ENDOCRINE FACTORS IN EXPERIMENTAL

PEPTIC ULCER AND HYPERTENSION

VOLUME I

THESIS SUBMITTED FOR THE DEGREE OF
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SUMMARY OF WORK

This thesis is divided into six parts:-

Part I is concerned with the peptic ulcers which develop in rabbits following the administration of pitressin. Histological and arteriographic studies establish the pathogenesis of the gastric lesions, and an increase in adrenal cortical function is suggested on the basis of a bio-assay method.

Part II shows that pitressin peptic ulcers in rabbits and rats are aggravated by the simultaneous administration of cortisone or corticotrophin. Ulcers are more numerous, some perforate, and bacterial invasion is increased. Similar lesions are produced both in the presence and absence of the adrenal glands.

Part III is concerned with the concept that stimulation of the pituitary and adrenal glands during stress produces "diseases of adaptation" in the sensitized (unilateral nephrectomy and increased sodium chloride intake) rat. Following exposure to cold stress peptic ulcer is not found, but hypertension develops. It is shown that the hypertension is not the result of increased adrenal cortical function following cold exposure, but is closely related to the increased consumption of sodium chloride.

Part IV is an investigation of the pathological effects of

several different stressors on sensitized rats.   Mild
hypertension develops in very few animals and there is no
voluntary increase in the consumption of sodium chloride diet.

Part V demonstrates the hypertensive pathological effects of
sodium-retaining steroids.   It is shown that similar lesions
can be produced by sodium chloride administration without
hormone overdosage.   The role of the kidney in this hyper-
tensive syndrome is established and it is shown that the adrenal
glands or the cortical hormones are not essential for its
development.

Part VI is a study of the hypertension associated with the
regeneration of adrenal cortical tissue in sensitized rats.
Factors relating to the pathogenesis of this syndrome are
elucidated by modifying the sodium chloride intake of the
animals, and by transplantation experiments in which the
regenerating adrenal tissue is transplanted into different areas
drained by the portal and systemic circulation.

The text is presented in Volume I and the illustrations in
Volume II.   An Appendix of technical methods is included in
Volume I.

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HORMONES AND DISEASE

Throughout the history of medicine various hypotheses have been advanced in an endeavour to integrate the numerous different bodily reactions which occur in physiological and pathological states.   About the middle of the 19th century the French physiologist, Claude Bernard, introduced his concept of the "milieu interieur," relating the constancy of the internal environment of the body to changes in the external environment. Subsequently, the American physiologist, Walter B. Cannon, introduced the term "homeostasis" to signify the maintenance of this constant state (1929).   He emphasized the role of the adrenal medulla and the autonomic nervous system in controlling the internal adjustments of the body to meet situations as varied as muscle work, temperature changes and nervous stimulation.

In 1936 Hans Selye developed his concept of the "General Adaptation Syndrome" and emphasized the primary importance of the hormones of the adrenal cortex in mediating this reaction to stressful stimuli.   In response to a great variety of noxious agents a remarkably uniform picture of changes developed.   He described in experimental animals an initial "shock phase," lasting from a few minutes to several hours, characterized by hypotension, decrease in muscle tone, hypochloraemia, hypoglycaemia, hyperkalaemia, gastro-intestinal erosions and other disorders.   This was followed by a "phase of counter-shock" in which many of the biochemical disturbances were reversed

resulting, for example, in sodium retention and elevation of the blood sugar. At this stage, in addition, there developed morphological changes, the main features of which were enlargement of the adrenal cortex and atrophy of the thymus, spleen and other lymphatic organs. To complete his concept Selye described "a stage of resistance" in which the organism became adapted to repeated application of the stressful agent. Later this was followed by "a stage of exhaustion" in which adaptive mechanisms failed with the reappearance of gastro-intestinal ulcers, thymic and lymphatic involution, loss of adrenal lipids and death of the animal.

It is an integral part of Selye's concept that the changes in this syndrome are mediated by an increase in the secretion of the cortex of the adrenal gland or, alternatively, result from undue sensitivity to adrenal cortical hormone. In either case hypercorticalism ensues. The increased cortical secretory activity follows increased secretion of adrenocorticotrophic hormone (ACTH) from the anterior pituitary gland, and is abolished by hypophysectomy (Selye, 1937). There is ample evidence that the secretory activity of the adrenal cortex is increased in every type of stress so far investigated. This is true both of laboratory animals and man. The reactions of the human adrenal cortex and adenohypophysis to stress and to exogenous ACTH administration have been recorded by Symington and his associates using histological, histochemical and purely

chemical standards (Currie and Symington, 1955; Symington and Davidson, 1956; Symington et al., 1956; Grant et al., 1957).

In 1946 Selye carried his hypothesis further and introduced the concept of "diseases of adaptation." He stated that the increased secretory activity of the adrenal cortices during stress, or an imbalance of the adrenal cortical hormones during stress, can cause metabolic abnormalities, and certain diseases, such as hypertension, arteriosclerosis, polyarteritis nodosa, rheumatic fever, rheumatoid arthritis, gastro-duodenal ulcers, diabetes mellitus and many others. It is his viewpoint that the hormones are the chief mediators in these diseases.

Although there is no doubt about the increased secretory activity of the adrenal cortex in response to stress, the metabolic and morphological consequences of this increased secretion remain a matter of controversy. These changes are abolished by hypophysectomy and by adrenalectomy, but this does not necessarily imply that the adrenal cortex plays a primary causative role in their production. "When a biologic response disappears following the removal of an organ it is unsafe to conclude that the missing response is a function of that organ" - W.B. Cannon.

An alternative concept has been advanced by Ingle (1952), by Sayers (1950), and by Engel (1951). This holds that the increased secretory activity of the adrenal cortices during stress serves to meet an increased need for the hormones and does

not represent hypercorticalism. The adrenal cortex plays a "supporting" or "permissive" role in certain metabolic and morphological adjustments to stressors and in the manifestation of certain diseases, but only rarely does the adrenal cortex play a primary role in the aetiology of disease. This concept excludes the part played by the adrenal gland in major endocrine disorders, such as Cushing's disease, the adrenogenital syndrome, and Addison's disease.

Ingle (1952, 1953, 1954a, 1956) has published numerous experiments on laboratory animals which support this concept of the permissive action of adrenal cortical hormone. An example may serve to illustrate his hypothesis. Following fractures, burns, surgery, and infections, experimental animals usually develop a negative nitrogen balance. Since the secretory activity of the adrenal cortices is increased during stress, and since large doses of either adrenal cortical extracts or of the 11-oxygenated steroids cause an increased nitrogen loss, it has been postulated by Selye that the post-stress negative nitrogen balance is caused by the secretion of increased amounts of hormones by the adrenal cortices. In Ingle's experiment (Ingle, Ward and Kuizenga, 1947) the urinary nonprotein nitrogen (NPN) of adrenalectomized and sham-operated, tube-fed male rats was studied following fracture of the hind limbs. The sham-operated animals, with their adrenal glands intact, exhibited the expected rise in urinary NPN. This failed to occur in the adrenalectomized

rats maintained solely on 1% saline as drinking fluid. When, however, the adrenalectomized animals were maintained on 4 ml. of adrenal cortical extract daily and submitted to fracture the urinary NPN increased to levels comparable to those seen in rats with intact adrenal cortices. The administration of adrenal cortical extract at this "supporting" dose level to control non-fractured adrenalectomized rats failed to produce changes in nitrogen metabolism similar to those observed in the stressed rats. It was concluded that the metabolic response to fractures requires the presence of adrenal cortical hormones for support, but is not caused specifically by the increase in secretion of these hormones. Ingle (1954b) has defined the terms implied in the concept of "permissiveness."

1. The metabolic response to a stimulus occurs in the presence of an endocrine organ.

2. The response fails to become overt when the organ is removed and no replacement therapy is given.

3. The response is again elicited by an appropriate stimulus when a steady intake of hormone is substituted for an endocrine organ. The hypothesis that the response is caused primarily by an increase in the secretory activity of the gland is made untenable by this third condition.

A similar pattern of metabolic changes follows surgery, burns, trauma, and other stressful situations in man. Moore and Ball (1952) have described fully the increased urinary

excretion of potassium and nitrogen, the decreased urinary excretion of sodium and chloride, and other biochemical changes observed in surgical patients in the post-operative period. They advanced the view that these variations in metabolic balance were the result of an increase in the secretion of adrenal cortical hormones consequent upon exposure to the stress of surgery. As in Ingle's animal experiments, this inter-pretation is no longer tenable in man. Robson and his associates (1956) have shown by balance studies in patients undergoing second stage adrenalectomy for mammary cancer that identical biochemical changes result in the post-operative period in the complete absence of the adrenal glands provided the patient is maintained on a steady intake of adrenal cortical hormone. It was shown subsequently that the alteration in balance of sodium, potassium and chloride is not dependent on an increase in secretion of the salt-active steroid, aldosterone (Dudley, Robson, et al., 1957).

In the terms of many experiments, both in animals and man, the bulk of the evidence favours the view that the corticoids make conditions suitable for the normal metabolic response to trauma and as such presumably tend to subserve homeostasis. Their role is important but is basically a secondary one, rather than that of a primary aetiological agent. If this view is accepted, then the various inter-reactions, both adrenal and extra-adrenal, which evoke these metabolic responses remain to be solved. Elucidation of these mechanisms is difficult because

of the lack of fundamental knowledge of steroid action at
cellular level. However, two recent papers provide some
information on the nature of these biochemical changes. It is
well known that one of the responses to trauma in the rat is
hyperglycaemia. It has been shown by Engel and Fredericks
(1957) that the adrenalectomized rat exhibits a normal hyper-
glycaemic response to trauma if maintained on constant or
"permissive" doses of glucocorticoids while fasting. A
similar response occurs in the untreated adrenalectomized rat
if it is adequately fed. In both cases the normal response of
hyperglycaemia is interpreted as being dependent on an
availability of liver glycogen to permit an hyperglycaemic
response, while the failure of fasted untreated adrenalectomized
rats to develop hyperglycaemia may be due to deficient glycogen
stores, rather than to hormone lack per se. Cuthbertson and his
colleagues (Cairnie et al., 1957) have investigated the negative
nitrogen balance which follows bone fracture and other forms of
trauma in rats. They observed an increase in heat production
and average total metabolism reaching a maximum at a time
corresponding to the maximum output of urinary nitrogen (NPN) in
rats fed a high-protein diet. In contrast, rats maintained on
a practically protein-free diet failed to exhibit the character-
istic sharp rise in urinary nitrogen excretion following fracture
and there was no rise in total metabolism as measured calori-
metrically. The failure of these reactions was interpreted as

being due to exhaustion of the labile protein reserve pool.
Both of these groups of experiments emphasize the importance of
"extra-adrenal" factors in metabolic responses hitherto
considered to be controlled primarily by the hormones of the
adrenal cortex, although the role of adrenal steroids in carbo-
hydrate and protein metabolism is appreciated.

It is apparent from this selection from a voluminous
literature that much of the work on the endocrine aspects of
injury has been devoted to the metabolic aspects of the response.
The morphological consequences have received less attention, yet
it is part of Selye's concept that the adrenal glands play a
primary role in a wide variety of human diseases. The improve-
ment in some patients with rheumatoid arthritis and other
diseases following the administration of ACTH, cortisone, or
hydrocortisone supports but does not prove the hypothesis. Only
a few reports are available that exposure of rats to stress under
unusual conditions produces pathological changes (Selye, 1943;
Selye, 1950; Sellers and You, 1956). This thesis is an
experimental investigation into the role of the hormones of the
pituitary gland and the adrenal cortex in causing disease, and is
concerned especially with the part played by the hormones in
peptic ulcer and hypertension.

PART I

THE EFFECT OF POSTERIOR PITUITARY HORMONE
ON THE STOMACH

THE EFFECT OF POSTERIOR PITUITARY
HORMONE ON THE STOMACH

Over the years many varied approaches have been made to the problem of peptic ulcer, both in the laboratory and the clinic. Numerous facets of the problem have been explored, including the possibility that the endocrine system may be involved in the aetiology of this common disease (Hurst and Stewart, 1929; Ivy, Grossman and Bachrach, 1951). Dodds and his associates first showed in 1934 that the pressor factor of the neurohypophysis produces lesions in the acid-secreting mucosa in a wide variety of experimental animals. Later, Cutting et al. (1937a), working in the same laboratory, demonstrated that the intravenous injection of posterior pituitary extract diminishes the quantity of gastric acid secretion and that this is associated with a 50% reduction in blood flow through the stomach. Dodds and his colleagues did not imply a causative relationship between their experimental findings and human peptic ulcer. The difference in the distribution of the gastric lesion in the animal and in man was emphasized.

In 1949 Barclay and Bentley demonstrated an arteriovenous shunt mechanism in the submucosa of the human stomach, and their observations were subsequently confirmed by others (Walder, 1950; Barlow, 1951; Barlow et al., 1951). It seemed possible

that pitressin might operate such a shunt in the experimental animal and so account for the reduced blood flow and gastric lesions reported by Dodds.   It was decided to test this hypothesis and to investigate some aspects of endocrine function in experimental peptic ulcer in rabbits using arterio-graphic and histological methods.

MATERIALS AND METHODS

Arteriographic Methods

Numerous methods are available for demonstrating vascular pathways, both functionally and anatomically.   Some require highly specialized micro-radiographic (Barclay, 1947) or cine-radiographic (Trueta et al., 1947) equipment while in others various plastic materials are used in conjunction with corrosion techniques (Trueta et al., 1947; Reynolds, 1949).   In the present experiments 3 simple methods were used, each demonstrating a different aspect of the vascular pattern of the stomach.

1.   Post-mortem method – Adequate post-mortem injection of the gastric vessels demonstrates the anatomical pattern of the entire vascular bed provided the particle size of the injection material is sufficiently small to pass through the capillaries.   For this purpose a 2% aqueous solution of Prussian blue was injected into the gastric vessels by means of an injection apparatus incorporating a maximum pressure safety-valve device (Figs. 1 and 2), modified from the

apparatus of Weeks and Tindley (1949).

2. Benzidine method - A modification of Pickworth's benzidine
   technique (Bacsich and Wyburn, 1939-42) was used to
   demonstrate vessels which contained blood at the time of
   the animal's death.

3. Carbon method - The gastric vessels which were functioning
   under the conditions of a particular experiment were
   demonstrated by injecting finely divided carbon (particle
   size less than 3μ) into the aorta of the anaesthetized
   rabbit.

With each method thick sections (100-150 μ) were prepared
for microscopic examination.   Technical details are amplified
in the Appendix.

Histological Methods

All stomachs, including those examined arteriographically,
were sectioned routinely and stained with haematoxylin and
eosin.   Injected tissues showed normal cutting and staining
properties, although H. & E. sections from stomachs fixed in
formol-sugar-saline stained rather less satisfactorily than
tissue fixed in 10% neutral formalin.

Thin slices of liver were fixed in Rossman's solution and
sections stained with the periodic-acid Schiff routine to
demonstrate glycogen.   Almond green and haematoxylin were used
as counterstains (see Appendix).

The adrenal glands were fixed in 1% formol-calcium and

embedded in gelatine.   Frozen sections were stained with Sudan IV, Sudan black and unstained sections were examined with polarized light for birefringent crystals (see Appendix).

Animals

Adult rabbits of both sexes weighing 2.0-2.5 Kg. were used. They were fed a compound feeding mixture (diet No. 18, Bruce and Parkes, 1946; see Appendix) with greens and tap water.   They were killed by ether inhalation.

Experimental Design

Two main series of experiments were designed (Table 1). The purpose of the first series (Groups 1, 2, 3, 4, 5A and 5B) was to investigate the sequence of early changes in the stomach at intervals during the first 5 hours following pitressin administration.   The effect of repeated hormone injection over a longer period was studied in the second series (Group 6). Pitressin (Parke-Davis) was injected subcutaneously into the tissues of the anterior abdominal wall.   Two injections of 200 units were given when the large dose of 400 units was administered.

RESULTS

THE DEVELOPMENT OF THE GASTRIC LESION

These observations are based on a study of the stomachs from Groups 3 to 5B (Table 1).   They indicate that the initial lesion was necrosis of the acid-secreting mucosa and that this was followed in a short time by haemorrhage into the necrotic

TABLE 1

Summary of experiments

Group	No. of animals	Dose of Pitressin (units)	Duration of study	Investigation
1	15*	-	-	Prussian blue
2	8*	-	-	Carbon
3	7	400	24 hrs	Histology only
4	7	400	5 hrs	Benzidine
5A	5	400	5 hrs	Carbon
5B	20	100	5 hrs	Carbon
6	8	100 (X 5)✝	10 days	Prussian blue

* The vascular pattern of the normal stomach was studied in groups 1 and 2.

✝ 100 units of pitressin were administered every second day for 10 days.

mucosa.   A time-response effect was established.

HISTOLOGICAL OBSERVATIONS

Fundal area of stomach

In animals killed 30 minutes after pitressin the fundal mucosa showed a pink mottled appearance.   At this early stage the superficial half of the acid-secreting mucosa was necrotic. All the cells of this zone were affected and the mucus-secreting surface cells were shed over extensive areas.   The parietal or acid-secreting cells within this zone showed varying degrees of nuclear and cytoplasmic degeneration (Figs. 3 and 4).   In contrast the epithelium in the deep half of the mucosa appeared normal (Fig. 5).   The mucosal blood vessels were also involved. The arterioles and capillaries deep in the mucosa contained normal-staining erythrocytes (Fig. 5), but in the superficial zone, capillaries immediately beneath the denuded epithelial surface contained only the envelopes of red corpuscles with no stainable haemoglobin or simply some yellow granular debris (Fig. 4).

One hour after pitressin small mucosal haemorrhages about 1 mm. in diameter were present, mainly in the tips of the gastric folds.   Microscopically the appearances of necrosis were similar to those seen at the previous stage but now focal haemorrhage separated the tips of the gastric glands (Fig. 6).

Two hours after pitressin mucosal haemorrhages were more numerous and at some points of the fundus they had coalesced to

form irregular areas about 1 cm. in diameter. Microscopically mucosal necrosis was more advanced and the majority of the epithelial cells were detached from their basement membrane but intact chief and parietal cells were present close to the muscularis mucosae. Intramucosal haemorrhage had increased in severity and occasionally haemorrhagic bullae were present in the superficial necrotic zone (Fig. 7).

Three, four and five hours after injection the acid-secreting mucosa was markedly haemorrhagic, necrotic and oedematous (Fig. 8). Histologically, destruction of epithelium was severe and in some areas the gastric cavity was lined only by masses of poorly stained erythrocytes (Fig. 9). Haemorrhage was present also around the basal portion of the gastric glands and at some points the muscularis mucosae was necrotic. A commencing inflammatory reaction characterized by pavementation and emigration of polymorphs developed at the 5 hour stage. Haemorrhagic submucosal oedema was prominent at this stage and also at the 4 hour period.

In animals killed 24 hours after injection the fundal tissues appeared less oedematous but areas of haemorrhage persisted. Microscopically well-stained erythrocytes infiltrated between the columns of necrotic epithelium and a mild polymorph reaction was present at the periphery of the necrotic areas. The capillaries of the acid-secreting mucosa were filled with erythrocytes of normal appearance. Haemorrhagic

submucosal oedema was less intense.

Oesophagus, pylorus and duodenum

In contrast to the appearances of the fundal area, there
was nothing to suggest involvement of the mucus-secreting
mucosa on gross inspection of the pylorus and duodenum of Groups
3 to 5B.   However 5 rabbits treated with 400 units of pitressin
(Groups 3 to 5A) and 1 rabbit receiving 100 units (Group 5B)
showed small focal lesions in these areas microscopically.   The
surface epithelium and tips of the mucous glands were necrotic.
There was no haemorrhage but in some instances the erythrocytes
in the related capillaries were poorly stained.   The occurrence
of such lesions was not related to the severity of the changes
in the fundus.

ARTERIOGRAPHIC OBSERVATIONS

Groups 1 and 2.   Normal stomach

The normal distribution of the blood vessels within the
gastric wall in man has been adequately described by Barlow
et al. (1951) and will be discussed later.   The Prussian blue
injection method was applied in the present work to illustrate
the vascular pattern in the normal rabbit's stomach (Group 1).
Observations were also made on stomachs from the kitten, cat,
and on human specimens removed at partial gastrectomy.   This
small study in comparative angiography is illustrated in the
series of Figures 10 to 16 and descriptive detail is amplified
in the legends.   Similar patterns were obtained in normal

rabbits (Group 2) using the carbon method.

Group 4. Benzidine studies

The results confirmed the histological observations on the distribution of blood in the vessels and tissue spaces of the stomach following pitressin. The significant changes in the vascular pattern were confined to the acid-secreting area of the fundus. The pylorus and duodenum were normal. At ½ and 1 hour after pitressin the superficial mucosal capillaries in the fundus were empty of benzidine-positive material while the erythrocytes in vessels deep in the mucosa stained intensely black (Figs. 17 and 18). Animals killed at the 4 and 5 hour stage showed the most marked alteration in the distribution of blood (Fig. 19). Patchy mucosal and submucosal haemorrhage were present. The vessels in the deep half of the mucosa were stuffed with blood which stained intensely black. The superficial mucosa was disrupted by areas of haemorrhage and there was extravasation of erythrocytes into the oedematous submucosa. In the non-haemorrhagic areas of the fundus the appearances were similar to those seen at the 1 hour period.

Groups 5A and 5B. Carbon studies

Five rabbits treated with 400 units of pitressin were investigated by the carbon technique (Group 5A). The hypertensive effect of the hormone resulted in considerable leakage of blood around the puncture site in the aorta. Each animal displayed the characteristic necrotic and haemorrhagic lesions

in the acid-secreting mucosa, but the difficulty experienced
in filling the vascular system with carbon largely vitiated the
experiment.   Further investigations with a reduced hormone
dosage were satisfactory.

Twenty rabbits (Group 5B) received 100 units of pitressin
subcutaneously and necrosis of variable severity affected the
acid-secreting mucosa in 13.   Necrosis was absent in the
remaining 7.   Rabbits killed in the early stage of superficial
mucosal necrosis (15-30 minutes) showed a striking variation in
the distribution of circulating carbon as compared with normal
controls.   Where superficial fundal necrosis was complete the
related mucosal capillaries were empty of carbon (Fig. 20).   In
contrast the mucosal vessels of the histologically normal pylorus
and duodenum were adequately filled with circulating carbon (Figs.
21 and 22).   Where a patchy necrotic lesion developed in the
fundus carbon was absent from the capillaries of the necrotic
areas but was present in the vessels of islets of healthy
epithelium, in the pylorus and duodenum, and in the vessels of
other abdominal viscera (Fig. 23).   It would appear therefore
that during the early stage of necrosis, ischaemia involved more
or less extensive areas of the acid-secreting mucosa while a
normal circulation continued through the pylorus and duodenum.

Rabbits were examined by the carbon technique at intervals
of 2, 3, 4 and 5 hours respectively, when the haemorrhagic
features of the pitressin lesion were becoming increasingly

prominent.   The circulation was successfully re-established throughout much of the acid-secreting mucosa, as indicated by the filling of the vessels with carbon.   Defective filling persisted in the surface capillaries in some areas of superficial fundal necrosis.   There was a conspicuous absence of carbon in the haemorrhagic areas characteristic of the late lesion.   Here the extravasated erythrocytes were so tightly packed as to prevent entrance of the particles.

Seven rabbits treated with 100 units of pitressin showed no histological evidence of fundal necrosis.   All were examined in the early stage of the lesion and a normal gastric angiographic pattern was obtained in 5.   The remaining 2 animals showed in one case partially and in the other completely defective filling of the fundal capillaries and a normal circulation in the pyloric and duodenal mucosae.   It is possible that they suffered from fundal ischaemia, the duration of which, prior to killing, was too brief for the development of mucosal necrosis.

LIVER AND ADRENAL GLANDS

It seemed probable that pitressin, apart from any specific action on the stomach, might act as a non-specific stressor (Selye, 1946) and evoke a stress response in the pituitary-adrenal system.   Accordingly, the bio-assay technique devised by Symington (1951) was applied to Groups 3 and 4 (Table 1). The animals were fed the diet up to the commencement of the experiment after which it was withdrawn.   The level of liver

glycogen was then correlated with the degree of adrenal lipid
depletion at intervals from 1 to 5 hours following pitressin.
It is fully appreciated that histological methods give
relatively crude estimates of the degree of chemical activity
in an organ.   Bearing these limitations in mind a depletion in
glycogen content of the liver lobule was demonstrated at the
3 hour period with a gradual repletion at 5 hours (Figs. 24, 25
and 26).   These changes were associated with a partial lipid
depletion of the zona fasciculata which was not fully restored
at the end of 5 hours.

THE ESTABLISHED GASTRIC LESION

A preliminary experiment (Group 6, Table 1) was carried out
to investigate the effect of repeated administration of pitressin.
Pitressin (100 units) was injected every second day for 10 days.
The day following the final injection the animal was killed and
the gastric vasculature demonstrated by the Prussian blue
technique.   Peptic ulcers developed in the fundal mucosa with
fibroblast proliferation and new capillary formation in the base
and edge of the lesions (Fig. 27).   These ulcers contrasted with
the haemorrhagic erosions of the early pitressin lesion.   Since
the character of these more "chronic" lesions forms a major
feature of Part II, they will be described more fully later.

DISCUSSION

Oliver and Schäfer (1894) discovered that the injection of

pituitary extract causes a marked rise in blood pressure. A
few years later, Howell (1898) showed that this property is
associated with posterior lobe secretion. A further advance
was made in 1909 when Dale showed that the administration of
posterior lobe extract causes a marked contraction of the
uterus. The work of Kamm and his associates (1928) proved the
presence of at least two distinct active principles, Pitressin
and Pitocin, which were afterwards separated in a highly
purified state. It is now established that the posterior lobe
of the pituitary gland has important physiological functions in
the control of the circulation and of smooth muscle tonus
generally, in the process of parturition, in water metabolism,
and possibly in fat metabolism.

Pitressin (Parke-Davis) is a solution of the pressor factor
of the posterior lobe of the pituitary and is practically free
from the oxytocic principle. A gross pharmacological dose of
the hormone was used in the present experiments for two reasons:

1. By reference to the previous work of Dodds et al. (1934) in
    which an equivalent high dose was used.

2. To produce lesions of sufficient extent to evaluate more
    readily the vascular factors involved.

From the results obtained it would appear reasonable to
consider the development of the gastric lesion in three separate
stages: a stage of necrosis, a stage of mucosal and submucosal
haemorrhage, and a stage of inflammation.

A remarkable feature of the pitressin lesion is the
rapidity of onset of necrosis in the acid-secreting mucosa.
Animals treated with 400 units showed necrosis in 30 minutes,
and even with 100 units extensive necrotic areas were present
at 15 minutes.   A prominent feature of this early stage was
the defective staining of erythrocytes in the superficial
mucosal capillaries, confirmed in routine sections and benzidine
preparations.   This contrasted with the normal tinctorial
properties of red corpuscles in the deep mucosal vessels.
These appearances suggested a cessation of capillary circulation
in the acid-secreting mucosa, since if a flow existed at the
early stage, the content of the superficial capillaries would be
constantly replaced by healthy erythrocytes from the deep mucosal
vessels.   Carbon arteriography confirmed the lack of a normal
circulation in the acid-secreting mucosa since no carbon was
propelled by cardiac action into the necrotic zone.   Ischaemia
of the fundal mucosa appeared to be the important primary factor
in the development of pitressin necrosis.   This would account
for the rapidity of onset of superficial mucosal necrosis, which
may become generalized throughout the fundus or confined to
irregular islets of mucosa with intervening healthy areas, the
pattern depending upon the distribution of the ischaemia.

Epithelial necrosis was localized in the first instance to
the superficial fundal mucosa and it is likely that some factor
supplemented the effects of ischaemia.   The earliest signs of

damage were observed in the surface mucus-secreting and immediately subjacent cells, which are in closest proximity to the gastric secretions. The arrest of the blood-flow, with the action of the gastric juice, would account for the rapid onset and the localization of the necrosis. Many of the cells in the deep mucosa showed no evidence of necrosis possibly because they were sufficiently far removed from the gastric juice to escape its adjuvant necrotizing effect and did not suffer irreversible damage from the ischaemic episode alone. Sheehan and Moore (1952), in their analysis of the sequence of changes which occur in the development of renal cortical necrosis, state that ischaemia lasting quarter to half-an-hour was sufficient to damage the glomerular filter with the production of albuminuria. Ischaemia of 2-3 hours' duration caused death of the proximal convoluted tubules. These observations accord with the results reported here. Ischaemia of a quarter to half-an-hour's duration is sufficient to cause necrosis of the superficial gastric epithelium in the presence of an acid medium.

The second or haemorrhagic stage of the lesion develops with the re-establishment of the mucosal circulation through a vascular system damaged by ischaemic and chemical necrosis. With continued haemorrhage through the weakened capillary walls, the fully developed lesion resembles a haemorrhagic mucosal infarct. Finally, in the third stage of the lesion, leucocytes

invade the necrotic tissue.

From these experiments there is no evidence to support the original hypothesis that the operation of an arteriovenous shunt mechanism formed the basis of the gastric lesion. An increase of submucosal channels carrying injected carbon and by-passing the mucosa was not seen. The absence of a fundal circulation could be explained either on the basis of a direct vascular spasm, or a prolonged increase in tonus of the muscle coat at the fundus so impeding blood flow. Both views are not inconsistent with the known physiological effects of pitressin. It is remarkable, however, that the ischaemia was confined to the region of the acid-secreting mucosa while a normal circulation proceeded through the remainder of the intestinal tract, a finding similar to that of Basu Mallik (1955) using pilocarpine nitrate in rats. Metz (1938-39), in direct observations on the gastric mucosa, noted that the initial response to pitressin was marked constriction of arteries and veins with blanching of the mucosa. It is interesting to note also that Wolf and Wolff (1943), in studies on their patient with a permanent gastric fistula, found that the administration of pitressin resulted in pallor of the mucosa, followed by secondary hyperaemia.

Bishton (1950) used a post-mortem arteriographic technique, similar in principle to the Prussian blue method, to study the vascular changes in gastro-duodenal ulcers produced by pilo-carpine hydrochloride in guinea-pigs. Within the limitations

of the method, he concluded that the lesions were the result
of mucosal ischaemia.   Basu Mallik (1955), in a carefully
controlled investigation of the factors involved in gastric
lesions produced by pilocarpine nitrate in rats, came to the
conclusion that they were caused by ischaemia following an
increase in muscle tone in the stomach associated with active
and prolonged peristalsis.   In investigations on acute histamine
erosions in guinea-pigs Williams(1951) suggested from the
character and distribution of the lesions that they were
primarily vascular in origin, although no arteriographic studies
were carried out.   It is not improbable that several of these
experimental procedures used to induce peptic ulcer can be
explained on a common pathogenesis involving increased smooth
muscle tone, either of vessels or gastric muscularis.

Recent work has shown that arteriovenous shunts exist in
the submucosa of the human stomach, but there is some doubt
(Doran, 1951; Crane, 1954) as to the validity of the original
experiments of Barclay and Bentley (1949).   The experiments of
Walder (1950) throw light on the existence of gastric vascular
shunts.   Perfusing fresh gastrectomy specimens at 37°C through
a cannula in the right gastro-epiploic artery, he introduced
glass spheres of 160 µ mean diameter into the perfusate and
collected a roughly comparable number in the outflow through the
right gastro-epiploic vein.   Communicating channels of such a
calibre must therefore exist within the gastric vascular system.

Their dissection was demonstrated by Barlow (1951) and Barlow et al. (1951) as more or less direct channels situated in the submucosa linking arteries destined to supply the mucosa with veins draining the submucosal plexus. Furthermore the anastomoses were distributed in equal numbers throughout all areas of the submucosa. The application of this knowledge of vascular anatomy to gastric physiology and pathology is still speculative.

It is not the purpose here to develop an hypothesis which involves posterior pituitary hormones in the aetiology of human peptic ulcer, although such a claim has been put forward in the past (Harvey Cushing, 1932). Also it would be premature here to discuss the role of the anterior pituitary and adrenal glands, although an increase in function was suggested in the results of the examination of the liver and adrenal glands in the present experiments. Rather the pitressin lesion has been regarded as a basis for further hormonal studies in peptic ulcer and this is elaborated in Part II.

SUMMARY

1. The subcutaneous injection of pitressin in the rabbit produces pathological changes in the acid-secreting mucosa of the stomach.

2. They are characterized by rapid superficial mucosal necrosis, followed by haemorrhage and inflammation.

3. The initial necrosis is the result of ischaemia of the fundal mucosa supplemented by the chemical destructive action of the

gastric juice.

4. Haemorrhage is produced by the re-establishment of the circulation through a damaged mucosal vascular system.

5. There is no evidence to support the hypothesis that gastric arteriovenous shunts play a significant role in the pathogenesis of the pitressin lesion.

PART II

THE EFFECTS OF CORTISONE AND ADRENOCORTICOTROPHIC

HORMONE ON THE STOMACH

THE EFFECTS OF CORTISONE AND ADRENOCORTICOTROPHIC
HORMONE ON THE STOMACH

Within recent years numerous reports have appeared of the re-activation or first appearance of peptic ulcers in patients treated with cortisone or adrenocorticotrophic hormone (ACTH) for some other disease. The morbidity and mortality from gastro-duodenal complications represent a recognized clinical hazard, even with the newer cortisone derivatives. The problem was reviewed by Gray and his associates (1956):-

"The incidence of ulcer production in any large series of patients being treated with adrenal steroids approximates 5% and has been reported as high as 17.5% depending upon the duration and dosage of steroid administered. There is a prevalence of duodenal ulcer over gastric ulcer in the cases reported. Peptic ulcer was found in 7.5% of 477 patients maintained on steroid therapy and in 16.6% in a small series of patient receiving the new synthetic steroids, prednisolone and prednisone. In a review of the literature there appears to be a high incidence of perforation (27.7%) and haemorrhage (33.8%)."

It was decided, therefore, to test the effects of these hormones on the stomach and, in particular, on pitressin-induced peptic ulcers (Crane and Duncan, 1956). Three series of experiments were designed.

1. A study of the effects of cortisone and ACTH on

pitressin lesions of the rabbit's stomach, using a
very much smaller dose of pitressin than previously.

2. An investigation of the effects of cortisone on
pitressin gastric lesions in adrenalectomized and
non-adrenalectomized rats.

3. A study of the effects of cortisone and ACTH on the
stomach of the intact rat, treated with and without
antibiotics.

THE EFFECTS OF CORTISONE AND ACTH ON PITRESSIN
LESIONS OF THE RABBIT'S STOMACH

MATERIALS AND METHODS

Experimental Design

Adult rabbits of both sexes weighing approximately
2.0-2.5 Kg. were used.   Histological studies were carried out
on 93 animals in 5 groups (Table 2).   A further 34 rabbits
were used for bacteriological and histological studies in 2
groups of 17 treated as in subgroups 1A and 2A.

Cortisone acetate (Roussel) or Acthargel (Armour) was
administered intramuscularly each day and pitressin (Parke-Davis)
injected intravenously _via_ the ear vein.   Treatment with
cortisone or ACTH was started 3 days before and was continued
throughout pitressin treatment.   Pitressin was injected daily
for 5 days.

The adrenals, spleen and thymus were removed and weighed
and this served to control hormone dosage (Table 3).

TABLE 2

Summary of experiments

Group	No. of animals	*Dose of Pitressin (units)	≠Dose of Cortisone (mg.)	≠Dose of ACTH (units)
1A	14	20	-	-
1B	6	10	-	-
1C	8	5	-	-
2A	25	20	15	-
2B	6	10	30	-
2C	7	5	15	-
3A	7	20	-	20
3B	6	20	-	40
4	8	-	15	-
5	6	-	-	20

* Daily for 5 days.

≠ Daily for 8 days.

TABLE 3

Mean body and organ weights in rabbits treated
with pitressin, cortisone and ACTH

Group	Body weight g. Initial	Final	Thymus mg.	Spleen mg.	Adrenals mg.
1A	2261	2268	2540	1006	302
1B	2261	2342	2120	1042	280
1C	2606	2564	2529	980	217
2A	2356	2356	750	630	148
2B	2359	2359	1213	465	163
2C	2506	2383	1350	497	138
3A	1987	1875	1347	1085	192
3B	1993	1792	1138	476	468
4	2098	2160	1280	666	215

Animals

The rabbits were fed a compound feeding mixture (diet No.18,
Bruce and Parkes, 1946) with greens and tap water, all of which
were available throughout the experiments.   They were killed by
ether inhalation.

Histological methods

The 127 rabbits examined histologically were treated in the
following manner.   The stomach with lower oesophagus and
proximal duodenum were excised immediately after death and
opened along the greater curvature.   After removal of stomach
contents, the specimen was pinned out on cardboard and fixed in
10% neutral formalin.   Some 15 blocks were taken from each
specimen, including fundal and body areas, pyloric antrum,
curvatures, oesophagus and duodenum.   The blocks were fixed for
a further 24 hours and processed to paraffin.   Haematoxylin and
eosin preparations were made and selected sections were examined
by van Gieson's method and by Gram's method for organisms.

Bacteriological methods

In the 34 rabbits studied bacteriologically a portion of
the diseased acid-secreting mucosa was excised immediately after
death and placed in a sterile container.   It was washed 3 times
in sterile normal saline, a fresh container being used for each
wash.   The specimen was cut into 3 portions.   One fragment was
used to inoculate the butt of a double strength agar blood plate
for anaerobic culture.   Another was used similarly to inoculate

a blood agar plate and then was dropped into Robertson's broth.
The third fragment was smeared on a slide for examination by
Gram's method.   In a few cases the gastric contents also were
examined.   All strains of <u>Clostridium welchii</u> were identified
by Nagler's reaction and, in addition, culture filtrates from
strains obtained from 19 of the 34 rabbits were sent to Dr.
Warrack of the Wellcome Research Laboratories for complete toxin
analysis.   The stomachs were also examined histologically as
described above.

<div align="center">RESULTS</div>

As a result of the administration of small doses of
pitressin, either alone or combined with cortisone or ACTH,
clearly defined lesions formed in the acid-secreting mucosa of
the rabbit's stomach.   The most common were mucosal erosions
and acute gastric ulcers.   In some animals bacterial invasion
of the lesions was pronounced and in a few others perforation
of an acute ulcer occurred.

<u>Erosions</u>

A lesion which involved the gastric mucosa without
penetration of the muscularis mucosae was termed an **erosion**.
They were present in 17 (61%) pitressin-treated rabbits (Group 1),
in 36 (95%) animals which received cortisone and pitressin
(Group 2), and in 13 (100%) where pitressin was given with ACTH
(Group 3).   There was considerable variation in the number and
depth of erosions in animals of any one group, but they were more

numerous in Groups 2 and 3 and here a deeper mucosal lesion was encountered (Figs. 28, 29 and 30). The degree of polymorph reaction also varied but in many instances it was depressed by cortisone or ACTH treatment. Where, however, bacterial colonization of the erosions was prominent a leucocyte response occurred and was of intensity equal to that of animals treated only with pitressin.

Acute ulcers

A lesion which penetrated the muscularis mucosae was termed an ulcer. Acute ulceration was observed in only 8 animals (28.5%) in Group 1; usually 1 or 2 ulcers were seen but in one animal there were 7 acute ulcers. In contrast 31 rabbits (86%) treated with both pitressin and cortisone (Group 2) showed multiple acute gastric ulcers; the presence of 6 to 12 ulcers was common and in one animal (Group 2A) 18 were identified (Figs. 28, 29 and 30). In 4 animals (Group 2A) the entire acid-secreting area was necrotic (Fig. 31). A similar increased incidence of ulcers was noted with pitressin and ACTH (Group 3); 12 animals (92%) showed gastric ulceration (Fig. 28).

The histological character of the lesions also differed. In the ulcers induced with pitressin alone there was an abundant polymorph reaction at the surface, while the base was composed of well-formed granulation tissue (Fig. 32). The surface epithelium at the edge of the ulcer showed regeneration and commencing re-epithelialization was sometimes seen at the margin.

There was never any involvement of the external muscle coat of
the stomach in this series (Group 1) and perforation was not
encountered.

In contrast, ulcers produced by pitressin and cortisone or
ACTH (Groups 2 and 3) showed a characteristic depression of
cellular reaction (Fig. 33).   The surface of the ulcer was
formed by necrotic remnants of the muscularis mucosae and
necrotic collagen fibres of the submucosa.   Furthermore, in
some ulcers there was also necrosis of the superficial layer of
the external muscle coat.   In the majority the cellular response
consisted of a scanty polymorph infiltration with scattered
collections of erythrocytes.   The formation of granulation
tissue in the base was markedly depressed and in some almost
completely absent.   Fibroplasia, when present, was seen usually
in the submucosa at the margin of the lesion deep to the
regenerating gastric epithelium.

Acute ulcers perforated in 3 animals in Group 2A (pitressin
and cortisone) but in none treated with pitressin and ACTH.   In
each case the perforated ulcer was situated in the fundal region
towards the greater curvature.   The perforations were loosely
occluded by omental adhesions and only a local peritonitis was
present (Fig. 34).

Bacterial invasion - Histological assessment

A microscopic assessment was made in Groups 1, 2 and 3 by
examining tissue sections stained by Gram's method.   Organismal

invasion, strictly limited to the surface of the necrotic tissue of erosions and acute ulcers, was seen in 4 (14%) of the 28 pitressin-treated rabbits - all of which received high doses of pitressin (Group 1A).   In contrast bacterial invasion was enhanced in animals treated with pitressin and cortisone.   Of the 36 rabbits with abnormal stomachs in Group 2 (Fig. 28), 14 (39%) showed different degrees of bacterial penetration of the gastric lesions.   In 4 rabbits in this group very intense bacterial invasion caused replacement of the entire acid-forming mucosa by a necrotic, friable, haemorrhagic tissue containing numerous gas bullae (Fig. 31).   Histologically, the mucosa and submucosa showed extensive necrosis and were disrupted by large gas spaces in the walls of which great numbers of organisms were present.   In the submucosa there was a marked acute inflammatory exudate.   In the remaining 10 animals bacterial invasion, although severe, was less extensive and the submucosa was involved only in one instance (Fig. 35).   Mucosal invasion was the main characteristic in the remaining 9 rabbits.   The depth of penetration varied, but in many erosions organisms penetrated almost to the muscularis mucosae.   The polymorph reaction was moderately intense and formed a zone separating the bacteria-laden necrotic tissue from the surviving gastric glands at the periphery of the lesion.

Examination by Gram's method showed 3 distinct morpho-logical types of bacteria in the enhanced invasion in Group 2

animals.   The most numerous organisms were short, rather thick Gram-positive bacilli (Fig. 36).   Long Gram-negative bacilli were also present, usually in smaller numbers but occasionally predominating, while small Gram-positive cocci were relatively scanty.

Bacterial invasion, although present, was not as prominent in rabbits treated with pitressin and ACTH (Group 3).   In one animal the mucosa was invaded to a moderate extent, while in 3 there was only superficial contamination.   In no instance was there evidence of submucosal bacterial invasion.

Bacteriological assessment

The organism most commonly isolated from the 34 rabbits examined bacteriologically was Cl. welchii but lactose-fermenting coliform bacilli were cultured almost as often.   On plate culture these two organisms usually showed a heavy growth whereas the other organisms which were isolated less frequently – micrococci, Streptococcus viridans, anthracoid bacilli, and proteus – were found only in small numbers.

The isolation of Cl. welchii was used to assess the degree of bacterial colonization of the lesions.   Positive cultures were further sub-divided according to whether the organism was isolated from direct plate culture and from Robertson's medium (indicating heavy colonization), or from the latter medium alone (slight colonization).   By such means (Fig. 37) a rough quantitative estimate of the degree of bacterial invasion was

obtained.   Examination of individual culture plates confirmed
the intensity of the invasion in rabbits treated with both
pitressin and cortisone (Figs. 38 and 39).   Plate and broth
cultures of some specimens of gastric tissue were sterile and
a number of plate cultures showed only very scanty colonies of
anthracoid bacilli.   This suggested that repeated washing of
the tissue before culture removed surface contaminants
adequately and that the organisms grown reflected fairly
accurately the degree of colonization of the diseased acid-
secreting mucosa.   The 19 strains of <u>Cl. welchii</u> which were
fully examined were all typical type A strains producing α ,
θ, κ and in some cases μ and γ toxins.   It was particularly
noted that all the strains produced collagenase (κ) and that
8 of the 19 produced hyaluronidase (μ).   A histological assess-
ment of bacterial invasion of the gastric lesions in each animal
closely paralleled the bacteriological assessment based on the
number of <u>Cl. welchii</u> grown in culture.

<u>Effects of varied dosage of pitressin,
cortisone and ACTH on the gastric lesions</u>

The experiments showed the effects of varying dosage of
pitressin, cortisone and ACTH.   14 animals of Group 1A received
20 units of pitressin daily and the stomach was normal in 3 and
abnormal in 11.   With 10 units daily (Group 1B) gastric lesions
were present in 4 of the 6 rabbits; and when the dose of
pitressin was reduced to 5 units daily (Group 1C) only 2 of the

8 rabbits showed mucosal erosions.

In contrast reduction of .the daily dose of pitressin in pitressin-cortisone treated rabbits (Group 2) did not diminish the number of abnormal stomachs. All animals in experiments 2B and 2C had gastric lesions and in some of them the lesions were as widespread as those with higher pitressin dosage (Group 2A). Increasing the daily dose of cortisone from 15 to 30 mg. (Group 2B) did not result in an increased incidence of perforation. Similarly, an increase in the dose of ACTH from 20 to 40 units daily in Group 3 failed to produce perforation of the acute gastric ulcers.

Controls

In these experiments the daily intramuscular administration of cortisone (Group 4) or ACTH (Group 5) for 8 days did not result in any gross or microscopical abnormality of the stomach.

<div align="center">SUMMARY</div>

The results of these experiments will be discussed at the end of this chapter and only a short summary will be given here.

The injection of small amounts of pitressin intravenously caused erosions and acute ulcers in the acid-secreting mucosa of the rabbit's stomach. The extent and depth of the lesions were increased by the administration of cortisone intramuscularly. Invasion of the stomach by bacteria, in particular by Cl. welchii and coliform bacilli, was increased. Perforation resulted in a few cases.

ACTH intramuscularly also increased the area and depth of the gastric lesions. Bacterial invasion was only slightly enhanced and perforation did not occur.

THE EFFECTS OF CORTISONE ON PITRESSIN GASTRIC LESIONS IN ADRENALECTOMIZED AND NON-ADRENALECTOMIZED RATS

A small experiment was designed to test the effects of pitressin and cortisone in another laboratory animal. The rat was chosen and the results largely confirm those established in the rabbit. In addition a small group of adrenalectomized rats was studied.

MATERIALS AND METHODS

Young adult albino rats of the Wistar strain weighing approximately 300 g. were used. They were fed a compound feeding mixture (diet No. 41; see Appendix) and drank tap water.

They were divided into 3 groups:

Group 6 - 9 rats received 10 units of pitressin (Parke-Davis) subcutaneously daily for 5 days.

Group 7 - 12 rats were injected with 20 mg. of cortisone (Roussel) intramuscularly daily for 8 days. Over the final 5 days 10 units of pitressin were injected subcutaneously each day.

Group 8 - 7 adrenalectomized rats were treated with pitressin and cortisone as in Group 7.

The operation of adrenalectomy is described in the Appendix.

The histological methods used were those described in the previous section.

RESULTS

The results are mainly confirmatory and will not be described in detail.   They are summarized in Figure 40. Pitressin alone (Group 6) caused mainly erosions of the fundal mucosa and only 3 rats (33%) showed acute ulcers.   With pitressin and cortisone (Group 7) a larger area of fundal mucosa was involved and the incidence of acute ulcer was increased to 66%.   Bacterial invasion also was enhanced and in some rats acute abscesses formed in the mucosa.   A similar pattern of changes was seen in the stomachs of adrenalectomized rats (Group 8) and the incidence of ulcer rose to 86%.   Perforation of an acute ulcer did not occur in any group.

THE EFFECTS OF CORTISONE AND ACTH ON THE STOMACH OF
THE RAT, WITH AND WITHOUT ANTIBIOTICS

In the preceding sections it was established that cortisone or ACTH intensified the pitressin gastric lesion in rabbits and rats, but had no effect on the stomach of control rabbits over the relatively short period of 8 days.   In 1951 Ingle, Prestrud and Nezamis reported the occurrence of small pyloric ulcers in rats treated with prolonged cortisone administration.   A similar effect was obtained with ACTH (Ingle, Prestrud and Li, 1951) but only gross observations were made on the stomach in both experiments.   It seemed important, therefore, to test the effect of prolonged hormone treatment using more critical methods.

In addition, it was decided to study the effect of antibiotic treatment in view of the bacterial invasion reported previously in this section.

MATERIALS AND METHODS

Young adult male rats of the Sprague-Dawley strain weighing approximately 300 g. were used. They were fed a diet of Archer Dog Pellets and tap water ad libitum.

Cortisone acetate (Merck & Co.) was injected subcutaneously in a 5 mg. dose each morning and evening. Depo ACTH (Upjohn) was given similarly in a 10 unit dose twice daily. Rats on antibiotics received crystalline penicillin G (Upjohn) 5,000 units and streptomycin sulphate (Merck & Co.) 5 mg. by subcutaneous injection each morning.

The rats were divided into 6 Groups of 6 rats as shown in Table 4. The effect of treatment was studied for 28 and 56 days.

The histological methods used were those described previously.

The left adrenal gland was weighed at the end of each experiment and served as an indicator of hormone dosage.

RESULTS

The results are shown in Table 4.

All the rats lost weight. Those treated with cortisone for 56 days (Groups 11 and 12) lost one-third of their initial body weight.

TABLE 4

Body weight, adrenal weight, and gastric lesions
in rats treated with cortisone, ACTH, and anti-
biotics, mean and standard deviation

Group	Treatment	Body weight g.		Left Adrenal mg.	Incidence of erosions
		Initial	Final		
9	Cortisone X 28 days	303 ±0.6	222 ±13.2	10.0 ±0.4	1 rat
10	Cortisone and antibiotics X 28 days	299 ±0.7	223 ±11.7	10.1 ±0.54	2 rats
11	Cortisone X 56 days	300 ±0	206 ±23.2	9.4 ±1.8	1 rat
12	Cortisone and antibiotics X 56 days	301 ±1.0	204 ±23.6	8.8 ±2.26	2 rats
13	ACTH X 28 days	305 ±6.5	272 ±14.6	100.2 ±23.2	None
14	ACTH X 56 days	308 ±6.7	254 ±30.6	112.0 ±23.2	2 rats

Marked adrenal atrophy followed cortisone treatment while ACTH administration resulted in gross adrenal hypertrophy.

Gastric lesions were seen in a few rats (Table 4). Our observations differ from those of Ingle and his associates. The lesions were erosions confined to the acid-secreting mucosa. Ulcers in the pylorus were not observed microscopically. Each rat affected had 1 or 2 gastric erosions, but in an animal treated with cortisone and antibiotics (Group 10) 5 were present. The character of individual lesions showed little variation. Necrosis was confined to the superficial part of the mucosa. The surrounding capillaries were congested but a polymorph leucocyte reaction was absent. Beneath the muscularis mucosae a few macrophages and fibroblasts were present (Figs. 41 and 42). Bacterial invasion of the lesions was of slight degree and antibiotic treatment did not modify this feature. Collections of yeast-like cells in the mouths of the pyloric glands were common in all groups (Fig. 43).

DISCUSSION

Erosions and ulcers

Localized lesions, either erosions or acute ulcers, result from the repeated administration of small doses of pitressin to rabbits and rats. Presumably they are produced by repeated ischaemic episodes similar to the mechanism described in Part I. When cortisone or ACTH is injected in conjunction with pitressin, the gastric lesion, although confined to the acid-secreting

mucosa, is more widespread and penetrates more deeply, and the incidence of acute ulceration is increased. Furthermore in the doses employed here it would appear that cortisone is rather more effective in this respect than ACTH. These hormones augment the gastrotoxic action of pitressin. This may be due to a true potentiation of the pitressin lesion, or to an inhibition of healing, or some other mechanism may be involved.

Green (1954) is of the opinion that the more widespread lesion may be a manifestation solely of depressed healing. There is no doubt that cortisone and ACTH depress the formation of granulation tissue in the base of the acute gastric ulcers which result from the administration of pitressin with one of these hormones. Numerous papers report a similar inhibition under diverse condition in man and laboratory animals (Ragan, Grokoest and Boots, 1949; Ragan, Howes et al., 1949; Creditor et al., 1950; Spain et al., 1950; Barber and Nothacker, 1952; Lattes, Blunt et al., 1953; Lattes, Jesser et al., 1953; Williams, 1954; Baker and Abrams, 1955; Curran, 1956). All agree that cortisone and ACTH diminish but do not completely depress the cellular responses of the host to the noxious stimulus. In the gastric ulcers of pitressin-cortisone or pitressin-ACTH treated animals in the present series the lack of granulation tissue in the centre of the ulcer crater was characteristic, while deep to the regenerating gastric epithelium

at the ulcer margin a scanty proliferation of fibroblasts, capillaries and macrophages was common. This localization of the reparative tissue is of interest in view of the work of Lattes, Blunt et al. (1953) on the healing of incised skin wounds in cortisone-treated rats. They observed that when epithelialization occurred a narrow zone of fibroblasts was seen beneath the basal layer of regenerated epithelium but not in the deep layers of the wound. They suggested that this localization might be related to the mucopolysaccharides produced by regenerating squamous epithelium at the surface of the wound. The distribution of granulation tissue in the pitressin ulcers modified by cortisone or ACTH closely parallels the observations of Lattes and his associates.

In addition to depression of healing it is possible that the more extensive gastric lesion of pitressin-cortisone treated animals may be due to a true potentiation of the ischaemic effect of pitressin. This would appear to be substantiated by the results of Groups 1C and 2C rabbits in which the dose of pitressin used was 5 units daily. With this small amount of pitressin alone (Group 1C), only 2 of 8 rabbits showed mucosal erosions; acute ulcers were absent and in particular there was no evidence of healed lesions. When, however, a similar level of pitressin dosage was supplemented by cortisone (Group 2C) all 7 rabbits had gastric lesions, 5 showed acute ulcers and erosions and 2 showed erosions only. Furthermore, there is evidence

that adrenocorticotrophic and adrenocortical hormones influence the dynamics of the circulation in man and experimental animals in pressor states other than those resulting from pitressin administration (Ingle, 1950; Selye, 1950; Brust et al., 1951; Kurland and Freedberg, 1951; Ramey et al., 1951) and also increase smooth muscle tone in the alimentary canal (Streeten, 1950; Streeten et al., 1957). It is possible that the more extensive gastric lesion may be a result, not only of depressed healing, but also of some other factor, possibly related to increased tonus of unstriped muscle in vessel or stomach wall. There is no absolute proof of the operation of such a mechanism in this series, however, and this aspect of the problem remains unsolved.

Perforation, as a complication of acute gastric ulceration, occurred in only 3 rabbits treated with pitressin and cortisone (Group 2A). It did not occur in rabbits receiving pitressin and ACTH nor in rats treated with pitressin and cortisone. This low incidence of perforation was not influenced by an increase in the dosage of cortisone or ACTH (Groups 2B and 3B). Lattes, Blunt et al., (1953) in experiments on the healing of fractures in cortisone-treated rabbits, found that the full depressant effect on healing was not exhibited until the fourth day of cortisone treatment. In a personally conducted experiment on a small group of rabbits in which combined pitressin-cortisone administration was continued for 8 days, no

perforations occurred and the microscopical appearances were in no way different from those described after 5 days combined treatment.

Bacterial invasion

Bacterial invasion of the diseased gastric mucosa, assessed by histological and bacteriological methods, was markedly enhanced in rabbits treated with pitressin and cortisone.   A similar feature was observed in rats.   This enhancement appeared less prominent when ACTH, in the dosage used here, was administered with pitressin.   Cl. welchii and coliform bacilli were predominant in the gastric lesions and in some rabbits the diseased mucosa was disrupted by gas, presumably formed by the bacteria.   The presence of Cl. welchii in the stomach of the rabbit is almost certainly due to the habit of this animal of ingesting its own excreta.   The same organism has been found in the gastric contents of man after partial gastrectomy (Howie et al., 1953; Duncan et al., 1954) but it is still uncertain how the organism reaches the gastric remnant or what clinical significance its presence may have.   It is not unreasonable to suppose that the presence of Cl. welchii in the gastric remnant after gastrectomy may be due to some degree of temporary post-operative reduction in gastric and intestinal motility. Recently there has been a revival of interest in the relation of Cl. welchii to various intestinal disorders including post-operative diarrhoea and necrotizing enteritis.   Series of cases

of the latter condition following partial gastrectomy have been described by Williams and Pullan (1953), and Dawson-Edwards and Morrissey (1955). The older literature was reviewed by Penner and Bernhein (1939) who emphasized the factor of shock in the aetiology of necrotizing enteritis. Bruce (1954) is of the opinion that such disorders are due to mucosal ischaemia which results from spasm of arterioles in the submucosa of the intestine. It was established that pitressin produces ischaemia of the fundal mucosa of the rabbit (Part I), and it seems probable that ischaemia so produced reduces oxygen tension in the mucosa and provides a suitable medium in which Cl. welchii can flourish in the gastric lesions of pitressin-treated rabbits. Similarly in man reduced oxygenation may favour the growth of these organisms. Although cortisone increased invasion of the gastric mucosa by this anaerobe, one can only speculate about the possible mechanisms involved. As discussed above cortisone may potentiate the ischaemic effect of pitressin and so further intensify the anaerobic conditions in the diseased gastric mucosa. In addition the enhanced bacterial invasion may be a result of the depression by cortisone of the cellular and humoral responses to infection. Finally there is the effect of cortisone in reducing the amount of ground substance produced in healing wounds (Lattes, Blunt et al., 1953; Lattes, Jesser et al., 1953; Curran, 1956). It could well be that hyaluronidase and collagenase elaborated by Cl. welchii might further reduce the

ground substance available for healing of the experimental gastric ulcers.

The hypothalmic - pituitary - adrenal - gastric axis

The concept of the "hypothalmic - pituitary - adrenal - gastric axis" was introduced by Seymour Gray and his associates from Harvard in 1950 and was presented in a more elaborate form in 1955-56. Since their hypothesis is apposite to the present discussion and to succeeding results it will be dealt with in some detail here.

It has long been believed by physiologists that two principal pathways exist for the stimulation of gastric acid secretion and pepsin. The first or neurogenic pathway is centred in the anterior hypothalamus and exerts its control _via_ the vagus nerve. The second mechanism is controlled by the release of the gastric hormone, gastrin, from the antral mucosa.

A second hormonal phase of gastric secretion mediated through the adrenal gland to the stomach by way of the posterior hypothalamus and pituitary was postulated by Gray _et al_. (1951) following the observation in man of an increase in gastric hydro-chloric acid and pepsin secretion during ACTH stimulation. There was a concomitant rise in the output of uropepsin in the urine. The hormonal pathways involved in these reactions are illustrated in Figure 44. This gastric response to ACTH stimulation is not dependent on an intact vagus nerve since it occurs in vagotomized patients (Gray _et al_., 1953), and is not

altered by the administration of anticholinergic drugs, such as atropine. Furthermore, it is not dependent on the antral phase of gastric secretion since a similar response occurs in patients following subtotal gastrectomy and antrectomy.

It is well known that peptic ulcer patients, especially those with ulcers of the duodenum, show an increase in the basal secretion of hydrochloric acid and gastric pepsin. Uropepsin values are also increased. Gray and his colleagues (1955-56) have shown that the results obtained from such patients approach the values obtained in continued cortisone or ACTH administration in normal subjects. Studies in patients submitted to the stress of surgical operation, in burns, and in emotional stress also showed a gastric response comparable to that obtained with ACTH stimulation. It does not seem unreasonable, therefore, in Gray's hypothesis to correlate these gastric responses in stressed patients, in normal subjects undergoing ACTH stimulation, and in the lean, worried, anxious "ulcer type" of patient seen so commonly in the clinic.

There is much suggestive evidence in the literature to support a correlation between stress, hormonal pathways and the stomach. Peptic ulcer is exceedingly rare in patients with long-standing Addison's disease (Jarvis et al., 1954), yet the development of chronic peptic ulcer in an Addisonian patient treated with adrenal steroids has been described (Gray et al., 1955-56). The gastric acidity is raised in Cushing's disease

but returns to normal following adrenalectomy (Kyle et al., 1956). Peptic ulcer not uncommonly complicates a severe stress, such as extensive burns (Curling's ulcer), and the high incidence of gastric complications following adrenal steroid therapy has already been noted.

An hypothesis which embraces many clinical observations and is supported by much experimental data (Welbourn and Code, 1953; Porter et al., 1953; Baker and Bridgman, 1954; Baker and Abrams, 1954) deserves most serious consideration. Illingworth (1956) in discussing the hormonal approach to peptic ulcer has stated:- "At present our understanding of this aspect of the ulcer problem is incomplete, but it has a fascination of its own, because it offers hope for further progress not only in the treatment, but possibly in the prevention, of peptic ulcer."

It is appropriate here to relate the results of Parts I and II of this thesis to current thought on the hormonal aspects of peptic ulcer. There is evidence which supports the view that there is an intimate relationship between the secretion of posterior and anterior pituitary hormones. It has recently been suggested that posterior lobe hormone, especially oxytocin, may influence the discharge of anterior lobe hormone complexes (Benson and Folley, 1957) and Verney has shown that under certain circumstances of stress there is an associated secretion of antidiuretic and adrenocorticotrophic hormones (Rydin and

Verney, 1938; O'Connor and Verney, 1942). Furthermore it is
believed that pitressin may act on the adenohypophysis as a
non-specific stressor (Selye, 1950). By such mechanisms
increased adrenal cortical activity could be expected. The
activation of the adrenal cortex by pitressin as shown by the
bio-assay technique of Symington (1951) in Part I, and the
observations that cortisone and ACTH intensify the gastric
lesions (Part II) suggest that adrenal steroids may play a
prominent role in the genesis of these experimental ulcers.
However, other observations indicate that neither an intact
pituitary nor adrenal cortex is essential. Cutting et al.
(1937b) showed that pitressin produces gastric lesions in the
hypophysectomized animal. In Group 8, pitressin induced peptic
ulcers in adrenalectomized rats maintained on 20 mg. of cortisone
daily, yet a similar dose of cortisone administered alone for
28 or 56 days produced only a few erosions (Groups 9 to 12) and
exerted a profound catabolic effect with marked weight loss.
If adrenal corticoids play a primary causative role it would be
expected that either cortisone or ACTH alone would produce frank
peptic ulceration, but on the contrary ulcers did not result
from prolonged treatment with either hormone (Groups 9-14). It
may well be that adrenocortical hormone plays a supporting or
"permissive" role as postulated by Ingle in many other experi-
mental situations (1953), rather than the role of a primary
causative agent.

Gray's concepts are complementary to the wider hypothesis of Selye concerning the "General Adaptation Syndrome" (1946). The latter described erosions and ulcers of the upper intestinal tract in the "alarm reaction" and "stage of exhaustion" of his syndrome. It seemed important to establish the validity of Selye's experiments relating stress to peptic ulcer. This is dealt with in Part III.

<div align="center">SUMMARY</div>

1. Cortisone or ACTH intensifies the pitressin gastric lesion in rabbits and rats. Healing is depressed and some ulcers perforate. Bacterial invasion is enhanced.

2. This reaction is not dependent on an intact pituitary gland or adrenal cortex.

3. Prolonged treatment with cortisone or ACTH in the rat produces a few gastric erosions but true ulcer does not develop. These changes are not influenced by antibiotic therapy.

4. Current thought on hormonal aspects of peptic ulcer is reviewed. It is suggested that adrenocortical hormone may act as a permissive agent rather than a primary aetiological factor in the genesis of these experimental lesions.

PART III

PATHOLOGICAL CHANGES IN ADRENALECTOMIZED AND

NON-ADRENALECTOMIZED RATS EXPOSED TO COLD

PATHOLOGICAL CHANGES IN ADRENALECTOMIZED AND
NON-ADRENALECTOMIZED RATS EXPOSED TO COLD

The relevant features of Selye's concept of the "General
Adaptation Syndrome" have already been surveyed in the Intro-
duction.   It will be recalled that according to this hypothesis,
adaptive mechanisms, especially those involving adrenal cortical
hormones, may fail during exposure to nonspecific forms of stress
and cause pathological changes which have been termed "diseases
of adaptation."   These include peptic ulcer, hypertension and
nephrosclerosis, rheumatic fever, rheumatoid arthritis, poly-
arteritis nodosa and many other conditions.   Features of some
of these diseases have been reproduced experimentally by over-
dosing animals with certain natural and synthetic steroids and
other hormones (Selye, 1955-56).   These changes are more
likely to occur if the experimental animal has been sensitized
by the removal of one kidney and is given a high intake of
sodium chloride.   Much of the experimental work in this field
has been performed on animals sensitized in this manner.

According to Selye a similar pattern of pathological
changes follows exposure to nonspecific forms of stress.   The
sensitized rat may develop hypertension and nephrosclerosis on
exposure to cold (Selye, 1943).   The development of gastro-
duodenal ulceration in the rat in various stressful situations
has been described (Selye, 1950).

In 1956-57 I accepted an invitation from Professor Dwight
J. Ingle to work in his laboratory on the pathological damage
produced by stress.   This seemed relevant to the problem of
peptic ulceration and the issues raised in the preceding parts
of this thesis.

In the first instance it was decided to study the patho-
logical changes which result from the exposure of sensitized
rats to cold.   The experiments were designed to test Selye's
hypothesis that the lesions are caused by a change in the
secretory activity of the adrenal glands.   Non-adrenalectomized
rats, and adrenalectomized rats treated with a constant intake
of adrenal cortical extract (ACE), were studied.   When the
animals were exposed to cold, pathological changes were produced
in both the presence and the absence of the adrenals, but the
role of the adrenal cortical hormones in causing these changes
was not absolutely established.   However, a hitherto unsuspected
variable was discovered which is highly relevant to previous
publications of other workers in this field.   It was shown that
the amount of high sodium chloride diet eaten by the animal is
an important factor in determining the extent of the pathological
changes.

MATERIALS AND METHODS

Male rats of the Sprague-Dawley strain were maintained on
Archer Dog Pellets until the experiment was begun.   Then all
the rats were sensitized in the manner of Selye by unilateral

nephrectomy (Appendix).   Some of the rats were adrenalecto-
mized, when both glands were removed with as much pedicle as
possible and without breaking the capsule (Appendix).
Following this operation, each adrenalectomized rat received
2 ml. of adrenal cortical extract (Upjohn) in 0.9% saline by
subcutaneous injection each morning and afternoon.   The sham-
operated rats received control injections of saline.   The rats
were given a special high (4%) sodium chloride medium carbo-
hydrate diet (Appendix) to complete the sensitization procedure.
The dry diet was packed into individual Franke animal feeding
jars which were weighed every 24 hours to calculate the daily
food intake.   In one experiment a fluid form of this diet was
fed by stomach-tube each morning and afternoon.

Temperature control

The rats were exposed to cold in a temperature chamber
designed and built under the supervision of the Mechanical
Development Division of the Upjohn Company.   It was cooled by
a sealed refrigeration unit which ran constantly, except for
automatically controlled defrosting for 15 minutes every 6 hours.
The desired temperature was maintained within $1^{o}C$ by a thermo-
static control of heating units built into the air ducts of the
machine.   The animals were housed in 12 individual metabolism
cages contained within the temperature chamber.   Twelve similar
cages were used outside the chamber for the maintenance of
control rats at room temperature ($25^{o}C$).   The initial

temperature of the refrigeration chamber was 25°C. This was lowered 2 degrees each day for 5 days, and then 1 degree every second day until the desired low temperature was reached, and then kept constant throughout the remainder of the experiment. All of the hair was shaved from each cold-exposed rat twice each week.

Histological methods

At the end of the experimental period each rat was anaesthetized with ether and exsanguinated, and autopsy was performed. Gross pathological changes were noted, and the heart, kidney, adrenal glands and thymus were weighed and fixed in Bouin's fluid. One adrenal was fixed in 10% neutral formalin. Sections of the hearts and kidneys were stained routinely with haematoxylin and eosin and Masson's stain. Selected examples were stained by the periodic acid-Schiff technique, Mallory's method, and Weigert's resorcin-fuchsin stain for elastic tissue (Appendix). Frozen sections of the formalin fixed adrenal glands were examined with Sudan black B and Sudan IV for lipids. The stomachs were inspected and suspicious areas taken for histological examination.

Experimental design

In Experiment 1, 24 unilaterally nephrectomized rats ate the dry high-salt diet ad libitum. Twelve of the rats were adapted to low temperature and kept at 3-5°C for 60 days, and 12 similar rats were kept at room temperature for the same

period of time.   These 2 groups were each sub-divided equally into adrenalectomized and non-adrenalectomized rats.   The adrenalectomized animals were each treated with 4 ml. daily of adrenal cortical extract in 0.9% saline, and the non-adrenalecto-mized rats were each given equal amounts of 0.9% saline.

Experiment 2 was a repetition of Experiment 1.

In Experiment 3, 8 unilaterally nephrectomized rats were adapted to the over-feeding of a fluid form of the high sodium chloride diet.   The food intake was increased 1 ml. daily until the level of 26 ml. per feeding was reached and then kept constant for 60 days.   This amount of fluid diet represented approximately as much dry diet as was eaten ad libitum by the cold-exposed rats.

RESULTS

The results are summarized in Tables 5 and 6.   The rats exposed to cold ate more food but gained much less weight than did the rats kept at room temperature.   The rats which were over-fed at room temperature became overweight.

Hypertensive changes in the heart, blood vessels and kidney were found in the cold-exposed animals, irrespective of the presence or absence of the adrenal glands.   Similar changes were also seen in the rats tube-fed the high sodium chloride diet at room temperature.   No other "disease of adaptation," in particular peptic ulcer, was found.

Ratings of renal and cardiac lesions

An attempt was made to assess the degree of renal and

TABLE 5

Body and organ weights of unilaterally nephrectomized adult male rats eating 4% NaCl medium CHO diet _ad libitum_ for 60 days, mean and standard deviation

Experiment	T°C	No. of rats	Body weight g. Initial	Final	Heart mg.	Kidney mg.	Thymus mg.	1 Adrenal mg.	Food g./rat/day
Non-adrenx, shaved	3	10	324 ±6.9	335 ±9.1	1712 ±93.0	2704 ±193.4	201 ±31.9	32.0 ±1.5	29.3 ±0.64
Adrenx, 4 ml. ACE, shaved	3	10	332 ±8.5	340 ±8.2	1650 ±66.3	2649 ±183.9	235 ±29.5	-	26.6 ±0.87
Non-adrenx, not shaved	25	12	339 ±9.1	471 ±8.9	1120 ±85.2	1926 ±56.0	301 ±39.5	25.5 ±0.8	15.2 ±0.32
Adrenx, 4 ml. ACE, not shaved	25	12	329 ±8.5	427 ±5.6	1198 ±100.8	1968 ±82.9	260 ±21.1	-	14.1 ±0.22
Non-adrenx, not shaved; tube-fed fluid NaCl diet	25	8	330 ±7.1	660 ±31.8	1576 ±100.8	2669 ±181.0	49 ±21.9	38.0 ±3.5	31.0

TABLE 6

Rating of pathology in unilaterally nephrectomized adult male rats eating 4% NaCl medium CHO diet ad libitum for 60 days, mean and range

Experiment	$T^\circ C$	No. of rats	Kidney gross	Kidney micro	Heart micro	Food g./ rat/day
Non-adrenx, shaved	3	10	2.6 (1.0-5.0)	3.65 (0.5-5.0)	1.6 (0-3.0)	29.3 ±0.64
Adrenx, 4 ml. ACE, shaved	3	10	3.0 (0.5-5.0)	3.5 (0.5-5.0)	1.5 (0-4.0)	26.6 ±0.87
Non-adrenx, not shaved	25	12	0.6 (0-2.0)	1.2 (0.5-3.0)	0.2 (0-2.0)	15.2 ±0.32
Adrenx, 4 ml. ACE, not shaved	25	12	0.75 (0-1.5)	2.37 (0.5-4.0)	1.4 (0-4.0)	14.1 ±0.22
Non-adrenx, not shaved; tube-fed fluid NaCl diet	25	8		2.78 (0.5-4.0)	1.1 (0-3.0)	31.0

cardiac damage in the different experimental groups. It is appreciated that these ratings are subjective observations and are a relatively crude method of assessment. The correlation between gross and microscopic damage, however, was fairly close in this and subsequent experiments, and appeared to be a reliable indicator of the variation from normal. The method of gross and microscopic assessment will be summarized here and the significance of the changes will be discussed after the presentation of the results.

The ratings of <u>gross renal damage</u> were based on the following scale:

0 - no abnormality.

1 - few small cortical nodules.

2 - moderately nodular, with some mottling.

3 - more nodules, mottling, with some grey spots on the surface of the kidney.

4 - rough, mottled, with grey and red spots.

5 - changes listed previously more severe, giving a "flea-bitten" greyish-yellow appearance.

The ratings of the <u>microscopic renal lesions</u> were based upon the following histological features:

0 - no abnormality.

1 - small foci of periglomerular fibrosis, a few hyaline casts, and some round cells in the interstitial tissue.

2 - active pelvic inflammation.

3 - glomerular lesions, no apparent vascular damage.

4 - hyalinization of glomeruli with hypertensive arteriolosclerosis.

5 - glomerular necrosis with hypertensive arteriolo-necrosis or changes resembling polyarteritis nodosa.

The ratings of the <u>microscopic cardiac lesions</u> were based upon the following histological features:

 0 - no abnormality.
 1 - hypertrophy of muscle.
 2 - focal macrophage reaction or fibrosis in myocardium.
 3 - changes listed previously with hypertensive arteriolosclerosis.
 4 - hypertensive arteriolonecrosis with or without necrosis of cardiac muscle fibres.

<u>Heart lesions</u>

There was no significant difference in the average weights of the hearts of control and adrenalectomized rats at room temperature (Table 5). The average weight of the heart was increased to approximately the same extent in the control and adrenalectomized rats exposed to cold. Over-feeding also caused a gain in heart size of non-adrenalectomized rats kept at room temperature but the gain was roughly proportional to the gain in body weight. One non-adrenalectomized rat exposed to cold had white patches on the heart, but otherwise no gross damage was seen in any heart.

Microscopically the non-adrenalectomized rats maintained at room temperature showed little or no heart damage. All other groups, including force-fed rats, showed a variety of cardiac lesions (Table 6). The most common finding was hypertrophy of the cardiac muscle fibres. In many cases this was accompanied by an increase in Anitschkow myocytes, especially around the adventitia of blood vessels and at the auriculo-ventricular

junction.   Granulomatous lesions took the form either of focal nodules or more diffuse collections of cells.   The latter were situated mainly in the subepicardium or subendocardium (Fig. 45). The cells were predominantly of the macrophage and lymphocyte series; polymorphonuclear leucocytes were scanty.   The focal lesions did not show the classical appearance of Aschoff nodules.

Areas of fibrosis were situated chiefly near the apex of the heart and were most common in cold-exposed and tube-fed rats.   They contained a few haemosiderin-laden macrophages. In several instances in rats exposed to cold, there was a clear association between myocardial fibrosis and proliferative thickening of the smaller coronary arteries (Fig. 46). Necrosis of arteriolar walls was seen occasionally in cold-exposed rats.   The largest coronary arteries appeared normal, apart from one adrenalectomized rat exposed to cold in which there was an eccentric thickening of the arterial wall due to a subintimal collection of foamy macrophages (Fig. 47).   This change was suggestive of an early atheromatous plaque.   Valvulitis was not seen in any rat, but in 14 animals foci of cartilagenous metaplasia were present at the base of the aorta at the origin of the aortic valve (Fig. 48).   Examples of this lesion occurred in all experimental groups and it cannot be said to represent a response to cold-stress.

Kidney lesions

The changes in weights of kidneys paralleled the changes in weights of hearts (Table 5). Exposure to cold and over-feeding caused striking gains in kidney weights but adrenalectomy and treatment with adrenal cortical extract did not.

The kidneys of non-adrenalectomized rats which ate ad libitum at room temperature showed some small pits and nodules (Table 6). The gross damage was somewhat greater for some of the adrenalectomized animals at room temperature; slight mottling and a few grey spots were noted. The over-fed rats of Experiment 3 showed a moderate amount of gross pathological changes. Gross damage was most severe in adrenalectomized and non-adrenalectomized rats exposed to cold; pits, nodules, grey and haemorrhagic spots were observed. Some of the kidneys appeared pale, very rough and "flea-bitten."

As shown in Table 6, the microscopic assessment of renal damage paralleled the degree of gross damage with the exception of the adrenalectomized animals maintained at room temperature. The severity of damage also varied in animals within a group as noted in the Table.

In some rats of all experimental groups mild hypertrophy of nephrons was commonly associated with a few hyaline casts in dilated tubules and small foci of lymphocytes as well as some plasma cells in the interstitial tissue. Near such foci peri-glomerular fibrosis was seen in some cases (Grade 1 renal lesion).

In 9 rats there was active chronic inflammation of the renal pelvis (Grade 2). This showed no predilection for any one experimental group but was not seen in any force-fed rat. Furthermore, pelvic infection appeared to be independent of the glomerular and vascular changes noted in the more severely damaged kidneys.

Glomerular lesions occurred in individual animals of all experimental groups, but were minimal in non-adrenalectomized rats at room temperature and were most severe in rats exposed to cold irrespective of the presence or absence of the adrenal glands (Table 6). Tuft damage varied from a slight increase in intercapillary material with adhesion to Bowman's capsule to complete glomerular hyalinization. Many of the cells of the glomerular tuft were swollen and "foamy" in appearance, and the whole structure appeared relatively bloodless. In some rats the milder type of damage was not associated with any qualitative change in the arterial or arteriolar walls as demonstrated by histological methods (Grade 3). With more severe damage, however, there was a clear correlation between glomerular lesions and proliferative arteriolosclerosis of the afferent arterioles (Grade 4) (Fig. 49). The latter type of lesion was seen in cold-exposed rats, adrenalectomized rats maintained at room temperature, and also in animals force-fed the high salt diet. The most severe kidney damage was noted in rats exposed to cold, both in the adrenalectomized and non-adrenalectomized groups

(Table 6). In these groups necrosis of the glomerular tuft with thrombosis of some capillary loops was associated with arteriolonecrosis of the afferent vessel (Grade 5) (Fig. 50). In one cold-exposed adrenalectomized rat this necrotic change also involved small arteries and produced an appearance resembling in some respects polyarteritis nodosa.

Gastric lesions

Peptic ulcers were not observed in any rat in response to cold stress. Two cold-exposed rats showed minute haemorrhagic points in the gastric mucosa, which on histological examination proved to be small superficial erosions of the acid-forming mucosa. In one rat the adrenal glands were intact, while the other was an adrenalectomized animal maintained on adrenal cortical extract.

Testes, prostate and seminal vesicles

In response to continued cold exposure a decrease in size of the testes, prostate and seminal vesicles was observed in both adrenalectomized and non-adrenalectomized rats. No quantitative measurements were made on these organs. Involution of the sex organs and their accessories in response to stress is a well-recognized experimental phenomenon and is considered to be due to a diminution in gonadotrophin secretion from the anterior pituitary gland (Selye, 1950).

Adrenal glands

The adrenals from cold-exposed rats were slightly larger

than the controls at room temperature. Mild adrenal hyper-
trophy occurred in force-fed rats (Table 5).

The zona glomerulosa in rats maintained on the sodium
chloride diet in the cold and at room temperature was narrow.
It was composed of small cells in which the cytoplasm was dense
and uniform. Lipid in the glomerulosa of both groups was
almost completely depleted, apart from a few granules associated
with the Golgi apparatus (Fig. 51). An increase in width of
the zona fasciculata and zona reticularis was seen in the cold-
exposed rats. Lipid staining in these zones was more abundant
and the cells were slightly larger than the room temperature
controls.

DISCUSSION
The role of the adrenal gland, adrenocortical hormone, and sodium chloride

These experiments confirm Selye's observations that
exposure of the sensitized rat to cold is associated with
increased damage to the heart and to the remaining kidney. They
emphasize, however, that the increase in food-intake is an
important factor determining the extent of this damage.
Sensitized rats kept at room temperature and tube-fed approxi-
mately the same amounts of diet eaten by the cold-exposed rats
(Experiment 3) had more damage than did rats eating ad libitum
at room temperature. However, pathological changes were greatest
in the rats exposed to cold, irrespective of the presence or

absence of the adrenal glands.   It was shown by Ingle and
Baker (1957) that lesions among cold-exposed rats are reduced
when the intake of the high-salt diet is restricted to a normal
level, although their incidence is rather greater than in rats
kept at room temperature.   It seems probable that cold-exposure
increases damage when the intake of the high-salt diet is
constant at either normal or high levels, but that when the
dietary load does change the extent of the variation is
important.

Treatment of the adrenalectomized-sensitized rat with
adrenal cortical extract at a level of 4 ml. per rat per day
apparently places the animal at a disadvantage in dealing with
a high salt load as compared to similar rats having their adrenal
cortices intact.   The adrenalectomized rats given this
substitution therapy and kept at room temperature had more
microscopic lesions in the hearts and kidneys than non-adrenalecto-
mized rats.   These differences were not evident grossly and were
not reflected by differences in organ weights (Table 5 and 6).
Crane, Porter and Ingle (1959) have reported on the effects of
adrenal cortical extract in the adrenalectomized, sensitized rat,
and showed a dose-response relationship between hormone
administration, blood pressure elevation and pathological changes
in the heart and kidney.   The unilaterally nephrectomized rat on
a high salt load is very sensitive to the level of adrenal cortical
hormone administered.   Doses of extract smaller than 4 ml. per

rat per day are insufficient to maintain life in adrenalecto-
mized cold-exposed rats, at least during the early stages of
adaptation to cold. It seems probable that the animal on a
fixed intake of adrenal cortex extract is unable to regulate its
balance of steroids towards the maintenance of homeostasis. The
observation in non-adrenalectomized rats that the zona glomeru-
losa was narrow, lipid depleted and composed of smaller than
normal cells may be indirect evidence that the adrenal cortices
undergo changes which favour the maintenance of homeostasis of
electrolytes. It may be that in the presence of a high salt
load the adrenal cortices can suppress their secretion of
sodium-retaining-steroids without the suppression of cortisone-
like steroids. These considerations are consistent with the
observations of Deane, Shaw and Greep (1948) and Bartter (1956)
relative to the role of the zona glomerulosa in the secretion of
sodium-retaining steroids.

The objective in this study was to design an experiment
having only one variable under investigation at a time, but this
was not successful. Food intake remained inconstant. When
rats are exposed to cold they must eat more food or they will
die. The possibility of tube-feeding all of the rats at the
level consumed voluntarily by the cold-exposed animals was
considered. This was unsuccessful since adrenalectomized or
non-adrenalectomized rats which are tube-fed die when exposed to
cold. The stressed rats cannot handle a high salt load imposed

suddenly twice daily, whereas they tolerate the same total amounts consumed gradually throughout the day. The present results do not exclude the possibility that adrenalectomized rats at room temperature given maintenance doses of adrenal cortical extract and a dietary load equivalent to that eaten by the cold-exposed rats would develop as severe pathological changes as rats exposed to cold.

The significance of the renal and cardiac lesions

A common finding in animals of all groups was foci of dilated tubules containing protein casts and lined by a rather flattened epithelium with basophilic cytoplasm. In many cases the related glomerulus showed periglomerular fibrosis and an increase of lymphocytes was present in the interstitial tissue (Grade 1 renal lesion). Such changes were described in the kidney of the aged rat by Ingle and Baker (1953) and more recently by Kennedy (1957). The latter showed that they develop at an earlier age, between one year and 18 months, in the unilaterally nephrectomized rat and are associated with a change in the ratio DNA/RNA in the kidney. Much younger rats were used in the present experiments and it was considered on morphological grounds that these focal lesions were the result of mild focal chronic pyelonephritis, although in view of Kennedy's work it is admitted that the mechanism of their production may be more intricate. These changes are not necessarily associated with obvious ascending infection in the medullary tubules and frank

pyelitis (Grade 2).   The latter was seen in only 9 rats in these experiments.

Blood pressure records were not taken in these studies. It seems most likely, however, that the cardiac hypertrophy and the renal and vascular changes of the more severely affected rats were associated with an elevation of blood pressure.   One might speculate that the more intense renal damage with proliferative arteriolosclerosis and arteriolonecrosis (Grades 4 and 5) represent a response in the rat comparable to that seen in benign and malignant hypertensive nephrosclerosis in man, although this does not imply a causal relationship.   This hypertensive renal damage was associated with hypertensive changes in the coronary arteries and replacement fibrosis of the hypertrophied myocardium (Grades 3 and 4 cardiac lesions).   Sellers and You (1956) have reported cardiovascular lesions with demonstrable lipid in the coronary arteries of normal rats fed a normal diet during exposure to cold for 10 to 18 months.   In the present experiments only one cold-exposed rat, an adrenalectomized animal, showed changes in a coronary artery suggestive of atheroma.

The significance of the gastric lesions

A correlation between exposure to stress and the development of peptic ulcer, as suggested by Gray et al. (1955-56) and by Selye (1950), was not shown in these experiments.   It is part of both concepts that the stress response is a general reaction

to a variety of noxious agents acting through the pituitary-adrenal system. Despite constant exposure to the stress of cold over a period of 60 days only 2 rats developed gastric erosions and true peptic ulcer did not occur. Although it is well known that peptic ulcers may develop in man during sustained emotional stress (the so-called "air raid ulcers") or following shock, surgery, burns and other forms of stress, the present studies lend no experimental support to the hypothesis that increased adrenal activity is a primary causative factor in their aetiology.

Conclusion

There is an impressive amount of evidence, much of it circumstantial, that the adrenal glands and their hormones are involved in a number of diseases of man. The positive findings of the present experiments involved the production of hypertensive lesions in rats under unusual conditions of stress. It is important to note that there was no evidence of acute rheumatism in the heart, arthritis, peptic ulcer, or other conditions which might be considered "diseases of adaptation." Selye maintains that alterations in pituitary-adrenal function during exposure to stress is the primary cause of these experimentally induced and many naturally occurring diseases. The present results lend more, but not absolute, support to the view that the role of the adrenal glands in the aetiology of disease is a permissive one which requires no departure from the normal

role of adrenal hormones in the economy of the body.

SUMMARY

1. Sensitized rats developed hypertensive lesions in the heart and kidney during exposure to cold. The damage was equally marked in non-adrenalectomized rats and adrenalectomized rats maintained on a constant intake of adrenal cortical extract.

2. Rats which were over-fed at room temperature showed lesions almost as severe as those seen in cold-exposed rats.

3. The role of the adrenal glands in causing these changes was not established with absolute certainty for two reasons. Food intake was not constant. The amount of adrenal cortical extract used as replacement therapy caused more damage in the adrenalectomized rats than in the non-adrenalectomized rats when the comparison was made at room temperature. It was postulated that the adrenal cortices may modify their secretion of hormones in order to facilitate the excretion of a high salt load.

4. No other "diseases of adaptation" were seen, and in particular peptic ulcer did not follow prolonged exposure to cold stress.

PART IV

PATHOLOGICAL CHANGES IN SENSITIZED RATS

EXPOSED TO STRESS

PATHOLOGICAL CHANGES IN SENSITIZED RATS
EXPOSED TO STRESS

As shown in the previous section, when the sensitized rat is exposed continuously to cold over a period of several weeks, severe renal and some myocardial damage results. During cold-exposure the animal approximately doubles its caloric intake to meet increased needs for heat energy and it is probable that the pathological results are dependent in part upon the increased dietary load, especially of sodium chloride. Although the literature on stress has become enormous, there is a paucity of evidence that exposure of normal animals to naturally occurring forms of stress causes disease. Indeed, there are only a few studies showing that exposure of sensitized animals to stress under highly artificial conditions causes pathological changes. Selye (1943) noted some renal abnormalities in sensitized rats given injections of dilute formalin and in similar rats forced to exercise, but a fully detailed report of results was not published.

In view of the results on cold stress, it seemed important to establish whether these lesions were in some way specific to cold exposure, or truly non-specific as proposed by Selye, and would result from the application of other stressors. Accordingly, in the next study, sensitized rats were exposed to various stressors (limb ligation, shock, surgery, haemorrhage,

burns, fractures, and injections of formalin) for a period of 8 weeks. Pathological changes were noted in the heart and kidney of only a few animals, and they were much less severe than those resulting from cold-exposure. A moderate elevation of blood pressure was present in only 3 rats. As in the cold stress studies, peptic ulcer did not occur.

MATERIALS AND METHODS

Male rats of the Sprague-Dawley strain were maintained on Archer Dog Pellets prior to unilateral nephrectomy. Thereafter, the animals were given the high (4%) sodium chloride medium carbohydrate diet ad libitum. As in previous experiments, the dry diet was tightly packed into individual Franke animal feeding jars which were weighed every 24 hours to record dietary intake.

Experimental design

Six normal rats were maintained on the stock diet. Eight groups of sensitized (unilateral nephrectomy, high salt diet) rats containing 6 rats per group were each exposed to one of the following conditions for 8 weeks:

1. Laparotomy was performed under ether anaesthesia once each week by opening and closing the abdominal wall from the diaphragm to the level of the bladder. The incision was made in a different site on each occasion.

2. Once each week, a strip of skin approximately 15 mm. wide extending from the neck to the base of the tail was excised

from the back under anaesthesia.   After 4 operations, the closure of the wound at each subsequent operation left the remaining skin very tight on the body of the rat, but after a few days of stretching and growth, the skin became loose again.

3. Haemorrhage was performed twice weekly by placing each rat in an activity-restriction cage, amputating the extreme tip of the tail, and immersing it in a heparin solution until a total of 8 ml. of blood was removed.   The loss of this amount of blood always left the rat pale and weak (normal total blood volume 20-22 ml.), but no deaths occurred.

4. A burn was caused once each week by immersing the body of the anaesthetized rat in water at $70^{\circ}C$ for 4-6 seconds.   The head, feet and tail were not burned.   The time of exposure for each animal was determined by the severity of the burn the preceding week.   The burns were not deep but caused marked hyperaemia of the skin and some surface scaling after a few days.   Following a burn, each rat became hyper-irritable and fought with its cage-mates, although it still permitted handling without forcible restraint.

5. A bone (tibia, or femur, or humerus, or the radius and ulna together) was fractured under ether anaesthesia once each week.

6. Ligation of one hind limb was done under ether anaesthesia the first week, the opposite limb the second week, and

alternatively thereafter for 8 weeks.   The procedure was
that described by Ingle (1943).   The ligatures remained
in place for 2 hours during each of the first 4 ligations,
and for 3 hours during each of the last 4 times.

7. A 1% solution of formaldehyde was injected subcutaneously
twice each day, 0.5 ml. per injection.

8. Six sensitized rats were maintained under normal conditions
for 8 weeks.

<u>Blood pressure measurement</u>

The level of blood pressure was recorded in groups 3, 4,
6 and 7 and in the 6 normal rats maintained on the stock diet.
The rats were anaesthetized with ether and the abdominal aorta
was cannulated below the level of the renal arteries.   The
cannula was attached by a short length of fine-bore rubber tube
filled with heparinized saline to a mercury manometer.   The
record was taken in all cases when anaesthesia was light as
judged by retraction of the rat's hind foot on pinching.

<u>Histological methods</u>

All rats were killed by exsanguination under ether
anaesthesia following blood pressure estimation.   Autopsy was
performed and gross pathological changes were noted.   The heart,
kidney, and portions of liver, spleen, pancreas, lung, stomach
and bowel were fixed in 10% neutral formalin.   The staining
methods used were those previously described apart from the
adrenal glands.   The latter were fixed in 1% formol-calcium,

cold 10% neutral formalin, and cold acetone.   Frozen sections
of gelatin embedded gland were examined with Oil red O for
lipids (Pearse, 1954), the Schultz method for cholesterol
(Symington, 1951), and with polarized light for birefringent
crystals.   Acid phosphatase was demonstrated in frozen sections
by the method of Gomori, and acetone processed material was used
for the Gomori alkaline phosphatase procedure (Pearse, 1954)
(Appendix).

RESULTS

The results are summarized in Tables 7 and 8.   The method
of estimating the severity and incidence of cardiac and renal
lesions was that used previously (pages 65 and 66).

The stressed animals showed a reduction in weight gain
over the experimental period, but this varied considerably from
one group to another (Table 7).   Their average daily food
consumption was slightly less than that of the control rats.

Heart lesions

The average weight of the heart of rats exposed to stress
ranged both above and below the mean for the control animals
(Table 7).   No gross abnormality was seen in any heart.
Microscopically the hearts from the control rats and from the
majority of the stressed groups were normal (Table 8).   Only in
some rats stressed by repeated burns or laparotomy was there any
cardiac abnormality.   This consisted of slight hypertrophy of
the cardiac muscle fibres with an increase in Anitschkow myocytes

TABLE 7

Body and organ weights of unilaterally nephrectomized adult male rats eating 4% NaCl medium CHO diet ad libitum for 56 days, mean and standard deviation

Experiment	Body weight g. Initial	Final	Heart mg.	Kidney mg.	Thymus mg.	Adrenal mg.	Food g./ rat/day
Control	302	446 ±14.7	1300 ±63	2100 ±94	561 ±36	26.0 ±1.3	19.2
Laparotomy	302	329 ±9.0	1230 ±23	1850 ±86	378 ±46	31.0 ±1.3	17.6
Skin excision	302	357 ±9.0	1360 ±47	1980 ±72	394 ±42	29.5 ±1.9	16.0
Haemorrhage	303	400 ±9.0	1280 ±39	1715 ±57	384 ±23	29.0 ±1.6	18.3
Burn	306	374 ±18.4	1425 ±59	2020 ±104	333 ±40	32.7 ±1.7	19.0
Fracture	302	372 ±3.7	1180 ±18	1900 ±94	440 ±42	32.6 ±6.3	15.6
Limb ligation	303	347 ±10	1308 ±57	1983 ±64	360 ±32	35.3 ±2.1	17.6
Formalin injection	305	433 ±11.1	1233 ±31	1995 ±33	368 ±28	30.3 ±1.9	17.9

TABLE 8

Rating of pathology in unilaterally nephrectomized adult male rats eating
4% NaCl medium CHO diet ad libitum for 56 days, mean and range

Experiment	Kidney gross	Kidney micro	Heart micro	Food g./rat/day	Blood pressure mm.Hg.
Control	0.5 (0-1.0)	0.75 (0.5-1.0)	0	19.2	124 ±2.7
Laparotomy	0.6 (0-1.5)	2.2 (0.5-3.0)	1.2 (0-2.0)	17.6	
Skin excision	0.58 (0.5-1.0)	0.9 (0.5-2.0)	0	16.0	
Haemorrhage	0.8 (0.5-1.0)	1.5 (1.0-3.0)	0	18.3	125 ±1.9
Burn	1.42 (0.5-2.5)	2.0 (0.5-3.0)	0.7 (0-2.0)	19.0	132 ±6.1
Fracture	0.5 (0-1.0)	1.0 (0.5-3.0)	0	15.6	
Limb ligation	1.92 (1.5-2.5)	1.25 (0.5-2.0)	0	17.6	136 ±4.6
Formalin injection	1.0 (0.5-2.0)	1.1 (0.5-2.0)	0	17.9	118 ±5.5

situated mainly around blood vessels.   In a few instances focal aggregates of cells, predominantly myocytes, were present in the myocardium (Fig. 52) or sub-endocardium.   These nodules did not show the classical features of the Aschoff node.   No abnormality was detected in the coronary arteries or their branches.   Valvulitis was not seen.   In 8 rats foci of cartilagenous metaplasia were present in the aorta at the origin of the aortic valve.   This change was not confined to rats of any one group and was seen in non-stressed animals.

Kidney lesions

The average weight of the kidney from the stressed groups was reduced to a variable degree as compared with the control rats (Table 7).   Grossly they showed slight nodularity and mottling with some grey and an occasional red spot on the surface.   Microscopically the most severe damage was seen in rats submitted to repeated laparotomy, or burning, and to a less extent haemorrhage (Table 8).   The most common finding in all stressed groups was some focal periglomerular fibrosis, hyaline cast formation, and infiltration of a few lymphocytes in the interstitial tissue (Grade 1 renal lesion).   In a few cases tubular damage was more severe and degenerative changes in the epithelium were noted (Fig. 53).   Frank pelvic inflammation (Grade 2) occurred in 7 rats; examples were seen in several stress groups but were absent from the control rats.   Damage to the glomerular tuft (Grade 3) was noted in rats stressed by

repeated fracture, laparotomy, haemorrhage or by burning
(Table 8).   The most extensive glomerular lesions occurred in
a rat repeatedly burned, and in which a blood pressure record
of 162 mm.Hg. was obtained (Fig. 54).   Damaged glomeruli were
shrunken and showed partial or complete adhesion to the capsule.
In some the subcapsular space was enlarged and the parietal
epithelium heightened.   The normal morphology of such glomeruli
was distorted further by an increase in structureless hyaline
material within the tuft leading to obliteration of the
capillary loops (Fig. 55).   These glomerular changes were not
associated with any abnormality of the renal arteries and
proliferative arteriolosclerosis and arteriolonecrosis (Grades 4
and 5) were not seen.

Blood pressure

Measurements were made in 4 groups of stressed rats and in
the control group (Table 8), and were found to be significantly
elevated in 3 rats.   One burned rat had a mean pressure of
162 mm.Hg., and 2 rats subjected to limb ligation had mean
pressures of 149 and 148 mm.Hg.

Stomach and other organs

There was no evidence of peptic ulcer or gastroduodenal
erosion in any rat.   Hypertensive vascular disease was absent
from the other organs (pancreas, etc.) taken for examination.

Adrenal glands

Some adrenal hypertrophy occurred in the stressed rats

(Table 7).   The glands of the control rats showed a uniform
pattern microscopically.   Zona glomerulosa was narrow and
composed of small cells with a homogeneous cytoplasm.   Lipid
depletion was virtually complete and the tests for cholesterol
and birefringence were negative.   Lipid globules, cholesterol
deposits and birefringence were most intense in the outer two-
thirds of the zona fasciculata and became less marked in the
inner aspects of the cortex (Fig. 56).

The adrenal glands of the stressed rats presented a
variable picture.   The most consistent alteration from the
control group was the enlargement of zona fasciculata and
reticularis.   Fine lipid droplets filled the cells throughout
the zones and in some rats the reactions for cholesterol and
birefringence were more intense.   In some glands the appearance
of the glomerulosa zone was identical with that seen in the
control rats, while in others the width of zona glomerulosa was
maintained and the cells contained lipid deposits, cholesterol
and birefringent crystals (Fig. 57).

Some variations in the pattern of distribution of the
phosphatases in the adrenal gland were noted.   Alkaline
phosphatase activity, as demonstrated by the method employed,
was observed in the cells and sinusoids of zona fasciculata and
reticularis of all rats, including controls.   Similar locali-
zation was seen in zona glomerulosa of the adrenal in the
majority of the rats, but in a few stressed animals glomerulosal

activity was confined solely to the sinusoids (Fig. 58).
Similarly an alteration in the pattern of acid phosphatase
activity was most apparent in the zona glomerulosa and consisted
of a variation in the width and intensity of the reaction (Figs.
59 and 60).

A clear correlation between the reactions of the adrenal
glands, especially zona glomerulosa, and the incidence and
severity of the renal and myocardial lesions could not be
demonstrated.

DISCUSSION

At least some of the stressors used in these experiments
caused an increase in the pathological changes in the kidney of
some sensitized rats as compared with the control sensitized
rats.   The average amount of damage and its upper range were
less in the present studies than previously seen in sensitized
rats exposed to cold.   Yet the stressors used here were as
severe as could be tolerated by the animal without causing death
and they were imposed repeatedly.   The accompanying increase in
food intake was an important factor determining the extent of
the pathological changes in cold-exposed rats.   In the present
studies the intake of the high salt diet was not increased above
the control values in the animals exposed to stress.

These experiments yield no information on the role of the
adrenal glands in the aetiology of the pathological changes in
the small numbers of rats affected.   The average increase in

adrenal weight was small in each experimental group. There
was no clear correlation between an alteration in the histo-
chemical pattern of the adrenal and the severity of renal and
myocardial damage. The average weight of the thymus, a
decrease in which is a good indicator of increased adrenal
cortical activity, was only moderately suppressed in the
stressed animals. There are no reports in the literature which
have a direct bearing on the role of the adrenal glands in
causing the pathological changes associated with exposure to
non-specific stressors, such as were used here. It would be
possible to compare stressed and non-stressed sensitized,
adrenalectomized animals treated with a constant intake of
adrenal cortical extract in order to test the hypothesis that a
change in the secretory activity of the adrenal glands is the
primary cause of the lesions, but this has not been done.

Only 3 rats showed a moderate elevation of blood pressure.
The incidence of glomerular lesions was greatest in these
animals but hypertensive vascular disease did not result. A
careful search was made for gross and microscopic signs of other
"diseases of adaptation." None were found. The stomach was
free from ulcers and erosions, there were no swollen joints,
rheumatic carditis, diabetes or any other pathological condition
outwith the heart and remaining kidney.

The basic conditions of these experiments (unilateral
nephrectomy and high load of sodium chloride) do not occur

naturally. An attempt was made to apply stressors as severe as cold-exposure, but they failed to produce lesions comparable in severity to those resulting from cold stress. This lends further support to the view that high dietary load is an important factor in determining the extent of the pathological changes. Whether or not these observations are interpreted to support or weaken the concept of Selye is a subjective matter. The complete absence of gastric lesions in these experiments is at variance with Gray's hypothesis relating stress, adrenal function and peptic ulcer.

SUMMARY

1. Sensitized rats were exposed to various stressors (limb ligation, surgery, haemorrhage, burns, fractures and injections of formalin) for 8 weeks.

2. Renal lesions with glomerulosclerosis were seen in a few rats, but only 3 animals showed a moderate blood pressure elevation without hypertensive vascular damage.

3. Dietary intake was not increased above control levels.

4. Peptic ulcer did not occur despite the severity of the stressors employed.

PART V

PATHOLOGICAL EFFECTS OF SODIUM-RETAINING STEROIDS AND SODIUM CHLORIDE LOAD IN RATS

PATHOLOGICAL EFFECTS OF SODIUM-RETAINING STEROIDS
AND SODIUM CHLORIDE LOAD IN RATS

It has been established that the hypertensive cardio-vascular and renal lesions which result from the exposure of rats to stress are more closely associated with a cold environment as the stressful agent.   Under these circumstances there is a voluntary increase in the consumption of sodium chloride diet, and evidence has been presented (Part III) which supports the view that the excess of sodium chloride may be an important factor in the causation of this form of hypertension.

It is well known that hypertension can be produced experimentally by the administration of sodium-retaining steroids. Treatment of the sensitized (unilateral nephrectomy and increased sodium chloride intake) rat with desoxycorticosterone (DOC) is now a classical method of inducing steroid hypertension.   Some of the renal and myocardial lesions from a small personally studied experiment are illustrated in Figures 61 and 62.   Other steroids with sodium-retaining properties, such as 2-methyl-9(α) fluorocortisol (Selye and Bois, 1956, 1957), 9(α)chlorocortisol (Ventura and Selye, 1957) induce similar changes.

It was discovered by Skelton (1953) that the chronic administration of methylandrostenediol (MAD) to the sensitized rat causes hypertensive lesions in the heart, blood vessels and kidney.   MAD is a synthetic steroid with anabolic, androgenic

and salt-retaining activity, and has been used therapeutically in the treatment of patients with wasting diseases and in post-operative convalescence.   In 1954 Salgado and Selye confirmed that MAD causes a syndrome of hypertensive vascular damage in the sensitized rat having intact adrenals, but reported that it does not do so when the adrenal glands are absent.   Crane, Porter and Ingle (1958) showed, however, that MAD causes hyper-tensive lesions in the adrenalectomized-sensitized rat treated with small supporting or "permissive" doses of adrenal cortex extract.   Examples of the pathological changes which were found are shown in Figures 63 to 68.   These changes occurred in rats on a 4% sodium chloride diet but without the marked increase in food-intake which is a critical variable in the response of the sensitized rat to cold.   Although these reactions to MAD were produced in the absence of the adrenal glands it was assumed by Crane, Porter and Ingle (1958) that the presence of adrenal cortical hormones was necessary to support the response since Salgado and Selye (1954) reported that MAD failed to cause such changes in the adrenalectomized-sensitized rat given no supportive therapy.

It may be said that there is general agreement among investigators that hypertension and its pathological lesions follow the administration of such steroids with sodium-retaining properties, and that this is most severe when one kidney has been removed and the animal is given an added intake of sodium

chloride.   There is disagreement, however, on the relative importance of the individual factors which make up the causal pattern of these experimentally induced lesions.   Selye and others describe the steroid as the primary cause and characterize unilateral nephrectomy and high salt load as "conditioning factors."

The present section is concerned with an alternative proposition that the primary cause of these pathological changes is the excess of sodium chloride or, more broadly, imbalance of electrolytes – which is more toxic when renal mass is reduced by unilateral nephrectomy or when sodium-retaining steroids are administered.   Meneely and his group (1953) showed that hypertension develops in normal rats fed excess sodium chloride over a prolonged period of several months.   The results of the present experiments indicate that high salt loads cause hypertension, renal and cardiovascular lesions which are much more severe in uni-nephrectomized rats, and that adrenal cortical insufficiency ameliorates but does not abolish these changes even when the intake of food and salt is constant.

MATERIALS AND METHODS

Dietary regime

Young male Sprague-Dawley rats, weighing approximately 300 g. were adapted to a fluid medium carbohydrate diet either _ad libitum_, or by stomach tube, 13 ml. each early morning and late afternoon (Appendix).   When sodium chloride was added to the

diet the load was expressed as per cent of the addition of water.   The rats were started on diet with 4% added salt and were adapted to further loading by increasing the level of sodium chloride 1% each week until the desired load was reached. The animals were kept in individual metabolism cages at room temperature (25°C).

Surgical methods, blood pressure measurement, and histological techniques have been described (Appendix).

Experimental design

Experiment 1.   Four groups of rats were prepared as follows:-

    A.   A sham operated group.
    B.   An adrenalectomized group.
    C.   A uni-nephrectomized group.
    D.   An adrenalectomized, uni-nephrectomized group.

Each group was maintained on a 4% NaCl dietary load for 56 days.

Experiment 2.   Similar groups (A, B, C, D) were adapted to a 12% salt load throughout 84 days.

Experiment 3.   A uni-nephrectomized group (C) and an adrenalectomized, uni-nephrectomized group (D) were adapted to a 16% salt load throughout 56 days.

Experiment 4.   Three groups of adrenalectomized, uni-nephrectomized rats (D) were adapted to a 4%, 12%, and 20% NaCl load respectively throughout 56 days.

Experiment 5. A group of normal rats was maintained on the diet without added salt for 56 days.

Tube-feeding was performed in Experiments 1, 2, 3 and 5; pair-feeding in Experiment 4.

The adrenalectomized rats were not given any supportive adrenal hormone therapy, apart from one instance described in the results.

RESULTS

The results are summarized in Tables 9 to 13 and Figures 69 to 72. They indicate that hypertension and its pathological lesions follow the administration of excess sodium chloride. This is most severe in the absence of one kidney and in the presence of the adrenal glands. The adrenal glands or the cortical hormones are not completely essential, however, for the development of hypertension.

Survival

Four adrenalectomized, uni-nephrectomized rats died after becoming anuric due to obstruction of the ureter of the remaining kidney with gravel; each had some degree of hydronephrosis. One adrenalectomized, uni-nephrectomized rat, tube-fed 12% salt, developed adrenal crisis after 7 weeks and was treated with 2.5 mg. of cortisone acetate on each of 5 consecutive days. It survived the last 4 weeks without further therapy but at autopsy had a blood pressure of 216 mm.Hg. and marked hypertensive nephrosclerosis and cardiovascular lesions. This animal is not

TABLE 9

Effects of 4% NaCl load in the presence and absence of the adrenals
Duration 56 days

Experiment 1	No. of rats	Body weight g. Initial	Final	Heart mg.	Kidney mg.	Blood pressure mm.Hg.	Kidney micro	Heart micro
Sham operated	9	328 ±7.6	432 ±6.6	1133 ±28	1382 ±33	137 ±2.0	0.33	0.22
Adrenalectomy	11	327 ±6.2	393 ±10.2	1195 ±21	1284 ±24	129 ±3.1	0.91	0.18
Uninephrectomy	9	329 ±7.8	432 ±4.8	1233 ±35	1959 ±52	146 ±3.2	1.77 (1.0-4.0)	0.44
Adrenalectomy Uninephrectomy	12	329 ±8.4	401 ±3.5	1160 ±24	1791 ±41	136 ±2.3	1.00	0.0

TABLE 10

Effects of 12% NaCl load in the presence and absence of the adrenals
Duration 84 days

Experiment 2	No.of rats	Body weight g. Initial	Final	Heart mg.	Kidney mg.	Blood pressure mm.Hg.	Kidney micro	Heart micro
Sham operated	6	324 ±5.5	441 ±2.4	1255 ±31	1467 ±62	144 ±6.3	0.83	0.66
Adrenalectomy	5	322 ±5.6	466 ±6.4	1346 ±37	1381 ±30	133 ±3.3	1.0	0.40
Uninephrectomy	7	322 ±3.2	459 ±9.3	1440 ±59	2406 ±112	163 ±5.6	3.57 (1.0-5.0)	2.14 (0-3.0)
Adrenalectomy Uninephrectomy	6	323 ±3.6	451 ±10.1	1361 ±27	2245 ±59	138 ±2.5	1.00	0.0

TABLE 11

Effects of 16% NaCl load in the presence and absence of the adrenals
Duration 56 days

Experiment 3	No. of rats	Body weight g. Initial	Body weight g. Final	Heart mg.	Kidney mg.	Blood pressure mm.Hg.	Kidney micro	Heart micro
Uninephrectomy	8	311 ±2.9	399 ±8.7	1331 ±44	2116 ±56	144 ±4.9	2.28 (1.0-5.0)	1.0
Adrenalectomy Uninephrectomy	9	314 ±1.8	423 ±4.6	1319 ±21	2040 ±42	127 ±3.1	0.88	0.0

TABLE 12

Effects of variable NaCl load in the absence of the adrenals
Duration 56 days

Experiment 4	No. of rats	Body weight g. Initial	Body weight g. Final	Heart mg.	Kidney mg.	Blood pressure mm.Hg.	Kidney micro	Heart micro
Adrenalectomy Uninephrectomy 4% NaCl load	15	333 ±4.3	403 ±15.6	1157 ±47	1741 ±71	132 ±2.3	0.53	0.57
Adrenalectomy Uninephrectomy 12% NaCl load	10	341 ±10	374 ±10.5	1178 ±30	1634 ±64	143 ±4.1	1.40	0.60
Adrenalectomy Uninephrectomy 20% NaCl load	7	335 ±5.5	388 ±15.0	1311 ±65	2045 ±162	154 ±8.9	2.29 (1.0-5.0)	0.80

TABLE 13

Effect of medium CHO diet without NaCl in normal rats
Duration 56 days

Experiment 5	No. of rats	Body weight g. Initial Final	Heart mg.	Kidney mg.	Blood pressure mm.Hg.	Kidney micro	Heart micro
Normal rats	11	311 ±0.9 452 ±2.9	1132 ±19	1210 ±33	128 ±2.4	0.36	0.54

included in the table of results (Table 10).

Body weight

All of the rats gained weight during the experiments. When the salt load was 4% (Experiment 1, Table 9) the non-adrenalectomized rats gained more weight than the adrenalectomized animals on the same caloric intake. When the salt load was high the the rate of gain was somewhat reduced in each group, and there was no clear difference between non-adrenalectomized and adrenalectomized rats. Gross oedema did not occur in any animal.

Urine volume

As shown in Figure 71 (Experiment 2) the amount of urine excreted by adrenalectomized rats was less than that excreted by non-adrenalectomized rats on the same salt load. Similar results were found in Experiments 1 and 3.

Blood pressure

Uni-nephrectomy was associated with elevation of the blood pressure in the salt-loaded rats; adrenalectomy, uni-nephrectomy with a lesser rise. The most severe hypertension occurred among non-adrenalectomized, uni-nephrectomized rats tube-fed 12% salt for 84 days (Table 10, Fig. 70). In some rats of all groups of adrenalectomized, salt-loaded animals there was a tendency for the blood pressure to rise, and this was most marked in the adrenalectomized, uni-nephrectomized rats of Experiment 4 which ate the 20% salt diet for 56 days (Table 12,

Fig. 72).

Kidney lesions

Uni-nephrectomy was associated with compensatory renal hypertrophy in both the presence and absence of the adrenals (Fig. 69). Increasing salt loads were associated with heavier kidneys in adrenalectomized and in non-adrenalectomized rats, and the heaviest kidneys were seen in hypertensive rats (Tables 9 to 12). Signs of gross renal damage were minimal in the normal rats on a low-salt diet and were increased by uni-nephrectomy and by salt-loading to the greatest extent when the adrenal glands were present.

Microscopically the renal lesions were assessed on the basis described previously (pages 65 and 66). The kidneys from rats maintained on the diet without added salt showed little deviation from the normal (Experiment 5, Table 13). Small scanty foci of periglomerular fibrosis, with some casts in dilated tubules lined by flattened basophilic epithelium, and a few round cells in the related interstitial tissue (Grade 1 renal lesion) were not uncommon in some rats of all salt-loaded groups. Only 2 rats in all these experiments showed active pelvic inflammation (Grade 2).

Significant elevation of the blood pressure was associated with significant glomerular damage and hypertensive arteriolo-sclerosis (Grade 4) and arteriolonecrosis (Grade 5) involving the glomerular afferent arterioles. In some rats a more

moderate elevation of blood pressure was associated with focal glomerular tuft damage but histologically the renal vessels showed no hypertensive changes (Grade 3).

The greatest increase in average renal damage resulted from the salt-loading of rats bearing intact adrenal glands and with renal mass reduced by unilateral nephrectomy (Experiments 1, 2 and 3, Tables 9 to 11). Examples of significant hypertensive damage in the kidney were most pronounced at the 12% and 16% sodium chloride loads. These renal lesions were patchy in distribution and unaffected glomeruli were readily identified. Damaged glomeruli were commonly enlarged so obliterating the subcapsular space, but in other instances the tuft was shrunken producing an eccentric dilatation of the space (Fig. 73). The swollen glomeruli contained numerous large cells with foamy cytoplasm and the tuft was relatively bloodless (Fig. 74). There was an increase in eosinophilic material within the tuft, giving a positive reaction with the Schiff stain and sometimes giving a fibrinoid reaction with the picro-Mallory method. Glomerular lesions of this nature were associated with hypertensive arteriolosclerosis (Fig. 75) or arteriolonecrosis (Fig. 76) affecting the afferent arteriole. Hypertensive glomerular and vascular lesions of this type were not seen in the sham operated groups or the adrenalectomized rats given no corticoid therapy in Experiments 1, 2 and 3 (Tables 9 to 11).

As noted above, one adrenalectomized, uni-nephrectomized rat,

tube-fed 12% salt, was given 2.5 mg. of cortisone acetate on each of 5 consecutive days during periods of adrenal crisis. The highest elevation of blood pressure was observed in this rat (216 mm.Hg.) in association with severe hypertensive damage to the glomeruli and arterioles of the kidney (Fig. 77).

Increasing levels of salt load to adrenalectomized, uni-nephrectomized rats given no corticoid therapy (Experiment 4) caused a progressive increase in the average amount of renal damage with a rise in the average level of blood pressure (Table 12, Fig. 72). One rat on the 20% NaCl load with a blood pressure of 198 mm.Hg. showed widespread glomerular damage with hypertensive necrosis of the afferent arterioles (Figs. 78 and 79). Five other rats on the 12% and 20% salt load showed glomerular lesions without hypertensive vascular lesions although in 4 the blood pressure ranged from 150 to 172 mm.Hg.

There was an increase in tubular damage in those rats showing hypertensive renal lesions. Casts lying within dilated tubules were more frequent. The related tubular epithelium was basophilic and the basement membrane thickened. In some distal tubules the renal epithelium was swollen and contained droplets showing a positive reaction with Schiff's reagent and stained red with the picro-Mallory method.

Heart lesions

An average increase in weight of the heart was associated with uni-nephrectomy and with salt-loading; the increase was

greater in the presence of the adrenals than in their absence
(Fig. 69).   Gross cardiac damage was not seen in any animal.

Microscopically the changes in the heart were rated as
previously (pages 65 and 66) on the basis of muscle hypertrophy
(Grade 1, cardiac lesion), the presence of focal areas of
myocardial fibrosis and macrophage reaction (Grade 2), and the
association of these changes with arteriolosclerosis (Grade 3)
and arteriolonecrosis (Grade 4) of the coronary vessels.   On
this basis significant hypertensive heart damage was most
prominent in the non-adrenalectomized, uni-nephrectomized rats,
tube-fed the 12% salt diet, and in this group the heaviest
hearts were seen (Experiment 2, Fig. 69).   There was focal
replacement fibrosis of the hypertrophied myocardial fibres
associated with hypertensive proliferative sclerosis of small
branches of the coronary arteries (Fig. 80).   Haemosiderin-
laden macrophages were present in some of the fibrosed areas.
A similar type of damage was observed in one non-adrenalectomized,
uni-nephrectomized rat adapted to the 16% salt load (B.P. 156
mm.Hg.), in the rat given cortisone acetate (B.P. 216 mm.Hg.) and
in an adrenalectomized, uni-nephrectomized rat adapted to the 20%
salt diet (B.P. 198 mm.Hg.).   In the other groups of rats there
was a low incidence of small focal areas of myocardial fibrosis
and macrophage reaction but in no instance was this related to
frank hypertensive vascular lesions.   The low incidence of this
type of damage was further reduced in the adrenalectomized rats.

Thymus

Adrenalectomy was associated with an average increase in the weight of the thymus above that of non-adrenalectomized animals. Salt-loading tended to suppress thymus weight in non-adrenalectomized rats and to a lesser extent in adrenalectomized animals.

Adrenal glands

There was tendency for salt loading to cause an increased adrenal weight, especially in uni-nephrectomized rats. The main alteration in the lipid pattern of the adrenal glands was the lipid depletion of zona glomerulosa in response to the sodium chloride load. This depletion was variable in degree but appeared most marked in the rats on the 12% salt diet. The cells of the zona fasciculata were completely filled with fine lipid droplets while the reticularis cells contained rather less lipid material.

Other organs

Vascular lesions were present in other intra-abdominal organs of the hypertensive rats, notably in the group of non-adrenalectomized, uni-nephrectomized rats maintained on the 12% salt diet (Experiment 2). The organs affected included the pancreas, spleen, stomach (Fig. 81) and small intestine (Fig. 82). In some instances the vascular damage resembled the "polyarteritis nodosa" lesions which result from the administration of sodium-retaining steroids, such as desoxycorticosterone. Hypertensive

damage to vessels was seen also in non-adrenalectomized, uni-nephrectomized rats on the 16% salt load. Such lesions were not seen in any adrenalectomized rat in these experiments, apart from the single animal given cortisone acetate.

DISCUSSION

The results show that loading rats with excess sodium chloride is associated with an elevation of blood pressure and significant hypertension and pathological changes in some of the animals. This occurs more readily in the uni-nephrectomized rat. It may be that the reduction of renal mass limits the ability of the rat to deal with a high salt load, and consequently makes signs of toxicity more likely to develop.

The adrenalectomized salt-loaded rat drinks and excretes less water than do similar rats with their adrenal glands intact. Salt-loading is less pathogenic in the adrenalectomized rat kept without cortical hormone in that higher loads of sodium chloride are required to produce pathological changes. This may be because some or all of the corticoids have an obligatory sodium-retaining action which makes it more difficult for the non-adrenalectomized rat or the hormone-treated adrenalectomized rat to excrete sodium _via_ the kidney, but the validity of this hypothesis has not been tested by balance studies. In addition certain corticoids, such as cortisone and hydrocortisone, act at cellular level and cause electrolyte displacement and excess, even in the absence of increased salt load (Knowlton, Loeb and

Stoerk, 1957).   The operation of endocrine factors at both
renal and cellular level might account for the relative ease
with which sodium chloride induces hypertension in the non-
adrenalectomized or hormone-treated animal as compared with the
untreated adrenalectomized rat.

Present evidence indicates that the processes leading to
hypertension and its pathological consequences can continue in
the absence of the adrenal cortical hormones.   Progressive
salt-loading of the adrenalectomized, uni-nephrectomized rat
caused a progressive increase in the average level of blood
pressure (Table 12, Fig. 72) with significant hypertensive
vascular changes and glomerular lesions at the higher salt loads.
Turner and Grollman (1951) have described the production of
experimental hypertension in the adrenalectomized dog, while it
has been known for a number of years that rats with an "endocrine"
kidney will develop hypertension with severe vascular disease in
the absence of the adrenal glands (Selye, 1950).

The pathological changes which accompanied hypertension in
the rats of the present experiments bear a close resemblance to
those which follow the administration of sodium-retaining
steroids, such as desoxycorticosterone or methylandrostenediol.
In a recent review of theories of hypertension Page, McCubbin
and Corcoran (1958) came to the following conclusions on the
basis of published evidence:- "The major, if not the only,
physiological activity of DOC is to provoke renal retention of

sodium, with moderate potassium excretion..... Present
evidence indicates that DOC has no intrinsic pressor action.
What it does is cause sodium retention and polydypsia by its
renal action, and hypertension is consequent thereon. With
patience, not uninephrectomized animals given DOC without excess
sodium can also become hypertensive; the same is true of
animals not given DOC but provided with large excesses of
sodium.   Thus uninephrectomy and DOC merely potentiate the
action of sodium by tending to decrease sodium elimination."
These conclusions are consistent with the present results. It
may be that the toxicity of abnormally high loads of sodium
chloride and the accompanying imbalance of other electrolytes
should be described as the primary cause of hypertensive
vascular disease in animals overdosed with adrenal steroids and
in sensitized rats exposed to the stress of cold exposure.
Although the hormones of the adrenal cortex bear some intimate
relationship to these pathological processes their role is
probably a supporting or "permissive" one in the aetiology of
these experimental syndromes.

Selye (1950) has emphasized the importance of the adrenal
gland and cortical hormones in adaptation to various stressful
situations.   Both tube-feeding and the administration of an
abnormal sodium chloride diet could be considered as stressors.
Yet these experiments show that adrenalectomized rats receiving
no exogenous adrenal cortical hormone can adapt to these

circumstances with only a small fall in the survival rate.

SUMMARY

1. Hypertension and pathological lesions in the heart, blood vessels and kidney follow the administration of sodium-retaining steroids, such as desoxycorticosterone or methylandrostenediol, to sensitized rats.

2. A similar syndrome of hypertensive damage can be reproduced by feeding excess sodium chloride in the diet.

3. These changes occur more readily in the absence of one kidney and it is most probable that reduction of renal mass limits the ability of the rat to deal with a high salt load.

4. Adrenalectomy ameliorates but does not abolish the hypertension. Adrenalectomized rats secrete less urine in response to a constant salt load than do rats with their adrenal glands intact.

5. "Sensitization" by unilateral nephrectomy and salt administration can be explained most satisfactorily as a device to accelerate the hypertensive effects of a sodium chloride load.

PART VI

THE PATHOLOGY AND PATHOGENESIS OF
ADRENAL REGENERATION HYPERTENSION

THE PATHOLOGY AND PATHOGENESIS OF
ADRENAL REGENERATION HYPERTENSION

The role of sodium chloride or, more broadly, electrolyte imbalance in the pathogenesis of several forms of experimental hypertension has been emphasized in preceding sections. It has been suggested also that the adrenal gland and the cortical hormones play a supporting role in the development of the hypertensive syndrome (Parts III and V).

In 1955 Skelton reported that hypertension, associated with pathological changes, develops if one adrenal gland is removed and the other gland enucleated and allowed to regenerate in rats sensitized by unilateral nephrectomy and given 1% sodium chloride to drink. In the autumn of 1957 experiments were started personally to determine whether Skelton's findings of adrenal regeneration hypertension could be confirmed and, if so, whether the syndrome could be modified by altering the experimental conditions by two separate procedures:

1. Substitution of tap water for 1% saline as drinking fluid.

2. Transplantation of the regenerating adrenal cortex to a site within the portal circulation, on the assumption that hormones formed by the regenerating tissue would be metabolized or degraded within the liver before reaching the peripheral tissues.

The results confirm Skelton's report, and indicate that the

two procedures outlined above protect the uni-nephrectomized rat from the hypertension which is associated with the presence of regenerating cortical tissue.

MATERIALS AND METHODS

Young immature female rats (Wistar strain) weighing approximately 100 g. were used; the sex and body weight advised by Skelton (1956). They were fed a stock diet _ad libitum_ (diet No.41; Appendix). They were allowed free access to drinking fluid, either 1% saline or tap water, but the daily fluid intake of each group of rats was measured.

The surgical techniques of unilateral nephrectomy, adrenalectomy, adrenal enucleation and transplantation are described in the Appendix.

The histological techniques were those used previously.

Experimental design

Five experiments were designed to investigate the pathological changes which develop at different time intervals in association with regenerating adrenal cortical tissue in uni-nephrectomized rats maintained on 1% saline and tap water. Each experiment consisted of a control group of uni-nephrectomized, uni-adrenalectomized rats, and a test group similarly prepared but with the other adrenal gland enucleated.

Experiment 1 consisted of control and test groups maintained on 1% saline for 14 days.

Experiment 2 was a repetition of Experiment 1 but the rats

were given tap water to drink.

Experiment 3 consisted of control and test groups maintained on 1% saline for 40 days.

Experiment 4 was a repetition of Experiment 3 but the rats were given tap water as drinking fluid.

Experiment 5 consisted of control and test groups maintained on 1% saline for 54 days.

A further experiment was designed to study the effect of transplantation of the regenerating adrenal cortex into the portal circulation in rats maintained on 1% saline for 50 days.

Experiment 6 consisted of control and test rats, and 3 further groups in which the enucleated adrenal gland was transplanted:

A. on to the mesenteric border of the spleen.

B. on to the surface of the liver between adjacent lobes.

C. on to the inferior mammary gland in the right groin.

RESULTS

The results are summarized in Tables 14 to 19. They show that uni-nephrectomized rats maintained on 1% saline and bearing regenerating adrenal cortical tissue develop hypertension with pathological changes in the heart, blood vessels and remaining kidney. The development of these features is prevented by the substitution of tap water as drinking fluid, or by the transplantation of the regenerating adrenal gland into the portal area despite the continued administration of saline.

TABLE 14

Adrenal regeneration in young sensitized female rats drinking 1% saline ad libitum, body and organ weights, mean and standard deviation.
Control - right nephrectomy, right adrenalectomy.
Test - right nephrectomy, right adrenalectomy, left adrenal enucleation.

Experiment	No.of rats	Time in days	Body weight g. Initial	Final	Kidney mg.	Heart mg.	Thymus mg.	R.adrenal mg.	L.adrenal mg.
Control	12	14	108 ±4.36	125 ±4.9	684 ±56	447 ±21.8	266 ±41	17.7 ±4.12	28.1 ±1.4
Test	11	14	112 ±5.66	138 ±11.5	759 ±82.4	496 ±51.4	369 ±118	17.9 ±3.15	14.8 ±3.46
Control	6	40	107 ±7.8	141 ±8.42	940 ±101	522 ±62.7	252 ±24	15.3 ±0.68	34.1 ±6.4
Test	6	40	111 ±6.86	136 ±29.7	1027 ±223	565 ±91.4	238 ±119	14.7 ±2.44	28.6 ±5.5
Control	6	54	165 ±10.5	197 ±13.7	1058 ±107	633 ±51	253 ±48.7	19.3 ±2.86	43.9 ±6.9
Test	6	54	170 ±6.85	189 ±16.3	1190 ±93.6	902 ±48	263 ±71.8	18.9 ±2.6	37.2 ±9.6

TABLE 15

Adrenal regeneration in young sensitized female rats drinking 1% saline ad libitum.
Control – right nephrectomy, right adrenalectomy.
Test – right nephrectomy, right adrenalectomy, left adrenal enucleation.

Experiment	No.of rats	Time in days	B.P. mm.Hg.	Saline ml./ rat/day	Kidney micro	Heart micro
Control	12	14	117 ±15.8	42	0.4 (0-1.0)	0
Test	11	14	137 ±15.4	42	0.9 (0-1.0)	0
Control	6	40	126 ±9.48	56	0.6 (0-1.0)	0
Test	6	40	153 ±19.1	72	3.1 (1.0-5.0)	1.9 (0-4.0)
Control	6	54	112 ±12	79	0.3 (0-1.0)	0
Test	6	54	153 ±20.2	91	2.5 (1.0-5.0)	1.8 (0-4.0)

TABLE 16

Adrenal regeneration in young sensitized female rats drinking tap water ad libitum, body and organ weights, mean and standard deviation.
Control - right nephrectomy, right adrenalectomy.
Test - right nephrectomy, right adrenalectomy, left adrenal enucleation.

Experiment	No.of rats	Time in days	Body weight g. Initial	Final	Kidney mg.	Heart mg.	Thymus mg.	R.adrenal mg.	L.adrenal mg.
Control	6	14	85 ±2.91	122 ±9.54	633 ±93	408 ±37.4	323 ±45.1	12.2 ±1.6	25.6 ±3.31
Test	12	14	89 ±4.58	126 ±8.24	798 ±86	472 ±42.4	436 ±84.8	13.5 ±2	14.5 ±2.39
Control	6	40	109 ±5.19	153 ±12.2	813 ±74.7	463 ±35.5	310 ±80.6	15.7 ±2.4	35.0 ±8.85
Test	6	40	109 ±5.19	159 ±11.1	928 ±124	545 ±84.5	277 ±58.2	17.8 ±1.61	29.9 ±4.92

TABLE 17

Adrenal regeneration in young sensitized female rats drinking tap water ad libitum.
Control - right nephrectomy, right adrenalectomy.
Test - right nephrectomy, right adrenalectomy, left adrenal enucleation

Experiment	No. of rats	Time in days	B.P. mm.Hg.	Water ml./ rat/day	Kidney micro	Heart micro
Control	6	14	116 ± 5.65	22	0.2 (0-1.0)	0
Test	12	14	121 ± 11.5	22	0.6 (0-1.0)	0
Control	6	40	114 ± 7.44	32	0.2 (0-1.0)	0
Test	6	40	135 ± 19.8	27	0.2 (0-1.0)	0

TABLE 18

Adrenal regeneration and transplantation in young sensitized female rats
drinking 1% saline ad libitum for 50 days, body and organ weights, mean
and standard deviation.
Control - right nephrectomy, right adrenalectomy.
Test - right nephrectomy, right adrenalectomy, left adrenal enucleation.
Transplant - right nephrectomy, right adrenalectomy, left adrenal
enucleated and transplanted.

Experiment	No. of rats	Body weight g. Initial	Body weight g. Final	Kidney mg.	Heart mg.	Thymus mg.	R.adrenal mg.	L.adrenal mg.
Control	6	115 ±13.7	168 ±16.5	855 ±94	582 ±38.7	277 ±40.6	18.0 ±4.65	45.9 ±2.72
Test	6	126 ±11.8	188 ±10	1242 ±242	755 ±106	313 ±87.1	20.9 ±1.23	47.0 ±3.51
Transplant-spleen	5	128 ±4.6	182 ±13.4	1116 ±66.4	670 ±97.4	384 ±79.3	22.6 ±2.2	36.4 ±10.5
Transplant-liver	5	131 ±10.4	197 ±22.7	1198 ±172	682 ±73.5	398 ±109	22.8 ±4.24	39.8 ±17.7
Transplant-groin	5	147 ±10.8	192 ±18.9	1148 ±225	755 ±81.2	287 ±32.6	20.6 ±2.61	40.6 ±14

TABLE 19

Adrenal regeneration and transplantation in young sensitized female rats drinking 1% saline ad libitum for 50 days.
Control - right nephrectomy, right adrenalectomy.
Test - right nephrectomy, right adrenalectomy, left adrenal enucleation.
Transplant - right nephrectomy, right adrenalectomy, left adrenal enucleated and transplanted.

Experiment	No. of rats	B.P. mm.Hg.	Saline ml./ rat/day	Kidney micro	Heart micro
Control	6	113 ±7.9	56	0.8	0
Test	6	151 ±19.6	70	2.0 (0-5.0)	1.3 (0-3.0)
Transplant- spleen	5	138 ±8.3	52	0.8	0
Transplant- liver	5	127 ±27	48	0.8	0
Transplant- groin	5	-	-	2.2 (1.0-5.0)	0 (0-3.0)

Hypertension and its lesions are not prevented by transplantation of the enucleated gland into the systemic circulation in the groin.

Body weight

There was no significant difference in weight gain in control, enucleated, or transplanted rats in any of the groups.

Fluid intake

The rats with adrenal cortices regenerating in situ drank more saline than the non-enucleated controls, especially at the 21 to 42 day period (Fig. 83). There was no difference between control and test rats when tap water was substituted, but these rats consumed less fluid than those maintained on saline (Fig. 84). The rats with adrenal cortical tissue transplanted into the portal area drank approximately the same amount of saline as the control rats (Table 19).

Blood pressure

There was a variable rise in average blood pressure in all groups of rats with regenerating adrenal cortices as compared with their respective controls (Tables 15, 17, 19). This was associated with significant pathological changes only in the rats enucleated in situ and maintained on saline for 40 to 54 days (Fig. 85), and in those rats with the regenerating gland transplanted into the groin. The development of severe hypertension with pathological lesions was prevented by the substitution of tap water as drinking fluid (Fig. 86), and by

transplantation of the regenerating cortical tissue into the portal area (Fig. 87).

Kidney lesions

An increase in the weight of the remaining kidney was observed in all rats bearing regenerating adrenal tissue (Tables 14, 16, 18). The heaviest kidneys were seen in the hypertensive rats. In the latter groups the kidneys were mottled, nodular, and showed grey and haemorrhagic spots beneath the capsule.

Microscopically the renal lesions were assessed on the ratings previously used (pages 65 and 66). Occasional small foci of periglomerular fibrosis, hyaline cast formation, and interstitial lymphocytic infiltration (Grade 1 renal lesion) were seen in some rats of all experimental groups. Only 2 rats showed active chronic pelvic inflammation (Grade 2).

Significant hypertensive renal damage was associated closely with the maximum elevation of blood pressure seen in rats with cortical tissue regenerating in situ or in the groin and maintained on saline for 40 to 54 days (Figs. 85, 86, 87). Histologically the type of glomerular lesion was very similar to that described in the experiments on salt-loading and steroid administration (Part V) and following cold exposure (Part III). The distribution of the lesions was focal and apparently normal glomeruli could be found readily. Affected glomeruli were enlarged and filled the subcapsular space. Some tufts contained

large swollen cells of "foamy" appearance but it was not possible to be certain whether these were of epithelial or endothelial origin. Such glomeruli were relatively bloodless and not infrequently the tuft capillaries were occluded by small thrombi (Fig. 88). The most severe type of glomerular damage was characterized by necrosis and the deposition of fibrinoid material both within the tuft and Bowman's capsule (Fig. 89). These glomerular lesions were associated with proliferative arteriolosclerosis (Grade 4) and arteriolonecrosis (Grade 5) affecting the renal arterioles (Figs. 89 and 90), and in some kidneys the larger arteries showed lesions resembling polyarteritis nodosa. Pathological changes in the tubules consisted of dilatation with protein cast formation and the presence of hyaline droplets in the tubular epithelium. Blood casts were present in the tubules of the most severely hypertensive rats (Fig. 89).

There was no evidence of hypertensive renal damage in any control rat irrespective of the type of drinking fluid, in enucleated rats maintained on tap water, or in rats given 1% saline and with the regenerating cortical tissue transplanted into the portal area.

Heart lesions

The weight of the heart was increased in the rats bearing regenerating adrenal cortical tissue. The heaviest hearts were seen in the enucleated rats maintained on 1% saline for 54 days

(Tables 14, 16, 18).

Microscopically the cardiac lesions were assessed on the ratings used previously (pages 65 and 66). Pathological changes were observed in the heart of rats with adrenal cortices regenerating in situ or in the groin and maintained on saline for 40, 50 and 54 days. They consisted of myocardial hypertrophy (Grade 1 cardiac lesion) commonly associated with replacement fibrosis (Grade 2). The branches of the coronary arteries showed proliferative arteriolosclerosis (Grade 3) and arteriolo-necrosis (Grade 4) in the most severely hypertensive rats. The latter type of vascular lesion was commonly associated with necrosis of the cardiac muscle fibres (Fig. 91).

Heart lesions were not seen in any control rat, enucleated rat maintained on tap water, nor in any rat with cortical tissue regenerating in the portal area.

Thymus

The thymus was enlarged in rats with cortical tissue regenerating for 14 days as compared with the control rats, irrespective of the type of drinking fluid. This difference was not obvious at the 40 and 54 day period (Tables 14 and 16). An increase in thymic weight was also observed in rats with the enucleated gland transplanted into the portal area for 50 days (Table 18).

Adrenal glands

At the original time of operation the right adrenal gland

was weighed following adrenalectomy, and the weight of the pulp
enucleated from the left gland was also measured.   Assuming
that the right and left adrenal glands were of similar weight it
was estimated that approximately 5 mg. of adrenal capsule and
adherent cortical tissue remained in situ or was transplanted
following enucleation.

The adrenal capsular tissue from some test rats was examined
immediately after enucleation.   These rats were then discarded.
The residual tissue consisted of fibrous capsule and vessels,
zona glomerulosa, and some rather crushed cells of superficial
zona fasciculata (Fig. 92).   The expressed pulp was composed of
the major part of zona fasciculata with the entire zona reticu-
laris and medulla (Fig. 93).

Following adrenalectomy the left adrenal gland of the
control rats underwent progressive enlargement.   There was no
significant difference in the increase in adrenal weight of
control rats on 1% saline or tap water (Tables 14 and 16).   The
control rats maintained on saline showed narrowing and lipid
depletion of zona glomerulosa when compared with normal glands
removed at adrenalectomy or the adrenal gland from control rats
maintained on tap water (Fig. 94).

Regeneration of cortical tissue occurred in all enucleated
rats, including transplants, irrespective of the type of drinking
fluid, but the final size of regenerated glands was usually
slightly less than their respective controls (Tables 14, 16, 18).

The control glands consisted, however, of both cortical and medullary tissue while the regenerated glands were composed only of cortical cells.

Microscopically in the regenerating glands the residual damaged cells of zona fasciculata became necrotic and were removed (Fig. 95), and regeneration appeared to proceed from the cells immediately beneath the adrenal capsule. When regeneration was advanced, at the 40 to 54 day periods, the majority of the glands in situ showed a zoning arrangement reminiscent of the normal, with large vacuolated cells containing abundant lipid (Figs. 96 and 97). Usually all the cortical cells were filled with lipid (Fig. 98), but in a few rats maintained on saline for 54 days a lipid-free subcapsular zone corresponding to zona glomerulosa in the saline controls was observed. There was no evidence of medullary tissue in any regenerating gland. The centre was composed of a fibrous vascular nodule. Occasionally fibrous bands radiated from this central core to join the capsule so separating the cortical cells into nodules and in these regenerated glands the zoning pattern was sometimes less obvious (Fig. 99).

Other organs

Hypertensive vascular lesions were observed in the spleen and pancreas of rats bearing adrenal cortices regenerating in situ or in the groin and maintained on saline. Occasionally in the mesentery a "polyarteritis nodosa" type of hypertensive

vascular change was noted (Fig. 100).

DISCUSSION

The results of these experiments confirm Skelton's original report (1955), and the more recent reports of others, that rats sensitized by unilateral nephrectomy and maintained on 1% saline develop hypertension with pathological lesions when one adrenal gland is removed and the other enucleated and allowed to regenerate. Skelton has stressed the importance of the regenerating adrenal gland in the aetiology of this experimental syndrome which he has termed "adrenal-regeneration hypertension." It seems probable, however, that regenerating cortical tissue is only a part of the pattern of causation, since if the 1% saline drinking fluid is replaced with tap water significant hypertension and its pathological effects are prevented.

It has been postulated by different groups of investigators (Chart, Ulsamer, Quinn, Howie, Sullivan and Gaunt, 1957; Masson, Koritz and Peron, 1958) that regenerating adrenal tissue might secrete excessive amounts of aldosterone and that either a relative or an absolute hyperaldosteronism could be the cause of the hypertension. Support for this hypothesis is based on the fact that aldosterone is produced by the subcapsular glomerulosa layer of the adrenal (Deane, Shaw and Greep, 1948; Giroud, Stachenko and Venning, 1956; Ayres, Garrod, Tait and Tait, 1958) and that some cases of primary aldosteronism in man are

accompanied by hypertension (Conn and Louis, 1956).   At
present, however, there are no reports in the literature which
support this hypothesis.   Masson et al. (1958) investigated
the ability of regenerating rat adrenals to form corticosteroids
but found no excessive amounts of aldosterone nor any significant
imbalance in the steroid mixture in vitro.   Ballard, Lipscomb and
Sayers (1958) found that regenerating adrenal cortices secrete
corticosterone and an unidentified steroid of low polarity at
subnormal rates in analyses of rat adrenal vein blood.

To date the reports in the literature show a lack of
agreement on the hypertensive actions of aldosterone in rats.
Gornall and his group (Kumar et al., 1957; Gornall et al., 1957)
reported that aldosterone in doses of 0.4 µg. daily produces
hypertension and pathological lesions in normal rats.   This was
not confirmed by Gaunt (Gaunt, Ulsamer and Chart, 1957) using
the same dosage with only minor changes in the experimental
conditions.   Gross, Loustalot and Meier (1957) produced hyper-
tension with much larger doses (0.5 mg.) of aldosterone acetate
in rats made sensitive by unilateral nephrectomy and the
administration of 1% saline as drinking fluid, but this dose is
outwith the physiological range.

An alternative hypothesis has been advanced by Page,
McCubbin and Corcoran (1958).   They suggest that the hypertension
is not a result of adrenal hypersecretion "but rather a delayed
response to a period of adrenal insufficiency, during which the

vessels have become sensitized and hyperreactive to the pressor effects of a normal steroid mixture." Using thymic weight as an indicator of adrenal activity, the increase in weight of this organ during the early stage of this syndrome (Experiment 1) suggests the presence of adrenal insufficiency, while its restoration to normal in the later stages (Experiments 3 and 5) suggests a return to eucorticalism at a time when the hypertensive syndrome is established. However, it is during this time that an increase in saline consumption occurs in the rats bearing regenerating adrenal cortices (Figs. 83 and 84), and the correlation between increased salt consumption and hypertension is just as close as the return to eucorticalism.

Transplantation of the regenerating adrenal cortex to the portal area prevents the development of the hypertensive syndrome. In these rats saline consumption is not increased. In addition thymic enlargement persists suggesting some degree of adrenal cortical insufficiency which might be attributed to the hepatic metabolism of steroids produced by the regenerating adrenal cortices.

It seems unlikely, however, that the hypertension can be attributed solely to toxic overdosage with sodium chloride. A differential in saline consumption was noted between control and test animals at two different levels at the 40 and 54 day period (Table 15). Animals with regenerating cortices which consumed an average of 72 ml. saline/rat/day for 40 days, developed

hypertension, while control rats drinking 79 ml. saline daily for 54 days did not show a rise in blood pressure. It may be that the defect in the rat with regenerating cortical tissue lies in its inability to deal with a sodium chloride load rather than the total load imposed. The exact role of the cortical tissue in this reaction remains to be elucidated.

SUMMARY

1. Hypertension and pathological changes in the heart, blood vessels and kidney develop in unilaterally nephrectomized rats maintained on 1% sodium chloride and bearing adrenal cortical tissue regenerating in situ or in the groin.

2. The hypertensive syndrome is prevented by the substitution of tap water as drinking fluid.

3. Hypertensive pathological changes are prevented by the transplantation of the regenerating adrenal cortical tissue into the portal area, either spleen or liver, despite the continued administration of saline.

CONCLUSION

CONCLUSION

In the Introduction the object of this Thesis was defined as a study of the role of the pituitary and adrenal glands in the aetiology of certain diseases, especially peptic ulcer and hypertension. Their experimental counterparts have been reproduced and modified by a variety of methods, such as the administration of pituitary hormones, natural and synthetic steroids, by sodium chloride overdosage, and during adaptation to stressful situations. Two main lines of thought have been pursued:-

1. The role of the adrenal corticoids in the aetiology of experimental peptic ulcer and hypertension.

2. The role of electrolyte excess or imbalance in the aetiology of certain forms of experimental hypertension.

The adrenal cortex, peptic ulcer and hypertension

It would be difficult to define "disease" and not imply that there is some failure of adaptive mechanisms. In addition, it would be difficult to define "stress" in a way which would exclude any cause of disease as being a stressor. Selye (1950) is the principal of many advocates of the concept that an alteration in adrenal cortical function as a result of exposure to stress is the primary cause of many human diseases, including peptic ulcer and hypertension. Examination of the results presented in this Thesis throws considerable doubt on the

validity of this concept.   As an alternative the evidence can be interpreted more satisfactorily as supporting the hypothesis that the hormones of the adrenal cortex play a permissive role in the development of certain morphological responses to stress and in the pathological manifestations of disease (Sayers, 1950; Engel, 1951; Ingle,1952).

The hormones of the adrenal cortex are necessary for the normal operation of many physiological processes, and so it is reasonable that the presence or absence of these hormones should affect the processes and signs of disease.   Peptic ulcer was produced experimentally by the administration of the posterior pituitary pressor factor and the pathogenesis of the gastric lesions was determined by arteriographic techniques.   An increased adrenal cortical response to this hormone was demonstrated (Part I).   Hypercorticalism per se is not ulcero-genic, yet the administration of corticoids or corticotrophin aggravated peptic ulcers induced with pitressin (Part II). Hypercorticalism induced by various stress procedures failed to cause peptic ulcer, but under the unusual circumstances of cold exposure hypertension developed (Parts III and IV).   Both experimental diseases occurred in the absence of the adrenal glands when the animals were given corticoid therapy.   The term "permissive" action seems preferable to "active" cause in this situation, although the word "permissive" does not represent insight into the exact mechanism by which the steroids

support these diseases.

A monistic approach, such as that proposed by Selye, to problems as complex as peptic ulcer and hypertension seems bound to be fallacious. A wide range of homeostatic mechanisms, psychic, neural, mechanical and humoral, govern the functions of the stomach and the blood pressure, in health and disease. The adrenal cortical hormones are a part of these regulatory mechanisms and as such fit into the pattern of causation. Recent reports support the view that the hormones of the adrenal cortex play a permissive role in the metabolic activity of the gastric parietal cells in man (Engel, 1955; Bruce, Card, Marks and Sircus, 1959). It seems likely that further research at this cellular level will yield fresh information on the problems of gastric physiology and pathology.

Electrolyte imbalance and hypertension

For a number of years it has been suggested that sodium chloride metabolism is abnormal in essential hypertension in man. The reduction in blood pressure achieved by restriction of salt intake and, more recently, the demonstration of the anti-hypertensive effects of natruretic agents such as chlorothiazide lend support to this view (Weller and Hoobler, 1959). However, the exact role which a possible disorder in salt metabolism plays in either the pathogenesis or the perpetuation of the disease in man is not clear.

In view of these clinical observations and impressions it

is of interest that the several different forms of experimental
hypertension here studied are linked on a common basis by
increased sodium chloride load. The original studies of
Selye (1943) are frequently quoted to relate the stress of cold
exposure, increased adrenal cortical function and hypertension.
As a result of carefully controlled experiments it is now
established that this form of hypertension is more closely
associated with an increased dietary salt load (Part III).
This view is strongly reinforced by the observations that other
severe stressors failed to induce similar pathological changes
when there was no voluntary increase in salt consumption (Part
IV). Overdosage with sodium chloride alone produced a hyper-
tensive syndrome similar in many respects to that induced with
powerful sodium-retaining steroids. The development of these
lesions was not dependent completely on the adrenal cortex or
its hormones, although the changes occurred more readily in
their presence (Part V). All of these changes were produced
in rats sensitized by unilateral nephrectomy. The results
reported in Part V agree completely with the view recently
expressed by Page and his colleagues (1958) that this method of
"sensitization" represents a device which accelerates the toxic
effects of sodium chloride load. This technique of
"sensitization" will still be used in this field of research.
Its continued use is justifiable now that its purpose is
established.

The causal relationship between adrenal cortical hormones and hypertensive pathological damage may be due mainly, but not completely, to the difficulty which the animal has with a sodium load.   It has been known for some time that the toxicity of sodium is determined in part by the ratio of sodium intake to that of potassium.   Meneely and his group (1956) showed that an accompanying rise in potassium intake partly ameliorates the pathological effects of high sodium chloride loads.   In addition, the results of Part VI demonstrate that the hypertensive pathological changes associated with adrenal cortical regeneration are prevented if tap water is substituted for saline as drinking fluid.   It seems possible that the toxicity of sodium may be based upon its distribution within and outside of cells, together with the accompanying imbalance of other electrolytes.   Tobian and Redleaf (1957, 1958) have demonstrated an increased sodium, potassium and water content of the arterial wall in experimental hypertension induced by either renal or endocrine mechanisms.   The movement of sodium and water into the cell and of potassium into the extracellular phase during acute pressor episodes has been reported by Friedman and his group (Friedman, Butt and Friedman, 1957; Friedman, Nakashima and Friedman, 1958; Friedman, Scherrer, Nakashima and Friedman, 1958).

The close temporal association between these cation shifts and blood pressure changes suggests a causal relationship at

cellular level in arterial smooth muscle.   The results reported in this Thesis lend strong support to the view that disturbances in electrolyte metabolism are an important factor in the pathogenesis of several forms of experimental hypertension.   As a result of metabolic balance studies a similar view has recently been expressed by Weller and Hoobler (1959) concerning essential hypertension in man.   Many physiological processes are influenced by their ionic environment and it may well be that cation changes affect energy-yielding processes or the actual contractile mechanisms in vascular smooth muscle so leading to an alteration in peripheral vascular resistance and blood pressure level.

APPENDIX

DIET

1. **Diet number 18 (Bruce and Parkes, 1946)**

Artificially dried grass	30%
Barley meal	20%
Ground nut meal	15%
Bran	15%
Linseed	10%
Meat and bone meal	8%
Minerals	2%

2. **Diet number 41**

Whole meal	46%
Sussex ground oats	40%
Fish meal	8%
Dried skimmed milk	3%
Dried yeast	1%
Cod liver oil	1%
Salt	1%

3. **Medium carbohydrate 4% sodium chloride diet**

Cellu flour	60 g.
Salt mixture U.S.P. XIV	40 g.
Dried yeast (Pabst)	100 g.
Wheat germ oil	10 g.
Cod liver oil	10 g.
Mazola oil plus 100 mg.K	10 g.
Mazola oil	190 g.
Casein	160 g.
Starch	300 g.
Dextrin	150 g.
Sucrose	140 g.
Sodium chloride	50 g.
To make fluid diet add water	1040 ml.

FIXATIVES

1. 10% neutral formalin.

2. Bouin's fluid.

3. Formol-calcium (Baker, 1944)

Formalin (40% formaldehyde)	10 ml.
Water	90 ml.
Calcium chloride	1 g.

4. Formol-sugar-saline (Bacsich and Wyburn, 1939-42)

Formalin (40% formaldehyde)	100 ml.
Sodium chloride, saturated aqueous solution	400 ml.
Cane sugar, saturated aqueous solution	400 ml.

5. Rossman's fluid

Picric acid, saturated alcoholic solution	10 ml.
10% neutral formalin	90 ml.

STAINING METHODS

Where the staining methods employed are those given in standard texts, only the reference to the source is appended. Where a modification of a technique was used, the method is given in full.

1. Lendrum's acid picro-Mallory method (Lendrum and McFarlane, 1940).

2. Masson's trichrome stain (Lendrum, 1947).

3. Weigert's resorcin-fuchsin stain (Conn and Darrow, 1949).

4. Periodic acid-Schiff method (Pearse, 1954).

5. Gram's method (Lendrum, 1947).

6. Calcium-cobalt method for alkaline phosphatase, after Gomori (Pearse, 1954).

7. Lead phosphate method for acid phosphatase, after Gomori (Pearse, 1954).

8. Oil Red O method (Pearse, 1954).

9. **Sudan methods**

   Solutions

   (1) Dissolve 2 g. of Sudan IV in a mixture of 50 ml. of absolute acetone and 50 ml. of 70% alcohol. Leave for 24 hours. Filter before use.

   (2) Dissolve 0.5 g. of Sudan black B in 100 ml. of 70% alcohol. Boil for 10 minutes in a reflux condenser. Cool and filter.

   Method

   (1) Cut 8μ frozen sections of gelatin-embedded adrenal glands.

(2) Rinse section in 70% alcohol.

(3) Stain for 10 minutes in Sudan IV or Sudan black B.

(4) Rinse in 2 changes of 70% alcohol.

(5) Rinse in water.

(6) Sudan IV sections may be stained with haematoxylin.

(7) Mount in glycerine jelly.

10. Schultz method for cholesterol (Symington, 1951)

Solutions

(1) 2.5% solution of iron alum in distilled water.

(2) Mixture of equal parts of glacial acetic acid and
      reagent concentrated sulphuric acid.

Method

(1) Immerse frozen sections of gelatin-embedded adrenal
      gland in the iron alum solution for 1-2 days.

(2) Rinse in water.

(3) Mount sections on a gelatin coated slide and allow
      to dry.

(4) Add a few drops of acid mixture to the section and
      cover with a coverslip.

ARTERIOGRAPHIC METHODS

1. Post-mortem method

The abdomen of the rabbit was opened immediately after death and the upper abdominal aorta and coeliac artery exposed. A fine glass cannula was tied into the origin of the coeliac artery and the contrast medium was injected by means of the safety-valve injection apparatus (Figs. 1 and 2). A 2% aqueous solution of Prussian blue (British Drug Houses) was used. A constant pressure of 180 mm.Hg. was maintained until the serosal surface of the stomach became uniformly blue in colour and until all blood had apparently been expelled by the injection mass through a small incision in the portal vein. Adequate injection usually took 2 or 3 minutes. After fixation in 10% neutral formalin blocks were taken from the oesophagus, stomach and duodenum. Paraffin sections were cut at 150 μ, passed through xylol and mounted in D.P.X.

2. Pickworth's benzidine method (Bacsich and Wyburn, 1939-42)

Solutions

(1) Sodium nitroprusside benzidine reagent - Prepare
0.5% solution of benzidine in 2% aqueous acetic
acid. Dissolve 0.1 g. of sodium nitroprusside
in 20 ml. of distilled water and add 25 ml. of
benzidine solution. Make up to 100 ml. with
distilled water and filter.

(2) Hydrogen peroxide solution - Add 2 ml. of hydrogen

peroxide (20 volume) to 400 ml. of distilled water.

Method

(1) Fix stomach in formol-sugar-saline for 48 hours.

(2) Wash stomach in running water for several hours.

(3) Cut frozen sections at 100 μ and wash in water.

(4) Immerse sections for 1 hour at 37°C in the sodium nitroprusside benzidine reagent.

(5) Rinse rapidly in water.

(6) Immerse in hydrogen peroxide solution for 1 hour at 37°C.

(7) Wash, dehydrate, clear in xylol and mount in D.P.X.

3. Carbon method

To demonstrate functioning vessels it was necessary to introduce into the vascular system during life some material which would be propelled by cardiac action into channels down to capillary size. A suspension of finely divided carbon of particle size less than 3 μ, prepared by Imperial Chemical Industries, was used. The rabbit was anaesthetized with ether and the abdominal aorta exposed. With a fine hypodermic needle 8 to 10 ml. of carbon suspension was injected slowly into the aorta 1 cm. above the origin of the coeliac artery over a period of 2 minutes. Carbon was also propelled into the other abdominal viscera and the degree of injection of their vessels acted as a guide to satisfactory filling of the gastric vessels.

Towards completion of the injection ether was administered liberally, and the oesophagus, stomach and duodenum were removed for fixation in 10% neutral formalin.   Blocks were processed to paraffin and sections cut at 150 μ.   The sections were passed through xylol and mounted in D.P.X.

SURGICAL METHODS

All surgical procedures were carried out under ether anaesthesia.   Clean but not sterile technique was used.   Under these conditions the incidence of post-operative infection is virtually nil but in most experiments antibiotics were given.   A single injection of 5000 units of penicillin and 5 mg. of streptomycin was administered and was not repeated.

1.   Adrenalectomy

A single midline skin incision in the lumbo-dorsal region was used to expose both adrenal glands.   The skin was retracted to one or other side to reveal the junction of the lateral abdominal muscles and the lumbar muscle mass.   A muscle incision was made at this junction and the kidney and adrenal gland were delivered into the wound.   With a fine curved haemostat forceps the adrenal pedicle was clamped with as much of the surrounding fat as possible, and the adrenal dissected free with its capsule intact.   A ligature was not applied and haemorrhage did not result.   The kidney was returned into the wound which was closed by a single linen suture.   Two linen sutures were used to close the skin incision.

2.   Adrenal enucleation

The adrenal gland was exposed as described.   A small incision was made in the capsule and the gland gently compressed with dissection forceps to express the pulp.   The adrenal

capsule with vascular pedicle intact was returned to the abdomen and the muscle and skin incisions sutured.

3. Adrenal transplantation

Bilateral adrenalectomy was performed and the left adrenal gland was placed in a Petri dish on filter paper moistened with saline. The lumbar incisions were closed as described. The abdomen was now opened through a ventral incision in the upper midline. The back of the rat was arched by placing a small block under the dorsum and by this manoeuvre the liver or spleen was easily delivered into the wound. The left adrenal was now dissected free from fat, the capsule incised, and the pulp expressed. The capsule was placed on the mesenteric border of the spleen or on the under surface of a lobe of the liver. A fine silk suture with atraumatic needle was used to secure the adrenal capsule to the splenic or hepatic capsule respectively. The abdominal muscle and skin incision were closed separately by continuous linen sutures. In a small group of animals the left adrenal capsule was transplanted into the right groin by suturing it subcutaneously to the mammary gland at that site.

4. Nephrectomy

In all experiments with unilaterally nephrectomized rats the right kidney was removed. The kidney was delivered into the lumbar wound as described. The renal capsule was ruptured and allowed to retract to the hilum of the kidney. The renal

pedicle was clamped with straight haemostat forceps and a firm linen ligature applied.   The kidney was removed and the muscle and skin incisions were closed separately by interrupted linen sutures.

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ENDOCRINE FACTORS IN EXPERIMENTAL

PEPTIC ULCER AND HYPERTENSION

VOLUME II

THESIS SUBMITTED FOR THE DEGREE OF
DOCTOR OF MEDICINE OF THE
UNIVERSITY OF GLASGOW

BY

W.A.J. CRANE, M.B., Ch.B. (Commendation)

Fig. 1. Injection apparatus for
post-mortem arteriographic method.
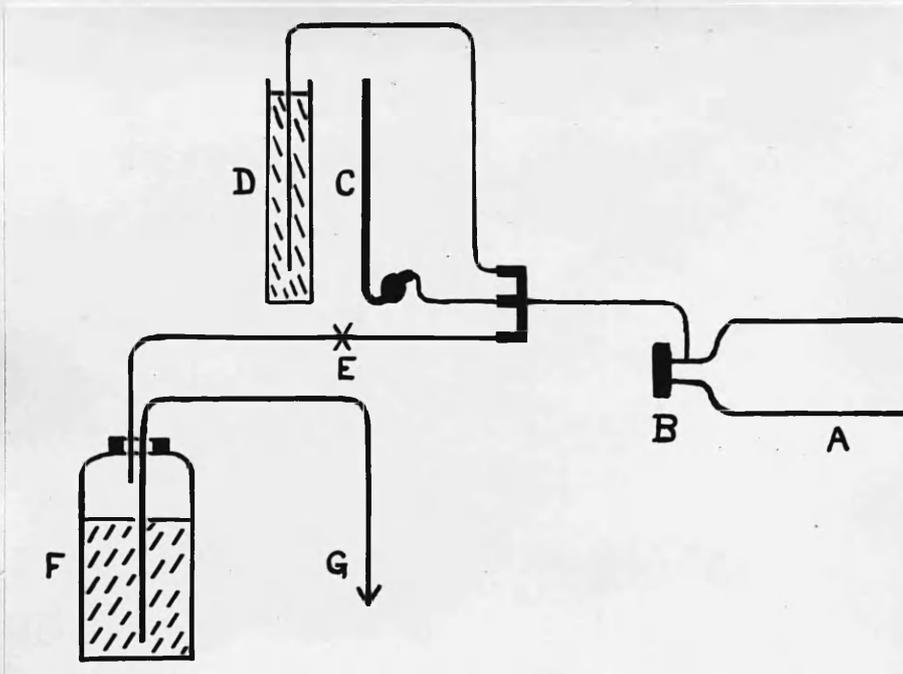
Fig. 2. Diagramatic representation
of injection apparatus.

A. Oxygen cylinder.   E. 3-way tap.
B. Coarse valve.   F. Injection mass.
C. Mercury manometer.   G. Cannula.
D. Mercury reservoir (safety valve).

Fig. 3.   The normal acid-secreting
mucosa of a rabbit's stomach.   Many
of the vessels are filled with
injected carbon.

Haematoxylin & Eosin. X 140.
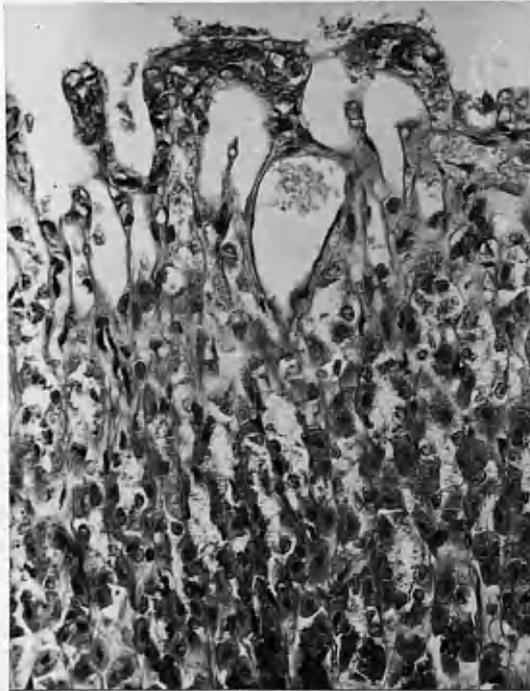
Fig. 4.   Superficial fundal mucosa.   Pitressin 30
minutes.   The surface columnar epithelium is shed and
the subjacent mucous neck cells and oxyntic cells show
early necrosis.   A dilated capillary contains
granular erythrocyte debris.

H. & E. X 350.
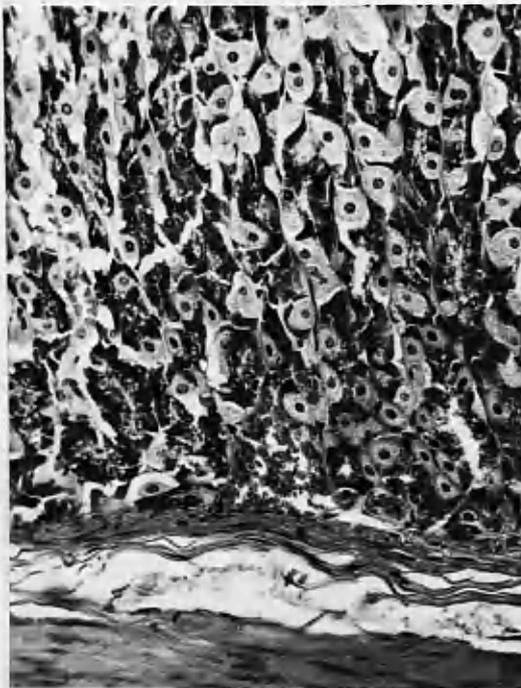
Fig. 5.   Deep fundal mucosa.   Pitressin 30 minutes.
Necrosis is absent in the area deep to that shown in
Fig. 4.   A venule contains normally staining
erythrocytes.

H. & E. x 350.

Fig. 6.   Fundus.   Pitressin 1 hour.   Degenerate
erythrocytes disrupt the necrotic superficial mucosa
at the summit of the gastric fold.

H. & E. x 60.

Fig. 7.   Fundus.   Pitressin 2 hours.   A haemorrhagic
bulla is present in the necrotic superficial mucosa.

H. & E. X 90.

Fig. 8. Stomach and duodenum. Pitressin
5 hours. There is marked oedema of the
fundal folds with mucosal haemorrhage.
The lesser curve, pyloric antrum and
duodenum are normal.
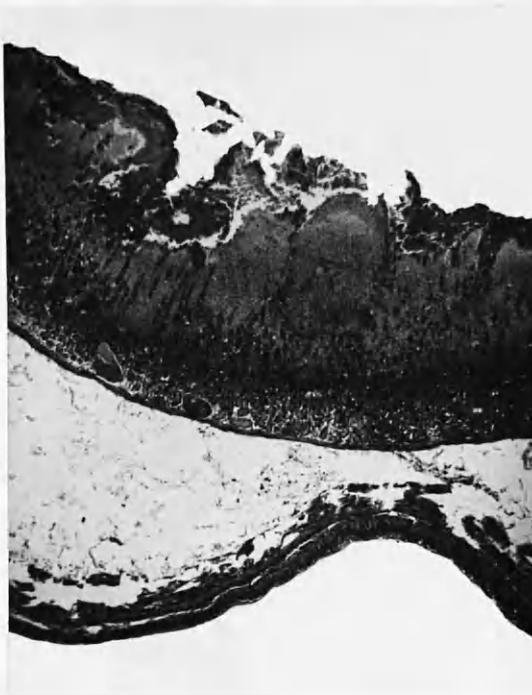
Fig. 9. Fundus.   Pitressin 5 hours.   The necrotic
mucosa is disrupted by haemorrhage, especially severe
towards the gastric lumen.   The submucosa is
oedematous.

H. & E. X 40.

Fig. 10.   Fundus of kitten.   An intricate capillary
anastomosis is present in the mucosa and muscle coat.
Venous drainage channels are seen in the submucosa.

Prussian-blue arterial preparation. 150μ. X 40.

Fig. 11. Duodenum of kitten.   The capillary pattern
in the duodenal villi, around Brunner's glands, and
in the muscle coat is shown.   Capillaries in the wall
of the bile duct are seen lower right.

Prussian-blue arterial preparation. 150μ. X 80.
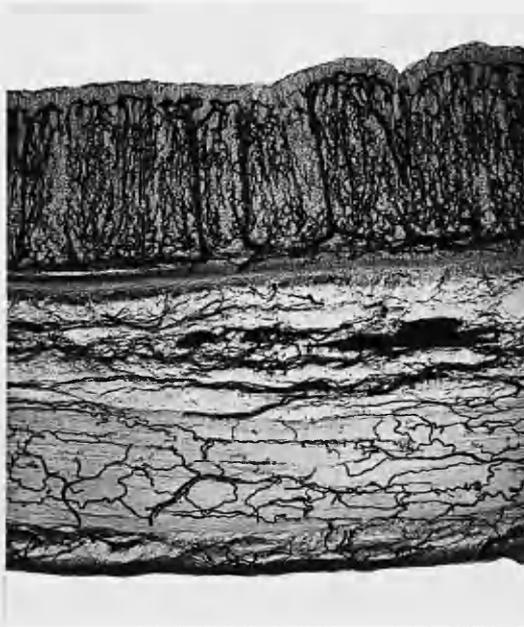
Fig. 12. Fundus of cat.   Anastomosing capillaries in
the mucosa drain from the surface via straight venous
channels.   Arteries and veins are filled in the
submucosa.   Further anastomoses are present in the
muscle coat.

Prussian-blue arterial preparation. 150μ. X 40.

Fig. 13.   Post-gastrectomy human specimen.   Fundus.
The mucosal pattern is that of anastomosing capillaries
drained by direct venous channels.

Prussian-blue arterial preparation. 150μ.X 60.
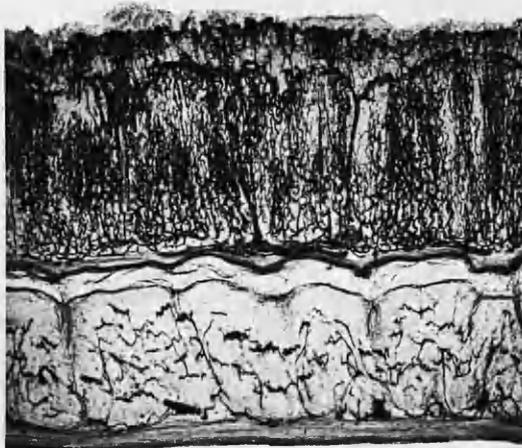
Fig. 14.   Fundus of normal rabbit.   The periglandular
capillary anastomosis in the mucosa is drained by
straight venous channels of wider calibre.

Prussian-blue arterial preparation. 150μ.X 40.

Fig. 15. Pylorus of normal rabbit. Capillary
anastomosis in the mucosa is less intricate. Drainage
again occurs by relatively straight venous channels.

Prussian-blue arterial preparation. 150μ. X 40.
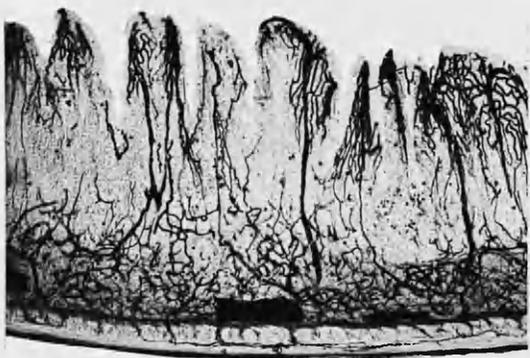
Fig. 16. Duodenum of normal rabbit. Venous injection
emphasizes the straight venous channels originating
from the capillary anastomoses at the tips of the villi.

Prussian-blue venous preparation. 150μ. X 40.
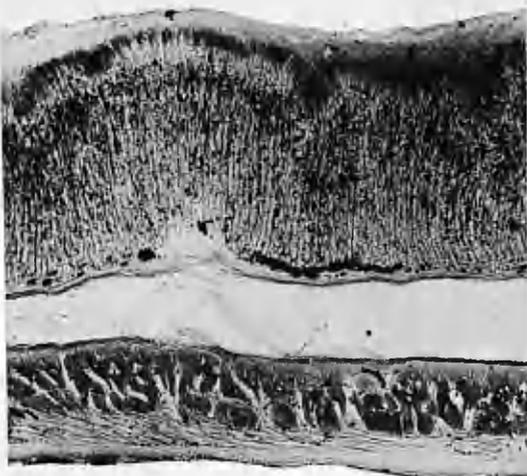
Fig. 17. Fundus. Pitressin
30 minutes. The distribution
of blood (black) is normal
apart from some deficiency at
the summit of the gastric
glands.

Benzidine. 100μ. X 40.
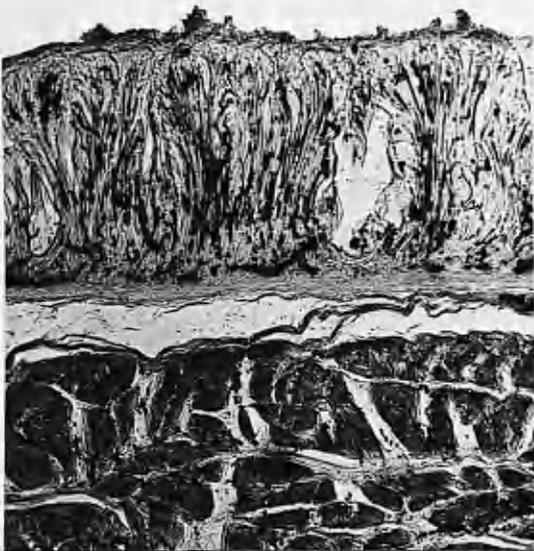
Fig. 18. Pylorus. Pitressin
30 minutes. The distribution
of blood is normal.

Benzidine. 100μ. X 40.

Fig. 19. Fundus. Pitressin
5 hours. There is gross
haemorrhage into the mucosa and
to a less extent into submucosa.

Benzidine. 100μ. X 40.
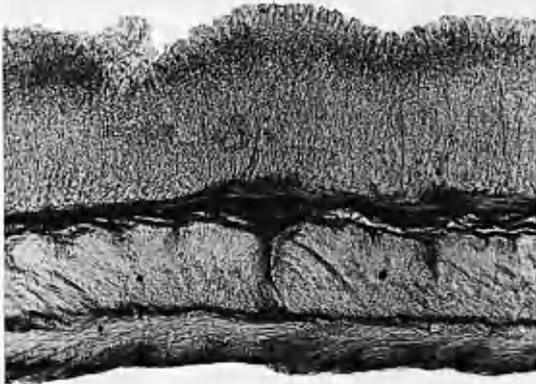
Fig. 20. Fundus. Pitressin
15 minutes. The blood vessels
contain no circulating carbon.
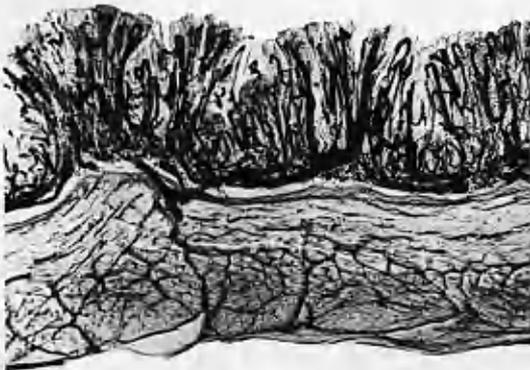
Carbon. 150μ.X 40.

Fig. 21. Pylorus from same
rabbit as in Fig. 20. The
vessels are well filled with
carbon.

Carbon. 150μ.X 40.
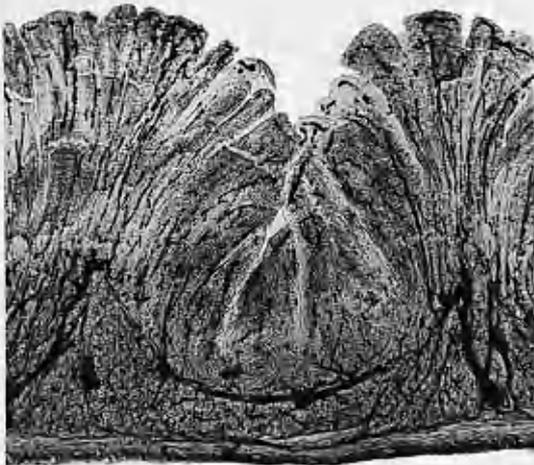
Fig. 22. Colon from same
rabbit as in Fig. 20. The
colonic vessels contain
circulating carbon.

Carbon. 150μ.X 40.
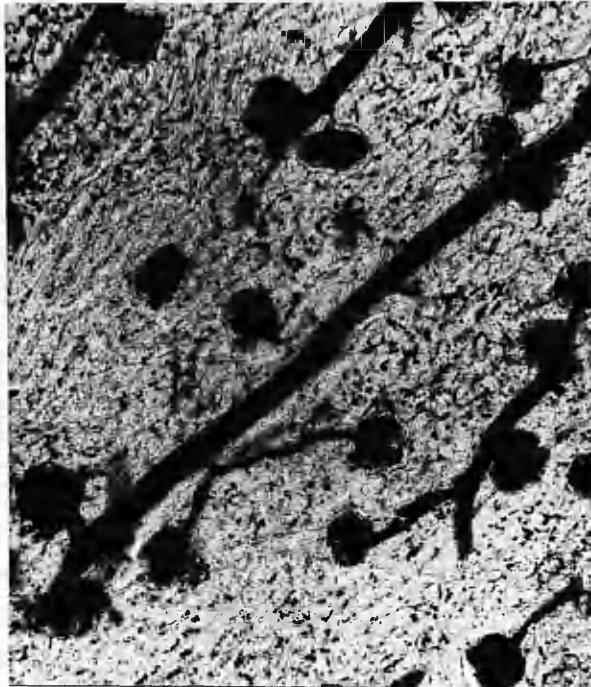
Fig. 23. Kidney.   Pitressin 30 minutes.   Arteries,
arterioles, and glomerular capillaries contain
circulating carbon.

Carbon preparation.   150μ.X 65.
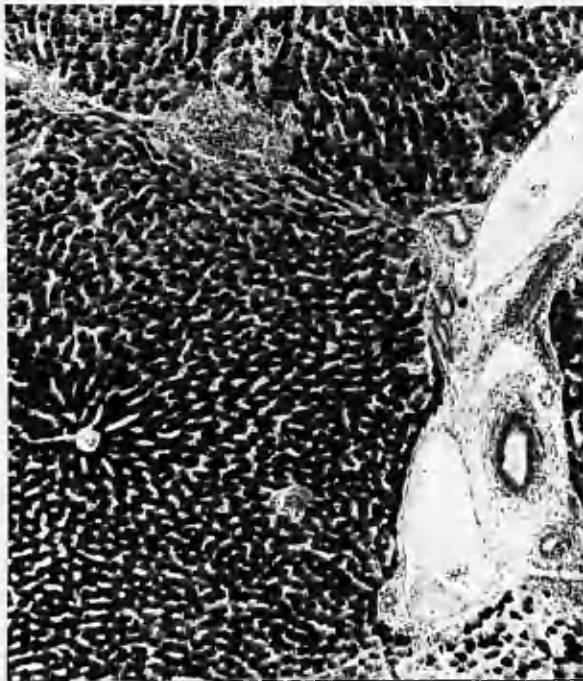
Fig. 24. Liver.   Pitressin 15 minutes.   The liver
cells contain abundant glycogen.

Periodic acid-Schiff. X 80.
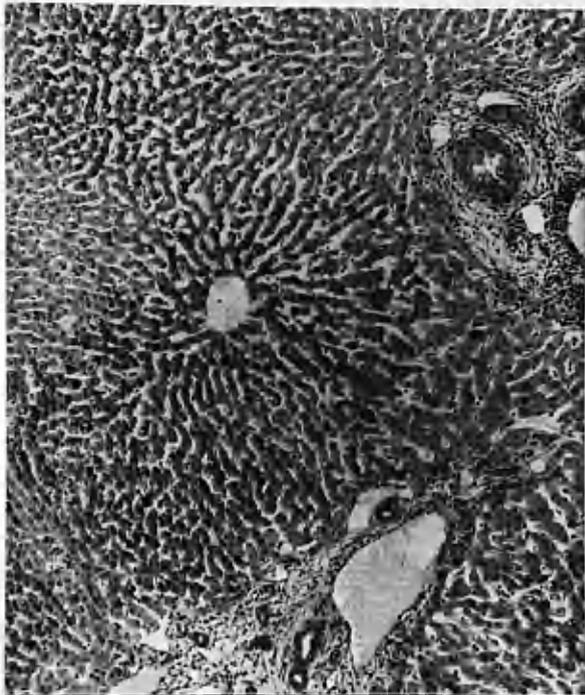
Fig. 25. Liver. Pitressin 3 hours. There is
marked depletion of liver glycogen. Compare
Figures 24, 25 and 26.

Periodic acid-Schiff. X 80.
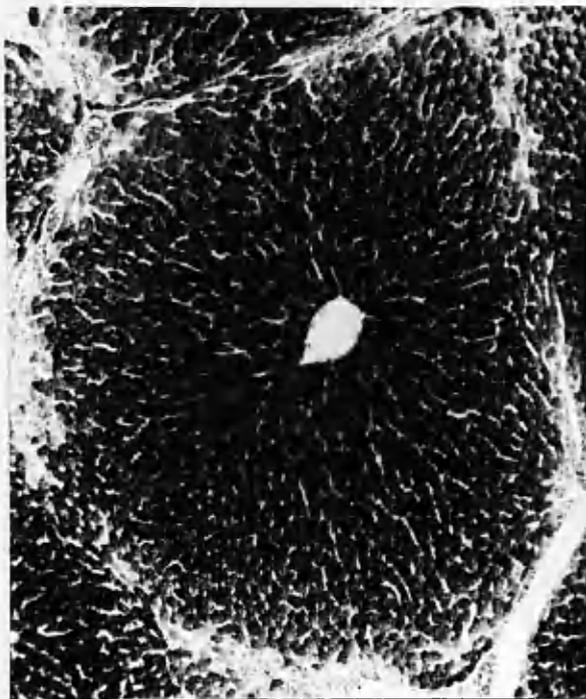
Fig. 26. Liver. Pitressin 5 hours. The liver
glycogen content of the liver is restored.

Periodic acid-Schiff. X 80.
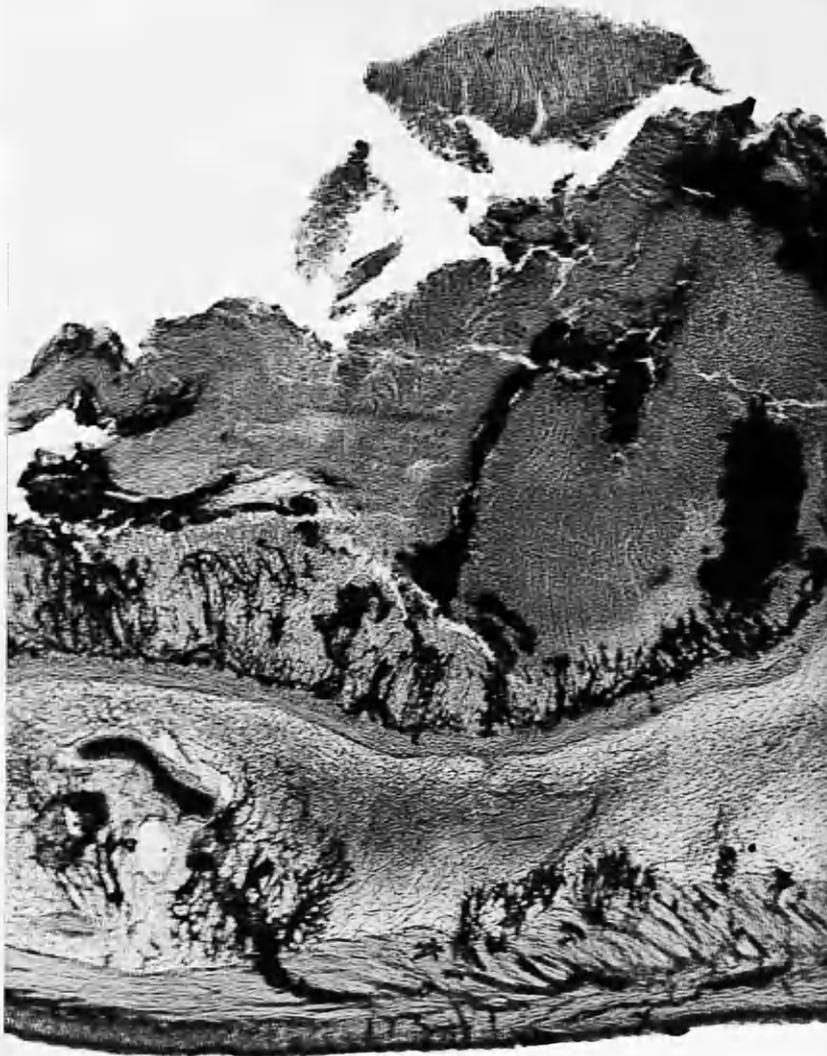
Fig. 27. Fundus.   Pitressin 10 days.
There is extensive mucosal necrosis with
distortion of the mucosal vascular
pattern.   New capillary formation is
seen in the submucosa.

Prussian blue
   arterial preparation. 150µ.X 25.
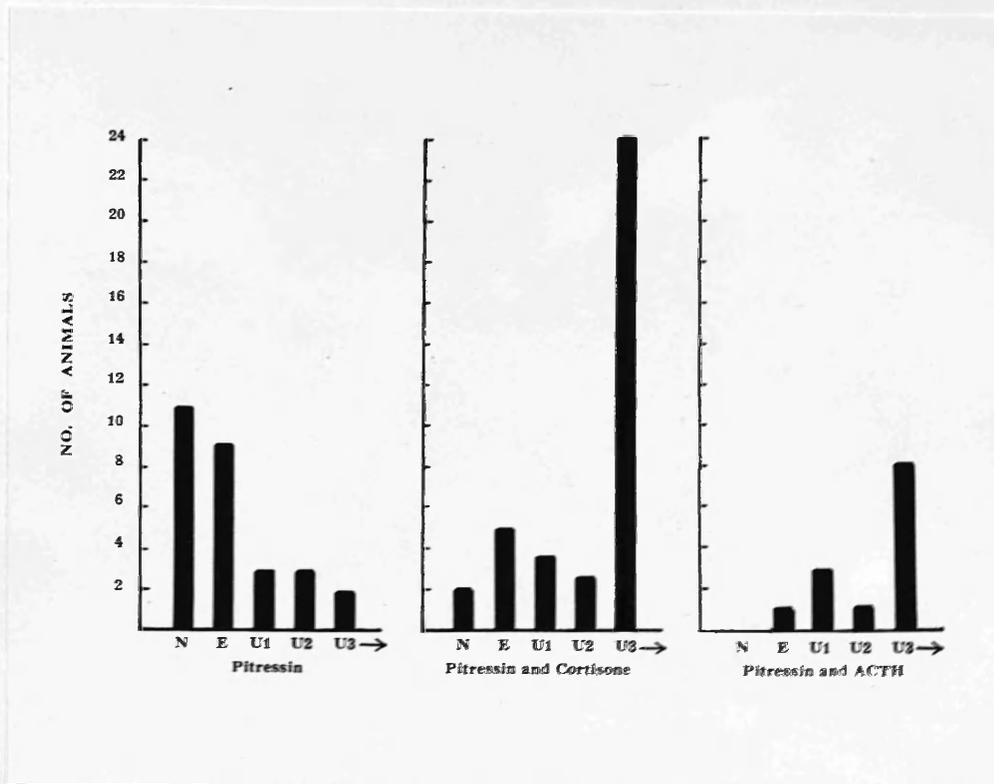
Fig. 28.   Incidence of gastric erosions
and acute ulcers in rabbits treated with
pitressin, pitressin and cortisone,
pitressin and ACTH.

N = stomach normal.

E = mucosal erosions only.

U1, U2, U3 ⟶   acute ulcers (1,2, 3 and
                more) with erosions.

Fig. 29. Stomach and duodenum.
Pitressin (Group 1A). The lesions
involve only a small area of the
acid-secreting mucosa.
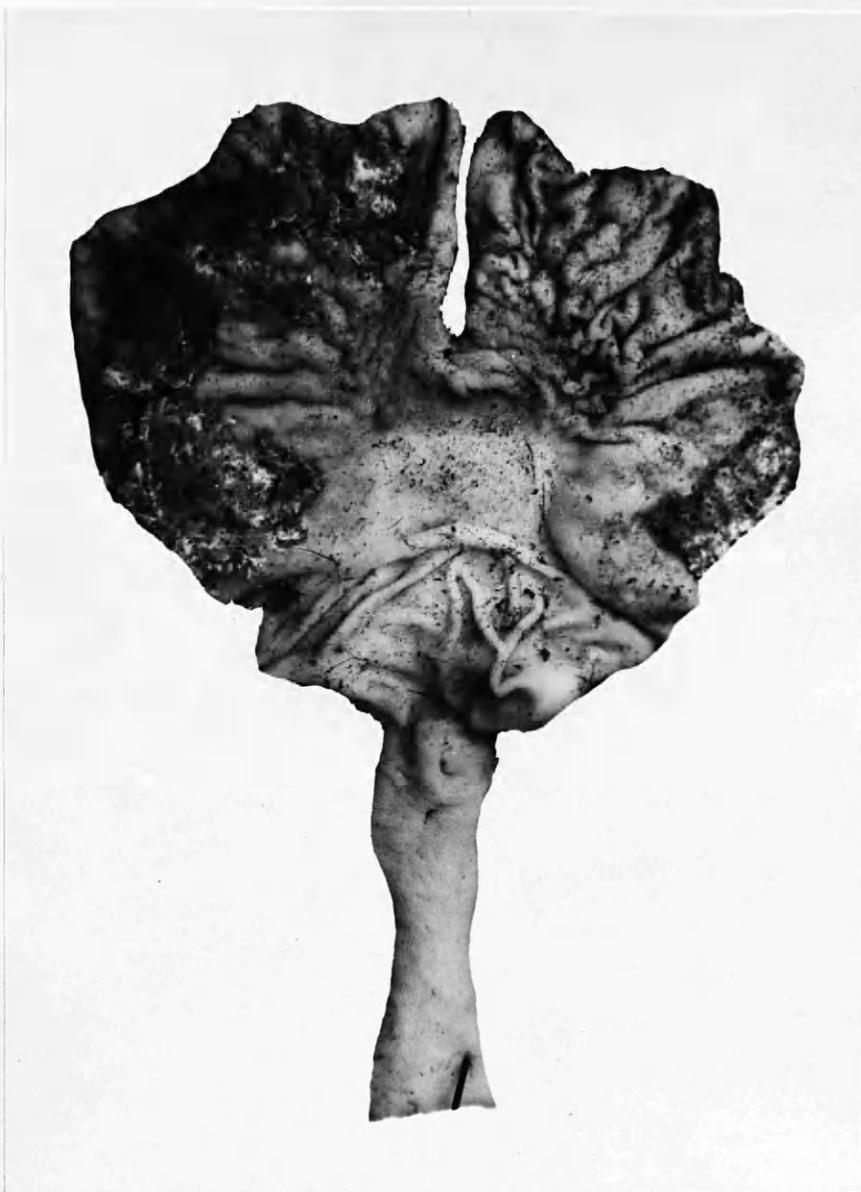
Fig. 30. Stomach and duodenum.
Pitressin and cortisone (Group 2A). A
much wider area of acid-secreting mucosa
is affected. The mucosal folds are
oedematous.
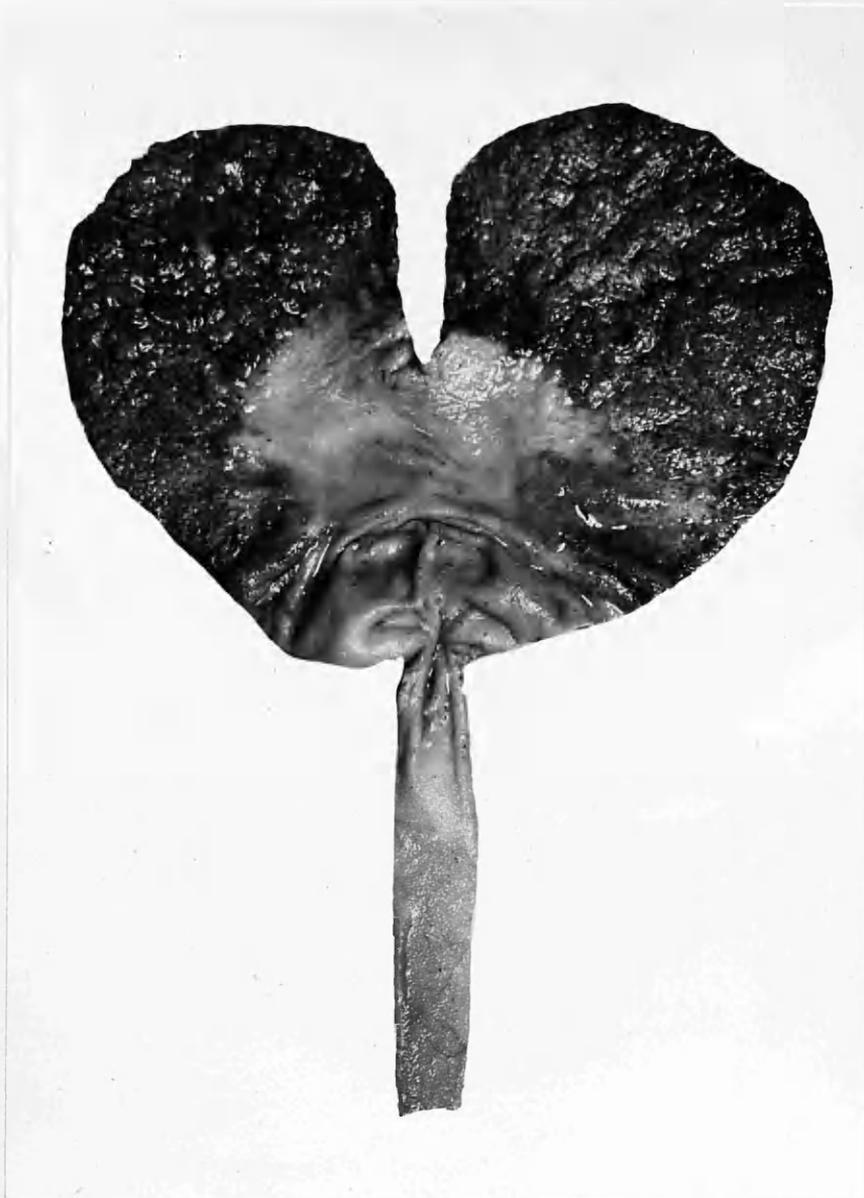
Fig. 31. Stomach and duodenum.
Pitressin and cortisone (Group 2A).
The entire acid-forming mucosa is
necrotic and disrupted by gas
bullae.
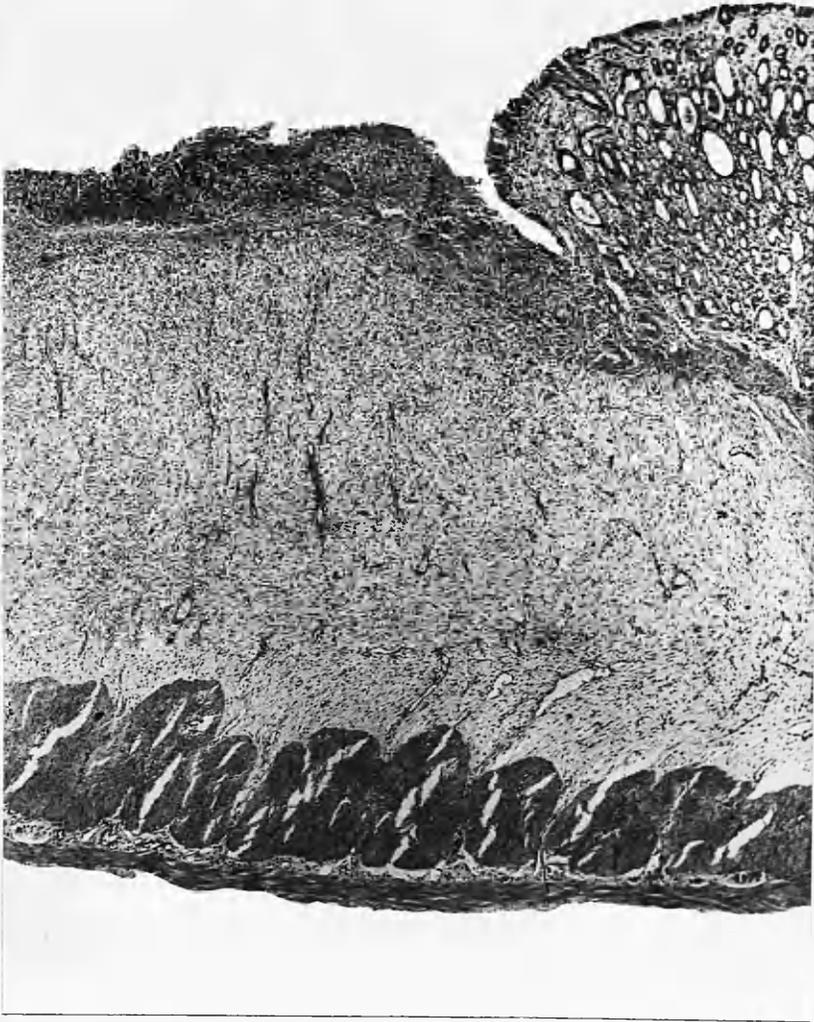
Fig. 32. The margin of an ulcer
produced by pitressin (Group 1A).
There is abundant granulation tissue
in the ulcer base. The muscle coat
is intact.

H. & E. X 45.
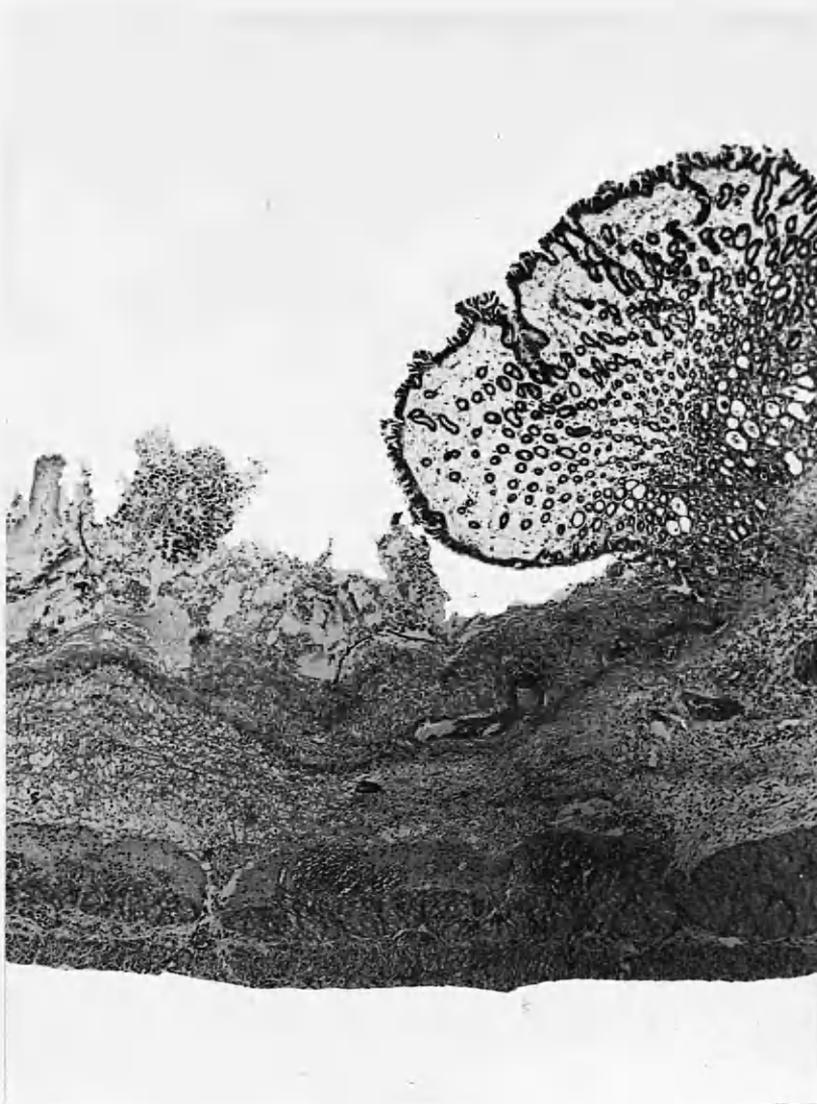
Fig. 33.   The margin of an ulcer produced
by pitressin and cortisone (Group 2A).
Fibroplasia in the centre of the ulcer
crater is absent.   A scanty cellular
reaction is seen at the extreme edge deep
to the oedematous mucosa.   There is
haemorrhage into the necrotic fibres of
the external muscle coat.

H. & E. X 45.

Fig. 34. Perforated fundal ulcer in rabbit treated with pitressin and cortisone (Group 2A). Only a local peritonitis is present.
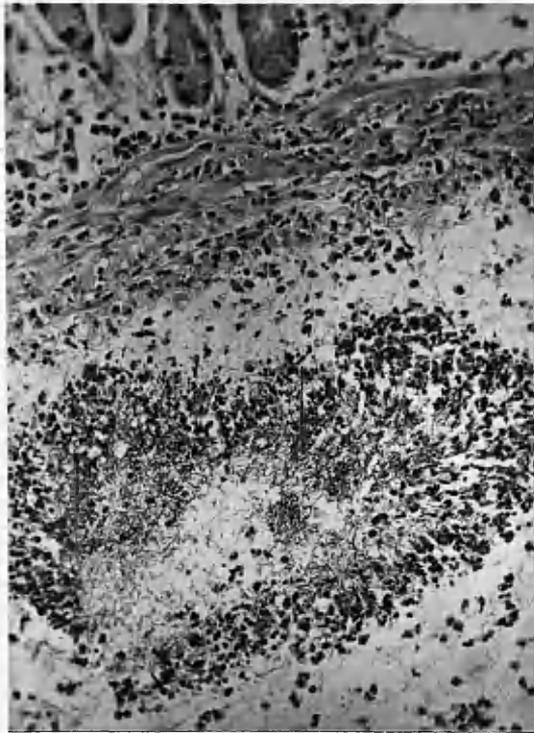
Fig. 35. A small abscess containing numerous
organisms lies deep to the muscularis mucosae.

H. & E. X 185.
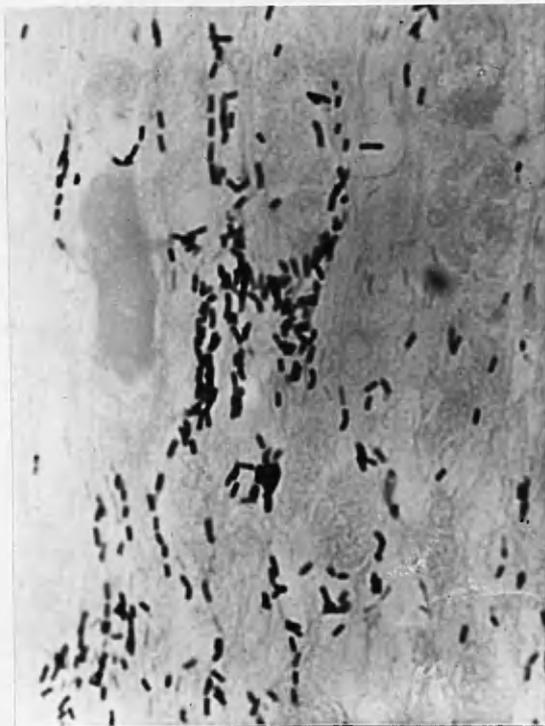
Fig. 36. Thick Gram-positive bacilli are
present in the necrotic fundal mucosa.

Gram.X 900.
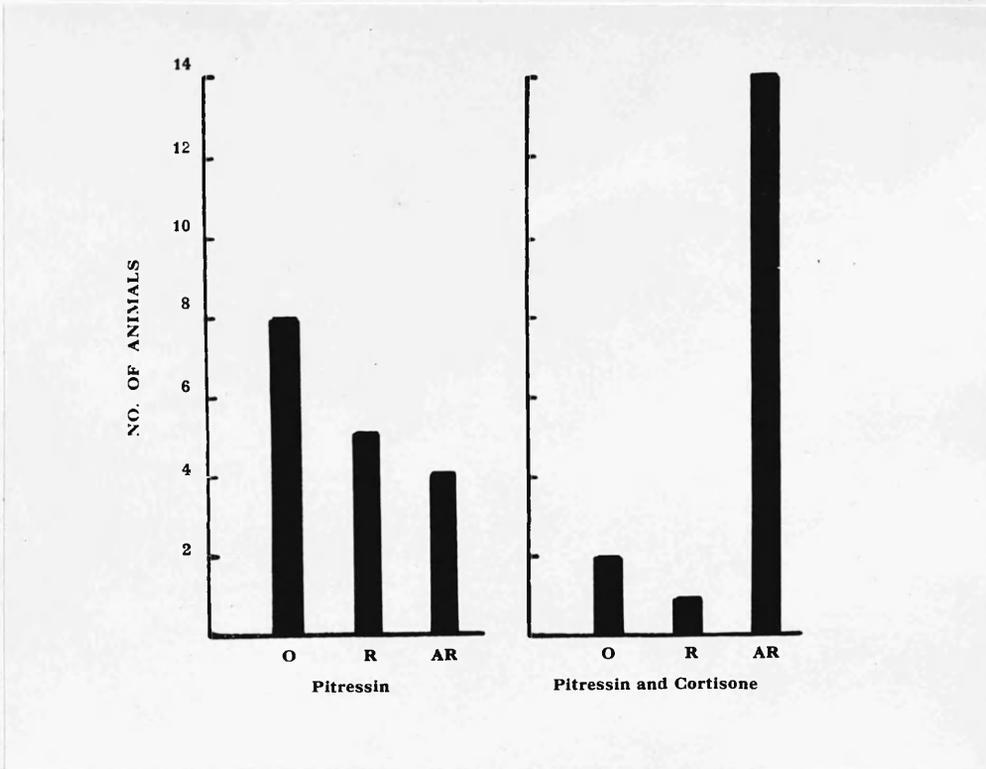
Fig. 37. Isolation of Cl. welchii from
gastric lesions of rabbits treated with
pitressin or pitressin and cortisone.

 O = no Cl. welchii.

 R = Cl. welchii in Robertson's broth
   only.

AR = Cl. welchii in anaerobic plate
   and Robertson's broth.

Fig. 38. Growth from gastric lesions
of a rabbit treated with pitressin and
cortisone (as in Group 2A). The plate
was cultured anaerobically for 24 hours
and then left under aerobic conditions
at room temperature for 24 hours.
There is a heavy growth of haemolytic
colonies of Cl. welchii with a more
scanty growth of coliform bacilli.

Fig. 39. Growth from gastric lesions of
a rabbit treated with pitressin (as in
Group 1A). The plate, cultured as
described in Fig. 38, shows only a scanty
growth confined to the butt and Cl.welchii
is absent.
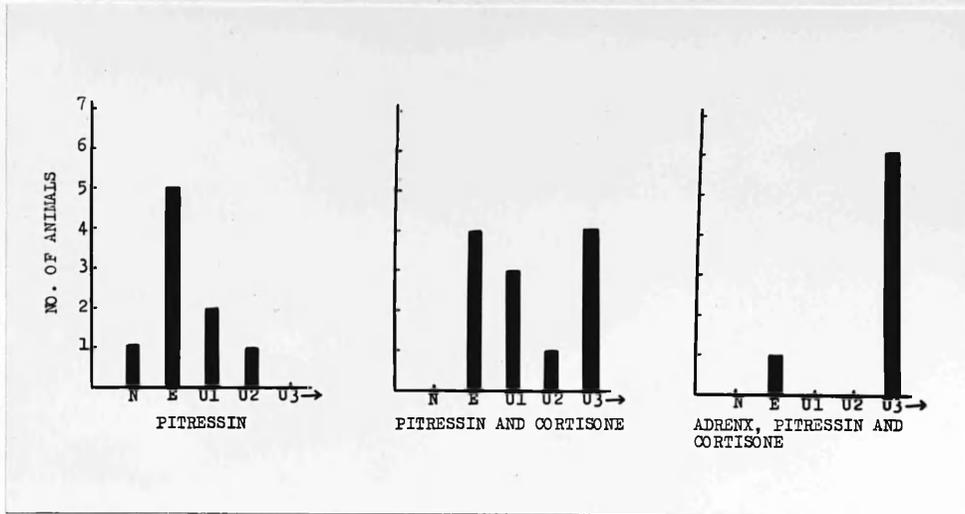
Fig. 40.   Incidence of gastric erosions and acute
ulcers in rats treated with pitressin, pitressin and
cortisone, and adrenalectomized rats treated with
pitressin and cortisone.

    N = stomach normal.

    E = mucosal erosions only.

    U1, U2, U3 ──→ acute ulcers (1,2,3 and more) with
                  erosions.

Fig. 41.   Fundus.   Cortisone 10 mg. daily with
antibiotics for 28 days.   There is necrosis of the
superficial mucosa.

                                    H. & E. X 130.

Fig. 42. Fundus. Cortisone 10 mg. daily with
antibiotics for 56 days. There is necrosis
with haemorrhage in the superficial mucosa.
Macrophages and fibroblasts are present in the
submucosa.

H. & E. X 130.
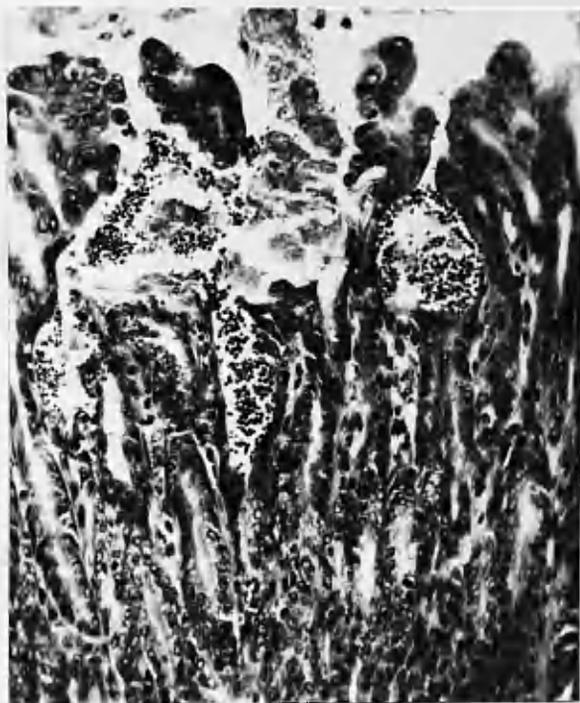
Fig. 43. Pylorus. Cortisone 10 mg. daily for
56 days. Yeast-like bodies are seen in the
mouths of the glands.

H. & E. X 225.
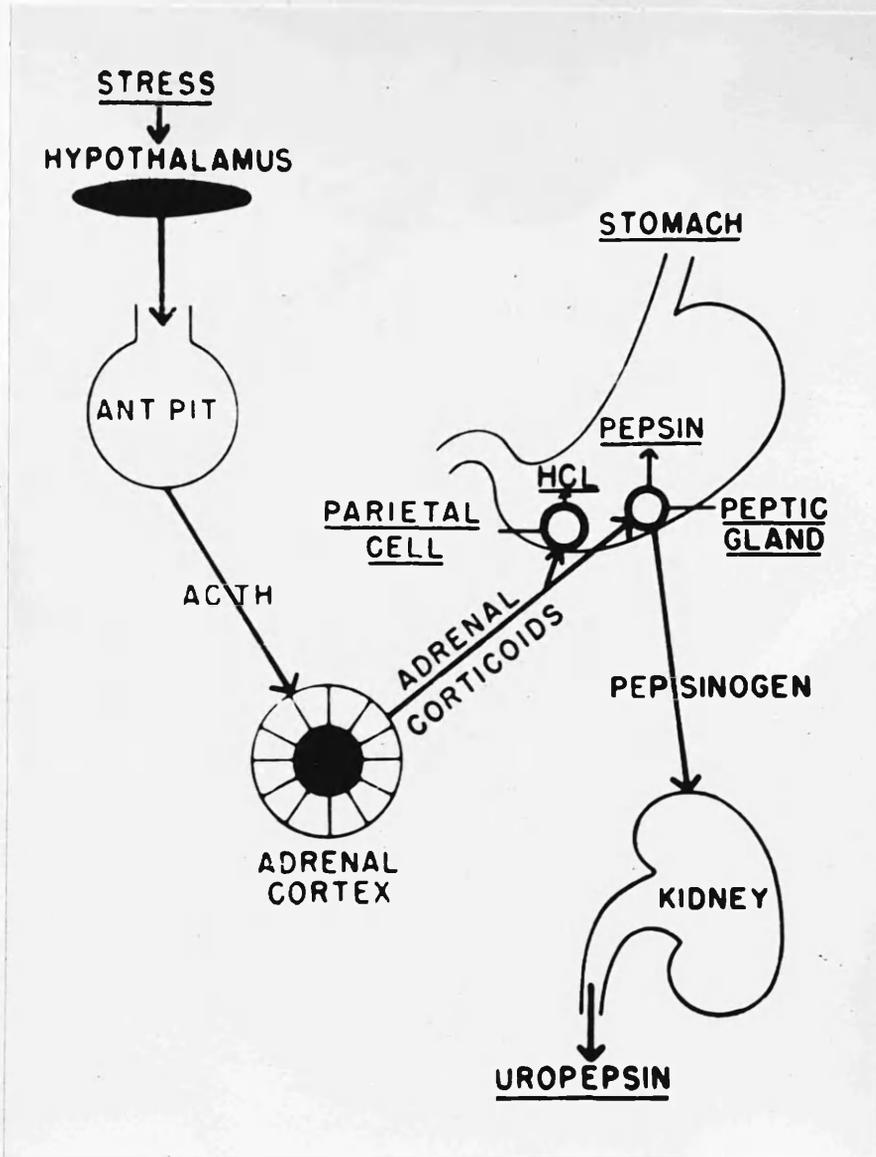
Fig. 44. Diagram to illustrate the functional relationship between the hypothalamus, anterior pituitary, adrenal gland and stomach, as postulated by Gray (from Gray et al., 1955-56).
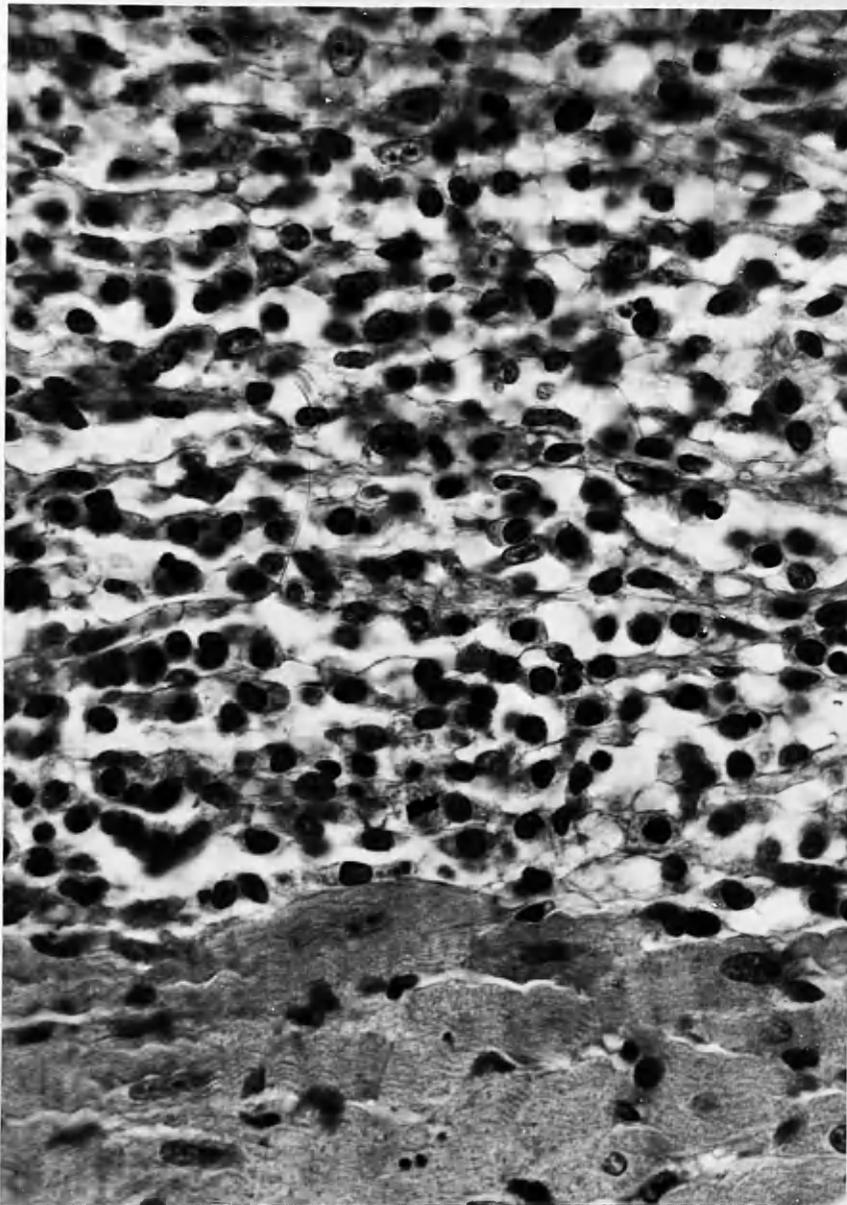
Fig. 45. Heart. Adrenx T25°C. A collection of cells, predominantly of macrophage type, is present in the myocardium.

H. & E. X 680.
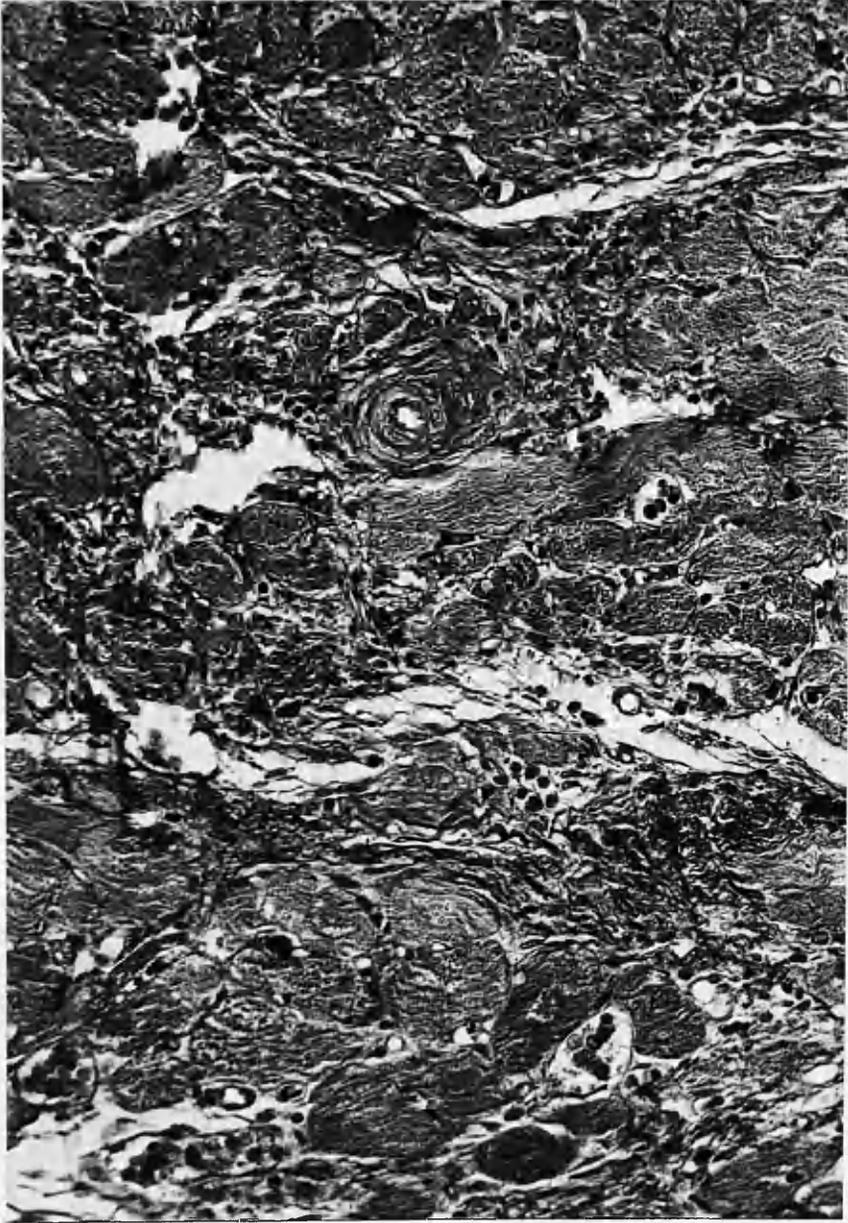
Fig. 46. Heart. Non-adrenx T3°C.
There is replacement fibrosis of the
myocardium and arteriolosclerosis of
a small arteriole.

Masson X 370.
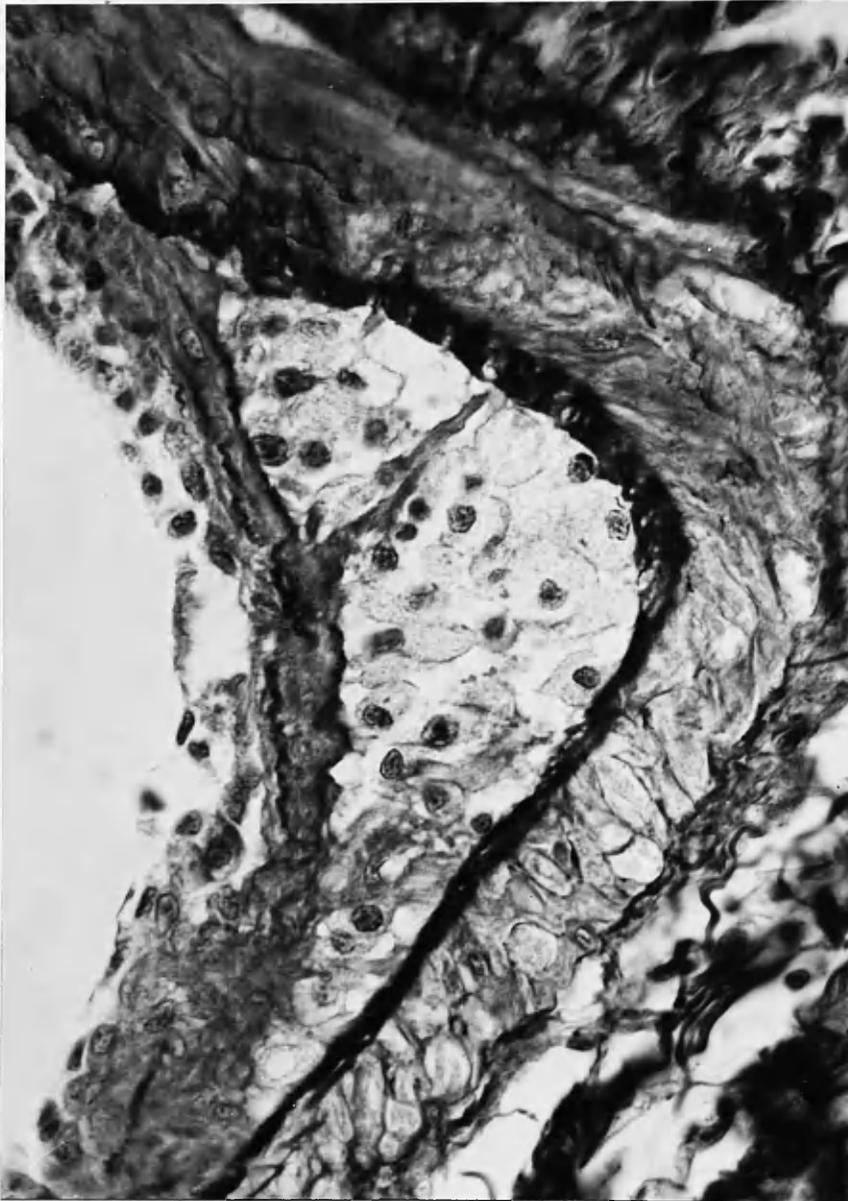
Fig. 47.   Coronary artery.   Adrenx
T3 C.   There is eccentric intimal
thickening caused by foamy cells lying
superficial to the internal elastic
lamina.

Weigert's resorcin-fuchsin
and Van Gieson. X 680.
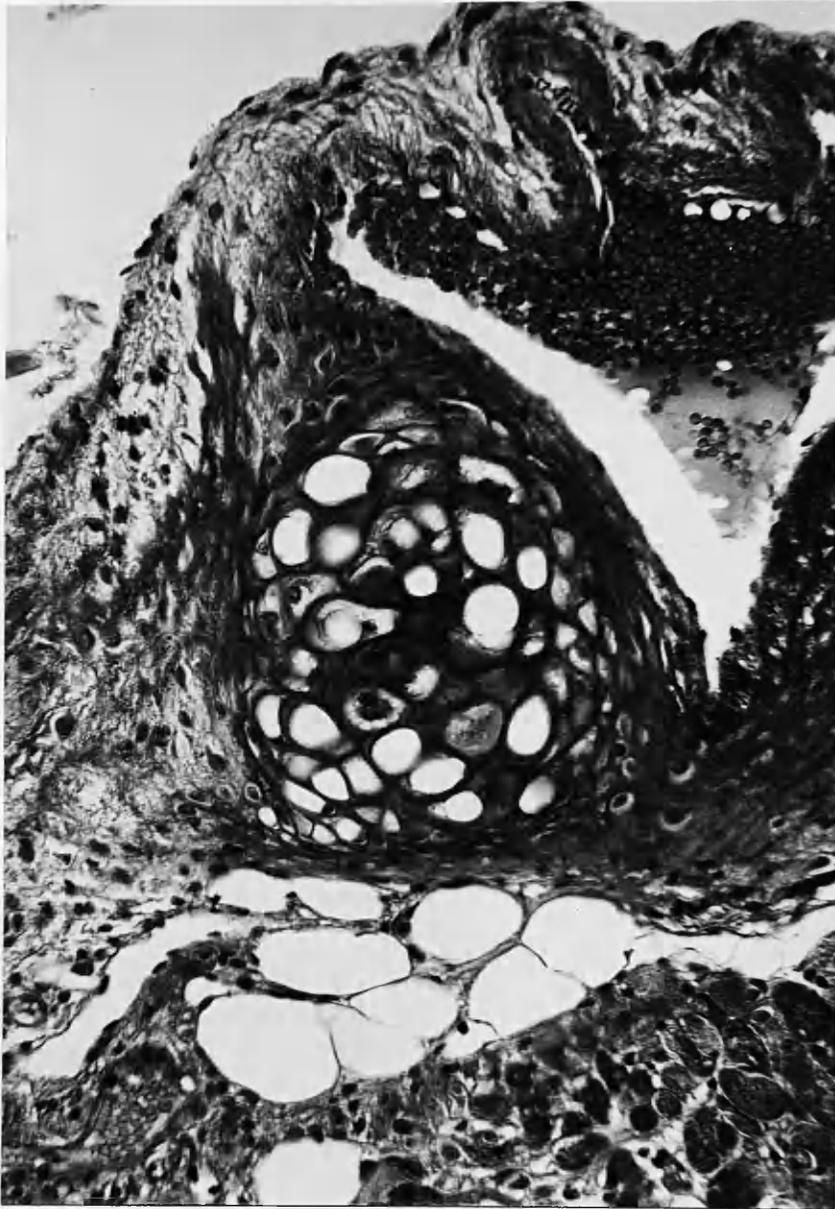
Fig. 48.   Aortic valve.   Non-adrenx
T25°C.   An islet of cartilagenous
metaplasia is present at the base of
the aortic valve.

Masson. X 345.
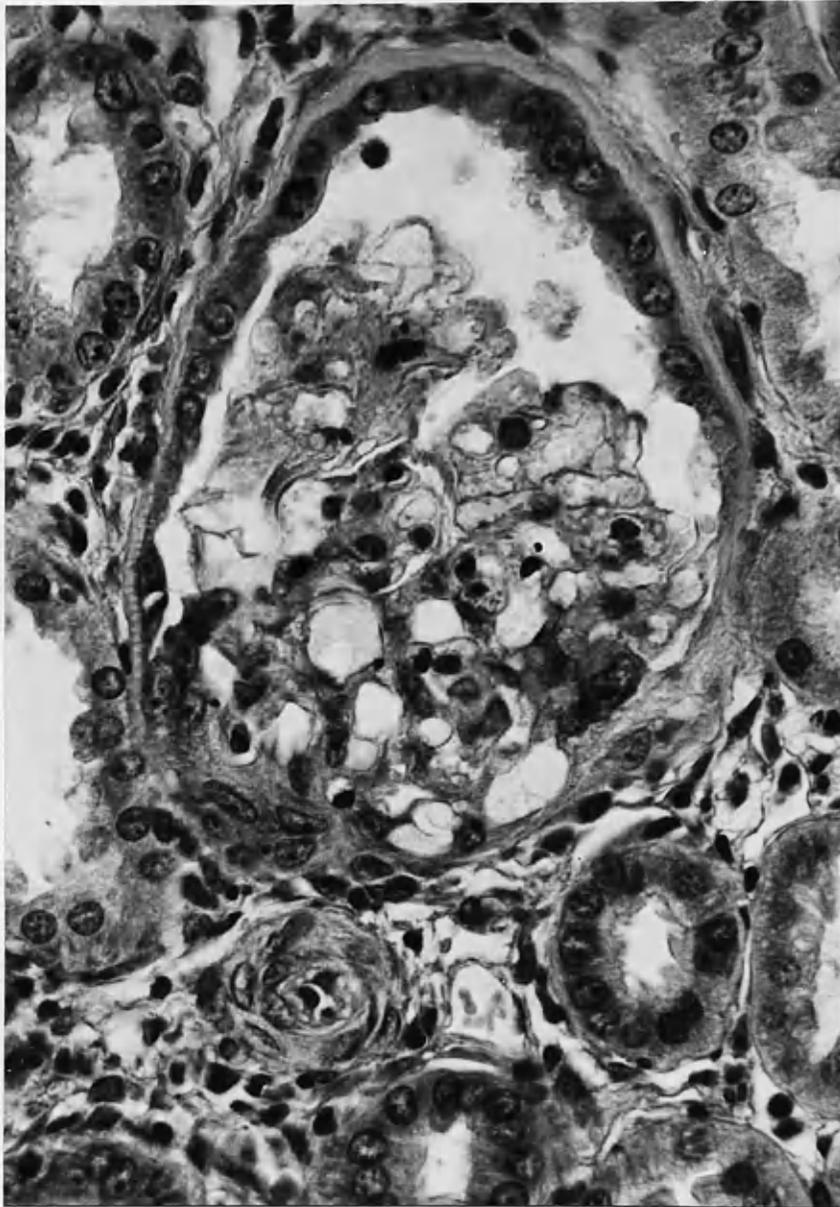
Fig. 49. Kidney.   Tube-fed T25°C.
The glomerular tuft is shrunken and
partially adherent to a thickened
Bowman's capsule.   The parietal
epithelium of the capsule is heightened
and shows mitotic activity.   There is
proliferative arteriolosclerosis.

H. & E. X 760.
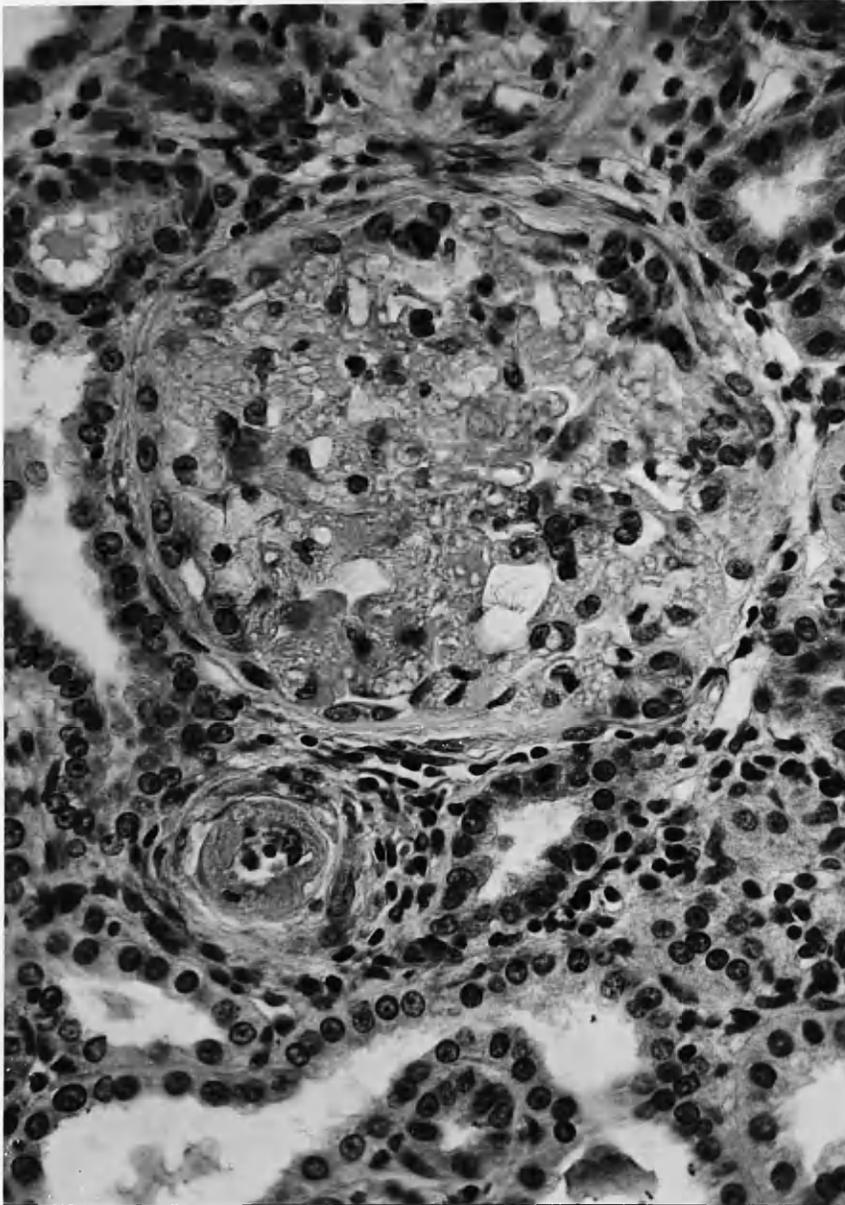
Fig. 50. Kidney. Non-adrenx T3°C.
The glomerulus is enlarged, bloodless
and fills the subcapsular space; some
of the cells have a swollen "foamy"
appearance. There is arteriolo-
necrosis of the afferent arteriole.

H. & E. X 500.
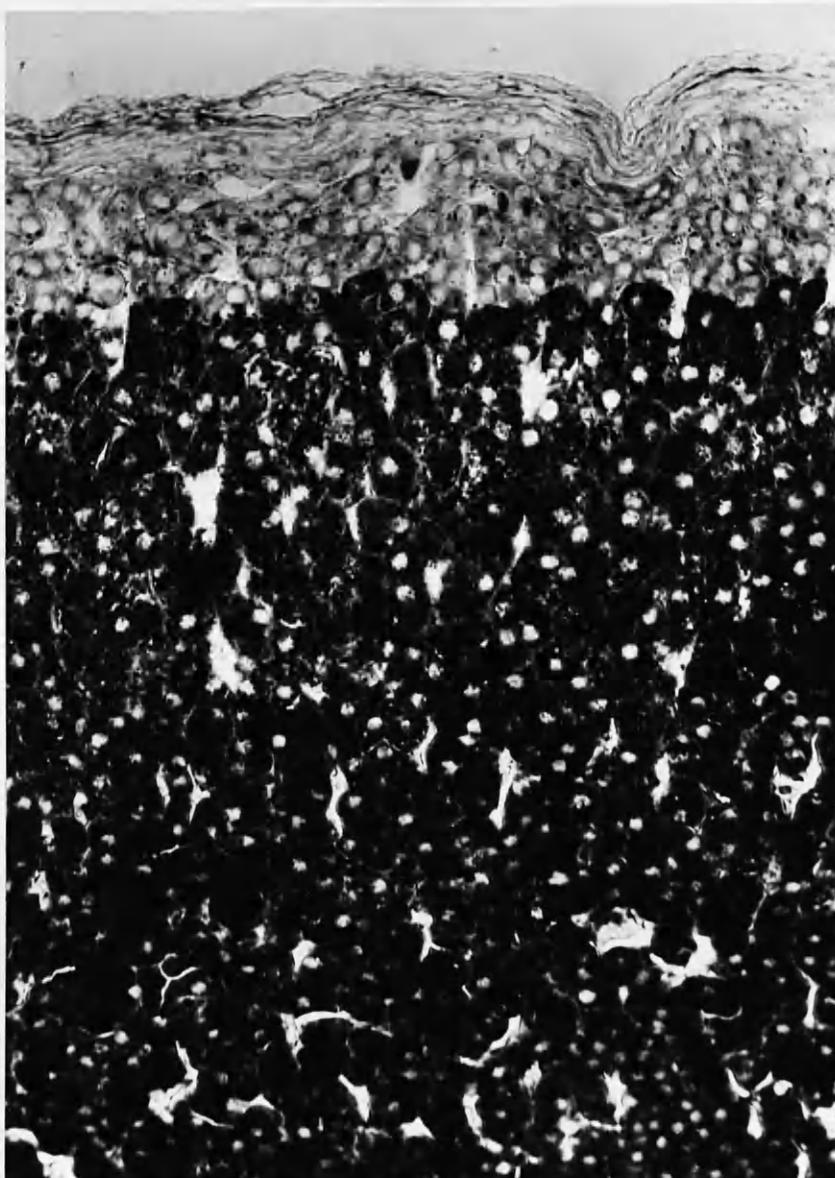
Fig. 51. Adrenal. Non-adrenx T25°C.
Zona glomerulosa is narrow and contains
almost no lipid.

Frozen section.
Sudan black. X 380.
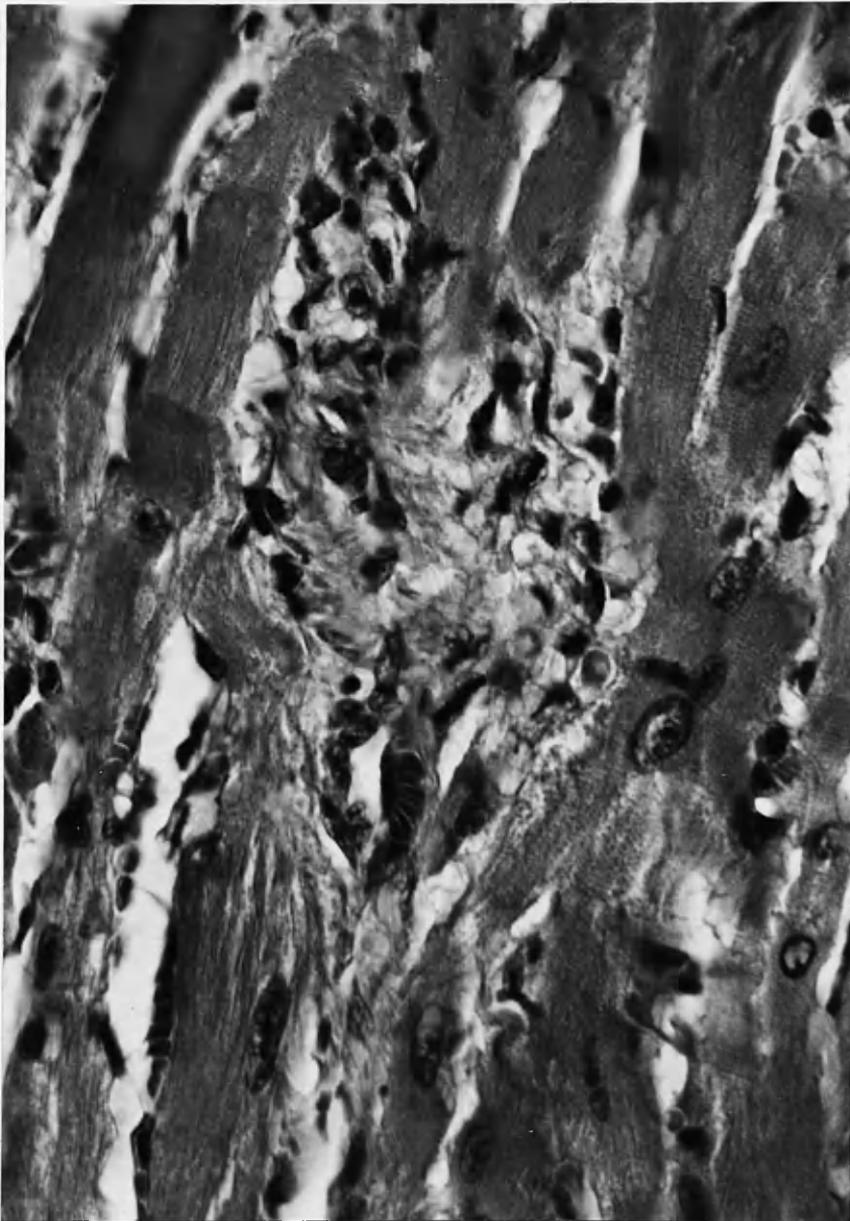
Fig. 52. Heart. Limb ligation. A small fibrous nodule containing Anitschkow myocytes is present in the interstitial tissue of the myocardium.

H. & E. X 760.
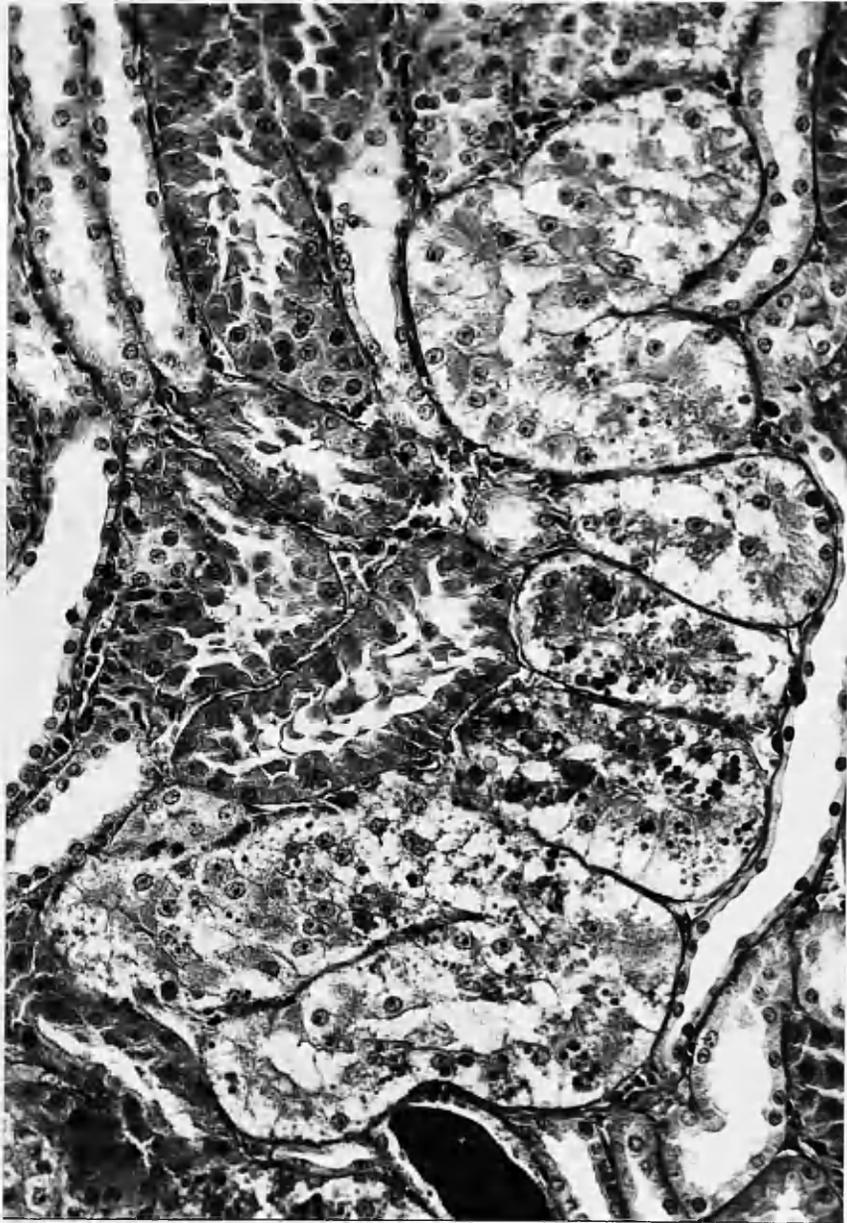
Fig. 53. Kidney. Burn. The
tubular epithelium shows vacuolation
and hyaline droplet change.

Picro-Mallory. X 345.
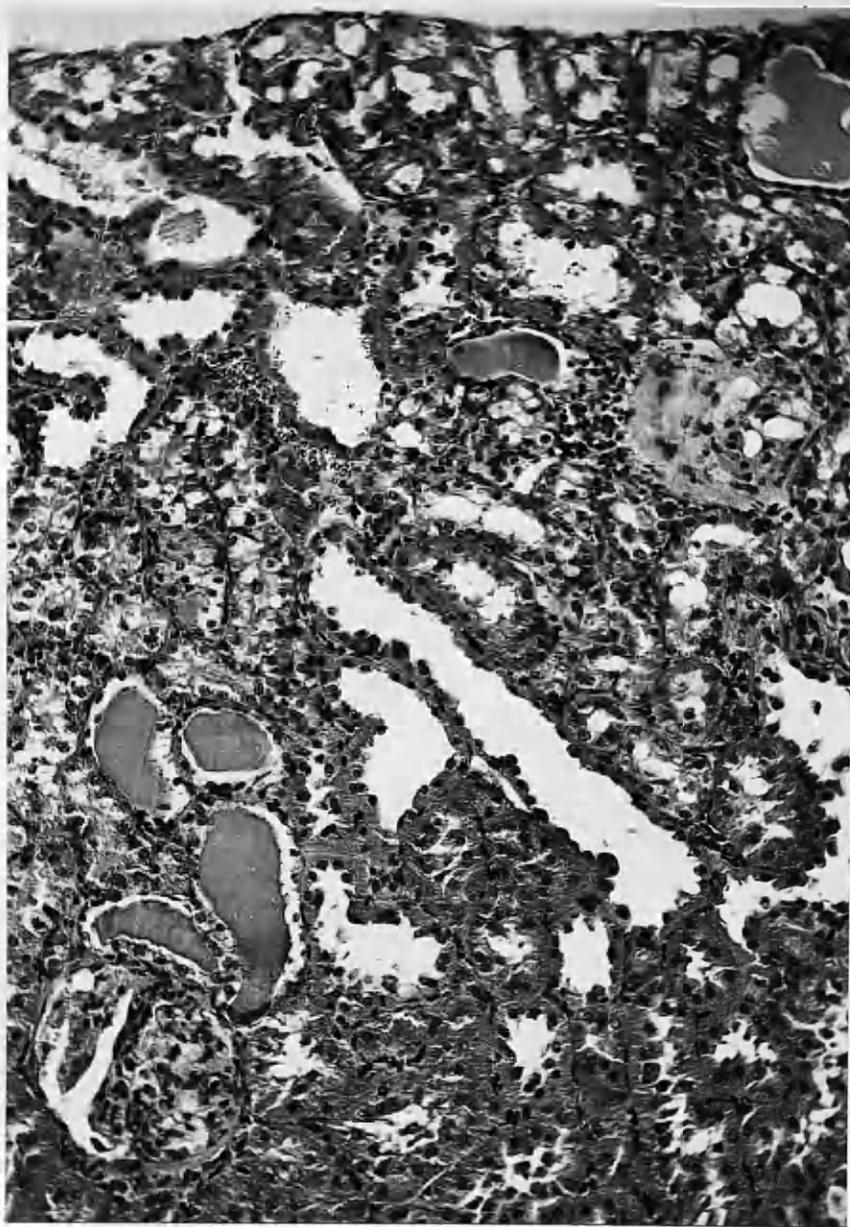
Fig. 54.   Kidney.   Burn.   There is
glomerular hyalinization, irregular
tubular dilatation, and hyaline cast
formation.

H. & E. X 225.
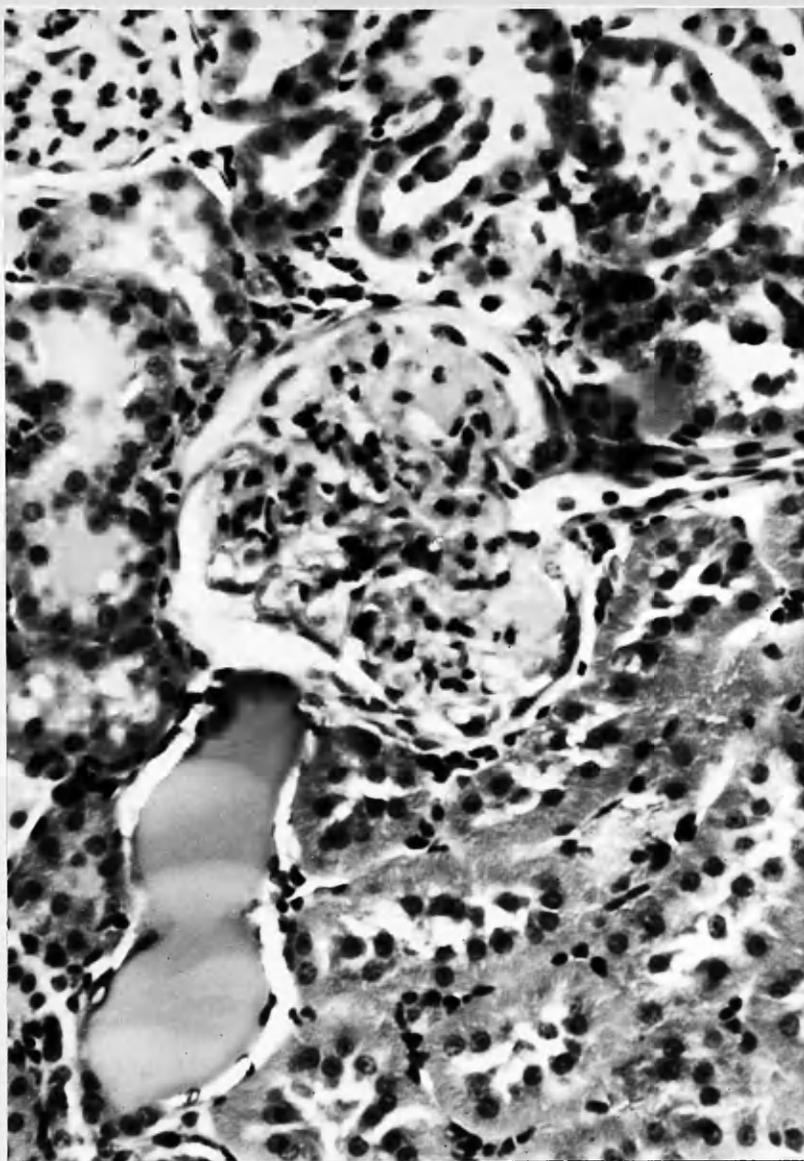
Fig. 55. Kidney. Laparotomy.
Early hyalinization is seen at the
vascular pole of the glomerulus
and the proximal tubule is filled
with a hyaline cast. The afferent
arteriole shows no arteriolosclerosis.

H. & E. X 300.
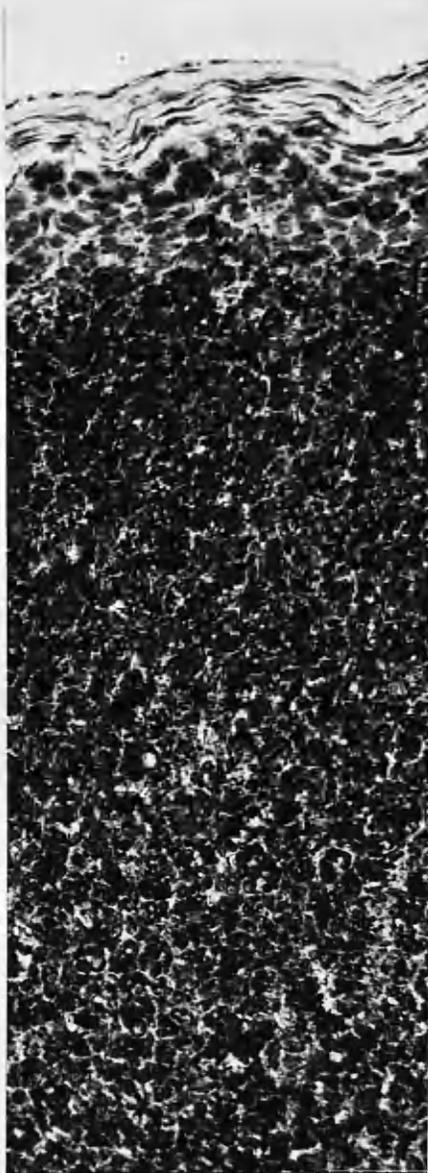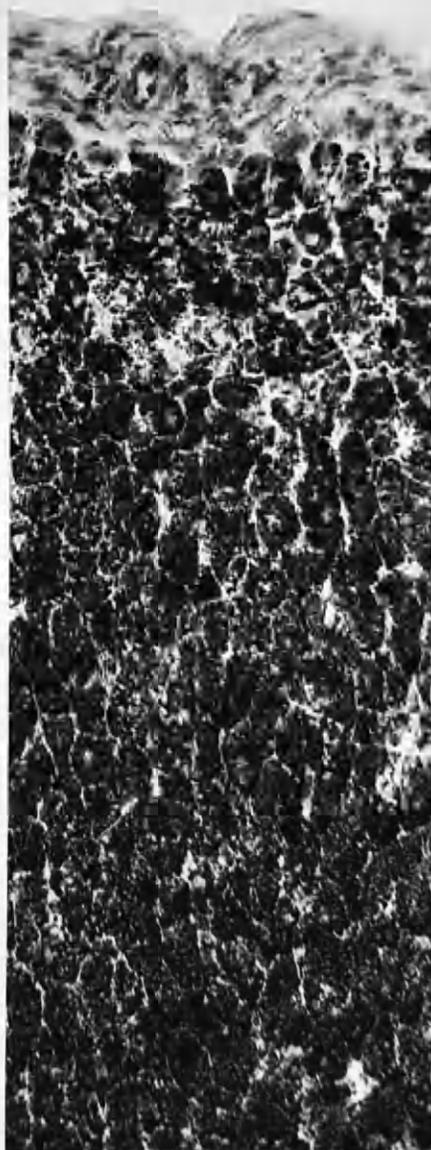
Fig. 56.   Fig. 57.

Fig. 56. Adrenal.   Control rat.   Zona glomerulosa is
narrow and contains only scanty lipid.   Fasciculata and
reticularis contain abundant lipid.

Fig. 57. Adrenal.   Laparotomy.   Zona glomerulosa is wider
and is well filled with lipid.   The cells of fasciculata
are larger and the lipid droplets finer.

Frozen sections. Oil red O and haematoxylin. X 380.
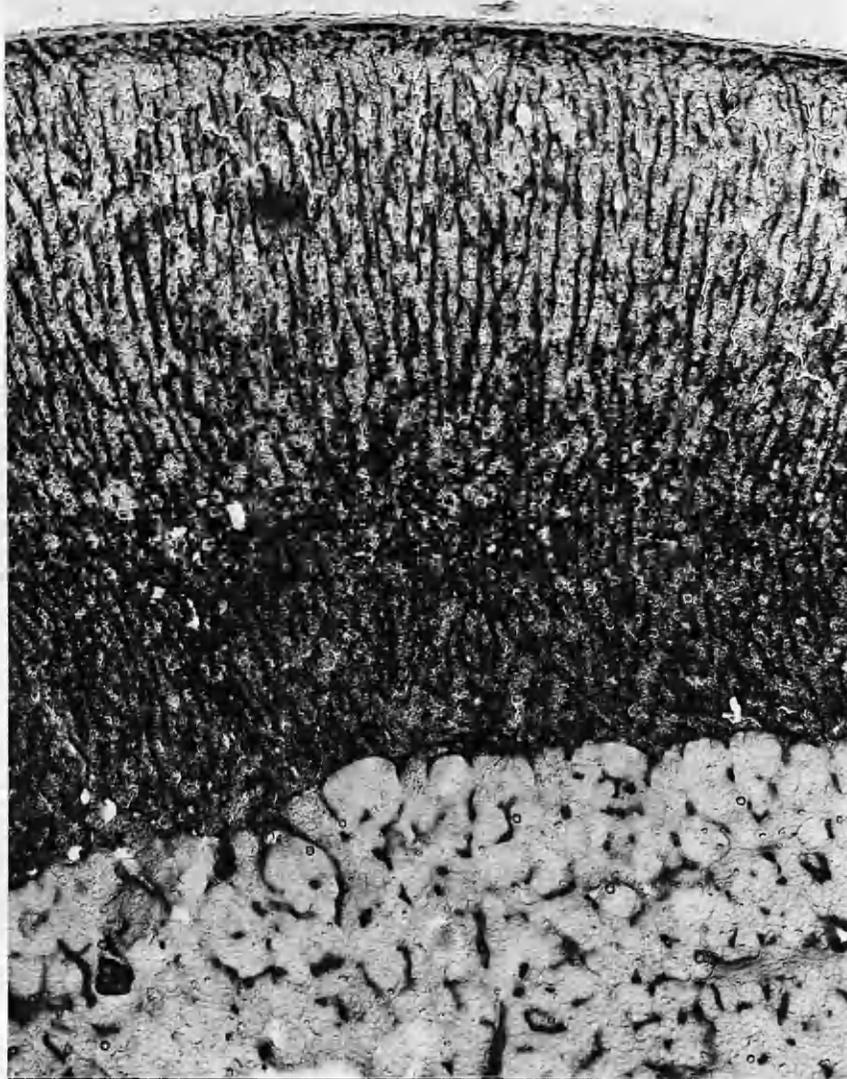
Fig. 58. Adrenal. Burn. Alkaline
phosphatase activity is present in the
cells and sinusoids of reticularis and
adjacent fasciculata zones. It is
confined to the sinusoids in zona
glomerulosa and the medulla.

Frozen section.
Gomori's alkaline phosphatase. X 95.
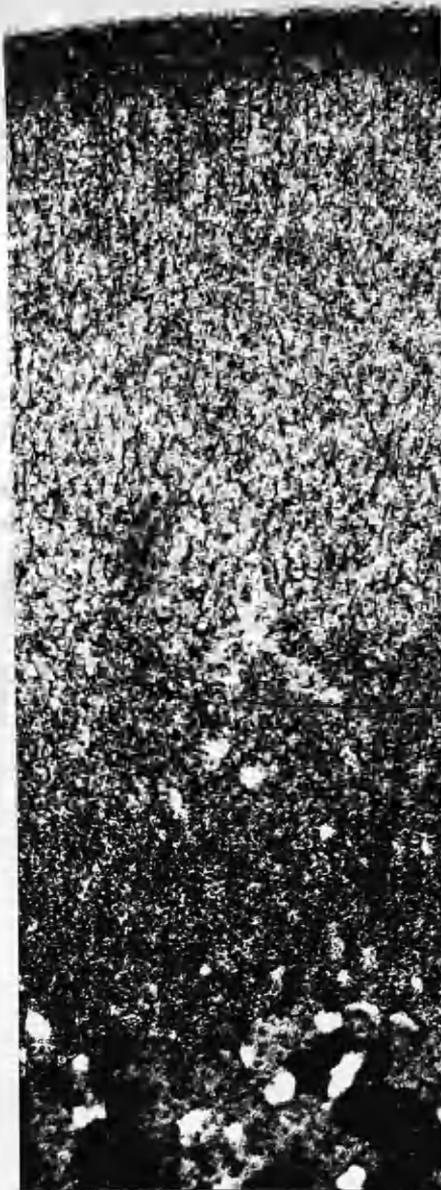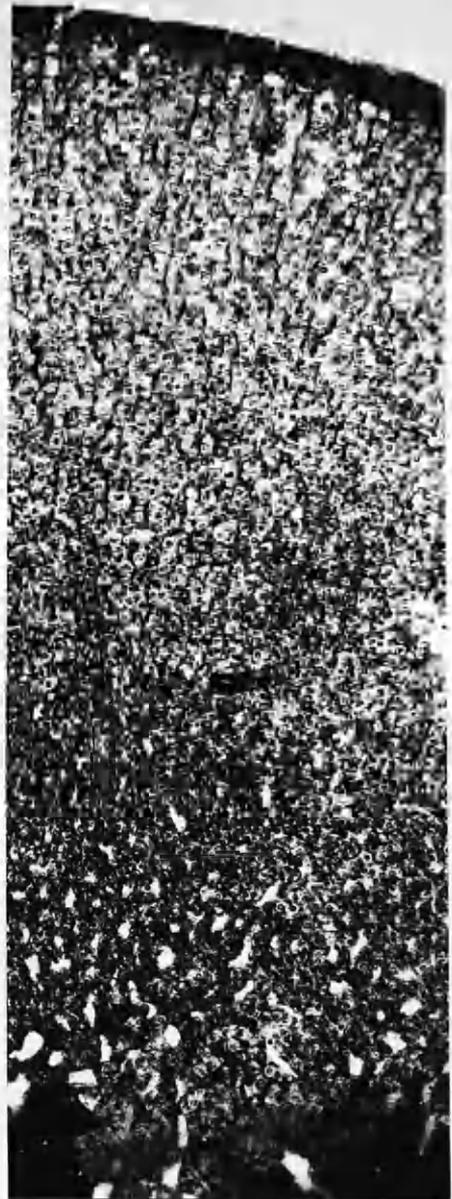
Fig. 59.   Fig. 60.

Fig. 59. Adrenal.   Formalin injection.   Acid phosphatase
activity is most intense in zona glomerulosa and reticularis
and in some of the medullary cells.

Fig. 60. Adrenal.   Formalin injection.   The pattern of
acid phosphatase activity differs from Fig. 59 in that the
zone of reaction in glomerulosa is narrowed.

                Frozen section.   Gomori's acid phosphatase. X 95.
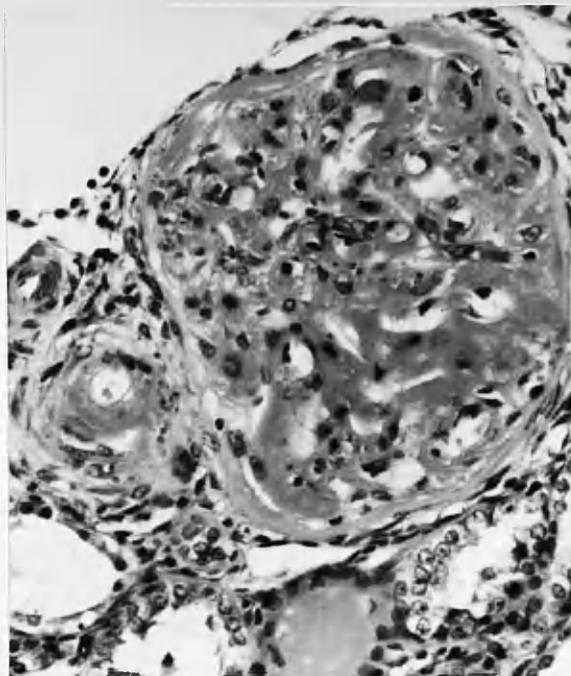
Fig. 61. Kidney. DCA 5 mg. The glomerular tuft
is necrotic and bloodless in appearance. There is
hypertensive arteriolonecrosis. B.P. 195 mm.Hg.

H. & E. X 260.
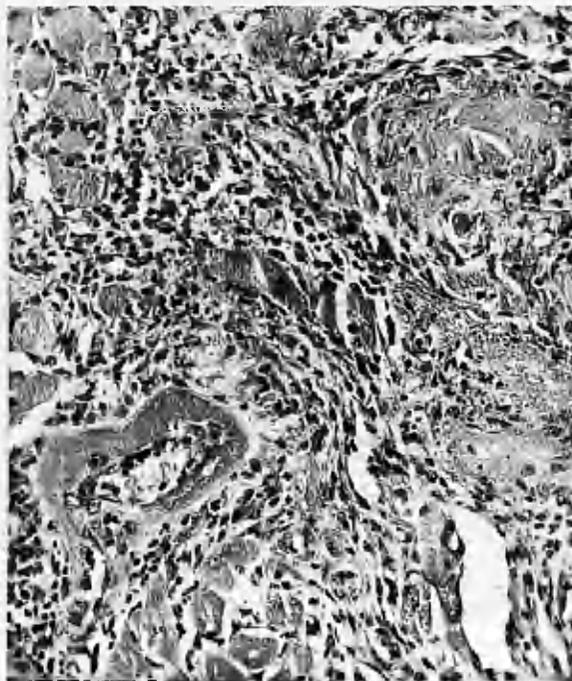
Fig. 62. Heart. DCA 5 mg. There is replacement
fibrosis of the hypertrophied myocardium with
hypertensive arteriolonecrosis. B.P. 188 mm.Hg.

H. & E. X 196.
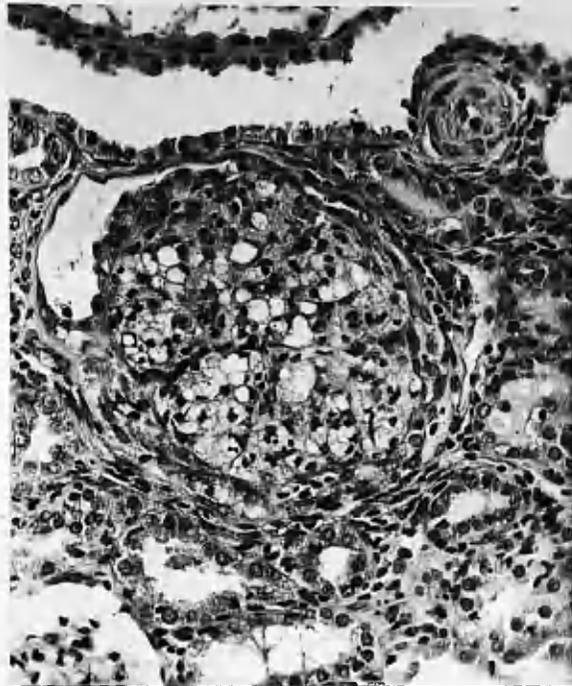
Fig. 63. Kidney.   Non-adrenx rat.   MAD 10 mg.
The glomerulus is enlarged and adherent to the
thickened capsule.   Many of the cells show a "foamy"
appearance.   Hypertensive sclerosis of afferent
arteriole.   B.P. 187 mm.Hg.

H. & E. X 210.
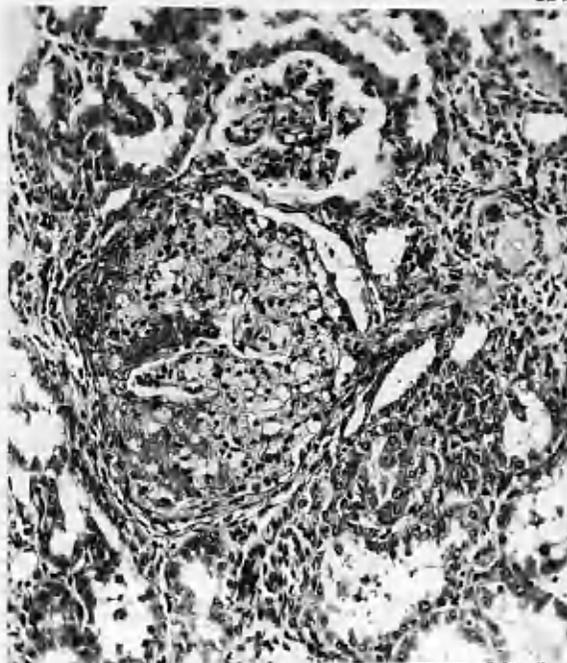
Fig. 64.   Kidney.   Adrenx rat.   MAD 20 mg. + ACE
1 ml.   The glomerulus is enlarged and partly
necrotic and haemorrhagic.   Thickening of afferent
arteriole.   B.P. 175 mm.Hg.

H. & E. X 145.
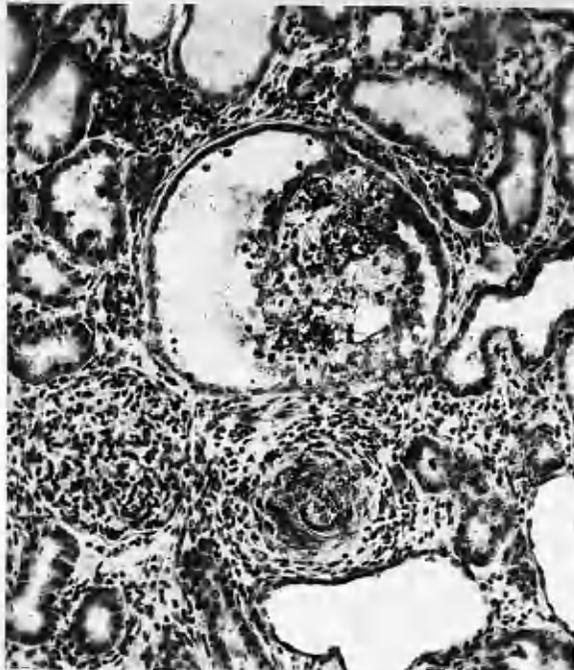
Fig. 65. Kidney. Non-adrenx rat. MAD 10 mg.
Lipid (black) is present in the hypertrophied
afferent arteriole, in the glomerulus and related
tubules. B.P. 177 mm.Hg.

Frozen section.
Oil red O & H. X 130.
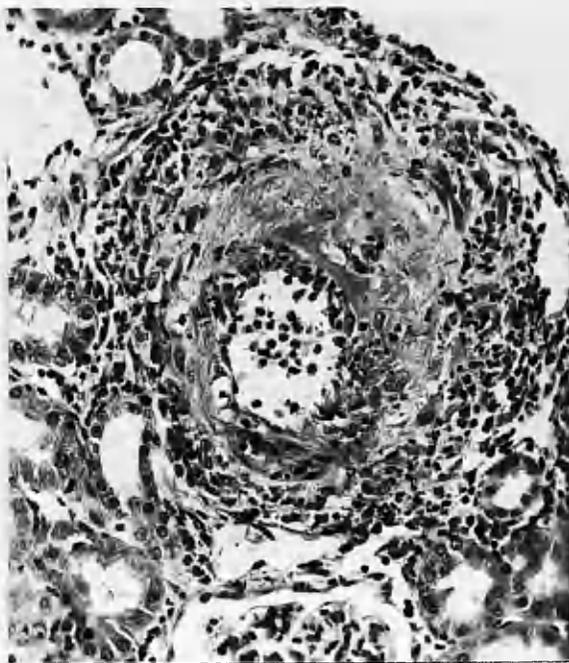
Fig. 66. Kidney. Adrenx-rat. MAD 20 mg. + ACE
1 ml. "Polyarteritis nodosa" involves a small renal
artery. B.P. 162 mm.Hg.

H. & E. X 200.
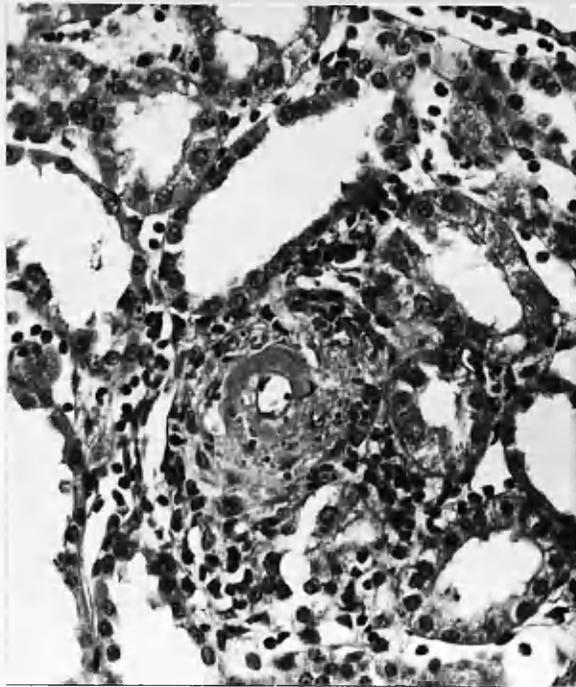
Fig. 67. Kidney.   Adrenx rat.   MAD 10 mg. + ACE
2 ml.   There is hypertensive fibrinoid necrosis of
a small renal arteriole.   B.P. 178 mm.Hg.

H. & E. X 290.
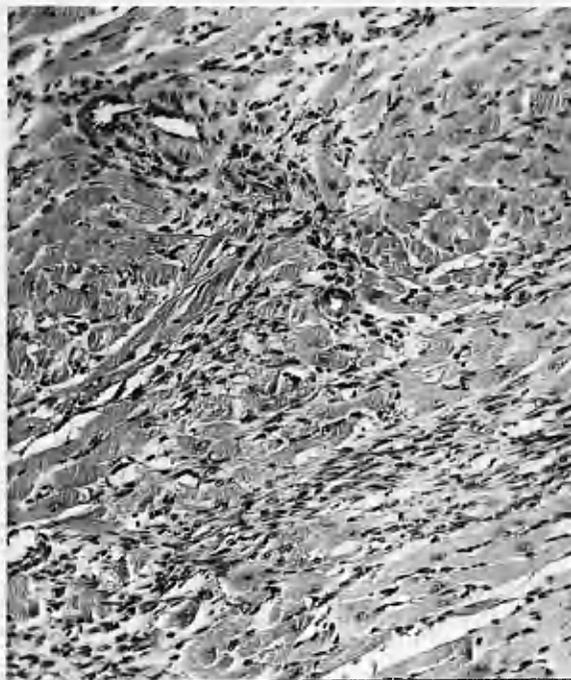
Fig. 68. Heart.   Non-adrenx rat.   MAD 20 mg.
There is replacement fibrosis of the hypertrophied
myocardium with hypertensive sclerosis of the
coronary arterioles.   B.P. 157 mm.Hg.

H. & E. X 130.
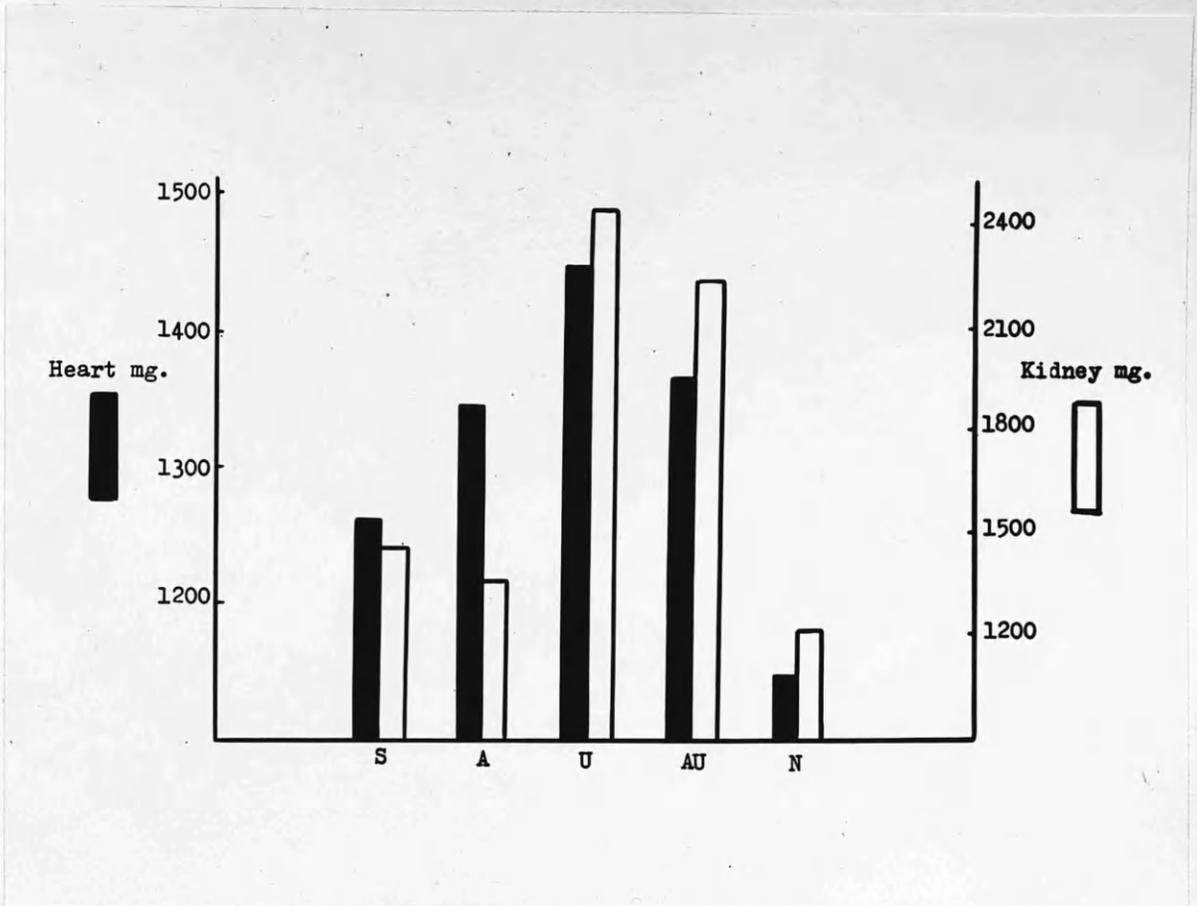
Fig. 69. Correlation between heart and kidney weights in rats tube-fed 12% NaCl medium carbohydrate diet for 84 days (Experiment 2).

    S = sham operated.

    A = adrenalectomized.

    U = uninephrectomized.

  AU = adrenalectomized, uninephrectomized.

    N = normal rats tube-fed medium carbo-
         hydrate diet without added salt
         (Experiment 5).
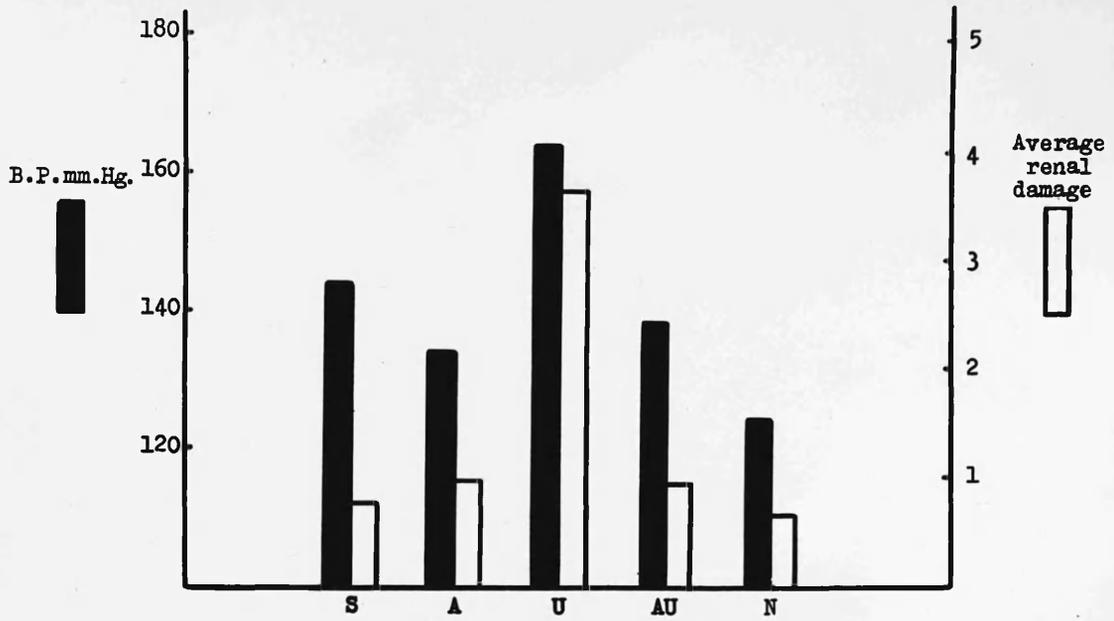
Fig. 70. Correlation between blood pressure
and renal damage in rats tube-fed 12% NaCl
medium carbohydrate diet for 84 days
(Experiment 2).

    S = sham operated.

    A = adrenalectomized.

    U = uninephrectomized.

    AU = adrenalectomized, uninephrectomized.

    N = normal rats tube-fed medium carbo-
        hydrate diet without added salt
        (Experiment 5).
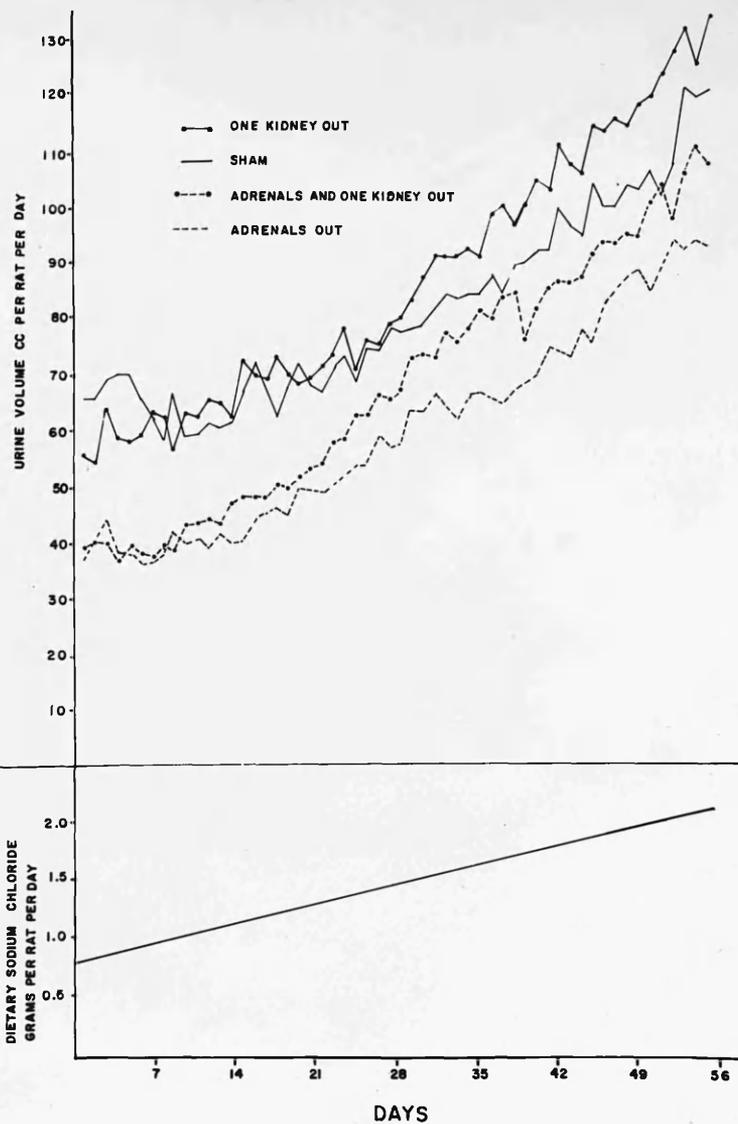
Fig. 71. Daily urinary volume and daily sodium chloride dietary intake in rats tube-fed 12% sodium chloride medium carbohydrate diet. (Experiment 2).
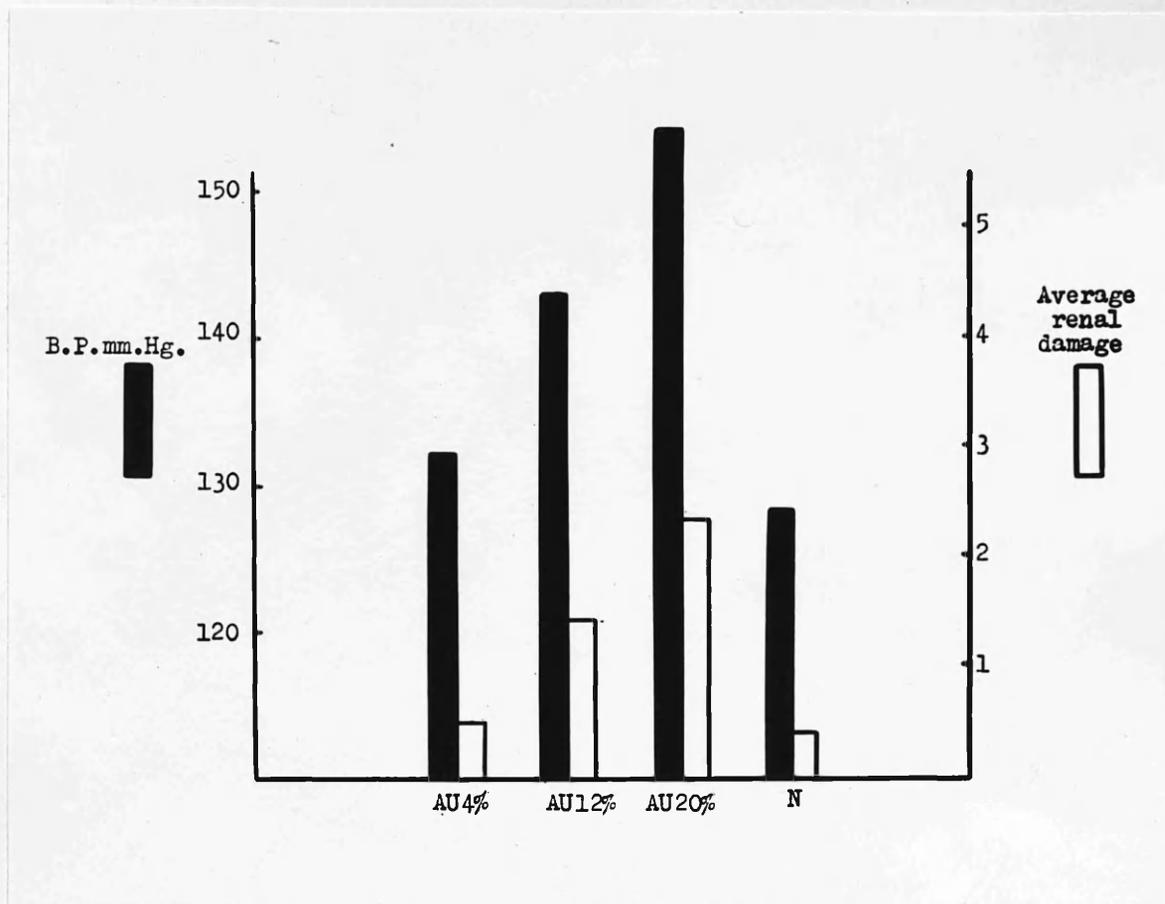
Fig. 72. Correlation between blood pressure
and renal damage in adrenalectomized,
uninephrectomized (AU) rats pair-fed 4%, 12%
and 20% sodium chloride medium carbohydrate
diet (Experiment 4), and normal rats (N)
tube-fed diet without added salt (Experiment 5).
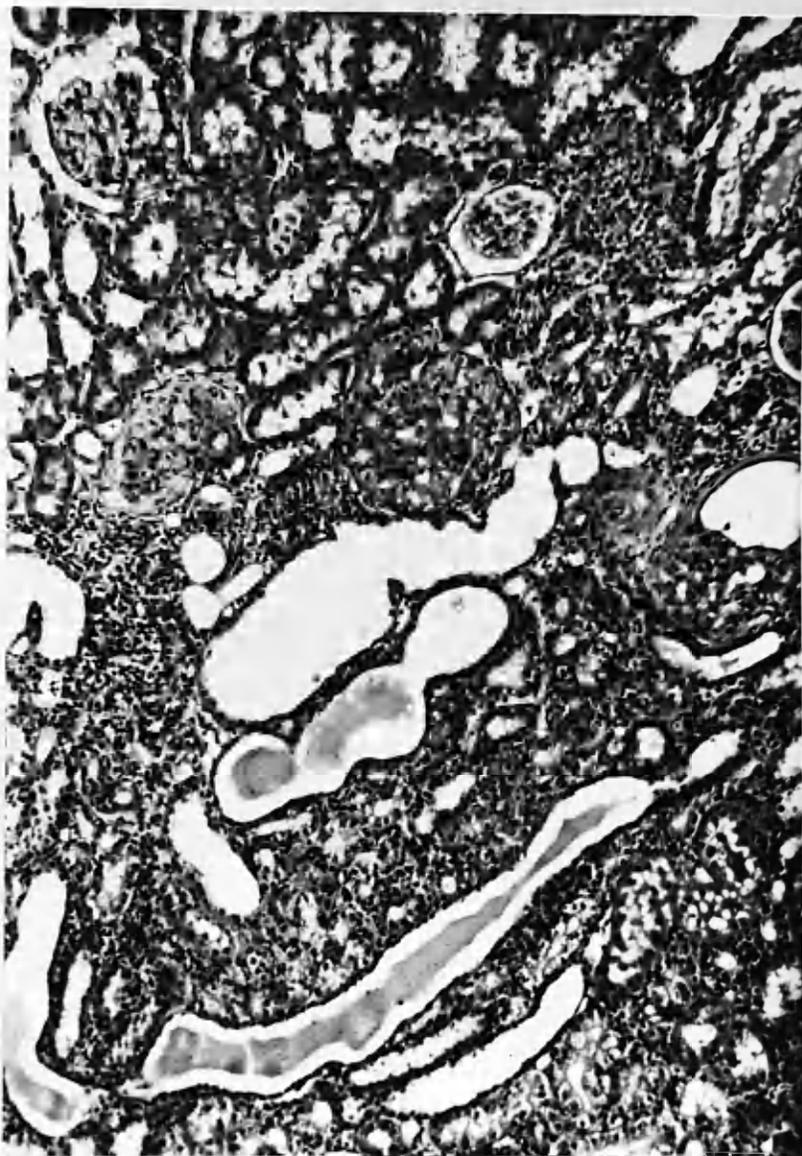
Fig. 73. Kidney. Non-adrenx,
uni-nephrx rat on 12% NaCl diet.
Some glomeruli are enlarged and
swollen, while others are normal
in appearance. The tubules are
dilated and contain protein casts.
B.P. 170 mm.Hg.

H. & E. X 120.
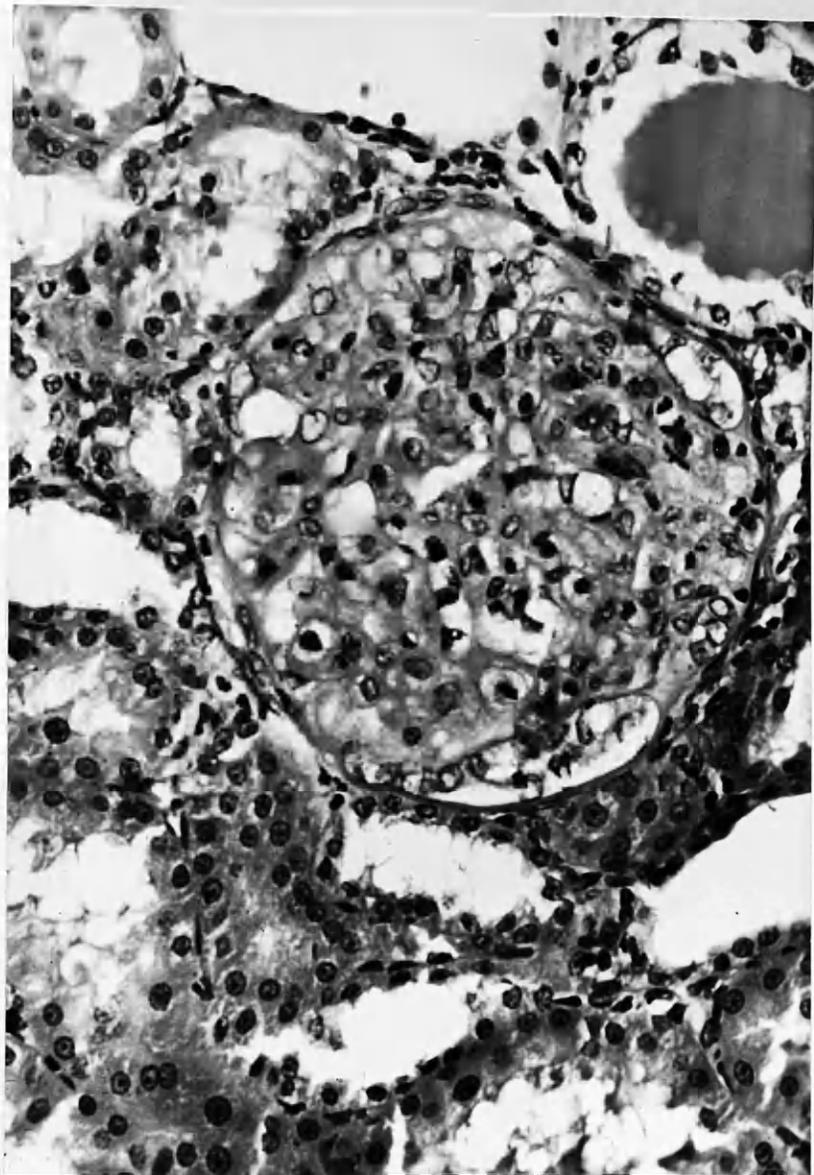
Fig. 74. Kidney.   Non-adrenx,
uni-nephrx rat on 16% NaCl diet.
The enlarged glomerular tuft contains
numerous "foamy" cells.   Glomerular
capillaries are not obvious.
B.P. 156 mm.Hg.

H. & E. X 450.
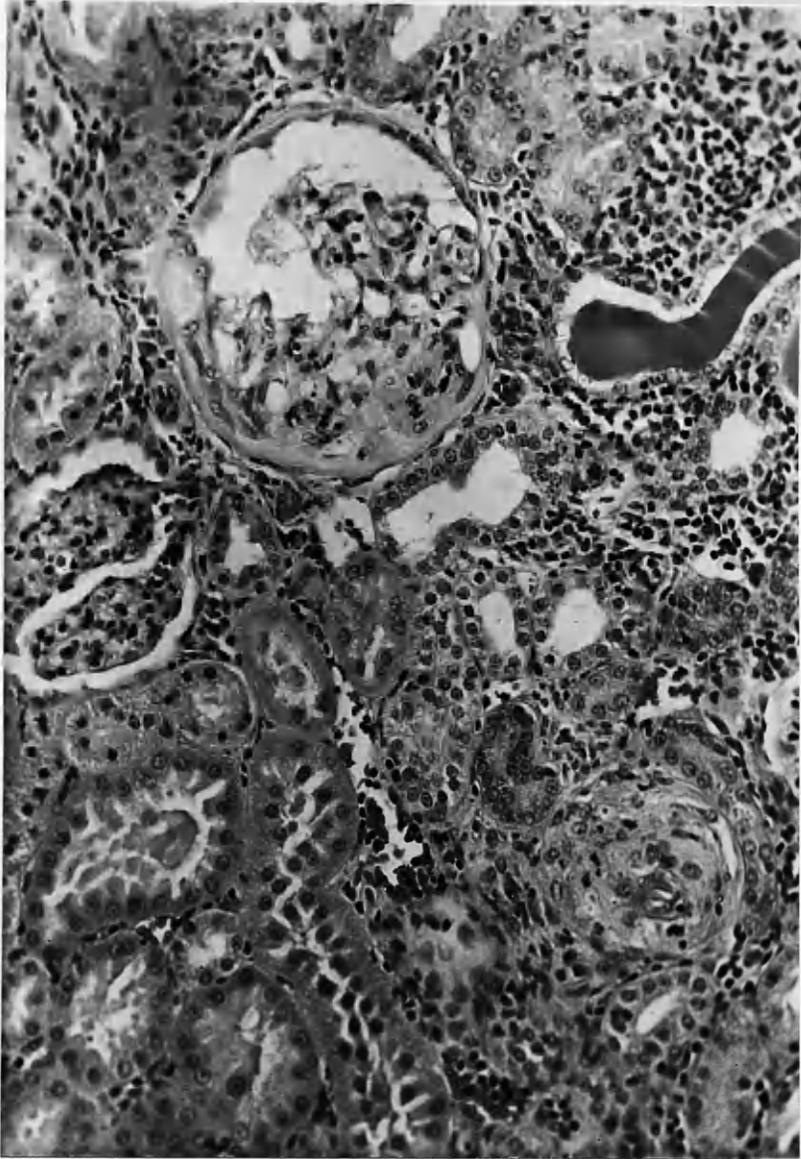
Fig. 75. Kidney. Non-adrenx,
uni-nephrx rat on 12% NaCl diet.
The glomerular tuft is shrunken and
fibrosed. The afferent arteriole
shows hypertensive sclerosis.
B.P. 164 mm.Hg.

H. & E. X 250.
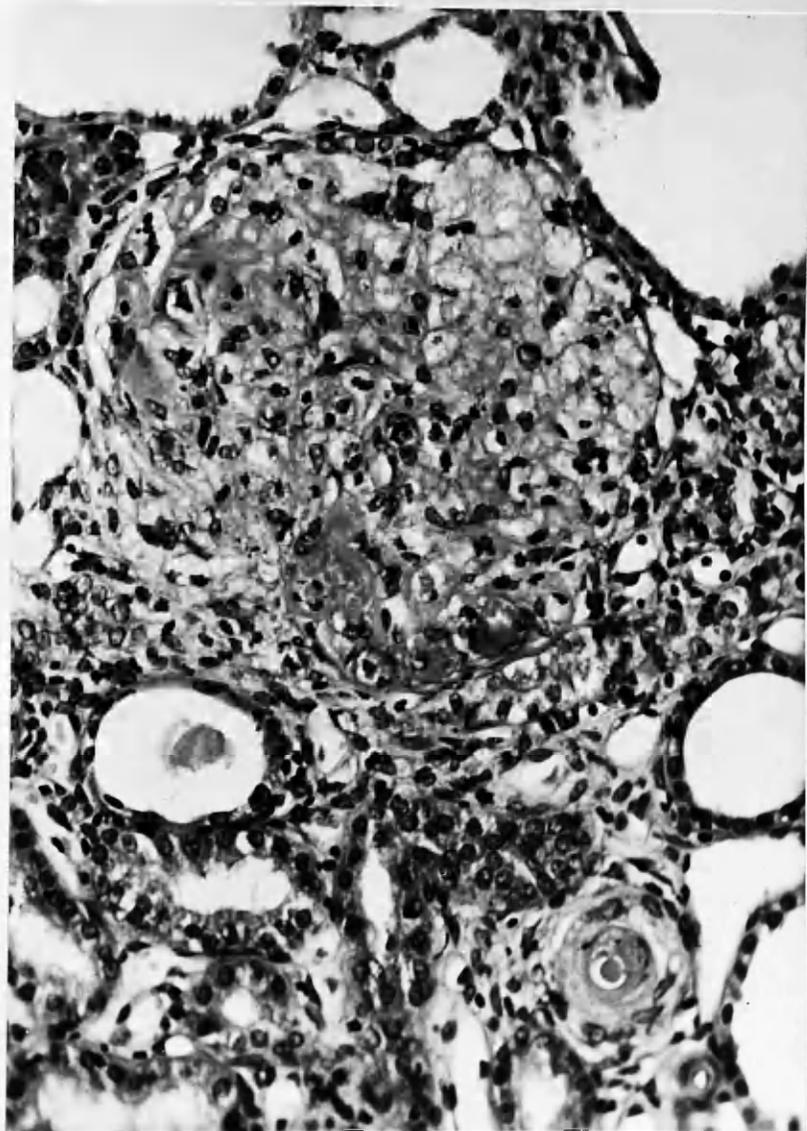
Fig. 76. Kidney. Non-adrenx,
uni-nephrx rat on 12% NaCl diet.
The glomerulus is enlarged and
contains numerous "foamy" cells.
There is hypertensive necrosis of
the afferent arteriole. B.P.
170 mm.Hg. Compare with Fig. 64.

H. & E. X 425.
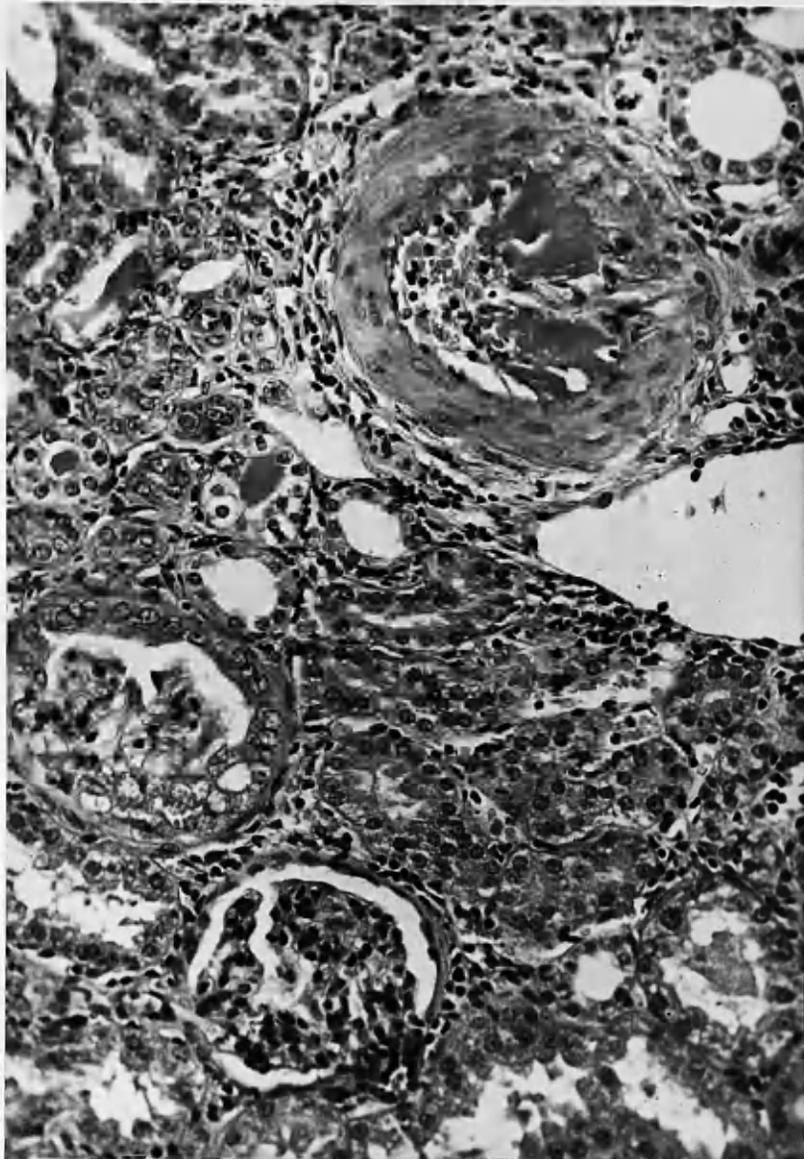
Fig. 77. Kidney. Adrenx,
uni-nephrx rat on 12% NaCl diet and
treated with 2.5 mg. cortisone
acetate for 5 days. There is
eccentric necrosis of a small renal
artery with glomerular damage.
B.P. 216 mm.Hg.

H. & E. X 285.
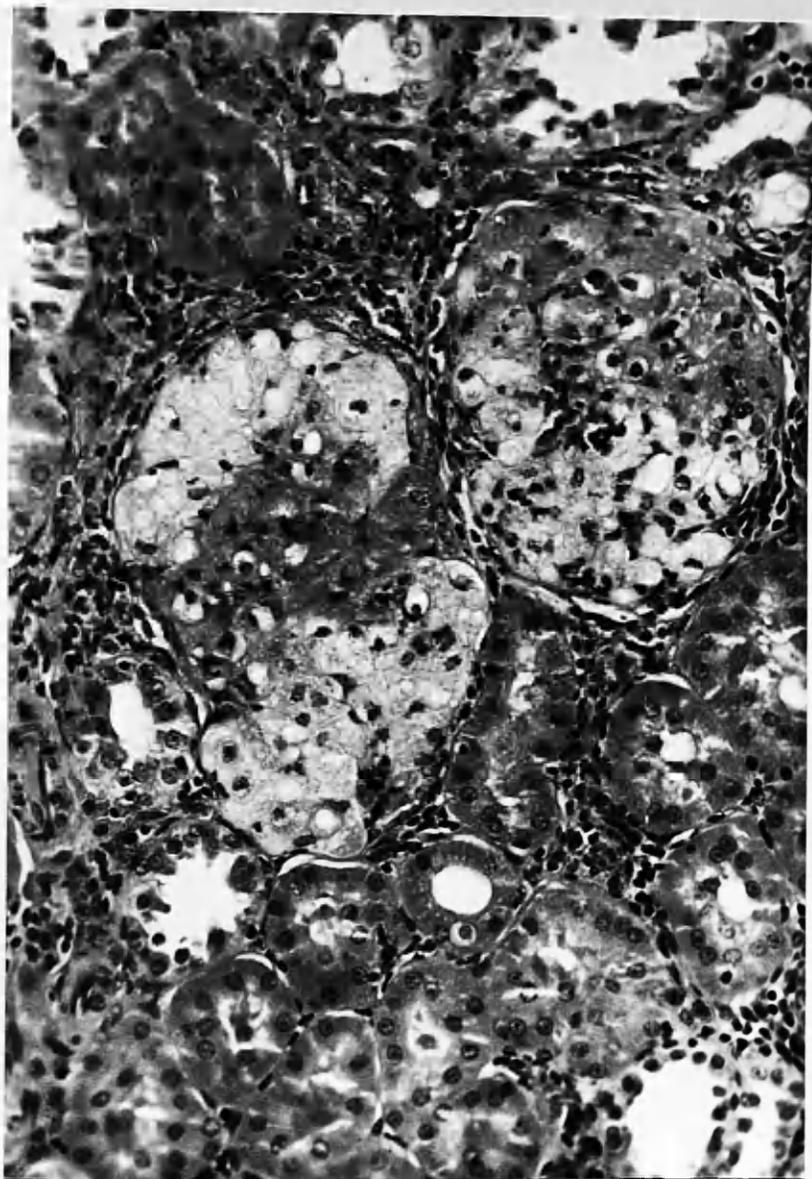
Fig. 78.   Kidney.   Adrenx,
uni-nephrx rat on 20% NaCl diet.
The glomeruli are enlarged, contain
numerous foam cells, and are
bloodless in appearance.
B.P. 198 mm.Hg.

H. & E. X 320.
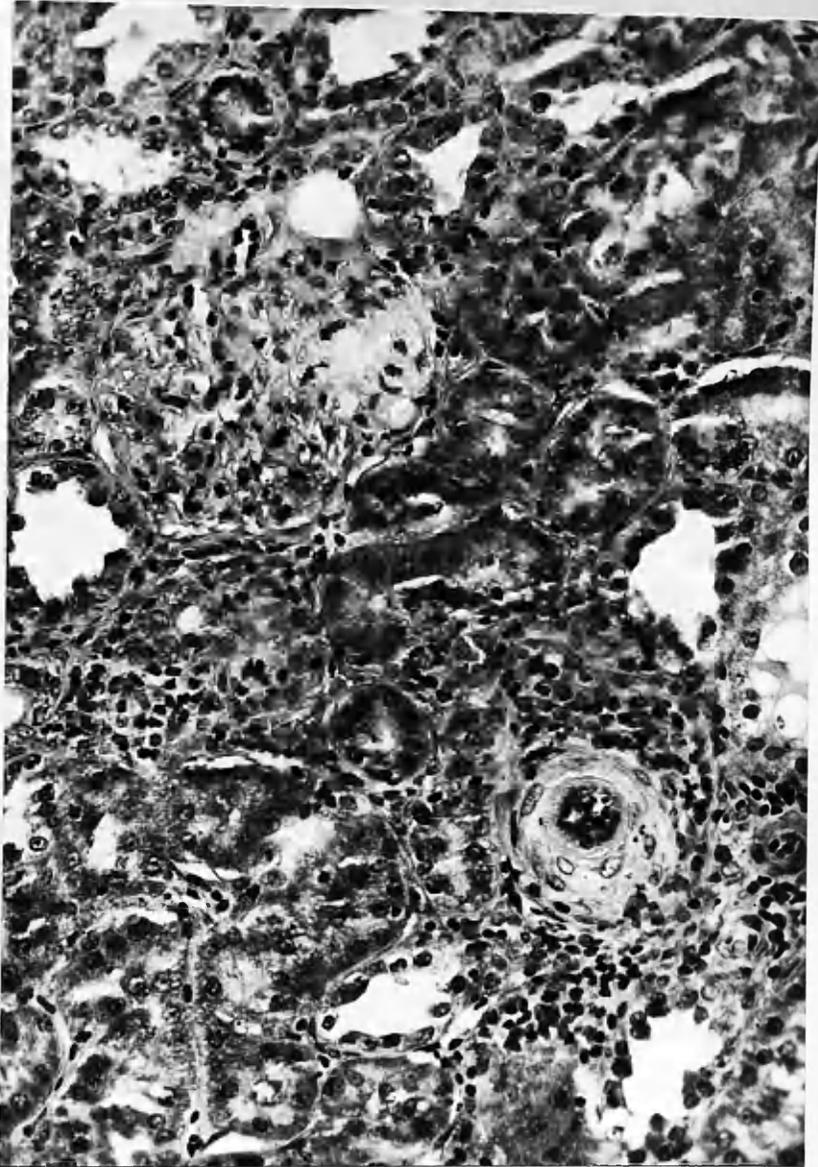
Fig. 79. Kidney. Adrenx,
uni-nephrx rat on 20% NaCl diet.
The glomerulus is damaged and the
afferent arteriole shows hyper-
tensive thickening with intimal
necrosis. B.P. 198 mm.Hg.

Masson.X 290.
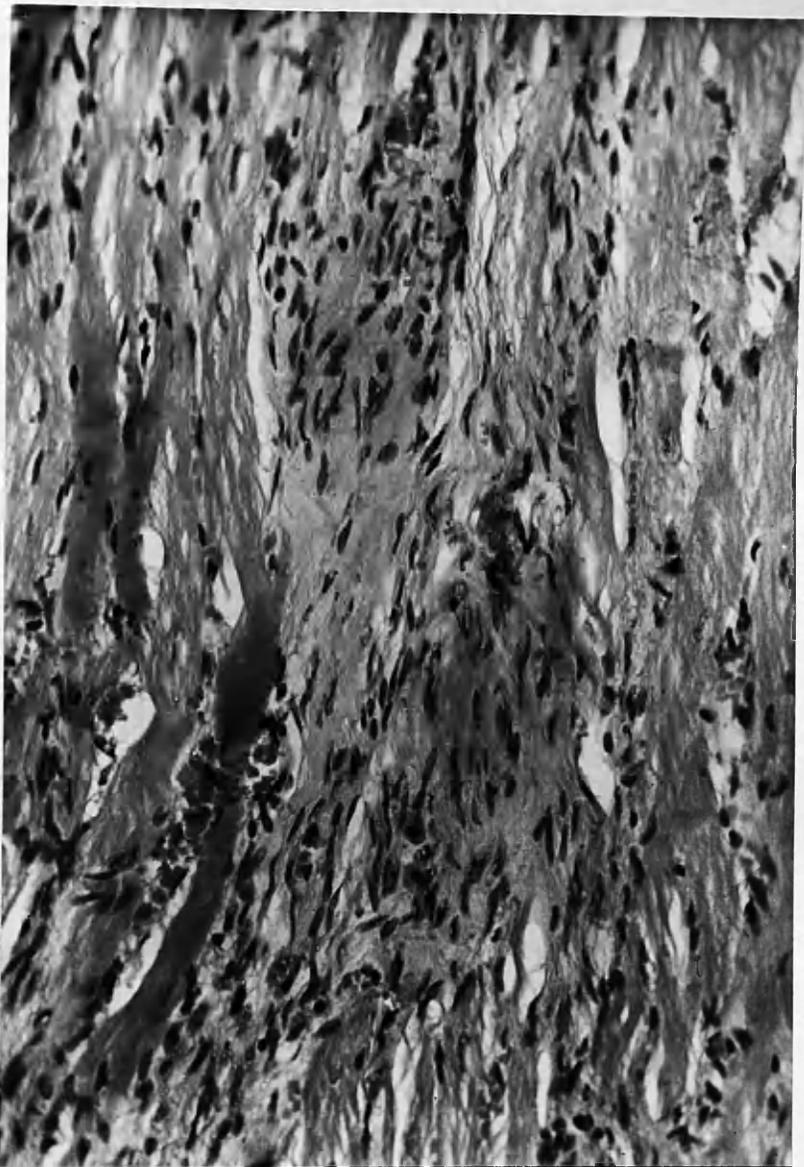
Fig. 80. Heart.   Non-adrenx,
uni-nephrx rat on 12% NaCl diet.
There is replacement fibrosis of
the hypertrophied myocardium.
B.P. 164 mm.Hg.

H. & E. X 200.

Fig. 81. Fore-stomach. Non-adrenx,
uni-nephrx rat on 12% NaCl diet.
"Polyarteritis nodosa" affects a
small artery in the submucosa.
B.P. 164 mm.Hg.

H. & E. X 300.

Fig. 82. Small intestine.
Non-adrenx, uni-nephrx rat on
12% NaCl diet.   There is
hypertensive necrosis of a small
submucosal arteriole.
B.P. 170 mm.Hg.

H. & E. X 330.
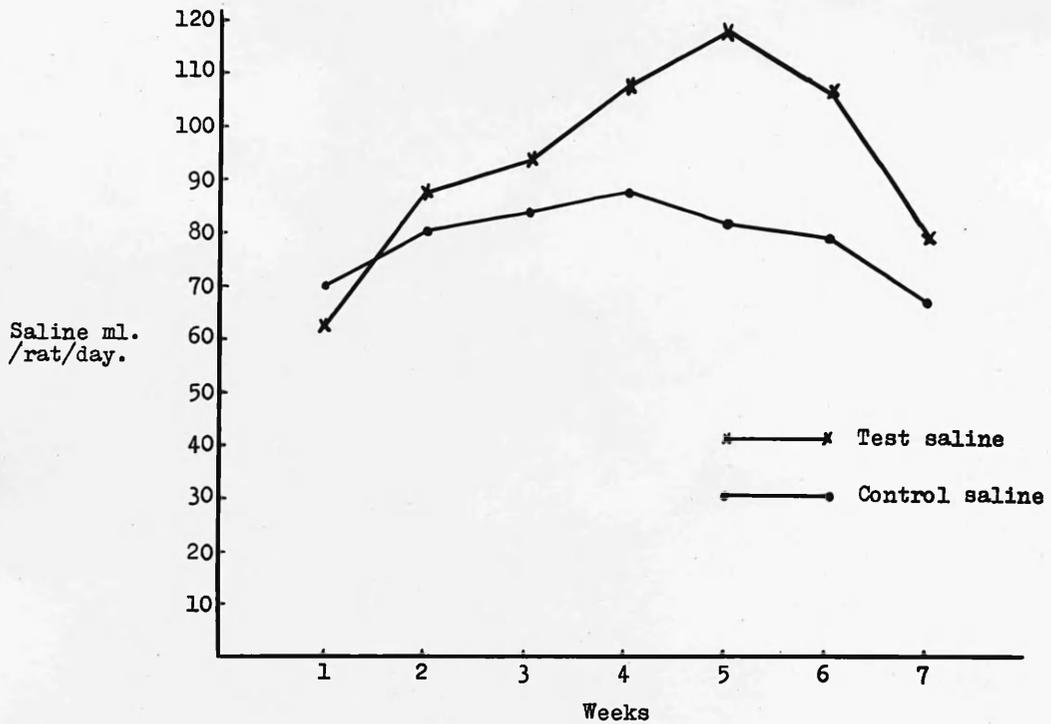
Fig. 83. Intake of 1% saline by unilaterally nephrectomized rats bearing regenerating adrenal cortical tissue compared with non-enucleated controls (Experiment 5).
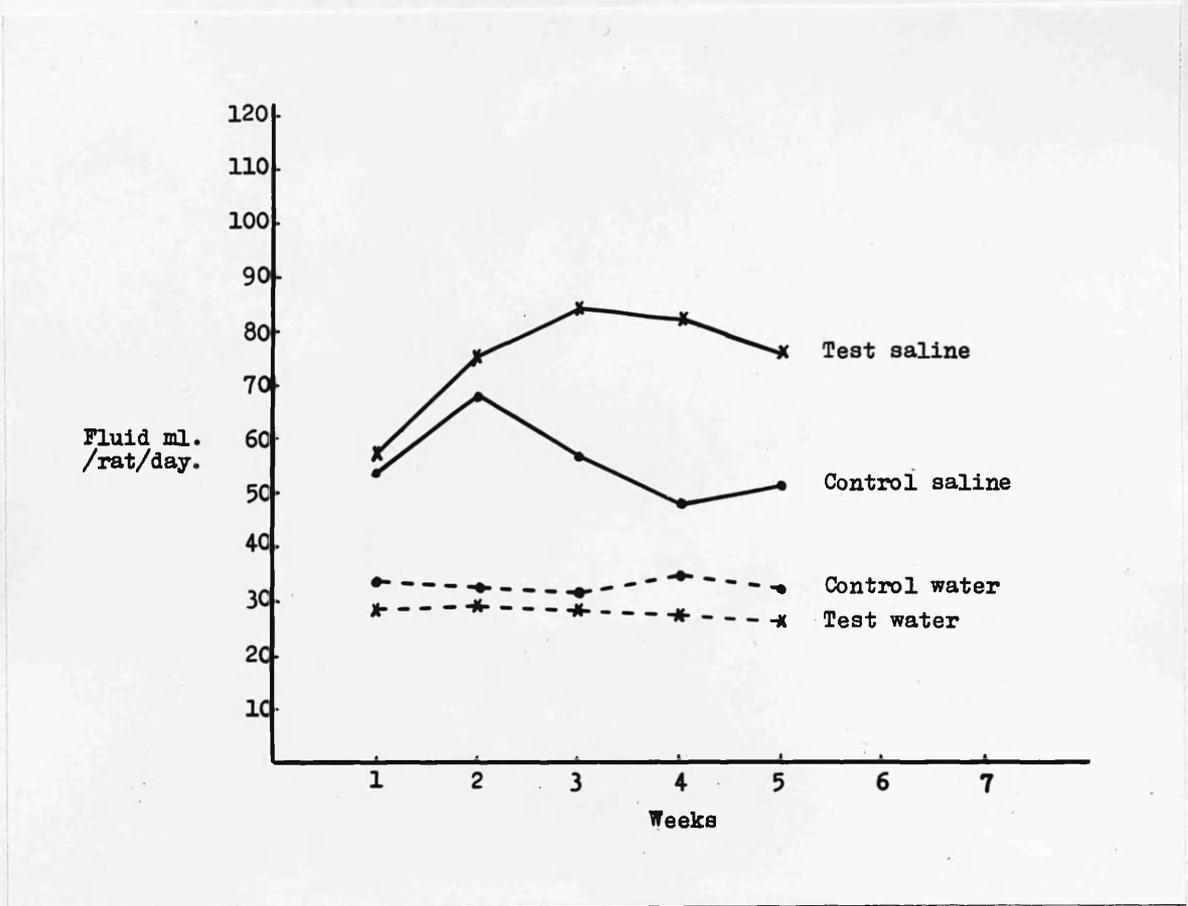
Fig. 84. Intake of 1% saline and tap water by unilaterally nephrectomized rats bearing regenerating adrenal cortical tissue compared with non-enucleated controls (Experiments 3 and 4).
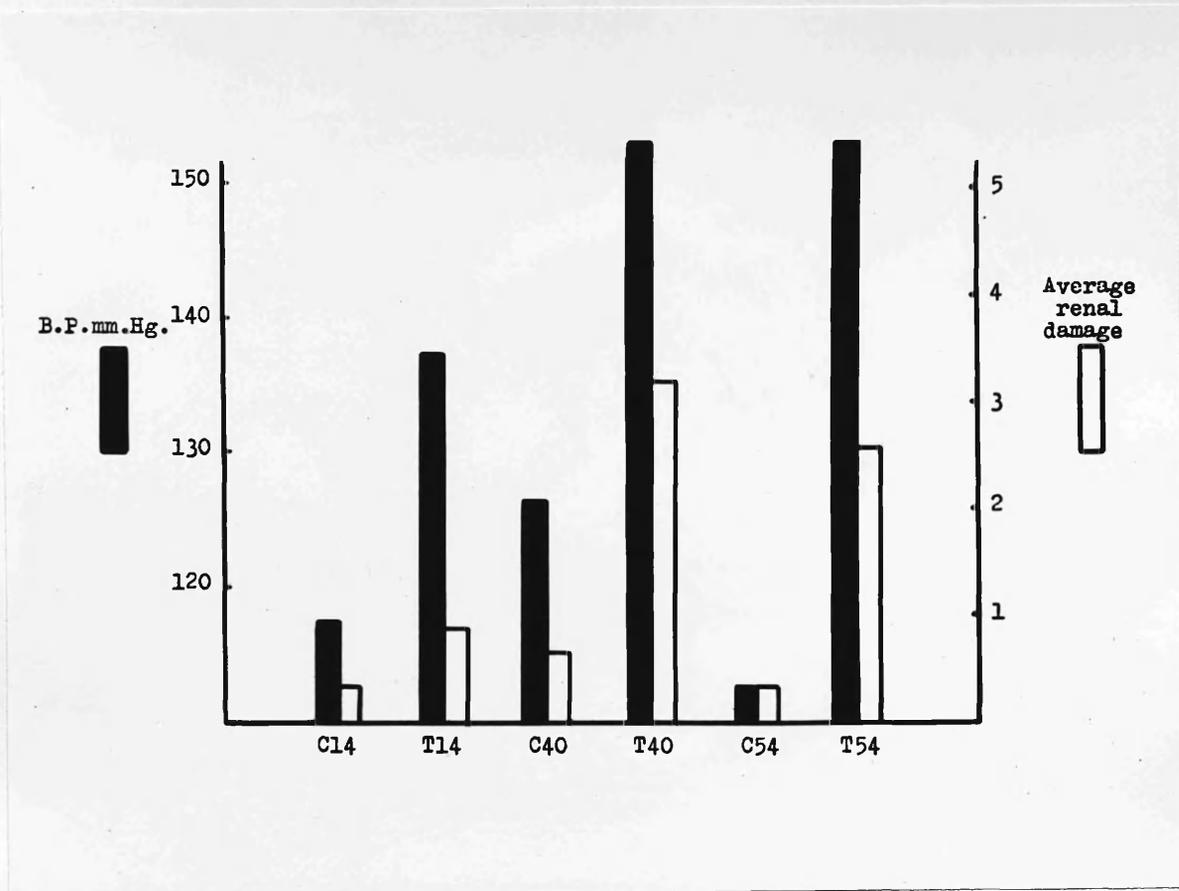
Fig. 85. Correlation between blood pressure
and renal damage in control and adrenal
enuclated rats maintained on 1% saline for
14, 40 and 54 days (Experiments 1, 3 and 5).

    Control (C) = right nephrectomy, right
                adrenalectomy.

    Test (T)   = right nephrectomy, right
                adrenalectomy and left
                adrenal enucleation.
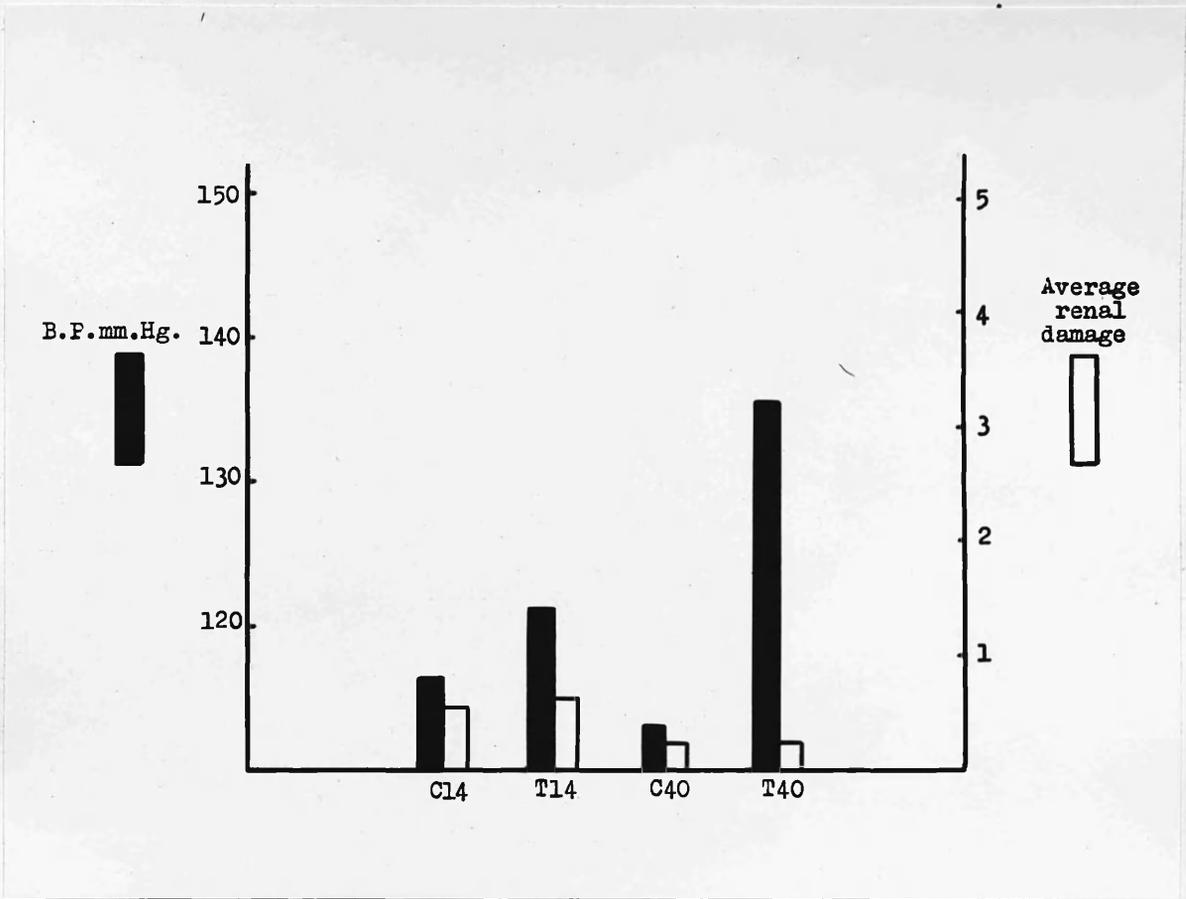
Fig. 86. Correlation between blood pressure and renal damage in control and adrenal enucleated rats maintained on tap water for 14 and 40 days (Experiments 2 and 4).

Control (C) = right nephrectomy, right adrenalectomy.

Test (T) = right nephrectomy, right adrenalectomy and left adrenal enucleation.
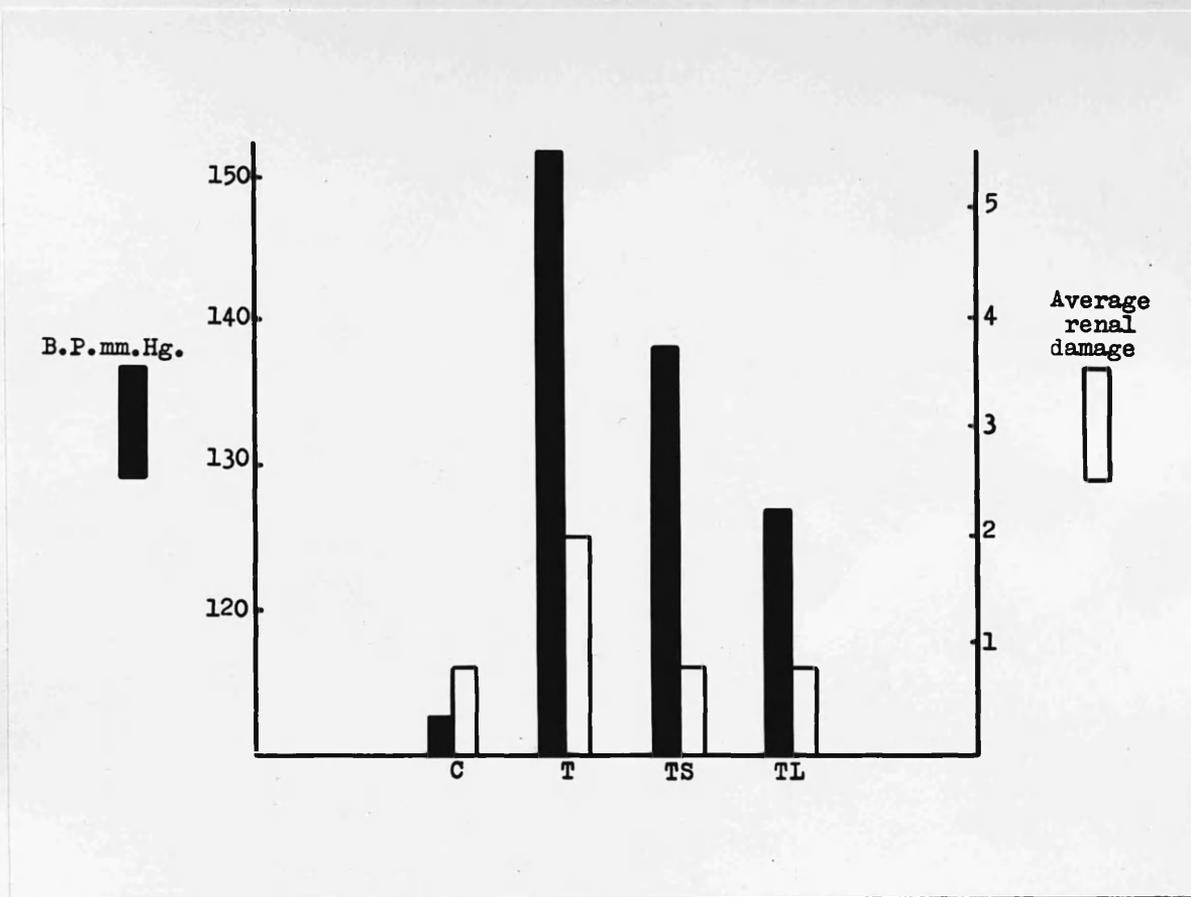
Fig. 87. Correlation between blood pressure and renal damage in control rats and rats with adrenal cortices regenerating in situ, in the spleen, and in the liver; maintained on 1% saline for 50 days (Experiment 6).

Control (C)   = right nephrectomy, right adrenalectomy.

Test (T)   = right nephrectomy, right adrenalectomy and left adrenal enucleation.

Transplant (TS) = splenic transplant.

Transplant (TL) = liver transplant.
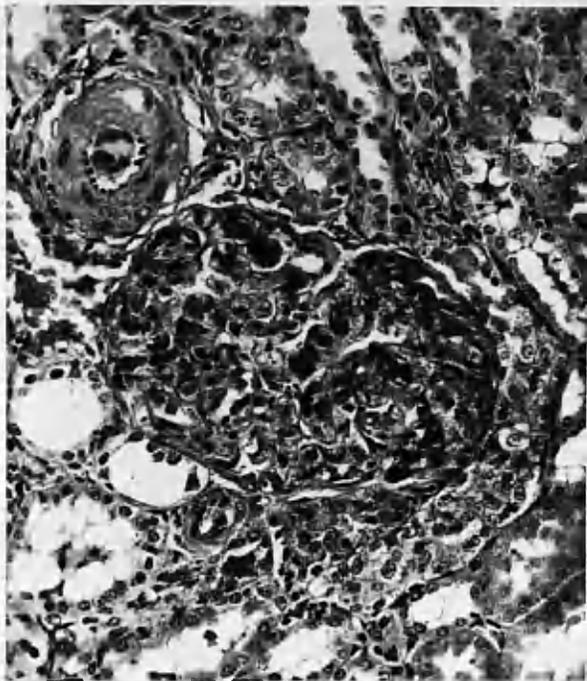
Fig. 88. Kidney. Adrenal regeneration in groin,
1% saline, 50 days. The glomerulus is enlarged
and the capillaries are occluded by small thrombi.

Picro-Mallory. X 240.
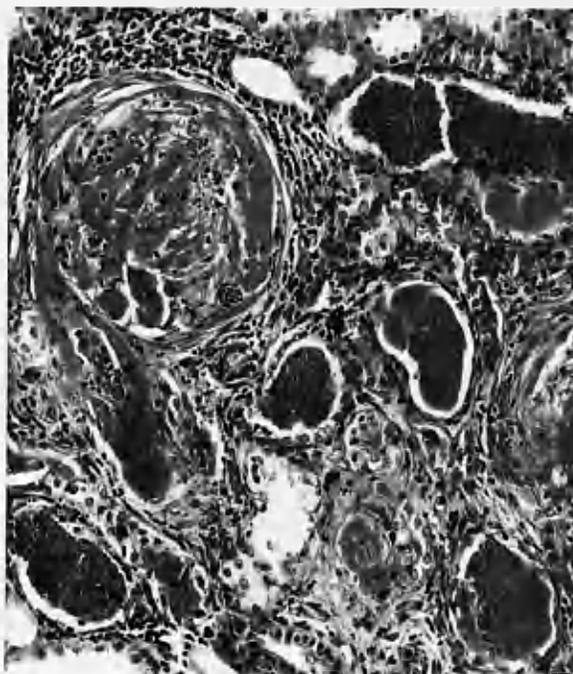
Fig. 89. Kidney. Adrenal regeneration in situ,
1% saline, 54 days. The glomerulus and afferent
arteriole are necrotic. The tubules contain
blood casts. B.P. 177 mm.Hg.

H. & E. X 200.
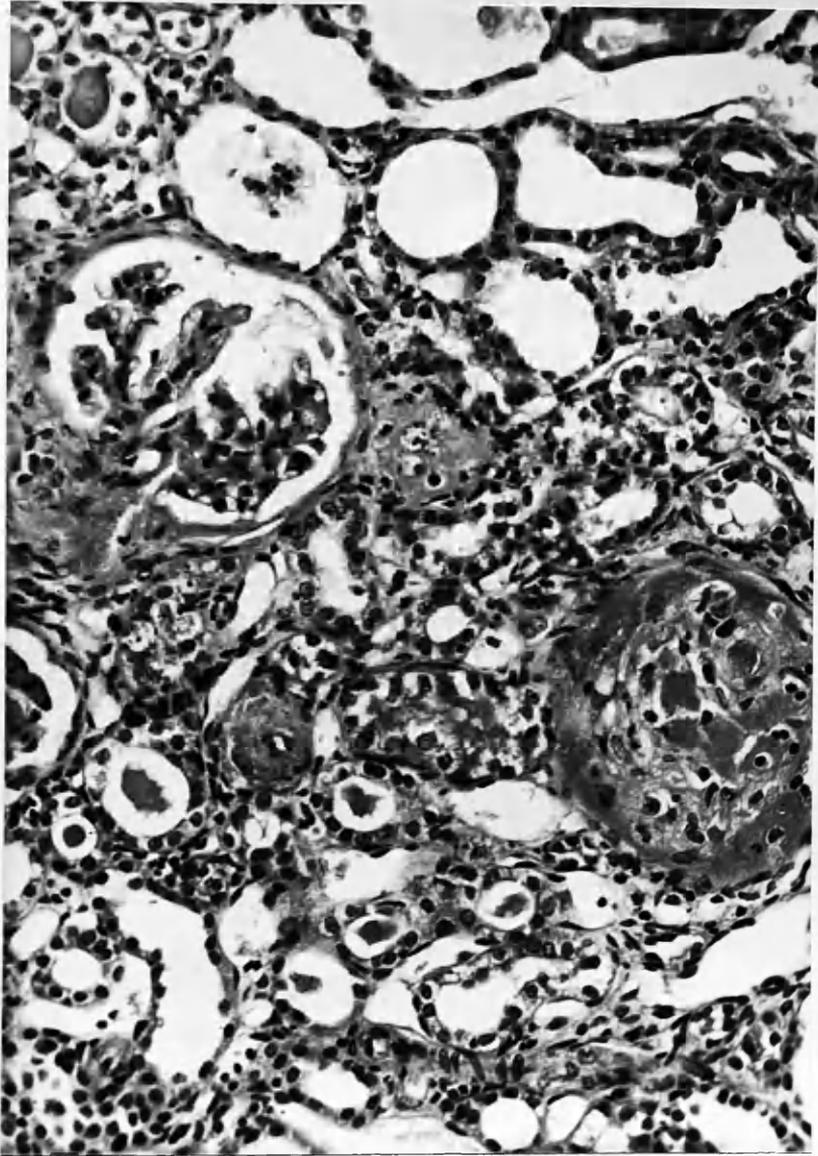
Fig. 90. Kidney. Adrenal
regeneration in situ, 1% saline,
40 days.   There is glomerular
damage, hypertensive arteriolo-
necrosis, and tubular dilatation.
B.P. 154 mm.Hg.

H.   & E. X 295.
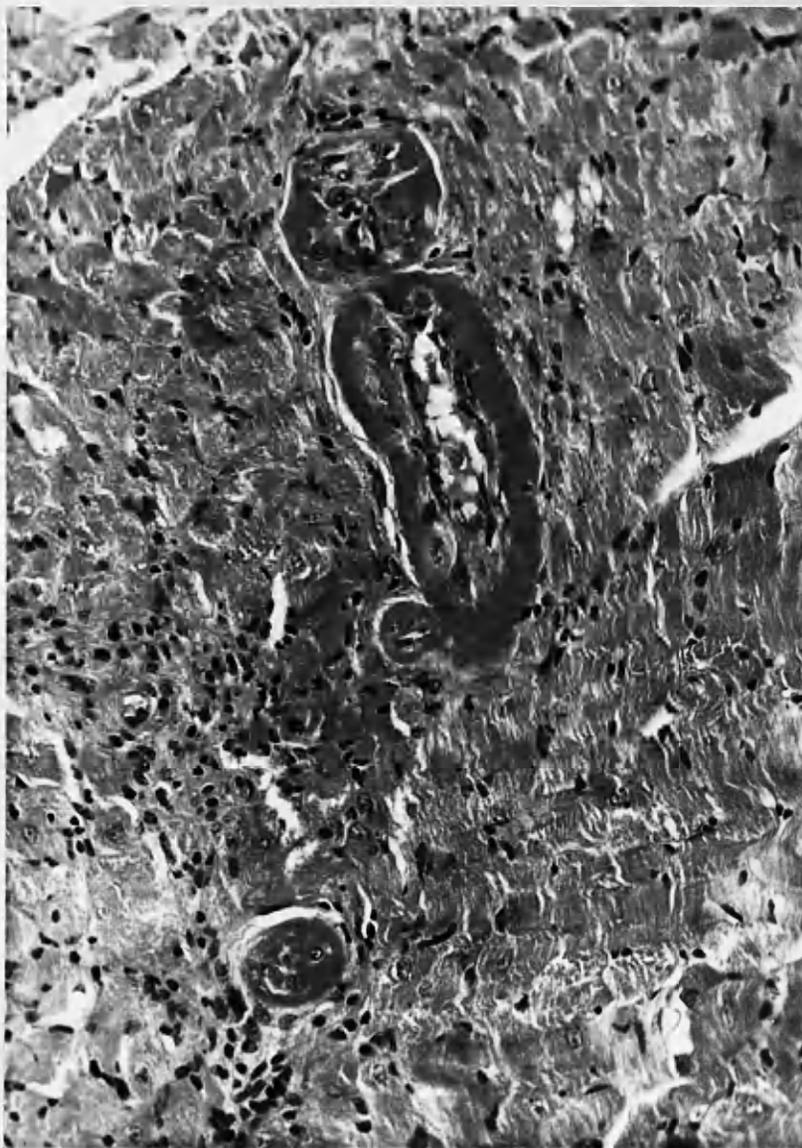
Fig. 91. Heart.   Adrenal
regeneration in situ, 1% saline,
40 days.   There is hypertensive
necrosis of the coronary vessels
with necrosis of small groups of
cardiac muscle fibres.
B.P. 156 mm.Hg.

H. & E. X 295.

Fig. 92. The adrenal capsular
tissue following enucleation consists
of fibrous capsule and vessels, zona
glomerulosa and some crushed cells of
superficial zona fasciculata.

Masson. X 85.
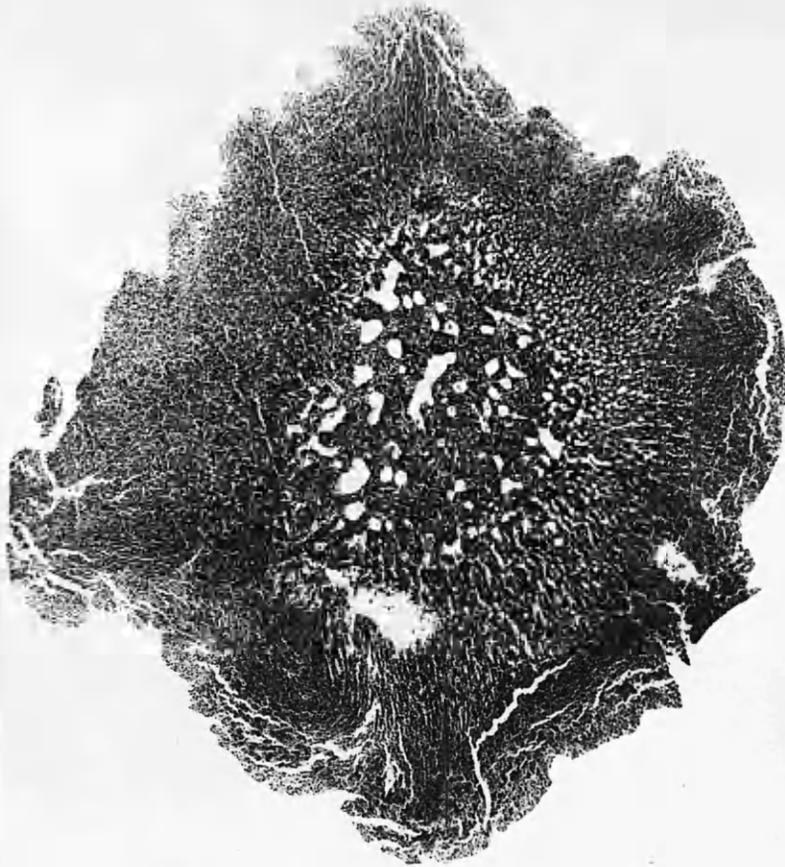
Fig. 93. The enucleated adrenal
pulp consists of the major part
of zona fasciculata, zona
reticularis and the medulla.

H. & E. X 23.
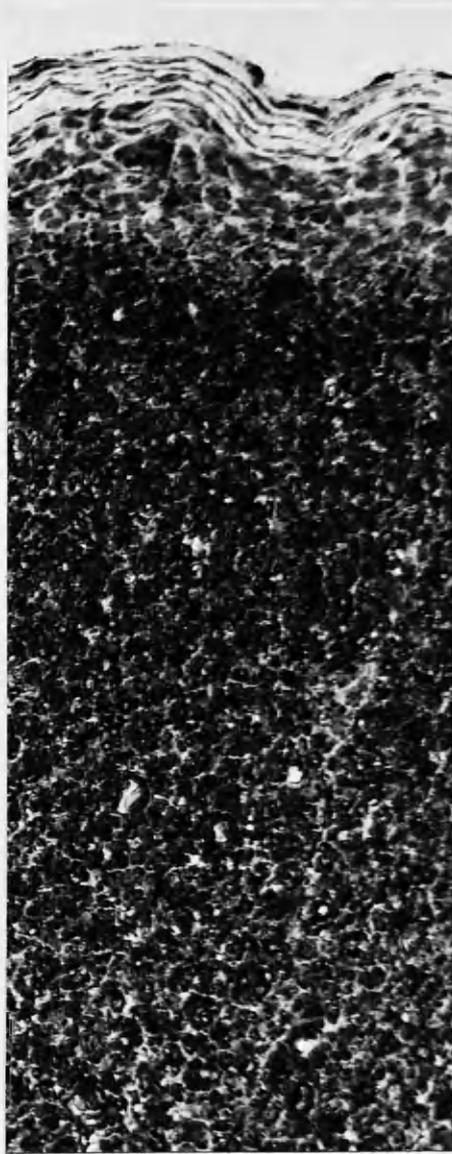
Fig. 94. Adrenal. Control rat
on 1% saline. Zona glomerulosa
is narrow and contains scanty
lipid. Zona fasciculata and
reticularis are filled with lipid.

Oil red O & H. X 200.
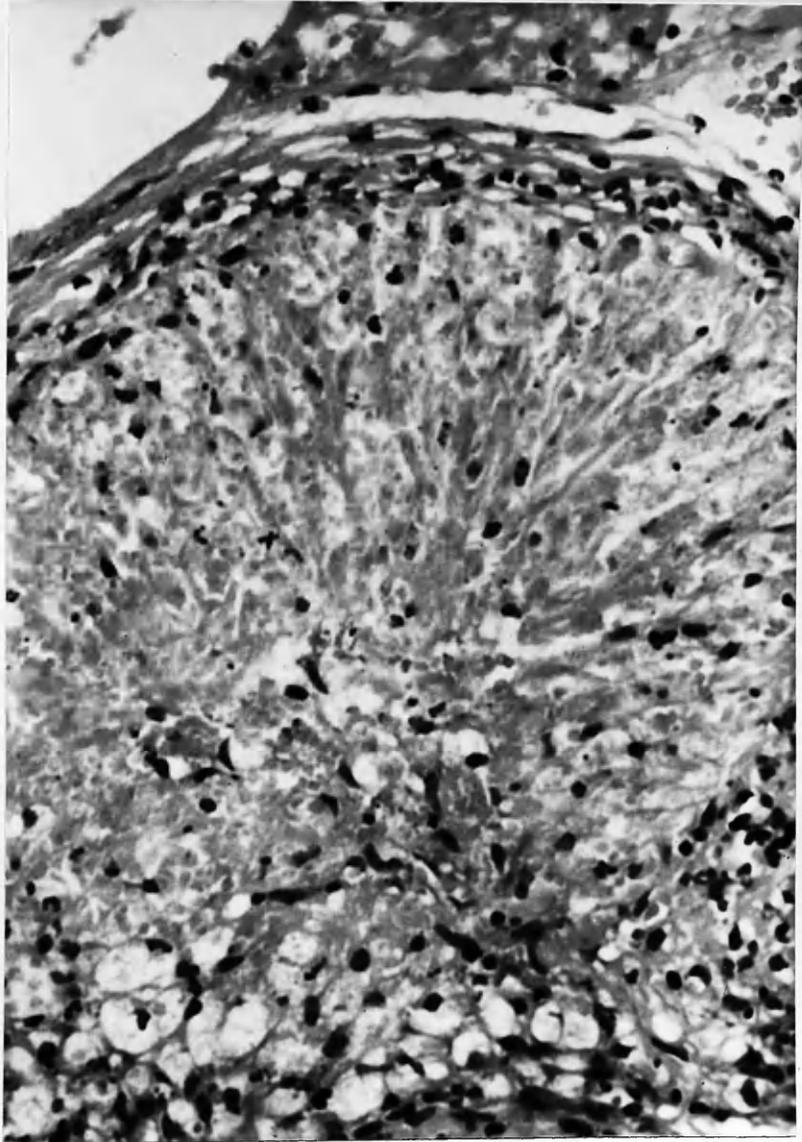
Fig. 95.   Adrenal 4 days after
enucleation.   All of the superficial
fasciculata cells and many glomerulosa
cells are necrotic.   Viable cells are
present immediately beneath the capsule.

H. & E. X 400.
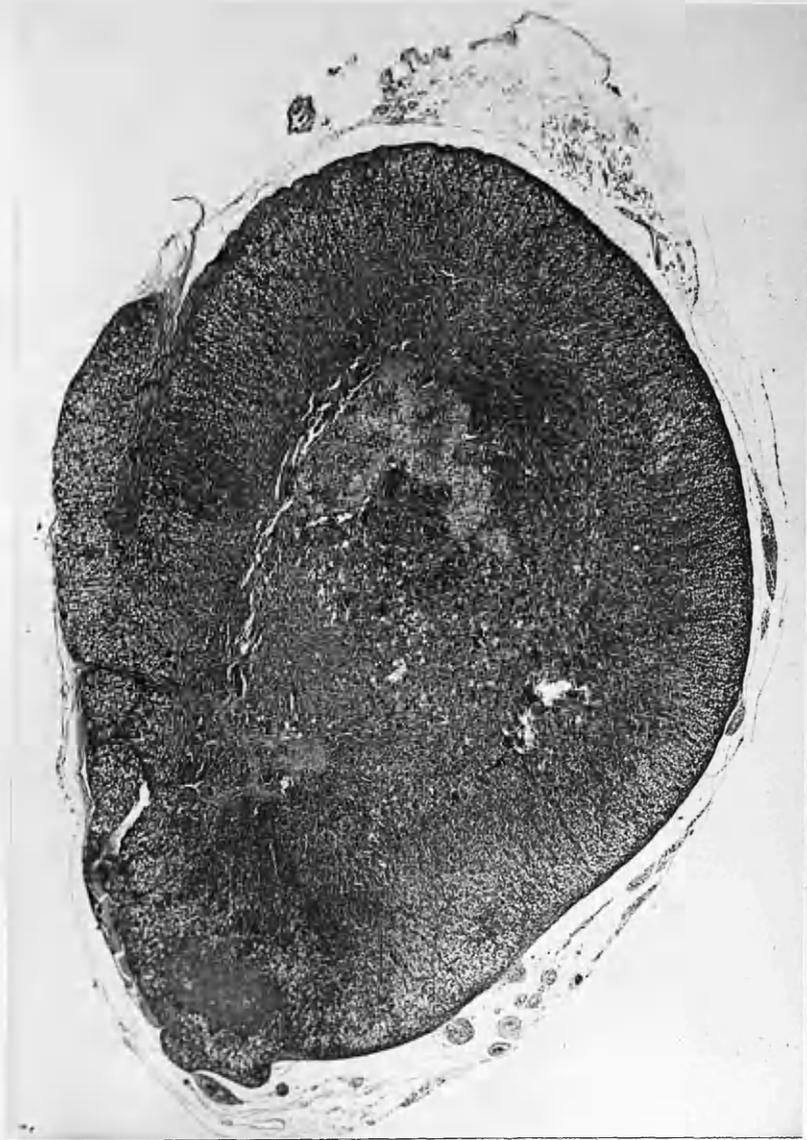
Fig. 96. Adrenal. Cortical
regeneration after 40 days, 1%
saline. Zoning reminiscent of
glomerulosa and fasciculata is
seen. The centre is composed
of reticularis cells and fibrous
tissue. B.P. 174 mm.Hg.

H. & E. X 17.
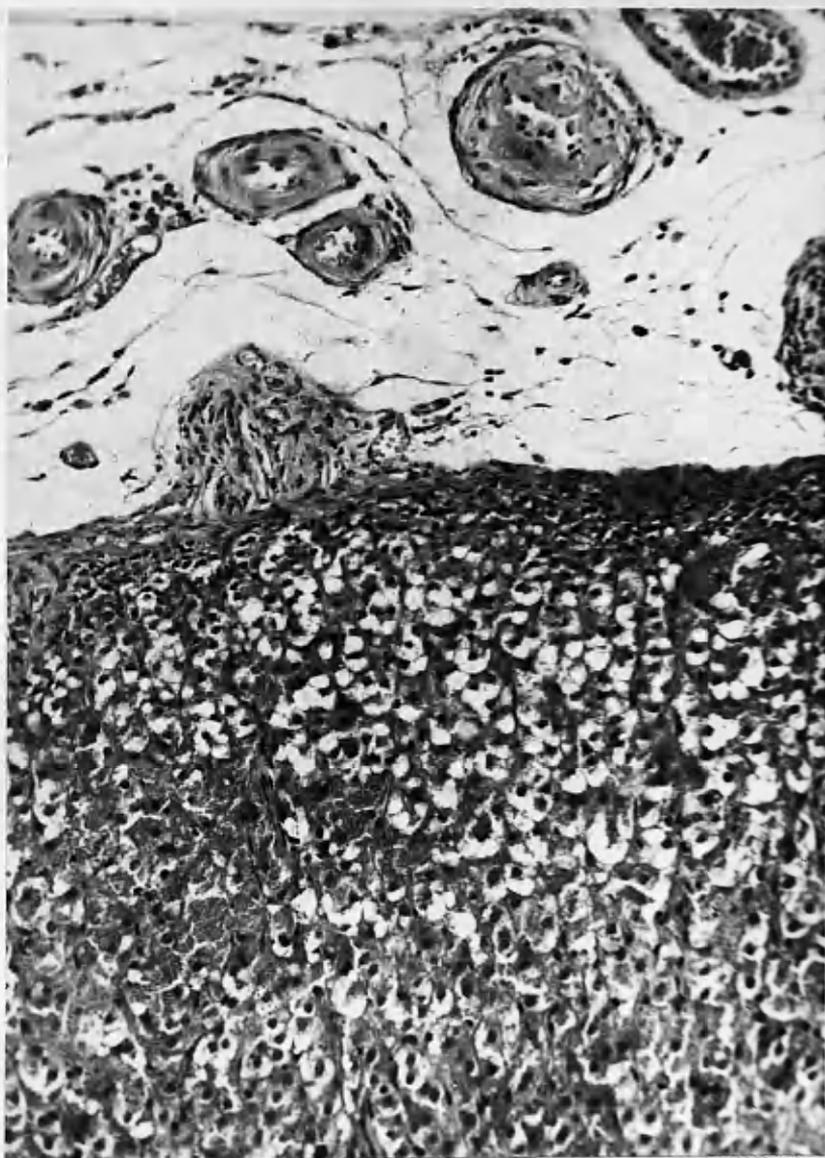
Fig. 97. Adrenal. Cortical
regeneration after 40 days, 1%
saline. A high power view of
Fig. 96 shows the narrow sub-
capsular glomerulosa layer, and
the large vacuolated fasciculata-
type cells. The capsular vessels
show hypertensive arteriolosclerosis.
B.P. 174 mm.Hg.

H. & E. X 195.
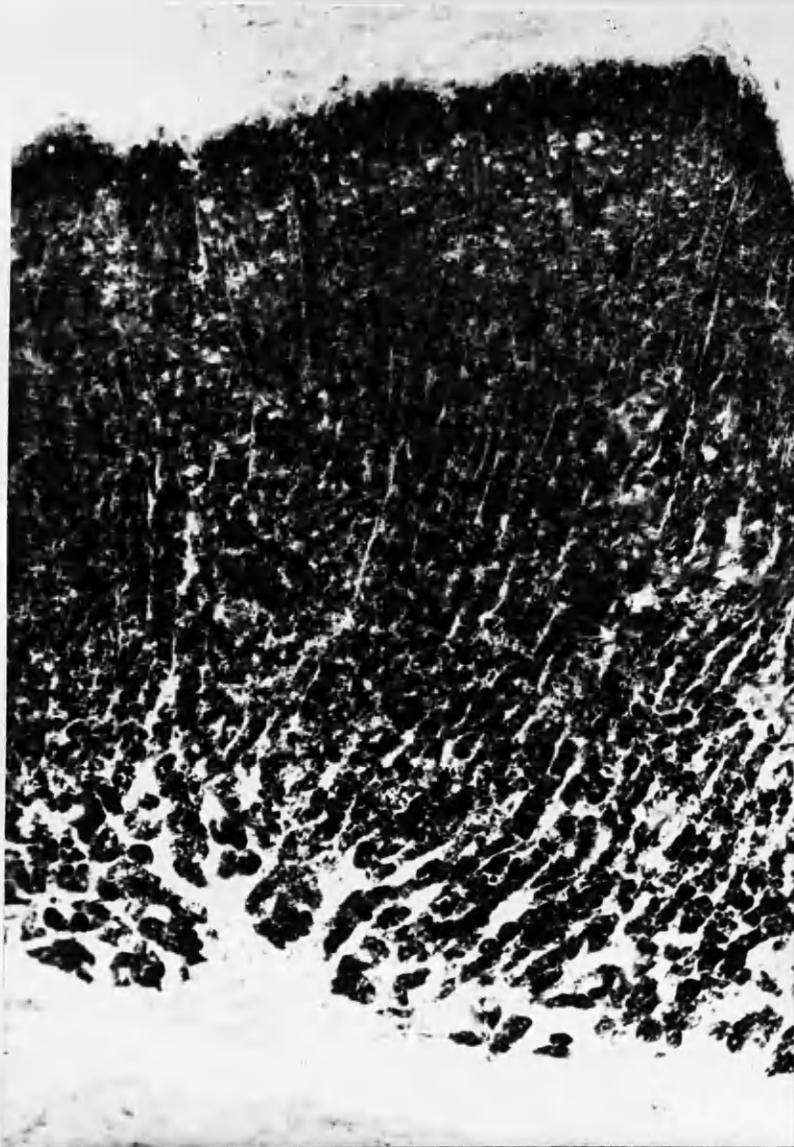
Fig. 98. Adrenal. Cortical
regeneration in groin for 50 days,
1% saline. A zoning arrangement
of the regenerated cortical cells
is apparent and they are well
filled with lipid.

Sudan black B. X 160.
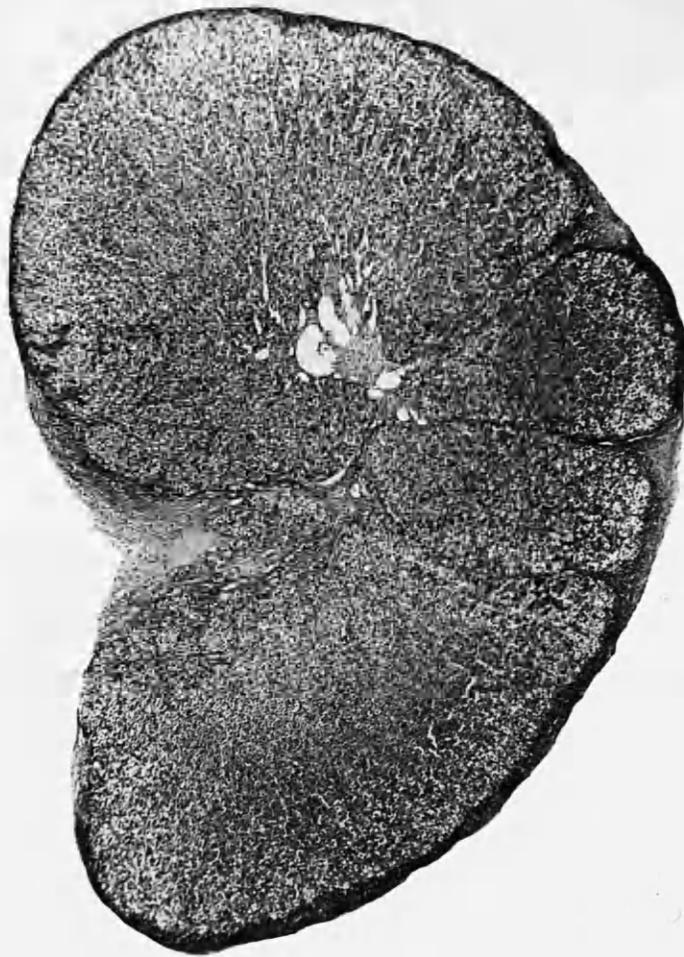
Fig. 99. Adrenal. Cortical
regeneration in groin for 50 days,
1% saline. The zoning arrangement
is rather less distinct and the
regenerated cells are separated into
nodules by fine fibrous bands.

H. & E. X 23.
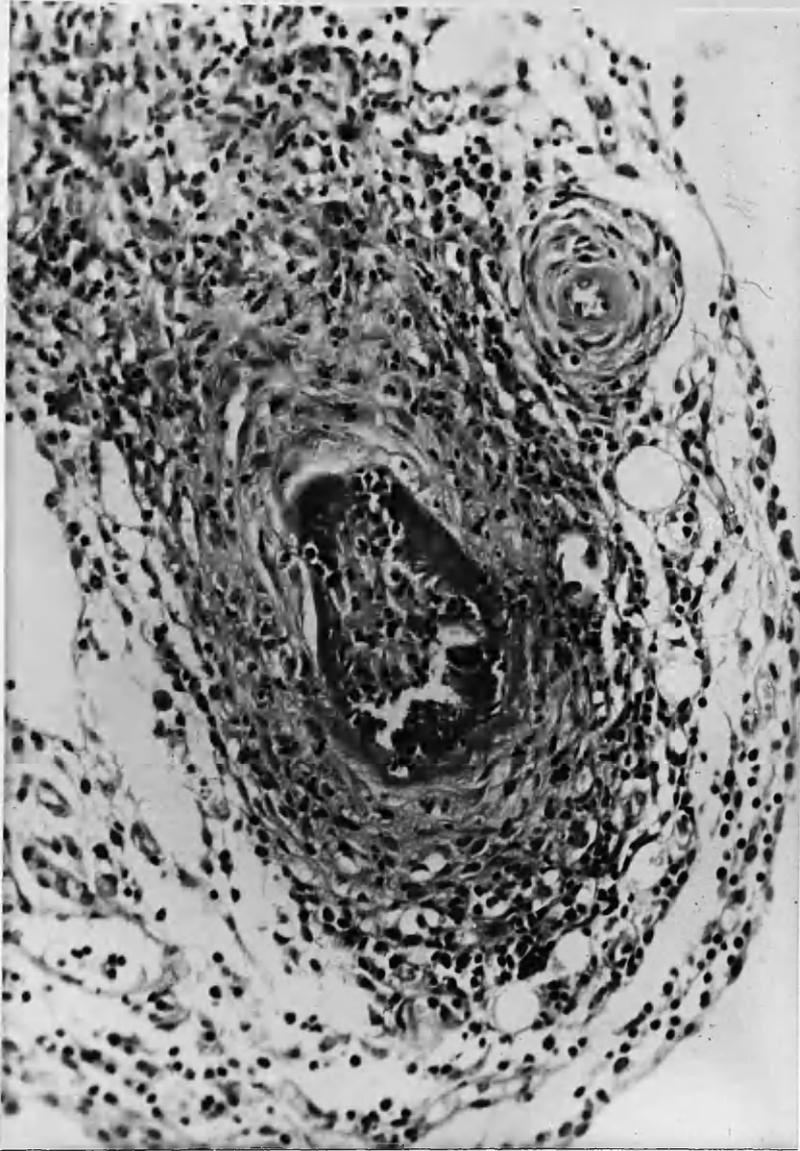
Fig. 100. Mesenteric artery.
Adrenal regeneration in situ for
40 days. The hypertensive type
of "polyarteritis nodosa" lesion
in the rat is characterized by
the deposition of intimal fibrinoid
and infiltration of the adventitia
with lymphocytes and macrophages.

H. & E. X 340.