HYPERTENSION OF RENAL ORIGIN IN RATS FOLLOWING LESS THAN ONE WEEK OF CHOLINE DEFICIENCY IN EARLY LIFE*

BY

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[WITH PHOTOGRAPHIC PLATE]

The production of arterial hypertension by dietary deficiency alone has not previously been demonstrated. Since the report by Griffith and Wade (1939) it has been well established that choline deficiency produces severe renal lesions in young rats (György and Goldblatt, 1940; Engel and Salmon, 1941; Handler, 1946; and others). A careful study of the blood pressures of rats maintained for long periods on diets low in choline has been made by Sobin and Landis (1947), who found no evidence of hypertension. The results of earlier studies by Honorato and Vadillo (1944) apparently support this same conclusion. Stimulated in part by these papers, by discussions with Professor Eugene Landis, and by the early studies of choline deficiency in our laboratories (Best and Huntsman, 1932), we decided to produce a severe renal lesion by this dietary means and then to study the blood pressures of the surviving animals maintained on full diets. Under these conditions, in our present series of observations all animals with severe residual renal damage developed hypertension. The average figure for ten animals in this group was 195 mm. Hg. This is the mean pressure recorded by arterial cannulation. The comparable figure for 36 control animals was 118 mm. Hg.

Methods

Weanling albino rats of the Wistar strain weighing 35-42 g. were fed a diet low in choline (T45) as described by our colleagues Lucas and Ridout (1948). The object was to produce the greatest degree of renal damage compatible with life. The animals were fed the choline-deficient diets for varying lengths of time. Most of the rats received the ration for five to six days. In the more recent experiments the animals were fed the choline-deficient diet for four five-day periods separated by two-day intervals during which the diet was supplemented with 0.35% choline chloride. All the animals received a stock diet for the remainder of the experiment. In the early studies the rats were sacrificed at the age of 3 to 4 months, but it was subsequently discovered that a higher percentage developed hypertension if allowed to live for six or seven months. Control animals were pair-fed with those receiving the choline-deficient diet. These controls received the T45 diet supplemented with 0.35% choline chloride. They were given the stock diet *ad libitum* at the same time as the

experimental groups, and were maintained on this until sacrificed.

Indirect blood-pressure determinations according to the method of Sobin (1946) were made at intervals on all rats in order to follow the progress of the experiment. At the end of the period of observation blood-pressure determinations were made by the direct procedure. The animals were anaesthetized with 90 mg. of sodium amytal per kilogram of body weight, administered intraperitoneally as a freshly prepared 10% solution in distilled water. The femoral artery was exposed and cannulated with a 24-gauge needle. Drops of 1% aqueous solution of procaine hydrochloride were placed on the artery during dissection to prevent spasm. The needle was attached to a fine-bore mercury manometer, and the system filled with Ringer's solution. Owing to the inertia of the system the readings obtained represented the mean of systolic and diastolic levels of blood pressure, and all direct blood-pressure measurements reported in this paper refer to this mean value rather than to systolic levels. The animals were killed by exsanguination. This procedure effectively emptied the hearts, which were weighed to the nearest 1/100 g., and these figures are expressed in the tables as percentages of the total body weights. In all but the earliest experiments, in order to obtain the greatest possible objectivity, the observations concerning blood pressure and heart weights were made without the observer (W.S.H.) knowing whether the animal belonged to the control or to the experimental group.

Paraffin sections of the viscera were made after fixation in either 10% neutral formol-saline or in Bouin's solution. Sections were treated with haematoxylin and eosin, Mallory's phosphotungstic acid haematoxylin (P.T.A.), Gomori's chromium haematoxylin and phloxine, and Masson's connective-tissue stain. In addition selected sections were stained for elastic tissue, for calcium, and for haemosiderin. Frozen sections of kidneys and large vessels were stained for fat and tested for cholesterol (Schultz reaction). Several sections from both kidneys of each animal were surveyed and the amount of renal damage classified as severe, moderate, or slight.

Observations were made on 62 rats surviving varying degrees of the renal haemorrhagic syndrome and on 36 control animals of similar age and weight.

Results

The findings in the experimental animals (Tables I-III) were divided into three groups according to the degree of

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residual renal damage noted microscopically. Group 1 (Table I) is made up of the 10 survivors of the haemorrhagic renal syndrome which had severe persistent kidney damage. All these animals had definite arterial hypertension. The average blood pressure was 195 mm. Hg and the average weight of their hearts was nearly double the normal value. Group 2 (Table II) comprises the 13 survivors of the acute

TABLE I.—Heart Weights and Blood Pressure (Determined by Direct Measurement) in 10 Rats Surviving Haemorrhagic Kidney with Severe Degrees of Residual Renal Damage

Animal No.	Heart Weight Percentage of Body Weight	Blood Pressure (Systolic-Diastolic Mean) (mm. Hg)
HJCa-737	0.64	230
HJO-448	0.57	210
HRCc-704	0.51	200
HJCb-747	0.50	200
HRCc-712	0.43	200
HRCc-530	0.88	200
HJCb-570	0.40	196
HJCb-732	0.66	180
HRCB-535	0.43	179
HRCO-518	0.48	150
Average	0.55	195

TABLE II.—Heart Weights and Blood Pressures in 13 Rats Surviving Haemorrhagic Kidney with Moderate Degrees of Residual Renal Damage

Animal No.	Heart Weight Percentage of Body Weight	Blood Pressure (Systolic-Diastolic Mean) (mm. Hg)
HJCa-746	0.40	200
HFB-105	0.40	195
HRCB-560	0.37	170
HJCa-744	0.40	170
HJCa-739	0.34	170
HRCb-552	0.34	165
HRCc-710	0.32	170
HRCc-711	0.35	160
HJCa-742	0.41	160
HJCa-743	0.37	160
AK-304	0.44	150
HJCa-759	0.35	140
HRC(c)-709	0.31	130
Average	0.37	165

TABLE III.—Heart Weights and Blood Pressures in 39 Rats Surviving Haemorrhagic Kidney with Slight Degrees of Residual Renal Damage

Animal No.	Heart Weight Percentage of Body Weight	Blood Pressure (Systolic-Diastolic Mean) (mm. Hg.)
HRCO-524	0.35	150
HRCO-522	0.34	130
HJO-416	0.33	153
HJB-467	0.32	158
HRCO-514	0.32	140
HRCO-519	0.31	150
HJO-405	0.31	120
AK-308	0.37	140
AK-303	0.41	150
HJO-424	0.39	140
HRCB-562	0.38	130
HRCO-503	0.38	130
HRCO-508	0.37	120
HFB-109-1	0.37	140
HRCO-512	0.36	140
AK-325	0.36	125
HRCO-521	0.35	135
HRCB-564	0.34	130
HJB-475	0.32	158
HJO-442	0.30	145
HJB-479	0.28	142
HJB-480	0.34	110
HJB-484	0.30	130
HJB-486	0.30	130
HRCO-507	0.29	125
HRCO-510	0.33	130
HRCO-513	0.30	120
HRCC-703	0.30	130
HRCC-713	0.31	160
HRCC-716	0.32	130
HRCC-717	0.34	160
HJCa-740	0.33	90
HJCb-749	0.31	160
HJCb-750	0.31	130
HJCb-751	0.35	185
HJCb-753	0.32	130
HJCb-756	0.31	140
HJCb-757	0.31	130
HJCb-758	0.30	100
Average	0.33	136

TABLE IV.—Heart Weights and Blood Pressures in 36 Normal Control Rats Fed Diets Containing Choline

Animal No.	Heart Weight Percentage of Body Weight	Blood Pressure (Systolic-Diastolic Mean) (mm. Hg)
A	0.33	110
B	0.30	100
C	0.32	115
D	0.32	130
E	0.30	130
K-1	0.34	100
L-1	0.30	105
M-1	0.33	130
N-1	0.30	130
O-1	0.32	110
P-1	0.35	130
A-2	0.30	130
B-2	0.28	140
C-2	0.30	135
I	0.26	120
J	0.30	130
HRCC-726	0.34	80
HRCC-727	0.30	82
HRCC-728	0.29	120
HRCC-729	0.32	170
HRCC-730	0.27	120
HRCC-731	0.28	120
HRCC-732	0.25	100
HRCC-733	0.27	130
HRCC-734	0.27	100
HRCC-735	0.26	130
HJCa-761	0.28	120
HJCa-762	0.31	110
HJCa-763	0.30	120
HJCa-764	0.31	135
HJCb-766	0.30	120
HJCb-767	0.29	125
HJCb-768	0.27	130
HJCb-769	0.26	90
HJCb-770	0.32	85
HJCa-765	0.30	130
Average	0.28	118

period of choline deficiency with moderate residual renal damage. Of these, 85% (11 of 13) had blood pressures of 150 mm. Hg or over. The average blood pressure for this group was 165 mm. Hg, and the average heart weight was one-third greater than that of the normal controls (Table IV). In the remaining 39 survivors (Group 3) only slight renal damage was found. There is little evidence that a significant degree of hypertension developed in the members of this group (Table III). Of 36 control animals of similar age and weight, only one had a blood pressure greater than 140 mm. Hg, and the average for the group was 118 mm. Hg (Table IV).

The kidney lesions took the form of tubular destruction combined with marked distension of other nephrons, many of which contained large amounts of eosinophilic colloid, particularly in the medulla. There was obvious reduction in the number of glomeruli in the severely damaged kidneys (Figs. 9 and 10), and the remaining glomeruli showed severe

FIG. 9.—Thick (100 μ) section of kidney of an animal with severe residual renal damage following a short period of choline deficiency when a weanling. The vessels have been injected with indian ink and tissue cleared in benzyl benzoate. Note the scarcity of glomeruli. ($\times 50$.)

degrees of thickening of the basement membranes throughout the capillary loops, while others were converted into little more than spheres of hyaline material. Fig. 1 (Photogravure Plate) illustrates the type and degree of kidney damage classified as severe. Figs. 3 and 5 present examples of kidney lesions classified as moderate and slight respectively.

The vascular lesions varied from early cellular proliferation of the media and adventitia of the arterioles (Fig. 6) in animals with only slight kidney damage and slight evidence of hypertension, to intimal hyalinization (Fig. 4) and fibrinoid necrosis (Fig. 2) in those with severe degrees of kidney damage and elevated blood pressure. Determinations of the non-protein nitrogen levels in the blood of two such animals showed values over 350 mg. per 100 ml. Photomicrographs of the kidney and a representative arteriole from a normal control animal are given for comparison (Figs. 7 and 8). No gross or microscopical evidence of atheromatous deposits was found in the larger arteries, nor could elastosis (Boyd, 1947) be demonstrated in these. Small deposits of cholesterol (Schultz-positive) in some of the hyalinized glomeruli were demonstrated in frozen sections of the most severely damaged kidneys. No evidence of inflammatory disease was found in any of the sections of hearts of the hypertensive animals.

Discussion

Reduction of the glomerular capillary bed incident to the kidney damage resulting from the deficiency of choline appears to be the significant factor determining the degree of hypertension eventually produced (Figs. 9 and 10). As these renal lesions are primarily tubular (Christensen, 1942; Hartroft, 1948) it may at first appear paradoxical that glomerular damage is such a prominent feature of the lesions in the surviving animals. However, these authors and others have pointed out that in severe cases of haemorrhagic kidney the glomeruli are secondarily involved in the later stages of the syndrome. When appreciable numbers of glomeruli are destroyed a train of events is apparently started leading to elevation of blood pressure. The hypertension in turn produces thickening of both the glomerular loops and the arterioles. It is likely that the mechanism producing hypertension in our animals is similar to that operating in rats after subtotal nephrectomy (Chanutin and Ferris, 1932). In the present experiments the factor responsible for reducing the amount of functioning renal tissue is dietary deficiency rather than surgery. The basic

mechanisms by which hypertension is initiated are probably similar to those demonstrated by Goldblatt *et al.* (1934). The effects of massive amounts of vitamin D in producing evidences of hypertension (Ham, 1940) are presumably exerted through similar pathways.

It is well known that there is great variation among the members of any group of young rats in the extent of renal damage resulting from choline deficiency. A small percentage of such animals never develop advanced renal lesions even if fed the diet for many weeks. In the early stages of the experiments here reported large numbers of weanlings were left on the choline-deficient diet for periods as long as 7 to 10 days. The mortality rates in these groups were high (over 80%). The small number of survivors were then given full diets, but appreciable residual renal damage was not present at necropsy several months later, and blood-pressure values during life were not significantly higher than normal levels. This finding indicated to us that a prolongation of the period of dietary deficiency had eliminated those animals most susceptible to this type of kidney damage. However, if these animals had lived they would presumably have been the ones most likely to develop hypertension. For this reason, in later experiments the young rats were not deprived of dietary choline for unbroken periods of more than five or six days. Accordingly, greater numbers survived this stage, and these included many in which a severe degree of kidney damage had been produced by choline deficiency.

In the experiments of Sobin and Landis rats were maintained on the choline-deficient diet for a period of months. During the initial period of acute deficiency 66% of the males and 20% of the females died. The growth of the surviving animals was distinctly slower than that of the controls, and the difference in weights at five months was conspicuous. Our experience in similar investigations confirmed the findings of these workers. The absence of hypertension in any of the animals maintained for prolonged periods on these deficient diets might be explained in either or both of two ways. Those animals most susceptible to the lack of choline may not have survived the initial period of acute deficiency. A second possibility is that the retarded growth of the surviving rats may have prevented the development of hypertension. In our present series, animals surviving the initial period of dietary deficiency were transferred to full diets, and growth from then on approached that of the normal controls. The higher growth rates may have made greater demands on the cardiovascular-renal systems of these animals with residual kidney damage, and thus have initiated the development of hypertension.

The clinical significance of our results is not apparent. Attempts to produce the haemorrhagic renal syndrome in any species other than the rat have not yet been successful. In this laboratory further investigations are being undertaken to increase the percentage of those rats surviving "haemorrhagic kidney" which have severe degrees of residual renal damage. If this can be accomplished we will have at our disposal a useful method for the production of experimental hypertension in rats in addition to those which involve surgical intervention or the injection of hormones or of nephrotoxic agents. The problem is to determine how long to deprive the young animals of dietary choline to produce consistently the maximal amount of irreversible renal damage compatible with life.

We may suggest that the wider application of the principle of subjecting young animals to acute deficiencies of one or more essential food factors, followed by periods of growth approaching or exceeding the normal in rate, may reveal other and hitherto unsuspected effects of the initial deficiency. We hope to explore some of these possibilities.

FIG. 10.—Comparable preparation to that shown in Fig. 9, from a normal control rat of similar age and weight. Note the large number of glomeruli. ($\times 50$.)

Summary and Conclusions

In this series of observations all the rats in which a severe degree of residual renal damage was produced by a short period of choline deficiency during early life later developed a definite hypertension.

The average blood pressure of this test series was 195 mm. Hg as determined by arterial cannulization, while that of the controls was 118 mm. Hg.

The average weight of the hearts of the hypertensive animals was almost double that of the controls. The histological picture of the arterioles of these animals was characteristic of advanced hypertension.

When only a moderate degree of residual kidney damage was produced by choline deficiency the hypertension, cardiac hypertrophy, and other pathological changes were correspondingly less.

There is no apparent immediate clinical application of these findings. Our results suggest that further work may provide a simple way of consistently producing hypertension in an experimental animal by dietary means.

The kindly interest of Professor William Boyd is gratefully acknowledged. We are also indebted to our colleagues Dr. J. H. Ridout and Dr. C. C. Lucas for their help, and to William Wilson for technical assistance. The photomicrographs were made by one of us (W.S.H.).

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SEVERE HYPERTENSION WITH RECOVERY AFTER NEPHRECTOMY

BY

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[WITH PHOTOGRAVURE PLATE]

Severe hypertension is a disease which may occasionally undergo partial regression under medical treatment or following surgical ablation of the appropriate sympathetic ganglia with splanchnicectomy. In both these instances this improvement may be sufficient to permit the survival of an occasional patient even for a number of years, but still with a considerable elevation of blood pressure. Any case, however, in which as a result of treatment the blood pressure falls to normal levels and remains there is extremely rare and therefore worth recording.

This desirable result has occurred in a young woman with severe hypertension referred for routine investigation of her renal tract. As a result of the investigations undertaken the left kidney was regarded as the source of her hypertension, a view which has subsequently been confirmed by the maintained fall of blood pressure following nephrectomy

and extirpation of the left adrenal gland. The details of the case are as follows.

The patient was a young woman aged 20, suffering from diplopia. She worked as a thread-spinner. There was a history, in July, 1947, of polyuria, two-hourly during the day, rising twice at night; in August to October of amenorrhoea, headaches, vomiting, and loss of weight; in October of diplopia on looking to the left; and in November of slight drowsiness and a little oedema of the ankles. There had been no headaches, paraesthesia, dimness of vision, or other palsies. There was nothing relevant in her previous history or that of the family. Her social conditions were good.

On examination her intelligence, nutrition, and colouring were good. There was slight oedema of ankles. The pulse was 80 regular, the vessels soft—? slight enlargement of heart—and the sounds pure. The electrocardiogram revealed nothing abnormal. B.P. 210/140. The blood picture was normal. There was paralysis of the left external rectus muscle. Both fundi showed papilloedema and dilated veins. The left retina showed as well some small haemorrhages and patches of exudate. The visual fields and the cerebrospinal fluid were normal. The urine had a specific gravity of 1008; there was a haze of albumin, but no cells or casts. The blood urea was 35 mg. per 100 ml.

On x-ray examination the skull and heart were normal, and the abdomen contained no calculi. Intravenous pyelograms showed that the function and outline of the right kidney were normal. The left appeared to function poorly and was not well outlined (Plate, Fig. 1).

Cystoscopic examination revealed no abnormality in the bladder, and both ureters were catheterized easily the full distance. Specimens from both kidneys and bladder were sterile and cell-free. The left retrograde pyelogram was of the small apelvical normal type (Fig. 2). Differential renal function: observation period, 20 min.; right kidney, 11 ml., pH 7, urea 0.55%; left kidney, 0.3 ml., pH 5.6, urea 0.8%; wastage, nil.

Dec. 16, 1947.—High spinal anaesthesia was carried out by Dr. H. H. Pinkerton, anaesthesia reaching to the level of the fourth dorsal segment. The blood pressure fell to 140/95 without distress. Intravenous pyelography was repeated under the spinal anaesthetic with no appreciable difference in the appearances or function of either kidney, and the opportunity was taken to do a muscle biopsy. In his report on this Dr. W. A. Taylor states that the peripheral arterioles show hyperplasia of the media without necrosis or fibrosis.

Dec. 23.—Under intratracheal gas-oxygen-and-ether (Dr. Beaton) the left renal area was exposed through a modified Fey's approach, the twelfth rib being removed. The kidney was small, pale, and contracted, with a slightly granular appearance on its subcapsular surface. The adrenal gland felt much firmer and the medulla appeared larger than normal. It was removed first with little appreciable effect on the blood pressure; the kidney was then removed, and within half an hour the blood pressure had fallen to 115/70 (see Chart 1). These procedures were completed without incident and the wound was closed without drainage. Convalescence was uneventful: the behaviour of the blood pressure and pulse rate during convalescence is shown in Chart 2.

Histological examination of the adrenal showed it to be 8 g. in weight—i.e., approximately 1 g. above normal. Careful serial slices revealed no evidence of other abnormality and no tumour could be found. The appearances of the kidney are illustrated in Fig. 3.

The pathological report (Dr. W. A. Taylor)

CHART 1.—Blood pressure record during operation. A. Incision made. B. Adrenalectomy completed. C. Renal pedicle clamped. D. Operation completed.

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FIG. 1.—Severe parenchymal damage in the kidney of a rat surviving the haemorrhagic renal syndrome of choline deficiency. H.E. stain ($\times 140$.)

FIG. 2.—Fibrinoid necrosis:arteriole in cortex of kidney in Fig. 1. H.E. ($\times 600$.)

FIG. 3.—Moderate parenchymal damage in kidney of rat surviving haemorrhagic renal syndrome. Note some relatively normal tubules. H.E. ($\times 140$.)

FIG. 4.—Hyaline degeneration in arteriole of cortex of kidney in Fig. 3. H.E. ($\times 600$.)

FIG. 5.—Slight parenchymal damage in kidney of rat surviving haemorrhagic renal syndrome. Note depressed cortical scar and distended tubules. H.E. ($\times 140$.)

FIG. 6.—Early cellular hyperplasia in arteriole in cortex of kidney in Fig. 5. H.E. ($\times 600$.)

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FIG. 7.—Normal renal cortex from a control animal fed diets containing choline throughout its life. H.E. ($\times 140$.)

FIG. 8.—Cortical arteriole from kidney of control animal. H.E. ($\times 600$.)

H. HOLDEN: SOLITARY PLASMOCYTOMA OF BONE

FIG. 1.—Radiograph before treatment, showing rounded opacity in region of right lung apex and almost complete destruction of right first rib.

FIG. 2.—Section of plasmocytoma. Plasma cells with deeply staining nuclei. ($\times 500$.)

FIG. 3.—Commencing regeneration of right first rib with disappearance of opacity three months after treatment.

FIG. 4.—Two years after treatment.