}}{\text{serum chloride in m.Eq./l.}}$$

and corrected by applying a Donnan constant of 0·96.

Inulin space was determined, using the method of Flear, Crampton and Matthews (1960). Two hindleg muscles (soleus and flexor digitorum longus) were removed from each animal immediately after evisceration. Similar muscles were removed from 12 additional control rats. All muscles were dissected out intact, a short length of tendon being left at each end. Alkali-stable inulin was prepared by heating with NaOH and repeated precipitation with alcohol (Weil, 1952). Muscles were left overnight in an inulin/saline mixture at 2–3 . Inulin concentrations were estimated by a colorimetric method using indolyl acetic acid (Heyrovsky, 1956).

RESULTS

Sodium (Table I)

In adrenal, skeletal muscle and mesentery from rats with adrenal-regeneration hypertension the concentration of sodium in fat-free, dry samples was found to be raised above normal but the differences did not reach conventional levels of significance. After 56 days treatment with hydralazine the sodium concentration in aortic samples was found to be significantly reduced ($P < 0{\cdot}02$). In the other tissues, with the exception of mesentery, there was a reduction in sodium concentration which did not reach acceptable levels of significance.

Potassium (Table II)

The concentrations of potassium in samples of regenerated adrenal ($P < 0{\cdot}01$), of heart ($P < 0{\cdot}01$) and of mesentery ($0{\cdot}02 < P < 0{\cdot}01$) were found to be significantly reduced in animals with adrenal-regeneration hypertension by comparison with normal. After 56 days treatment with hydralazine the mean potassium concentration in the samples of aorta, slightly lower than normal in hypertensive animals, was found to be further reduced. However, the extent of the reduction was of uncertain significance ($0{\cdot}1 < P < 0{\cdot}05$). The potassium content of regenerated adrenal and of mesentery also fell but in kidney, heart and skeletal muscle samples there was no evidence of change.

Chloride (Table III)

The concentrations of chloride in fat-free, dry samples of tissue from aorta ($P < 0{\cdot}01$), heart ($P < 0{\cdot}01$), skeletal muscle ($P < 0{\cdot}01$), mesentery ($0{\cdot}05 < P < 0{\cdot}02$) and adrenal of rats with adrenal-regeneration hypertension were lower than normal. Treatment with hydralazine for 56 days resulted in an increase in the concentration of chloride in skeletal muscle ($P < 0{\cdot}01$), adrenal and heart muscle. The concentration of chloride in kidney, aorta and mesentery was slightly lowered.

Chloride space

The calculated chloride space of all 6 tissues of animals with adrenal-regeneration hypertension was lower than that of normal controls, although in the case of the regenerated adrenal and the mesentery, the differences failed to reach

TABLE I.—*Tissue Sodium Concentrations expressed as m. Eq./100 g. Fat-free, Dry Tissue (±Standard Deviation)*

Group	Kidney	Aorta	Adrenal	Heart	Muscle	Mesentery
a. Normal (12)	38·7 ±10·9	39·0 ±8·7	25·1 ±7·1	18·1 ±8·9	9·5 ±3·5	25·1 ±26·3
b. Untreated (12) adrenal-regeneration	36·7 ±7·9	34·7 ±14·3	30·8 ±22·8	15·6 ±1·7	10·4 ±2·5	29·2 ±22·7
c. Treated (11) adrenal-regeneration	36·5 ±8·4	24·8 ±4·9	20·2 ±7·2	14·9 ±1·6	9·6 ±2·0	29·6 ±17·7
Significance of difference between *a* and *b*	..	..	..	..	..	..
Significance of difference between *b* and *c*	..	$P < 0{\cdot}02$	..	..	..	..

TABLE II.—*Tissue Potassium Concentrations expressed as m. Eq./100 g. Fat-free Dry Tissue (±Standard Deviation)*

Group	Kidney	Aorta	Adrenal	Heart	Muscle	Mesentery
a. Normal (12)	30·8 ±2·2	15·3 ±2·1	42·6 ±6·3	34·1 ±1·9	40·0 ±3·6	50·6 ±38·7
b. Untreated (12) adrenal-regeneration	30·1 ±4·1	15·3 ±4·5	33·9 ±5·4	31·7 ±1·2	40·1 ±2·4	18·3 ±9·8
c. Treated (11) adrenal-regeneration	31·3 ±3·9	12·2 ±2·1	29·1 ±7·7	32·3 ±2·6	41·6 ±1·9	16·3 ±6·2
Significance of difference between *a* and *b*	..	..	$P < 0{\cdot}01$	$P < 0{\cdot}01$	..	$0{\cdot}02 < P < 0{\cdot}01$
Significance of difference between *b* and *c*	..	$0{\cdot}1 < P < 0{\cdot}05$	..	..	..	..

TABLE III.—*Tissue Chloride Concentrations expressed as m. Eq./100 g. Fat-freeDry Tissue (±Standard Deviation)*

Group	Kidney	Aorta	Adrenal	Heart	Muscle	Mesentery
a. Normal (12) . . .	46·7 ±6·92	137·8 ±12·0	173·3 ±12·3	41·2 ±7·80	26·8 ±6·12	80·9 ±69·4
b. Untreated (12) adrenal-regeneration	47·0 ±12·1	72·4 ±31·6	138·4 ±86·6	25·6 ±7·03	16·6 ±3·73	62·8 ±53·5
c. Treated (11) adrenal regeneration	41·4 ±12·91	64·3 ±26·20	154·3 ±78·50	29·1 ±9·41	23·1 ±5·56	47·1 ±23·30
Significance of difference between *a* and *b*	..	$P < 0·01$	..	$P < 0·01$	$P < 0·01$	$0·05 < P < 0·02$
Significance of difference between *b* and *c*	..	..	..	..	$P < 0·01$	..

TABLE IV.—*Chloride Space* $\left(\frac{0·96 \times \textit{Tissue chloride}}{\textit{Serum chloride}}\right)$ *as Per Cent ± Standard Deviation*

Group	Kidney	Aorta	Adrenal	Heart	Muscle	Mesentery
a. Normal (12) . . .	10·9 ±1·92	44·6 ±14·4	38·9 ±10·6	19·5 ±2·60	6·7 ±1·53	11·1 ±4·97
b. Untreated (12) adrenal-regeneration hypertension	8·2 ±0·71	17·9 ±4·44	30·8 ±11·27	5·10 ±1·26	3·48 ±0·79	7·65 ±3·30
c. Treated (11) adrenal-regeneration hypertension	7·70 ±1·29	22·50 ±11·2	33·60 ±9·21	5·45 ±2·30	4·54 ±1·20	5·08 ±1·57
Significance of difference between *a* and *b*	$P < 0·01$	$P < 0·01$	$0·10 < P < 0·05$	$P < 0·01$	$P < 0·01$	$0·10 < P < 0·05$
Significance of difference between *b* and *c*	..	..	..	..	..	..

acceptable levels of significance. By contrast, treatment of a further group of animals with hydralazine for 56 days did not significantly change the calculated chloride space in any of the 6 tissues examined.

Inulin space

Inulin space of animals with established adrenal-regeneration hypertension (338·4 ml./kg.) was higher than in normal controls (287·5 ml./kg.) but the difference did not reach levels of significance. The corresponding measurements made in animals treated with hydralazine for 56 days (409·1 ml./kg.) revealed a rise in inulin space, the figures being significantly higher than those for untreated hypertensive animals and significantly higher than normal. In each group the variability of results was considerable.

TABLE V.—*Inulin Space (ml./kg.) ±Standard Deviation*

a. Normal (12 rats, 24 muscles)	*b.* Untreated adrenal-regeneration hypertension (12 rats, 24 muscles)	*c.* Treated adrenal-regeneration hypertension (11 rats, 22 muscles)
287·5 ±120·0	338·4 ±85·8	409·1 ±114·0

Significance of difference between	
a and *b*	−
b and *c*	+
a and *c*	+

DISCUSSION

Whether in established rat hypertension the cells of the aorta (Tobian, 1960) and arterioles (Tobian, Janecek, Tomboulian and Ferreira, 1961) come to contain increased amounts of sodium and potassium is still debated. The present results do not support this suggestion directly but indicate that antihypertensive treatment may effectively lower the overall aortic concentrations of these two ions. The influence of treatment on ion concentrations in other tissues is less consistent, but is in the same direction.

Observations made with antihypertensive drugs have shown that the necrosis of blood vessels which develops early in severe rat hypertension is related to the raised blood pressure (Gardner, 1960) and have suggested that a local change in muscle cell metabolism may be concerned with subsequent necrosis. The demonstration that the prevention of vascular necrosis is accompanied by a decrease in aortic sodium and potassium but not by a significant change in chloride concentration may be presumptive evidence that the changes in aortic electrolytes are predominantly intracellular. If this is accepted, then it appears reasonable to suggest that an alteration in intracellular electrolyte concentrations may be the factor which, with sustained raised blood pressure, leads to arteriolar muscle necrosis.

The changes in calculated chloride space which accompany the evolution of adrenal-regeneration hypertension are more difficult to explain. Figures for

plasma, and, by inference, extracellular fluid chloride concentrations are not altered by antihypertensive treatment (Gardner and Brooks, 1962). The decreased tissue chloride concentration of rats with adrenal-regeneration hypertension is unlikely to be accounted for by a reduction in the customarily small intracellular chloride content and may therefore be a consequence of cellular overhydration.

This view may be compatible with the demonstrated change in inulin space, the increased calculated volume of which is further increased by antihypertensive treatment. The inulin measurements support the suggestion of a raised extracellular fluid volume, the high sodium concentration of which is not reduced by treatment (Gardner and Brooks, 1962). It should be noted that the figures for inulin in normal skeletal muscle are considerably higher than those obtained by Flear, Grampton and Matthews (1960) and by Creese, D'Silva and Hashish (1955) but the results are comparable with those obtained for rat diaphragm by Creese (1954). No similar figures are available for skeletal muscle in adrenal-regeneration hypertension but the difference in the present results from those obtained by Ledingham (1953, 1957) by a different method, for skeletal muscle in renal hypertension, is very considerable. The explanation for this remains uncertain. The differences between chloride space and inulin space are sufficiently clear to suggest that chloride measurements alone, as a guide to extracellular fluid space in adrenal-regeneration hypertension, should be used with caution. Whichever view of extracellular compartmental fluid volume is accepted, it is likely that the change in tissue electrolyte concentrations is a further manifestation of a disturbance in the cell mechanism which regulates ionic exchange, whether this be operative at the cell wall (Conway, 1955), within mitochondria, or throughout the cytoplasm. The disturbance may determine arteriolar contractility and thus hypertension. It is possible that studies on arterial muscle cell metabolism, and in particular, on oxidation reduction enzyme behaviour, may help to explain the mechanism of these changes more fully.

Such studies are more likely to elucidate the mechanism of vascular necrosis if they are undertaken before the time of arterial muscle cell death rather than late in the evolution of experimental hypertension when secondary vascular and renal effects may conceal the underlying disturbance. Much of the evidence relating to vascular electrolytes in rat hypertension has been obtained at a late stage in the disease process. For example, it has been suggested that, in animals with a raised vascular content of sodium, the increased water content of the affected vessel walls causes a mechanical obstruction to blood flow (Redleaf and Tobian, 1958). This view has not been confirmed by direct observation and appears less probable as a cause of obstruction than vasoconstriction related to ionic imbalance. Evidence is also available to show that, simultaneous with these electrolyte shifts, there is a rise in the vascular content of sulphated acid mucopolysaccharide (Crane, 1962). The relationship between mucopolysaccharide accumulation and electrolyte regulation is not yet determined but the presence of mucopolysaccharide excess offers a more acceptable mechanism for small vascular obstruction to blood flow in established hypertension than does the demonstrated increase in water content (Redleaf and Tobian, 1958). Nevertheless, the experimental evidence on which both descriptions are based has been obtained with established and not with evolving hypertension. These changes may therefore be the result rather than a cause of the hypertensive process.

SUMMARY

Adrenal-regeneration hypertension was induced in male rats. One half of the animals was treated with the antihypertensive agent hydralazine. Treatment prevented the development of arteriolar necrosis and of necrotising arteritis.

The concentrations of aortic sodium and probably the aortic potassium in dry, fat-free samples of tissue, were significantly reduced in the animals in which treatment effectively prevented vascular necrosis. Aortic chloride concentrations remained unchanged in the treated animals and chloride space was not altered. Skeletal muscle inulin space, however, was increased.

The results are believed to support the suggestion that muscle necrosis in rat hypertension is one result of a sequence of events in which the development of sustained raised blood pressure and the altered metabolism of arteriolar muscle together result in cell death.

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