HISTOLOGICAL STUDIES IN
HYPERTENSIVE RATS

by

June R. Logan

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ABSTRACT

Eleven rats were made hypertensive by intramuscular injection of desoxycorticosterone acetate. An elevation in blood pressure of over 40 mm. Hg. was maintained for periods of 1.3 to 8.3 months before the animals were sacrificed. Histological sections were prepared of kidneys, adrenals, heart, liver, pancreas, and duodenum. Microscopical examination revealed slight degrees of tubular and glomerular degeneration in the kidneys of animals with hypertension of 3 to 8 months duration. No change in the juxtaglomerular apparatus was observed. Vascular disease was almost completely absent from any of the organs studied. It was concluded that an elevation in blood pressure can be maintained for periods up to 8 months without obvious renal or vascular damage. Hypertension in this case was probably due to arteriolar spasms causing increased peripheral resistance. It was suggested that appearance of arteriolosclerosis might mark the onset of an irreversible phase of hypertension.
ACKNOWLEDGEMENTS

The author wishes to express her thanks to Dr. A.H. Hutchinson for the kindly advice and helpful criticisms given by him throughout the investigation.

To Dr. J.A. Allardyce, under whose direction this work was carried out, a particular debt of gratitude is owing for continued interest and constructive suggestions.

Dr. E.C. Black is thanked sincerely for the gracious advice, suggestions, and aid he offered in the later stages of the problem.

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Thanks are also due to all my fellow students, and particularly to Mr. J. Salter and Mr. R. Rixon for their provision of experimental animals, and to Mr. R. Devito for aid with the photography involved.
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INTRODUCTION

The complexity of human hypertension warrants investigating various methods of simulating such a condition in experimental animals. Much of the previous work on experimental elevation of blood pressure has involved mechanical disturbance of renal function. Observations from both clinical and experimental data indicate that the adrenal cortex is a factor in the maintenance of blood pressure. It was decided to follow any histological change in animals rendered hypertensive by injection of desoxycorticosterone, a hormone of the adrenal gland. The problem was formulated in consultation with Professor J.A. Allardyce, the experimental animals to be provided by Mr. Salter and Mr. Rixon who will be reporting later on amelioration of high blood pressure induced by desoxycorticosterone.

A comparison of microscopical findings with those previously reported in experimental hypertension and their relation to clinical observations was hoped to add to the present concepts regarding the initiation and maintenance of the hypertensive state. The results obtained concerning lack of renal damage are in agreement with clinical findings in primary or essential hypertension without renal insufficiency. In contrast to post-mortem findings in human and experimental chronic hypertension which almost invariably revealed widespread arteriolosclerosis, high blood pressure was maintained for periods up to eight months without obvious vascular disease.
HISTORICAL REVIEW

I. Clinical hypertension.

Clinically, primary arterial hypertension without obvious renal impairment is distinguished from chronic nephritis with hypertension. However, certain patients show symptoms of primary hypertension terminating in renal insufficiency. This latter syndrome has been termed the malignant phase of essential or primary hypertension. (Ellis, 1938).

Moritz and Oldt (1937) found generalized vascular disease in the kidneys of patients with essential hypertension. Arteriolar sclerosis was most marked, with intimal hyalinization in the smaller arterioles and endothelial hyperplasia in the larger arterioles. These workers concluded that renal arteriolar sclerosis may be the initiating factor in human benign hypertension.

Post-mortem examination of kidneys of patients with malignant hypertension revealed widespread vascular lesions, especially of the afferent arterioles to the glomeruli (Ellis, 1938). Focal degenerative changes were present in the glomeruli. The walls of the afferent arterioles exhibited hyaline infiltration with, occasionally, complete obliteration of the lumen. Intimal hypertrophy was present in the smaller arteries. Severe albuminous degeneration and hyaline droplet formation were observed in the renal tubules. Similar vascular
lesions were found in other organs of the body, particularly the adrenals, pancreas, and intestine. The renal involvement was regarded as a terminal manifestation of the widespread vascular disorder and not the initiating factor in the disease.

Schroeder and Fish (1940) investigating the effect of nephrectomy upon hypertension associated with renal disease, found that removal of the diseased kidney does not consistently alter the course of arterial hypertension in a favorable direction.

Castleman and Smithwick (1943) studying renal biopsies from hypertensive patients, found varying degrees of vascular disease ranging from no obvious impairment to pathological involvement of every vessel. Thus, it appeared that in some cases hypertension precedes obvious signs of renal vascular disorder.

Rinehart, Williams, and Capeller (1941) reported nodular or adenomatous hyperplasia of the adrenal cortex to be a frequent finding in cases of essential hypertension. A patient with clinical signs of hypertension who subsequently developed hypoadrenalism exhibited a marked decrease in blood pressure (Perara, 1945). Replacement therapy with desoxycorticosterone caused the blood pressure to rise to its original high level.

II. Experimental hypertension.

Because of the association of hypertension with nephritis in man, the first attempts at producing experimental elevation of blood pressure involved structural
damage to the kidney or partial nephrectomy. Drury (1932) devised a method of producing persistent elevation of blood pressure by altering the blood flow through the kidney. Renal insufficiency of any desired degree was obtained by constricting one renal artery and later removing the contralateral kidney. Goldblatt, Lynch, Hanzal and Summerville (1934) introduced bilateral constriction of the renal arteries as a method of producing ischemia and resultant nephrosclerosis. A clamp was especially constructed for this purpose. Moderate constriction of both main renal arteries caused a persistent elevation of blood pressure approximating the benign phase of human essential hypertension. Microscopically, the kidneys showed thickened basement membranes of the glomerular tufts and thickened Bowman's capsules. Hyaline degeneration was present in some arterioles. Severe constriction of the main renal arteries resulted in hypertension with terminal renal insufficiency, clinically regarded as the malignant phase of essential hypertension (Goldblatt, 1938). Gross examination revealed petechiae and larger hemorrhages in many internal organs, particularly the gastrointestinal tract. Histologically, severely hyalinized and necrotic arterioles were evident in the esophagus, stomach, intestine, pancreas, mesentery, myocardium, and urinary bladder. In contrast to malignant hypertension in man, the kidneys of the experimental animals showed no arteriolar necrosis. This was explained on the basis of the low intravascular pressure within the kidney due to clamping of the renal artery,
whereas, in man, sclerosis and constriction of the preglomerular arterioles would undoubtedly cause high intrarenal vascular tension. Goldblatt concluded that renal insufficiency as well as elevation of blood pressure was necessary for the development of necrotic arterioles and associated hemorrhages in various organs.

Child (1938) described extensive kidney damage in dogs after severe constriction of both renal arteries. Parenchymatous degeneration and multiple small infarcts were observed. Petechial hemorrhages were present in various organs but, in contrast to Goldblatt's findings, the source of hemorrhages appeared to be in the capillary bed, the arterioles and larger arteries being intact. However, animals with moderate constriction of the renal arteries and a longer period of hypertension upon postmortem examination revealed pathological vascular changes only in the arterioles of internal organs and in the glomerular components of the kidney. Kidney damage consisted of hyalinization of the glomeruli, interstitial fibrosis, and tubular degeneration. A few glomerular tufts were adhered to their capsules and proliferation of the capsular epithelium was not uncommon.

Blalock and Levy (1939) investigated the effect on blood pressure of gradual complete occlusion of the superior and inferior mesenteric arteries. In these experiments no sustained elevation in systemic blood pressure was observed.

Graaf and Page (1940) demonstrated that compression
of the kidney parenchyma alone is sufficient to produce an
elevation in blood pressure. The kidneys of dogs were
wrapped in cellophane and the resulting inflammatory
reaction caused formation of an exudate and subsequent
adherence of the omentum to the renal capsule. Microscopic
examination of the kidneys revealed focal cortical and
tubular atrophy and cortical scarring of the ischemic type.
The renal blood vessels showed no intimal or medial changes
and the glomeruli were fairly well preserved. The arterioles of the adrenal gland exhibited marked medial hypertrophy and some hyalinizing changes. Widespread focal
necrosis was evident in the myocardium. The coronary
vessels showed medial hypertrophy and narrowed lumens but
no thrombi were observed.

Friedman, Jarman, and Klemperer (1941) induced
a condition of sustained hypertension in rats by wrapping
one kidney in cellophane, the opposite kidney remaining
intact. Inflammatory lesions were produced in the injured
kidney but extensive change in the vascular system was
observed in only a few instances. Vascular disease
appeared to have no causal relationship to the elevated
blood pressure.

Halpert and Grollman (1947) constricted the kidneys
of dogs with a figure eight ligature. Histologically,
changes in the arteries of all the organs examined were
minimal or absent. Enlargement of the heart was found
to be due to actual hypertrophy of the muscle fibres as
well as to cardiac dilatation. In the kidney all stages of
glomerular degeneration were present. The convoluted and collecting tubules of the necrotic glomeruli were completely obliterated or were distended with homogenous material staining bright pink with eosin. The conclusion was made that neither damage of renal tissue nor intrarenal vascular changes nor ischemia are primary prerequisites for the induction of hypertension but that a reduction in total number of functioning nephrons is the initiating factor.

That the elevation of blood pressure produced by the above experimental methods is not of direct nervous origin was demonstrated by Collins (1936). He showed that denervating the kidneys before clamping the renal artery did not interfere with the development of hypertension.

Page and Sweet (1937) demonstrated that hypophysectomy resulted in a marked reduction in blood pressure perhaps because of loss of adrenal stimulation through the adrenotropie hormone. Adrenalectomy was found to cause a gradual reduction in chronic hypertension induced by the Goldblatt method (Collins and Wood, 1938).

Selye (1942) produced nephrosclerosis and cardiac hypertrophy in chicks by overdosage with desoxycorticos-terone, a hormone of the adrenal cortex. Renal lesions included cloudy swelling of the tubules and hypertrophy and hyperplasia of both parietal and visceral layers of Bowman's capsules. Some glomeruli were enlarged because of hyperemia while others showed obvious signs of sclerosis due to hyaline infiltration. The blood vessels appeared intact. The blood pressure rise in these animals was not
Darrow and Miller (1942) investigating the effects of desoxycorticosterone overdosage in rats, observed necrosis of cardiac musculature and hypertrophy of renal tubules. No lesions were noted in the liver, skeletal muscle, or diaphragm.

A syndrome comparable to malignant hypertension in man was produced in rats by high dosage with desoxycorticosterone and sodium chloride (Selye, Hall and Rowley, 1943). Selye (1943) induced hypertension with kidney disease by subjecting rats to damaging agents such as prolonged exposure to cold. Hypertrophy of the adrenals glands was a characteristic feature of this treatment. Further studies on overdosing with desoxycorticosterone and sodium chloride by Selye and Hall (1944) confirmed the previous findings of Darrow and Miller.

In contrast to the large doses of desoxycorticosterone administered by previous workers, Fitch (1947) obtained a significant rise in blood pressure in rats by a single intramuscular injection of 0.05 mgm. of this hormone.

III. The juxtaglomerular apparatus as a source of renin.

Tigerstedt and Bergmann (1898) obtained a pressor substance, "renin", from normal kidney tissue of rabbits. After successfully inducing hypertension by means of renal ischemia, Goldblatt (1934) postulated the presence of a humoral pressor substance as a mechanism whereby the blood pressure is elevated.
Goormaghtigh (1937) described large afibrillar cells under the intima of the afferent arterioles of the renal glomeruli. These cells appeared to be derived from smooth muscle cells which had lost their myofibrils and acquired acidophilic granules. Around the vascular pole of the glomerulus they formed a ring-shaped structure termed the "juxtaglomerular apparatus". Morphologically, the epitheloid cells were found to resemble the large clear cells of the arteriovenous anastomoses, of the glomi of the skin, and of the glomus coccygeum.

In rabbits moderate constriction of the renal artery was followed by an increase in number and size of the granulated cells of the juxtaglomerular apparatus (Goormaghtigh, 1939). These cells appeared to invade the glomerular tuft and the walls of the cortical arterioles. Variations in the cytological features of the afibrillar cells as well as their proliferation with the onset of hypertension led Goormaghtigh (1940) to postulate the existence of glandular activity responsible for the elaboration of a vasopressor substance.

Kaufmann (1941) described hypertrophy and hyperplasia of the "Goormaghtigh" cells in post-mortem examination of patients with essential hypertension. Castleman and Smithwick (1943) in their studies on renal biopsies found no change in the juxtaglomerular apparatus.

Kaplan and Friedman (1942), in studies on the mesonephros and metanephros of the hog fetus, found the amount of renin extracted to decrease with tubular necrosis
and to increase with tubular hyperplasia despite the condition or number of functioning glomeruli present. The absence of Henle's loop and extensive collecting duct epithelium in the mesonephros suggested that the convoluted tubules were probably responsible for the production of renin. The afibrillar cells described by Goormaghtigh were not observed in either type of kidney.

Similar to the glomic vessels, the preglomerular portion of the afferent arteriole was found to be richly innervated (Oberling, 1944). This author observed individual variations in the preglomerular or juxtaglomerular apparatus in hypertensive patients but no parallelism between the degree of development and elevation of blood pressure. On the contrary, in malignant hypertension the afibrillar cells appeared to degenerate along with the glomerular components. The proliferated cells reported by Goormaghtigh were thought to be histiocytes or mastocytes of the reticulo-endothelial system. It was suggested that the juxtaglomerular apparatus represents a delicate regulatory mechanism of the glomerular circulatory system and that its degeneration may parallel the onset of the irreversible phase of hypertension.

In the "endocrine kidney", in which urine secretion is stopped by restriction of the renal blood supply, the juxtaglomerular cells showed no hyperactivity while only the convoluted tubules remained functionally active. The convoluted tubules were assumed to be the source of the vasopressor substance. (Selye, 1947)
Dunihue (1947) reported the increased renin activity present in a variety of hypotensive states to be accompanied by hypertrophy of the juxtaglomerular apparatus. He suggested that renal anoxia may be a causal factor in hyperplasia of the afibrillar cells.
MATERIALS AND METHODS

Salter and Rixon made available for this study eleven rats in which chronic hypertension had been induced by the intramuscular injection of desoxycorticosterone acetate. All blood pressure recordings were made by Salter and Rixon according to the method described by Fitch (1947).

The rats were killed by exposure to illuminating gas. The adrenals, kidneys, heart, and portions of liver, pancreas and duodenum were immediately removed and placed in a fixative. Upon removal the heart of each animal was weighed in the fixative.

The following fixatives were used:

(1) Bouin's fluid

Saturated aqueous picric acid 75 cc.
Neutral formalin 25 cc.
Glacial acetic acid 5 cc.

(2) Formol-saline

10% solution of neutral formalin in physiological salt solution

(3) Modified Zenker Stock

Potassium bichromate 25 gms.
Mercuric bichloride 50 gms.
Distilled water 1000 cc.

Both Bouin's fluid and Formol-saline were washed out in 3 or 4 changes of 70% alcohol.

Tissue fixed in Modified Zenker Stock was washed
in running water for 12 hours.

The tissue was dehydrated in a graded series of alcohols and cleared in toluene. Cedar oil was tried as a clearing agent but was found too difficult to remove from dense tissue such as kidney. When using the periodic-acid-Schiff’s-reagent technic described below, it is necessary to clear in toluene. Paraffin wax, melting point 57 deg. C., was used for imbedding and blocks were sectioned at 5 or 6 microns. Sections at varying intervals throughout each organ were selected for mounting. A gum arabic adhesive was used in fixing sections to the slides.

Sections were stained with hematoxylin and eosin or with periodic-acid-Schiff’s-reagent.

Standard Alum-hematoxylin, a modification of Harris’ hematoxylin, was found to give good results. The hematoxylin was removed from the cytoplasm in 1% N HCl in 70% alcohol. After staining with eosin in 95% alcohol immersion in methyl hydrate was more satisfactory than in absolute ethyl alcohol.

Periodic-Acid-Schiff’s-Reagent (PAS) Histological Technics (McManus, 1948a).

1. Paraffin sections to water.
2. 0.5% periodic acid in water 5 minutes.
3. Rinse in distilled water.
4. Schiff’s reagent, 15 minutes.
5. Rinse in three changes of sulfurous acid, each 2 minutes.
6. Wash in running water 3 to 5 minutes.
7. Stain in hematoxylin 20 to 30 seconds.
8. Wash in running water 5 minutes.
9. Dehydrate in two changes 95% alcohol.
10. Two changes of absolute alcohol.
11. Clear in toluene and mount in balsam.

Preparation of Schiff's Reagent.
1. Weigh out basic fuchsin - one gram.
2. Weigh out anhydrous sodium bisulfite - one gram.
4. Add fuchsin and stir.
5. Cool to 50 C.
6. Filter.
7. Add 20 ml. N HCl (98.3 ml. of HCl, S.G. 1.16, made to 1000 ml. with distilled water).
8. Cool to 25 C.
9. Add sodium bisulfite.
(Keep in dark. The fluid takes about two days to become orange or straw colored; then it is ready for use).

Sulfurous Acid Rinse.
1. 10% sodium metabisulfite - 6 ml.
2. Normal HCl - 5 ml.
3. Distilled water - 100 ml.

Schiff's reagent must be replaced when the solution begins to show a red coloration. The first sulfurous acid rinse is discarded after each set of slides and fresh
solution used as the third rinse. Sections previously stained with hematoxylin-eosin may be bleached in 1% HCl in 70% alcohol and successfully treated with periodic-acid-Schiff's-reagent.

Periodic acid breaks the 1,2 glycol linkage (R-CHOH-CHOH-R) of carbohydrates to yield aldehyde (R-CHO-R-CHO). Schiff's reagent acts with the aldehyde to produce a red to violet color; that is, the leucofuchsin is recolored. This reaction cannot be used as a specific histochemical test for carbohydrate since serine, threonine, and hydroxylysine form aldehyde when treated with periodic acid. Also, many tissue proteins and lipids contain a carbohydrate prosthetic group. However, this technic is valuable in pathological histology as it indicates the first signs of hyaline degeneration. Histologically, the PAS technic causes selective staining of the basement membranes of the glomeruli and of the renal tubules. The outlines of the smooth muscle cells of the arterioles and the capillary walls are similarly colored a bright red or purple. A less intense reaction is evident in the brush border of the cells of the proximal convoluted tubules and slight coloration may be produced in the cytoplasm of these cells. In pathological states characteristic coloring is obtained of the hyaline of arteriolosclerosis, hyaline droplets in the tubular epithelium, and granular cells of the renal arterioles. Hyaline casts in the renal tubules stain with varying degrees of brilliance.

(McManus, 1948b)
Glycogen, mucin and hyaluronic acid may be demonstrated by the PAS technic in paraffin sections. In the human duodenum the free surface of the intestinal epithelial cells shows strong coloring. This is the site of alkaline phosphatase activity as is the brush border of the proximal convoluted tubules of the kidney. In frozen section of the human adrenal gland PAS positive material is found in the reticularis and in the outer portions of the medulla. (McMans, 1948a)

As possible changes in the Golgi element of renal cells during hypertension have not yet been investigated, Aoyama's method of silver impregnation was attempted. However, impregnation of the Golgi body was not obtained, there being diffuse reduction of silver throughout the cell. Silver methods are capricious and often fail inexplicably (Baker, 1945).
The rats maintained an elevation of blood pressure of at least 40 mm. Hg. for periods ranging from 1 to 8 months. The following table indicates the degree and duration of hypertension and the amount of desoxycorticosterone acetate (DCA) administered.

Table I: Chronic Hypertension

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Weight</th>
<th>Normal blood pressure</th>
<th>Hypertension</th>
<th>Duration</th>
<th>Dosage</th>
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<td>275</td>
<td>142</td>
<td>200</td>
<td>1.3</td>
<td>0.10</td>
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<tr>
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<tr>
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<td>230</td>
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<td>135</td>
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</table>
The rats maintained an elevation of blood pressure of at least 40 mm. Hg. for periods ranging from 1 to 8 months. The following table indicates the degree and duration of hypertension and the amount of desoxycorticosterone acetate (DCA) administered.

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<tr>
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<th>Duration</th>
<th>Dosage</th>
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<td>1.8</td>
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</tr>
</tbody>
</table>
Gross examination

During the course of hypertension Rat 9 was the only experimental animal that exhibited signs of oedema, the others appearing normal in all respects. Upon gross examination of the internal organs no pathological changes were evident. The heart weights showed no significant differences between hypertensive and normal animals.

Histological observations

Kidney

Microscopically, the kidneys of the hypertensive rats showed varying degrees of focal degenerative change. In all cases damage appeared to be confined to single renal units, there being no widespread areas of pathological degeneration. Renal lesions of the same type were not uncommon in control rats.

Hyaline degeneration appeared to be the predominant pathological change in the kidney. The periodic-acid-Schiff's-reagent technic was found extremely useful in detecting the first signs of hyaline infiltration. In section, hyaline appeared as a homogenous, refractile, structureless material staining red to violet.

In the normal kidney the basement membranes of the tubular and glomerular epithelium gave a positive reaction with the PAS routine and the brush border of the cells of the proximal convoluted tubules stained with less intensity. The cell cytoplasm reacted slightly as evidenced by a light pink color. (fig. 1)
Pathologically, hyalinization of the glomerular components was the most common finding. All animals showed varying degrees of hyaline thickening of the basement membranes of the glomeruli and of the visceral and parietal layers of Bowman's capsules. Occasionally the glomerular tuft was diffusely adherent to the capsule (fig. 2). A few completely necrotic glomeruli were observed, the capillary tuft being replaced by homogenous hyaline material (fig. 3). Hypertrophy of the epithelial cells of the parietal layer of Bowman's capsule was frequently observed, especially in Rats 3 and 10 (fig. 4). A focal area of round cell infiltration in conjunction with a sclerosed glomerulus was evident in Rat 15 (fig. 5).

Tubular degeneration was noted to be chiefly confined to the distal convoluted and collecting tubules. The damaged tubules usually belonged to the same renal unit as a sclerosed glomerulus. Thickening of the basement membrane was the initial degenerative change, followed later by hyaline droplet formation in the tubular epithelial cells. Tubular necrosis was evidenced by complete hyalinization of the cells (fig. 5). Increased permeability of the glomerular capillaries to protein led to formation of hyaline casts which eventually occluded the lumen of the tubules (fig. 6). Extreme distension of a renal tubule by pink staining colloid material was particularly striking in Rat 4. The epithelial cells lining this tubule have degenerated (fig. 7). The cells of the intercalated segment exhibited hyperplasia in the region of
sclerosed glomeruli in a few instances (fig. 8).

The only degenerative change present in the renal blood vessels was intimal hyalinization of the arterioles (fig. 9). The main renal arteries and the intrarenal arteries were intact, no areas of degeneration attributable to ischemia being observed.

The afferent arteriole to the glomerulus was noted particularly for evidence of the juxtaglomerular apparatus as described by Goormaghtigh (1937). In almost every arteriole studied the media was comprised of typical smooth muscle cells (fig. 10). In a very few instances, usually one or two per section, two types of atypical cells were observed in conjunction with the afferent arteriole. These cells were situated subintimally along the course of the arteriole and at the point of capillarization. In contrast to the smooth muscle cells of the media they lacked myofibrils and possessed spherical vesicular nuclei. One type of cell was large and round presenting a swollen appearance. The cytoplasm was clear, staining lightly with hematoxylin (fig. 11). Found more rarely than the clear afibrillar cell was a large ovoid cell staining red with periodic-acid-Schiff's-reagent routine (fig. 12). No hypertrophy or hyperplasia of these cells was observed in the experimental animals.

In all experimental animals the amount of non-functioning renal tissue was relatively slight. Rats 5 and 9 exhibited no more degenerative change than present in control animals. Renal lesions in Rat 0, with hyper-
tension of 8 months duration, were only slightly more severe than those present in animals having an elevation in blood pressure for periods of 3 to 4 months.

Heart

Examination of the heart revealed no signs of cardiac dilatation or hypertrophy of muscle cells in any hypertensive animal.

In Rat 0 occasional sclerosis of the arterioles in the myocardium was observed. Damage consisted of hyaline infiltration of the intima with resultant narrowing of the vessel lumen (fig. 13).

A typical atheromatous lesion was observed at the base of the aorta in Rat 5. Rupture of the capillaries in the vessel wall appeared to initiate the formation of a fibrotic plaque consisting of connective tissue cells and mixed blood elements. The plaque was encompassed by the endothelial layer of the aorta and a large blood sinusoid formed by hemorrhage of the intimal vessels (fig. 14). Degenerative processes were indicated by infiltration of fat cells into the fibrous tissue. The large swollen appearance of these cells in section caused them to be named "foam" cells. In most cases the cytoplasm was disintegrated, the cells being enclosed in a hyaline capsule (fig. 15).

In no other hypertensive animals were there any signs of pathological change.
Adrenal

The size of the adrenal gland showed individual variation, the cortex being uniformly larger in female rats. There was no apparent relation between adrenal size and degree or duration of high blood pressure. Granules staining orange to red with the PAS routine were observed in the cells of the reticularis and outer portions of the medulla (fig. 16). This PAS positive substance was present in varying amounts in both control and experimental animals.

Vascular lesions were absent from the adrenal gland. The cortical and medullary cells appeared unchanged.

Liver

With the periodic-acid-Schiff's-reagent technic brilliant staining of the glycogen granules in the hepatic cells was obtained. The granules were not distributed uniformly but were accumulated more densely in some cells (fig. 17).

No vascular lesions were observed in the liver.

Duodenum

The free surface of the intestinal mucosa and the mucin of the goblet cells were colored by the PAS technic (fig. 18).

A thorough study failed to reveal any signs of vascular damage in the duodenum.
Duodenum

The free surface of the intestinal mucosa and the mucin of the goblet cells were colored by the PAS technic (fig. 18).

A thorough study failed to reveal any signs of vascular damage in the duodenum.

Pancreas

The pancreatic acini and the islands of Langerhans were Pas negative, the basement membranes staining faintly pink.

No degenerative changes were observed in the pancreatic blood vessels.
DISCUSSION

I. Factors in maintenance of hypertension.

Damage to renal tissue accompanied by an elevation in blood pressure has been produced in rats by overdosage with desoxycorticosterone acetate and sodium chloride (Selye, Hall, and Rowley, 1943). Extensive tubular damage was evident upon histological study, the cells of the proximal and distal convoluted tubules exhibiting hypertrophy and hyperplasia. Vascular changes were confined to the glomeruli, some being enlarged while others showed definite sclerosis with hyaline infiltration. In appearance the kidney was compared to that of the "large white kidney" of human arteriolosclerosis. Since, in these animals no attempt was made to discover the minimum dosage of adrenal hormone necessary to produce elevation of blood pressure with or without kidney damage, it is impossible to determine the causal relationship of hypertension to renal disease. In the present study hypertension was produced by single injections of 0.05 mgm. desoxycorticosterone acetate repeated if necessary; the maximum dose being 7.5 mgms. over a period of three weeks, as compared to 6 mgms. daily for twenty days administered by the previous workers. Histologically, lack of extensive renal or vascular damage was the most striking feature. Thickening of the tubular basement membranes and hyaline infiltration of the glomeruli were observed to a limited extent in the kidneys of animals.
with hypertension of three to eight months duration. Two animals with blood pressure recordings of over 300 mm. Hg. for 1.3 and 1.8 months revealed no more degenerative change than present in control rats. Thus, it appears that desoxycorticosterone acetate will induce hypertension in amounts insufficient to cause obvious damage to renal tissue.

Varying degrees of vascular and renal disease have been reported in experimental hypertension induced by other methods. Bilateral constriction of the renal arteries of dogs was found by Goldblatt (1938) to produce extensive kidney damage with widespread vascular lesions throughout the body. Similar lesions, comparable to malignant hypertension in man, were induced in rabbits by the same method (Wilson and Pickering, 1938). These workers concluded that renal insufficiency was a necessary factor in producing necrotizing arteriolitis as observed in human malignant hypertension. Wilson and Byrom (1939) succeeded in maintaining elevated blood pressure in rats by partial occlusion of one renal artery, the opposite kidney remaining intact. Histological changes comparable to malignant hypertension were observed but renal failure was thought to be due to the vascular strain imposed by a rapidly developing hypertension. Child (1938) suggested that, although the initiating factor is unknown, induced arteriolar disease might account for the maintenance of hypertension. Friedman, Jarman, and Klemperer (1941) using unilateral renal injuries to induce hypertension, found anatomic changes in the vascular system to have no causal relation to
elevation of blood pressure, intrarenal vascular involvement being rarely observed. In agreement with the latter workers, the writer observed negligible vascular changes in the organs studied although the rats were maintained hypertensive for periods up to eight months, comparable to the duration of chronic hypertension reported by Child. It appears evident that vascular degenerative changes, particularly of the arterioles, causing increased peripheral resistance is not necessarily a factor in maintaining chronic elevation of blood pressure.

In comparison with histological observations in experimental animals, post-mortem findings in cases of essential hypertension almost constantly revealed intrarenal vascular damage as well as widespread arteriolar sclerosis (Moritz and Oldt, 1937; Ellis, 1938). Renal biopsies presented the only contradictory evidence in human hypertension, Castleman and Smithwick (1943) reporting lack of renal vascular damage in some patients with clinical signs of primary arterial hypertension.

It is possible that a degree of vasoconstriction throughout the arteriolar system which is undetectable microscopically might be sufficient to produce a significant elevation in blood pressure. The maintenance of hypertension in this case would be caused by a constant source stimulation rather than by passive resistance due to arteriolosclerosis. However, in view of the previous work a more prolonged hypertension might be expected to cause degenerative changes in the blood vessels, arteri-
olosclerosis perhaps marking the onset of an irreversible phase of hypertension.

II. Study of juxtaglomerular apparatus.

In the rat the juxtaglomerular apparatus was not a prominent feature of the glomerular components in observation of random sections taken throughout the kidney. Upon careful examination large clear cells resembling those described by Goormaghtigh (1937) were observed subintimally along the course of the renal arterioles (fig. 11). Of less frequent occurrence but situated in the same relative position was a more ovoid cell staining red with the periodic-acid-Schiff's-reagent technic (fig.12). These cells are probably identical with the granular cells of the renal arterioles found by McManus (1948b) to stain selectively with periodic-acid-Schiff's-reagent technic. The difficulty in comparative study of the juxtaglomerular apparatus has been discussed by Oberling (1944). He found, in man, the "preglomerular apparatus" to be irregularly developed in the afferent arterioles with the cells generally accumulated on one side of the vessel. Thus, for each glomerulus only one plane of sectioning will give a complete picture of the apparatus. Considerable species difference in the degree of development of the myo-epithelial elements has been reported. In the rabbit, in which Goormaghtigh first observed the afibrillar cells and their proliferation in the ischemic kidney, the juxtaglomerular apparatus is extremely well developed. The cells are
smaller and less numerous in the human kidney but Kaufman (1941) found a hyperplasia in hypertensive patients. In the metanephros of the hog fetus Kaplan and Friedman (1943) found no indication of the "Goormaghtigh" cells. Similarly, the juxtaglomerular apparatus is absent from the kidneys of children under two years of age.

In agreement with the present concept of renin formation in the kidney, the writer found no parallelism between the development of the afibrillar arteriolar cells and the degree of hypertension. Evidence indicates that the convoluted tubules are responsible for the elaboration of the vasopressor substance (Selye, 1946; Kaplan and Friedman, 1943).

III. Involvement of adrenal gland.

Histological examination of the adrenal gland offered no clue as to the mechanism whereby desoxycorticosterone acetate causes an elevation in arterial blood pressure. Involvement of the adrenal gland in both clinical and experimental hypertension has long been recognized. Hyperplasia of the adrenal cortex has frequently been reported in cases of human hypertension. Selye (1943) found hypertrophy of the adrenal cortex a constant feature in rats made hypertensive by exposure to non-specific damaging agents. Although changes in the adrenal glands were not observed in animals rendered hypertensive by renal ischemia removal of the cortex resulted in a gradual decrease in blood pressure (Collins
and Wood, 1938). The site of action of the cortical steroids on the kidney is believed to be confined to the tubules, desoxycorticosterone functioning in the retention of sodium and the excretion of potassium (Ingle, 1942). Overdosage with desoxycorticosterone was found to produce cardiac lesions identical with those caused by potassium deficiency (Darrow and Miller, 1942).

It is interesting to speculate on the production of atheromatous lesions in Rat 5 although no conclusions can be drawn from such an isolated case. The amount of body cholesterol is thought to be a factor in such lesions, atherosclerosis being produced experimentally by a diet high in cholesterol (Anderson, 1946). Selye (1945) has found chronic overdosage with desoxycorticosterone acetate to raise the blood cholesterol level. Thus, one might postulate that oversecretion of an adrenal hormone increasing the blood cholesterol level is a factor in causing atherosclerosis and resultant hypertension.
SUMMARY

Histological sections of kidney, adrenal, liver heart, pancreas, and duodenum of hypertensive rats were studied. The most striking feature was lack of widespread vascular disease even in an animal with chronic hypertension of eight months duration. A slight degree of kidney damage was observed, consisting mainly of thickening of the basement membranes of the tubules and hyalinization of the glomeruli. Two rats exhibited hypertension with no more degenerative change present than in control animals. The afibrillar arteriolar cells described by Goormaghtigh (1937) were noted but no relation to the hypertensive state was evident. An atheromatous lesion was present at the base of the aorta in one animal. No parenchymal changes were observed in any of the other organs studied.

The significance of the results in relation to clinical hypertension and to experimental hypertension induced by other methods is discussed.
CONCLUSIONS

From histological studies in hypertensive rats it was concluded that:

1. Desoxycorticosterone acetate will induce hypertension without obvious damage to renal tissue.

2. Hypertension can be maintained for periods up to eight months without extensive vascular involvement. The appearance of arteriolosclerosis perhaps marks the onset of an irreversible phase of hypertension.

3. The juxtaglomerular apparatus appears to play no part in elaboration of a vasopressor substance.
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Plate I

Figure 1. Section of normal kidney showing prominent staining of the basement membranes of the tubular and glomerular epithelium, with a lighter reaction given by the brush border of the cells of the proximal convoluted tubules. Periodic-acid-Schiff's-reagent. (100)

Figure 2. Section of kidney showing thickening of the basement membranes of the parietal layer of Bowman's capsule and of the glomerular epithelium. Periodic-acid-Schiff's-reagent. (440)
Figure 1.

Figure 2.
Plate II

Figure 3. Section of kidney showing hyaline degeneration of a capillary tuft. Lateral to the necrotized glomerulus is a normal Bowman's capsule. Periodic-acid-Schiff's-reagent. (440)

Figure 4. Section of kidney showing proliferation of the cells of the parietal layer of Bowman's capsule. Periodic-acid-Schiff's-reagent. (440)
Plate III

Figure 5. Section of kidney through focal area of degeneration. Thickening of the tubular basement membranes with hyaline degeneration of an epithelial cell is evident. Surrounding the sclerosed glomerulus is an area of round cell infiltration. Periodic-acid-Schiff's-reagent. (440)

Figure 6. Section of kidney showing hyaline cast formation with occlusion of the lumen of a convoluted tubule. Periodic-acid-Schiff's-reagent. (440)
Plate IV

Figure 7. Section of kidney showing extreme distension of a renal tubule with colloid material in the region of a sclerosed glomerulus. Periodic-acid-Schiff's-reagent. (100)
Figure 7.
Plate V

Figure 8. Section of kidney showing proliferation of the cells of the intercalated segment in the region between two Malphighian corpuscles. Periodic-acid-Schiff's-reagent. (440)
Plate VI

Figure 9. Section of kidney showing intimal hyalinization of an arteriole. Periodic-acid-Schiff's-reagent. (440)
Plate VII

Figure 10. Section of kidney showing afferent arteriole to glomerulus comprised of typical smooth muscle cells. Periodic-acid-Schiff's-reagent. (440)

Figure 11. Section of kidney showing large afibrillar cell situated subintimally in the afferent to the glomerulus. Periodic-acid-Schiff's-reagent. (440)
Plate VIII

Figure 12. Section of kidney showing large red-staining cell situated subintimally in the afferent arteriole to the glomerulus. Periodic-acid-Schiff's-reagent. (440)

Figure 13. Section of heart showing intimal hyalinization of an arteriole in the myocardium. Periodic-acid-Schiff's-reagent. (440)
Plate IX

Figure 14. Section through heart at the base of the aorta showing a subendothelial fibrotic plaque with a hemorrhagic lesion. Periodic-acid-Schiff’s-reagent. (100)

Figure 15. Section of heart at base of the aorta showing subendothelial infiltration of foam cells. Periodic-acid-Schiff’s-reagent. (100)
Plate X

Figure 16. Section of adrenal gland showing a PAS positive substance in the reticularis and outer regions of the medulla. Periodic-acid-Schiff's-reagent. (100)

Figure 17. Section of liver showing brilliant staining of glycogen granules. Periodic-acid-Schiff's-reagent. (440)
Figure 16.

Figure 17.
Plate XI

Figure 18. Section of duodenum showing red-staining mucin in glandular cells of the mucosa. Periodic-acid-Schiff's reagent. (440)