T H E S U P R A R E N A L G L A N D

ITS FUNCTION IN HYPERTENSION AND THE COUNTER-SHOCK MECHANISM

by

THOMAS SYMINGTON, B.Sc(Hons)., M.B., Ch.B.

-----ooOoo-----

THESIS PRESENTED TO THE UNIVERSITY OF GLASGOW FOR THE DEGREE OF DOCTOR OF MEDICINE (M.D.)

-----ooOoo-----

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

TABLE OF CONTENTS.

INTRODUCTION.

THE SUPRARENAL GLAND.

ITS FUNCTION IN HYPERTENSION AND THE COUNTER-SHOCK MECHANISM.

INTRODUCTION.

The work on which this thesis is based began in 1944 when I had the opportunity of investigating two adrenal tumours subsequently diagnosed as phaeochromocytoma. These two routine surgical specimens were the first of six cases which I was able to collect and, so far as I know, they constitute the largest series of recorded cases (Blacklock, Ferguson, Mack, Shafar and Symington, 1947). A full clinical and pathological description of them is given in the appendix (II).

Pathological examination of the tumours provided an exercise in histological technique, and the following technical and pathological problems were encountered and constitute Part 1 of the thesis.

1. The Chrome Reaction in the Diagnosis of Adrenal Medullary Tumours with an in-vitro Demonstration and Explanation of the Reaction.

The chrome reaction played an important part in the pathological diagnosis of chromaffin tumours and a positive reaction was pathognomonic of them. Sometimes, the material was received many hours after operation or death, and the chrome reaction was negative; in such circumstances, unless the histological picture was characteristic or familiar to the observer, the diagnosis became extremely difficult.

2. The Disintegration of the Phaeochromocyte Cell and Liberation of the Pressor Substance into the Blood.

The clinical manifestations of phaeochromocytoma vary a great deal but it will be seen from the following table that the cases were representative of four recognised clinical groups.

Group 1 - The adrenosympathetic syndrome with paroxysmal hypertension (Cases 1 and 2).

Group 2 - Persistent hypertension (Case 3).

Group 3 - Asymptomatic (Cases 4 and 5).

Group 4 - Malignant phaeochromocytoma (Case 6).

Cases 1 and 2 had features typical of the adreno-sympathetic syndrome (Group 1). When the histological appearance of the two tumours was compared, the mode of disintegration of the inactive granular phaeochromocyte cell was seen and liberation of the pressor substance, directly into the blood stream, demonstrated.

3. The Association of Phaeochromocytoma with Hypertension.

Examination of the literature from 135 published cases (Tables 1, 2 and 3) showed that the hypertension was mainly paroxysmal in type in Group 1. If the patient survived long enough there was a tendency for the blood pressure to become persistent, although superimposed paroxysms were still present. Thus Group 1 hypertension could be subdivided as follows:-

Group 1A - Paroxysmal hypertension with interval blood pressure normal.

Group 1B - Paroxysmal hypertension with raised interval blood pressure.

In Group 2, as illustrated by Case 3, the hypertension was persistent, no superimposed paroxysms were present and the clinical features of this group were indistinguishable from those of essential hypertension.

Although the attacks of paroxysmal hypertension in Group 1A were

shown to result from liberation of adrenalin directly into the blood, the cause of persistent hypertension associated with Group 1B and Group 2 was not obvious, and attempts to solve this problem formed the basis of the animal experiments in Part 2 of this thesis.

4. The Use of Cortical Extract in the Post-Operative Treatment of Phaeochromocytoma.

It is well recognised that patients suffering from chromaffin tumours may be extremely shocked following a severe paroxysmal attack. If this occurs during the operation, as in case No. 1 in the series, the chances of recovery are impaired. In the treatment of such cases, adrenalin therapy alone, even in large doses is not satisfactory; but administration of adrenal cortical extract in large amounts seems to have a beneficial result. It is quite obvious that there will be an acute medullary insufficiency following removal of the tumour, but it is not clear why cortical extract should have a beneficial effect in counteracting the post-operative shock.

The four points mentioned above are considered in detail in Part 1 and the unsolved problems form the basis of subsequent animal experiments when attempts were made, in rats, to simulate the action of chromaffin tumours by injecting adrenalin in various forms. No vascular lesions were seen in any of the animals, but one unexpected finding at this stage completely altered the course of the investigations. The animal experiments had been in progress for about 9 months, without any positive findings, when my attention was drawn to an article on the osmic acid fixation of the suprarenals (Bennett, 1940). When the next animal died after an injection of adrenalin the supra-

renals were fixed in osmic acid. I was surprised to find the suprarenal cortex had responded to the injections of adrenalin by liberation of osmophil-positive material, and chrome fixation showed that this material was not adrenalin but true cortical secretion. In 1936 Grollman suggested that the major function of adrenalin "is to protect or act synergistically with the more vital and destructible cortical hormone." But Soffer (1945) referring to Grollman's statement writes - "the evidence adduced for this hypothesis is meagre and at best decidedly equivocal." Part 2 of the thesis supplies this evidence and shows that the suprarenal cortex and medulla, although different embryologically, are anatomically and physiologically interconnected.

The identity of the osmophil-positive cortical secretion was established in Part 3 using a new histological bioassay technique. It was shown to be sugar-active corticoid material liberated during the counter-shock phase of the alarm reaction. This finding was an important one, since it brought the investigation into line with Selye's adaptation syndrome and offered an explanation for the use of cortical extract in the control of shock following removal of a chromaffin tumour.

The possibility that hypertension and rheumatoid arthritis are diseases of adaptation and result from chronic endocrine overdosage, is considered in Part 4. Definite vascular lesions resulted from desoxycorticosterone acetate implants into rats and there is a striking similarity between those lesions and the ones produced by Byrom and Wilson (1939) using a modified Goldblatt clamp. A close relationship appears to exist between renal ischaemia and suprarenal cortex, and an

attempt is made to represent this in diagrammatic form (page 89).

The last section (Part 5) describes an attempt to provide direct evidence for the adaptation theory of hypertension. Although unsuccessful in this respect the experiments uncovered changes in the animal suprarenals similar to those seen in the Waterhouse-Friderichsen syndrome and the investigation has helped to clarify the aetiology of this puzzling condition.

PART 1.

PHAEOCHROMOCYTOMA.

The following technical and pathological problems, mentioned in the introduction, will now be discussed in more detail:

1. THE CHROME REACTION IN THE DIAGNOSIS OF ADRENAL MEDULLARY TUMOURS WITH AN IN-VITRO DEMONSTRATION AND EXPLANATION OF THE REACTION.

Various methods have been employed to demonstrate the pressor substance in adrenal medullary cells. Osmic acid vapour (Cramer, 1918) and silver impregnation methods give excellent results, but the osmic vapour method has the disadvantage that it also demonstrates lipoid material in the adrenal cortex (see Parts 2 and 3), and has led to erroneous interpretations (Cramer, page 44). The silver impregnation technique was found to be quite satisfactory following proper fixation. When formol-corrosive was used, no granules were seen in the cells, but chrome-fixed material stained by a modification of Bielschowsky's silver impregnation method showed the adrenalin granules as black deposits (Fig. 1). Nevertheless, the method of choice in the pathological investigation of phaeochromocytoma is undoubtedly chrome-fixation followed by Giemsa staining.

In 1865, Henle found that the medullary cells of the adrenals turned brown on being left in a solution of potassium dichromate for hardening purposes, and believed that this was due to the formation of a chemical compound of chromic acid with some substance in the suprarenals. Ogata and Ogata (1916), using test tube experiments with dichromate, showed that the brown precipitate was an inorganic

substance which they believed to be chromium dioxide, according to the following reaction:

$$K_2Cr_2O_7 + 4H_2O - 3(O) = Cr_2(OH)_6 + 2KOH$$

$$Cr_2(OH)_6 + K_2Cr_2O_7 = \underbrace{CrO_3Cr_2O_3 + 3H_2O}_{\text{Sesqui and trioxides of chromium.}} + K_2CrO_4$$

They showed also that the reaction was most marked in a neutral medium, acid or alkali interfered with it.

Fixation of Tissue: Thin slices of tumour tissue should be fixed in a solution containing potassium dichromate, and fixatives such as Orth, Müller and formol-dichromate give good results. Müller fixation was used in two cases in my series (Nos. 1 and 2). Thin slices of tumour tissue were placed in 10% neutral formalin for 4 to 6 hours then transferred to Müller's fluid for 14 days, with frequent changes. Although the method gave an excellent chrome reaction, fixation of tissue was poor, and the tumour cells were disrupted at the sinusoids and vacuolated spaces (Fig. 2). In view of this, formol-dichromate fixation was used in the remaining cases when thin slices of the tumour were placed in a solution of equal parts 10% neutral formalin and 5% potassium dichromate for 48 hours. The solution was changed once during that period and the tissue treated subsequently with 5% potassium dichromate for 14 days. Dehydration and paraffin impregnation were carried out in the usual manner. This method gave excellent fixation as well as providing suitable material for the chrome reaction (Fig. 3).

Staining of Chromaffin Tissue: Brownish-yellow granules were seen in the cytoplasm of the cells after chrome-fixation, and when

the method described by Schmorl (1928) was used subsequently, excellent results were obtained. Details of the method are as follows:- the sections were treated for 24 hours with a dilute solution of Giemsa stain prepared by adding 100 drops of Giemsa solution to 100 c.c. of distilled water. They were then washed in distilled water until the purple colour of the section assumed a pinkish hue. Differentiation was carried out quickly in 0.25% acetic acid, and more slowly in 93% alcohol, after which they were cleared and mounted in the usual manner. Using this technique the granules in the cytoplasm of the cells stained dark-green, the nuclei violet, and the red cells pink. Since differentiation with 0.25% acetic acid tends to be too rapid, it was suggested that this stage could be omitted (Sevki, 1934), and in the present series it was found that better control of differentiation could be obtained using 93% alcohol alone. Rapid differentiation with acetic acid or over-differentiation with 93% alcohol caused the chromaffin granules to lose their green colour and return to their original brownish-yellow colour. This result of over-differentiation has been observed by others (Edward , 1937; Hick, 1933). Neither Schmorl's method nor Sevki's modification gave good nuclear differentiation and this was achieved in the present series by treating the sections with an aqueous solution of 0.5% potassium permanganate for two minutes, decolorising in 1% oxalic acid, and then staining with dilute Giemsa stain as above. The nuclei of the cells stained in this way appeared more distinct and were pale-blue in colour, the granules in the cytoplasm now appeared olive-green, and there was not the same tendency for them to revert to the original brownish-yellow

colour. In addition, differentiation could be more easily controlled.

Duration of a Positive Chrome Reaction after Death: Since the presence of a positive chrome reaction plays such an important part in the diagnosis of chromaffin tumours, it was important to know the length of time the reaction persisted in the tumour cells after post-operative removal or death. The present series of cases shows that the granules were clearly demonstrable up to 5 hours after death (Fig. 3, Case 4), but not after 12 hours (Case 5). Thus, materials should be examined within 5 hours of operation or death for the demonstration of a positive chrome reaction.

Explanation and in-vitro Demonstration of the Chrome Reaction: Chromaffin tissue elaborates a pressor substance, adrenalin, which is also a powerful reducing agent. If this tissue is fixed in a solution of potassium dichromate, the latter is reduced to a mixture of sesqui and trioxides of chromium, which are deposited in the cytoplasm of the chromaffin cells as brownish-yellow granules. When the section is stained with Giemsa solution, a physico-chemical combination results between these granules and the methylene blue of the stain, and the colour produced will depend on the proportion of both substances present. The sections, when removed from the stain, have a purple colour due to a predominance of the methylene blue-eosin components of the Giemsa, but after washing with water, the red blood cells and fibrous septa take up the eosin and stain pink. The excess methylene blue is removed by differentiation with 93% alcohol and eventually an optimum concentration of chromium oxide-methylene blue is formed. This yellow-brown-blue mixture gives rise to the

green colour of the granules as shown in the following equation:-

$CrO_3Cr_2O_3$ + Methylene Blue $\rightleftharpoons$ ($CrO_3Cr_2O_3$) (Methylene Blue)

Yellow Brown Blue Green

The fact that the change is reversible is shown by a return to the original "dun" colour if the section is further differentiated by 93% alcohol until all the methylene blue is removed. Thus it is seen that the oxides of chromium deposited in the phaeochromocyte cell act as a mordant so that the cell can retain the methylene blue component of the Giemsa stain. The part played by potassium permanganate is not understood. It appears to oxidise the chromium oxides to a stable form which, acting as a more effective mordant, renders the chromium-oxide-methylene blue mixture more stable. In an endeavour to prove those points, attempts were made to reproduce the reaction in-vitro using cloth for the purpose. The first difficulty was in finding a suitable medium which would retain the chromium salts after their reduction. Linen and cotton material proved useless but white flannel was satisfactory. A small piece of flannel (Fig. 4) was soaked for two hours in 10 c.c. of a solution of 1:1000 adrenalin hydrochloride, then placed in a mixture of equal parts 5% potassium dichromate and 10% neutral formalin for two days. A brown colour was produced similar to that seen in sections of the tumour. The cloth was washed in water, treated with 0.5% potassium permanganate, decolorised in 1% oxalic acid, and stained with dilute Giemsa for two days. Differentiation was carried out in the usual way when the typical green colour was produced, and prolonged differentiation failed to reproduce the original "dun" colour unless the permanganate

step was omitted. The process was repeated, but the original treatment with adrenalin omitted, and a blue colour was produced similar to that seen in tumours with a negative chrome reaction.

The flannel in the experiment acted in the same manner as the phaeochromocyte cell, first holding the adrenalin solution and later the oxides of chromium which were formed. It was not possible to reproduce the action with cotton or linen, which are carbohydrate materials and, since flannel is a protein substance, the result suggests that adrenalin must be linked to a protein molecule before a positive chrome reaction can be produced. In view of this it is extremely probable that adrenalin is held normally in the medullary cell as a protein complex. The experiments were repeated with other reducing agents: 10 mg.% sodium hydrosulphite and ascorbic acid. Both gave the typical green colour with Giemsa but in neither was the reaction so marked as with adrenalin. Accordingly, it must be appreciated that the chrome reaction is not necessarily specific for medullary or rather chromaffin tissue, but depends on the presence in the cells of a powerful reducing agent (adrenalin or its precursor), which has the ability to reduce potassium dichromate and fix the chromium oxides so formed in the cell. Since the medulla of the suprarenals, carotid body and para-aortic glands are the only tissues which elaborate such a reducing agent, the reaction has come to be regarded as specific for chromaffin tissue.

2. <u>THE DISINTEGRATION OF THE PHAEOCHROMOCYTE CELL AND THE LIBERATION OF THE PRESSOR SUBSTANCE INTO THE BLOOD.</u>

Interesting results were obtained when the clinical and

pathological aspects of two operation cases (Nos. 1 and 2) in the series were compared. Both patients gave a history typical of the adreno-sympathetic syndrome. One of them (No. 2) received adequate premedication, the anaesthetist encountered no difficulties, and there were only two slight rises of blood pressure during the operation. The systolic blood pressure rose to 180 mm.Hg. when the incision was made and again to 220 mm.Hg. when the tumour was handled. The patient's condition was satisfactory following the operation and her convalescence uneventful.

When the adrenal tumour was examined from this case, it was found that many of the blood-capillaries were composed of irregular spaces lined by a reticular basement membrane, while others consisted of large sinusoidal spaces lined only by tumour cells (Fig. 5). The cytoplasm of the cells was finely granular (Fig. 6), except in the neighbourhood of the sinusoidal spaces, where they were vacuolated and free from granules (Fig. 7). The presence of granular phaeochromocyte cells in the sinusoidal spaces (Fig. 8) and efferent veins (Fig. 9) has aroused considerable interest. It is extremely unlikely that their presence was due to incomplete fixation as was believed originally when Müller-fixed material was used, since they were found in Müller, formol-corrosive and neutral formalin-fixed material, and it it very probable that they were dislodged into the blood vessels when the tumour was handled.

The other case (Case No. 1) did not receive adequate premedication and, as a result, the anaesthetist had to deal with a nervous and apprehensive patient, who developed a hypertensive attack during the

operation. He was extremely collapsed after the operation, and his systolic blood pressure registered less than 50 mm.Hg. The vascularity and general appearance of the cells of the tumour were similar to those described, but Giemsa staining showed that only a few cells were granular. Some of them were vacuolated, but many had a homogeneous green coloration of the cytoplasm. No granular tumour cells were seen in the blood vessels, but the serum in the sinusoidal spaces and efferent veins was stained green (Fig. 10). Since in-vitro experiments (page 10) showed that adrenalin could produce this characteristic colour with Giemsa's stain, it indicated that the fluid in the adrenal vein was adrenalin. It was possible to follow the disintegration of the cells in the few areas where granular phaeochromocytes were seen (Fig. 11a and b). The cell at first had the appearance of a mulberry, but as the pressor substance was liberated, clear spaces appeared in the cytoplasm; eventually these spaces coalesced and a large non-staining inclusion vacuole was formed. This cell does not represent an intermediate phase in the secretion of the phaeochromocyte, as suggested by Howard and Barker (1937), but a terminal one.

When the pathological and clinical aspects of the two cases are compared, it is seen that in one, the tumour was in a state of inactivity, represented by the granular appearance of the phaeochromocyte; in the other, the patient had a paroxysmal reaction and the tumour was seen to be in a stage of great activity, granular phaeochromocytes were present in varying stages of disintegration and resulted in the liberation of the pressor substance directly into

the blood stream. The arrangements and character of the sinusoidal spaces facilitated this action. Thus, the pressor substance formed by the chromaffin cells exists there in a granular inactive form which disintegrates to liberate the pressor substance directly into the blood via the sinusoidal spaces. The liberation of adrenalin accounts for the paroxysms of hypertension which occur in the adreno-sympathetic syndrome.

3. THE ASSOCIATION OF PHAEOCHROMOCYTOMA WITH HYPERTENSION.

Disintegration of the phaeochromocyte cell, with liberation of the pressor substance into the blood stream , gives rise to attacks of paroxysmal hypertension which are the basis of the adreno-sympathetic syndrome. In the earlier cases (Manasse, 1893) no mention was made of the relationship between these tumours and hypertension, but Fishberg and Oppenheimer (1924) noted 5 cases of chromaffin tumours with adequate blood pressure measurements or other evidence of hypertension, and Rabin (1929) added a further 4 cases in which the hypertension was independent of renal disease. In a review of 53 cases Eisenberg and Wallerstein (1932) found 25 of them associated with hypertension and recognised that the hypertension might be paroxysmal or persistent in character. Laubry and Bernal (1934) also described two distinct phases in the hypertension of chromaffin tumours; in one "the blood pressure was normal between attacks, and in the other the crises of pressure increased and occurred with greater frequency. Eventually it became persistent and manifestations of renal lesions and subsequent organic changes developed." Progression

of the paroxysmal to permanent variety has been recognised also by Boman and Wells (1937), and Van Epp et al (1940) believe that the paroxysmal variety "may progress so far as to cause death from irreparable vascular damage." In a review of 135 published cases shown in the following table, I found the blood pressure was paroxysmal in 76, persistent in 8 and normal in 16. The term hypertension was mentioned in 12 cases, but it was not possible, from the data available, to decide whether it was paroxysmal or continuous in type. No mention of blood pressure was made in 23 cases and it was impossible to classify them.

Relationship to Hypertension in 135 Cases.

(Blacklock and Symington, 1947)

	Present series from literature since 1936, including our own six cases.	Edward (1937).	Eisenberg and Wallerstein (1932).	Total.
No. of Cases	49	34	52	135
Paroxysmal hypertension	39	21	16	76
Persistent hypertension	4	3	1	8
Hypertension only mentioned, no B.P. readings given	0	5	7	12
No mention of hypertension	1	1	21	23
Normal blood pressure or hypotension	5	4	7	16

Table compiled from cases shown in Tables 1 to 3.

Of the 76 cases of paroxysmal hypertension accurate blood pressure readings were available in 53 and, of these, 20 showed a persistently raised pressure, rising further during paroxysmal attacks. The figures differ slightly from those recorded by Green (1946). I considered hypertension present when the systolic pressure was 140 mm. or over and the diastolic 100 mm. or over. Green, on the other hand, considered hypertension present when the interval blood pressure was either (a) 140 mm. systolic and 90 mm. diastolic, or (b) 95 mm. diastolic. From the above data it is evident that paroxysmal hypertension can be subdivided as follows:-

(A) Cases in which the interval blood pressure was within normal limits.

(B) Cases in which the interval blood pressure was persistently elevated.

If a patient in Group (A) lived long enough there was a tendency for the interval blood pressure to become elevated, but paroxysms of hypertension were still superimposed and their recognition served as an aid to diagnosis.

Persistent hypertension without any evidence of paroxysms was seen in 8 cases. The blood pressure and clinical features of this group are indistinguishable from those of essential hypertension and unless a palpable tumour is present the diagnosis in this group is usually made at post-mortem.

In view of the remarkable success of surgery, it became important to discover the extent of vascular damage done by these tumours,

and the final stage at which surgical intervention was likely to prove successful.

The following points were noted as they applied to cases in the paroxysmal and persistent group.

1. The return to normal of blood pressure and retinal changes in successful operation cases.
2. The histological appearance of kidney, heart and spleen in cases dying with or without operation.

Details of cases giving this information are shown in Tables 4 to 6. Whereas the pathological information from the last point would serve as an indication of vascular damage, the clinical information from the first would give an indication of the reversibility of the vascular lesions.

GROUP 1. CASES OF PAROXYSMAL HYPERTENSION.

(A) Interval Blood Pressure Normal. (Table 4).

Only 9 cases in this group gave the required information about the retinal vessels or kidney damage and they are shown in Table 4. In all successful operation cases in this group there was an immediate return to normal of the systolic and diastolic blood pressure. Information was given about the retinal vessels in 8 cases, 4 of them survived the operation and the vessels returned to normal in 2 (Rodin, 1945; Biskind et al), but no mention was made of the post-operative state of the retina in the other 2 (Shipley and Pincoff, 1929; Engel, Mencher and Engel, 1942, Case 1). The remaining 4 patients died without any surgical interference (Howard and Barker, 1937; Wells

and Bowman, 1937; Baker and Reinhoff, 1937; and Holst, 1938).

In the published reports the histological data is inadequate to permit a satisfactory assessment of the vascular changes. Howard and Barker, and Neusel and Weisel (1932) found no trace of sclerosis of the vessels or glomerular lesions; and Baker and Reinhoff (1937) noted only "moderate arteriosclerotic lesions of the kidney in a female of 40 years."

Cardiac hypertrophy of a moderate degree was found in all cases but exact weights are given in two cases only (Howard and Barker, 340 g.; Bowman and Wells, 325 g.). Occasional mention is made of changes in other organs. The lungs were usually haemorrhagic and small sclerotic vessels were noted in the spleen and pancreas in one case (Howard and Barker), but the lesions lose their significance since they occurred in a man of 69 years.

It is rather difficult from the above data to determine the extent of vascular damage, but the post-operative return of blood pressure to normal in all surviving cases and of the eyes to normal in two, suggests that whatever vascular damage is present in this group it is reversible.

(B) Interval Blood Pressure Elevated. (Table 5).

There were 20 cases in this group and 10 were subjected to operation. Eight of the 10 survived and in 7 of them the blood pressure returned to normal. In the eighth it fell from 200-300/100-110 to 140/100 mm.Hg. (Bauer and Leriche, 1934), but the patient was still having typical paroxysms 8 years later. The

authors give no explanation for this but it is possible that active chromaffin tissue was present elsewhere, as this has been noted previously (McKenzie and McEachern, 1938). The 2 remaining patients died ½ hour (Holst, Case 2) and 1 day (Volhard, 1931) after operation.

Severe eye changes were seen in 13 patients. Eight of them were operation cases but the state of the retina after operation is mentioned only in 4. The retinal changes returned to normal in 3 of them (Evans and Stewart, 1942; Green, 1946; and Spalding, 1947), but it remained unaltered in the fourth (Beer, King and Prinzmetal, 1937). However, it is interesting to note that this case was well 3 years later and her blood pressure normal (reported by Biskind et al).

Histological examination of the kidneys was reported in 8 cases. No glomerular or vascular lesions were found in 2 (Palmer and Castleman, 1938; Labbé, Tinel and Doumer, 1922) but definite vascular damage was present in the remainder (Paul, 1931, Cases 2 and 3; Beer, King and Prinzmetal, 1937; Gutmann, 1947; Labbé, Violle and Azerad, 1929; McCullagh and Engel, 1942, Case 2). Paul (Case 3) found hyalinised glomeruli in wedge-shaped areas which were related to hyalinised arterioles. There was narrowing of the lumen of these vessels. A similar picture was presented by Gutmann and both cases are significant since they occurred in patients of 23 and 38 years respectively.

One case in the present series appeared to be in the transition stage between groups 1(A) and 1(B), although the diastolic blood pressure (80-85 mm.) between attacks did not satisfy the requirements

of the second group (B.P. 140/100). Microscopic examination of the kidneys showed changes similar to those described above; the glomerular capillaries were generally patent, but some glomeruli were enlarged and adherent to the capsule (Fig. 12) and a few were sclerosed. The afferent arterioles were thickened and the lumen of the vessel narrowed (Fig. 13), while the arcuate and interlobular arteries showed marked splitting of the internal elastic lamina (Figs. 14 and 15). Those changes are significant since they occurred in a young man of 21 years.

Cardiac hypertrophy was marked in most cases in this group and in one (Evans and Stewart) the electrocardiograph showed changes which returned to normal after operation.

Many of the records, as can be seen, are incomplete, but I think the conclusion to be drawn is that vascular damage is liable to occur in any case of chromaffin tumour with paroxysmal hypertension and a raised interval blood pressure. However, the return of blood pressure to normal and resolution of eye changes in some of the operation cases described, suggest that the vascular damage is not necessarily irreparable as stated previously (Van Epp, page 15), and so operation at this stage has a reasonable chance of bringing about a normal return of blood pressure.

GROUP 2. PERSISTENT HYPERTENSION. (Table 6).

This group consists of 8 patients all of whom had chronic hypertension. The clinical features are essentially those of benign or malignant hypertension and Case 3 in my series was diagnosed by a physician of long experience as possibly one of malignant hypertension.

The case histories are incomplete in 3 cases (Hick; Kirshbaum and Balkin, 1942, Case 2; Edward) but long histories of hypertension are given in the remainder. The blood pressure was normal originally in 2 of them (Thorn et al, 1944; Kremer, 1936) but rose gradually to hypertensive levels over a period of years. Eye changes were noted in 3 cases (Kremer; Oppenheimer and Fishberg, 1924; Thorn et al) but they were reversible in the only one subjected to operation (Thorn et al), and in this case the blood pressure returned to normal 270 days after operation. The vascular lesions seen in the kidneys, spleen and pancreas of 3 cases (Kremer; Rabin; Fishberg and Oppenheimer) were similar to those seen in Group 1(B). The arcuate and interlobular arteries of the kidneys were arteriosclerotic and the afferent arterioles of the kidney showed conspicuous hyalinisation in one case (Rabin). Most of the glomeruli were normal, a few were sclerotic, while some of them had hyaline material in the tufts (Rabin; Kremer).

There was well marked cardiac hypertrophy which was greatly in excess of that seen in the other groups.

The vascular lesions were similar to those seen in Group 1(B) (page 19) and, although operation was performed in one case only, it was significant that both blood pressure and eye changes returned to normal in it. In spite of this, it is impossible to say that the vascular changes seen in this group are reversible.

The review of the literature shows the advisability of operation in all cases of chromaffin tumours, irrespective of whether the blood pressure is paroxysmal or persistent, but the success of the

operation in early paroxysmal cases calls for operative interference as early as possible before hypertension has become persistent.

Cause of the Different Types of Hypertension Associated with Chromaffin Tumours: The cause of all types of hypertension associated with chromaffin tumours has been ascribed to hyperadrenalaemia (Eisenberg and Wallerstein; Hick) but proof of this was lacking until Beer and Prinzmetal and later Strombeck and Hedberg (1939) showed an excess of adrenalin in the blood during a paroxysm. The existence of hypertension in some cases of cortical carcinoma further complicated the issue. Peyron (1930) endeavoured to explain those findings by saying that the hypertension of medullary tumours was paroxysmal whereas it was persistent in cortical carcinoma. From the above review the number of chromaffin tumours associated with persistent hypertension disproves this.

Before discussing the cause of the various types of hypertension associated with chromaffin tumours it is necessary to consider the normal secretion of adrenalin from the suprarenal gland. It is known that the splanchnic fibres to the adrenal medulla are preganglionic in type. They end directly around the medullary cells and the chemical transmitter between the preganglionic fibres and the medullary cells is acetyl-choline (Sampson Wright, 1940). How this substance liberates adrenalin from the medulla is not clear but, it is possible, from the present investigation (page 11) that it has the power to disintegrate the inactive granular stages of adrenalin and liberate the active pressor substance directly into the blood. In

chromaffin tumours a similar state of affairs presumably exists but in a more exaggerated form. Thus, cold, emotion, excitement or any factor known to have a sympathetic action sets into motion the train of events already mentioned (page 11). Whether the hypertension produced is paroxysmal or persistent would appear to depend on a number of factors:-

(a) Frequency of the attacks.

(b) Presence or absence of continuous secretion of adrenalin from the tumour.

(c) The degree of hyperadrenalaemia.

(d) The ability and rapidity with which the patient can tolerate or detoxicate adrenalin.

In Group 1(A) (page 17) the paroxysmal hypertensive attacks were usually well spaced and associated with a degree of hyperadrenalaemia which exceeded the ability of the body to detoxicate it. In the interval the blood pressure returned to normal and remained so until the next attack.

In Group 1(B) (page 18) a clear-cut explanation is not so obvious since all four factors seem to play a part. The attacks occurred with greater frequency and it has been suggested that "the high pressure may be due to a persistent oversecretion of adrenalin" (Editorial B.M.J. 26th April, 1947). Until the blood adrenalin is shown to be raised permanently it can only be suggested that chronic hyperadrenalaemia may be a factor in the aetiology of Group 1(B) hypertension associated with chromaffin tumours. The recent improve-

ment in the methods of estimating blood adrenalin (Shaw, 1938) should be a great advantage in this respect. Meantime, it is to be noted that experimental evidence by Freeman et al (1940) shows that continuous intravenous injections of adrenalin in dogs cause a great rise in blood pressure which gradually falls to sub-normal levels during the transfusion.

The cause of persistent hypertension seen in Group 2 (page 20) is even more obscure. Paroxysms did not occur in those patients and their blood pressure rose steadily over a number of years (Kremer; Rabin; Thorn et al). The arterial and renal changes were marked and the degree of hyalinisation of the afferent arteriole was comparable to that seen in essential hypertension. Indeed it is possible that the changes might be attributable to the renal humoral mechanism. Since Goldblatt caused experimental renal ischaemia by clamping the renal artery various suggestions have been put forward to explain the renal ischaemia of essential hypertension in the human subject. Narrowing of the renal artery by atheromatous plaques, and disturbance of balance between the venous and arterial pressure within the kidney, are only two of the many theories (Van Dellan et al, 1945). Again it has been suggested that neurogenic hypertension is the initial form of essential hypertension and that the early changes result from spasm of the renal arterioles, following sympathetic stimulation. Since the post-ganglionic fibres of the sympathetic act by liberating adrenalin at their terminals, it might be expected that phaeochromocytoma with hyperadrenalaemia would cause an extreme

degree of spasm of the renal vessels with subsequent renal ischaemia similar to that induced experimentally by Goldblatt, and it was with this idea in mind that I commenced the experimental work which forms Part 2 of this thesis.

4. THE USE OF CORTICAL EXTRACT IN THE POST-OPERATIVE TREATMENT OF PHAEOCHROMOCYTOMA. (Figs. 16, 17 and 18).

Although an acute adrenal medullary insufficiency must necessarily result after removal of an adrenal medullary tumour, adrenalin alone does not provide adequate post-operative therapy especially if the patient is shocked following the operation. This point was well demonstrated in Case 1 of the series. The patient had a severe paroxysmal attack during the operation and was extremely shocked; yet large doses of adrenalin intravenously and intramuscularly did not assist in any way.

Recently it has been shown that pre- and post-operative administration of suprarenal cortical extract is beneficial and this is now part of the accepted treatment of those cases. Nevertheless, no reason has been put forward so far to explain why an apparent medullary insufficiency should benefit by the administration of cortical extract.

The following explanation is a possibility. When the tumour is removed at operation the suprarenal cortex is excised also, and so a cortical as well as medullary insufficiency results. In two cases in the thesis (Cases 4 and 5) the tumour was small and in both it was separated from the cortex by a fibrous capsule which was

present in the region of the zona reticularis (Fig. 16). With the expansion of the medullary neoplasm, the remaining cortex narrows and eventually is no more than a rim of tissue partly inside and partly outside the thick fibrous capsule (Figs. 17 and 18). It has been suggested that the external layer of the cortex is the germinal one (Bennett, 1940) and if this is so, the thin rim of cortex on the surface of the tumour would be still potentially active. This explanation gains support from subsequent experimental work (page 72).

PART 2.

THE VASCULAR RESPONSE OF THE RAT TO ADRENALIN INJECTIONS.

PART 2.

THE VASCULAR RESPONSE OF THE RAT TO ADRENALIN INJECTIONS.

Two problems emerged from the investigation on phaeochromocytoma:-

1. The part played by adrenalin in the chronic hypertension of chromaffin tumours.
2. The use of cortical extract to control the shock which followed a severe paroxysmal reaction during operative removal of the tumours.

Animal experiments were undertaken in the hope of clarifying those problems, and rats were the animals of choice since they had been used previously and served as a basis for comparison (Selye, 1947; Byrom and Wilson, 1939). Evidence of hypertension was based on histological changes in the vessels since a satisfactory method of blood pressure measurement was not available in unanaesthetised rats. Nevertheless, it was considered advisable to find the effect of large amounts of adrenalin on the blood pressure of the animals and a visual method (Griffith and Farris) was used on ether anaesthetised normal rats. The apparatus is shown in Fig. 19. The manometer (Fig. 21) is designed so that air passes along the rubber tube (R) when the screw (C) is turned, and the resulting pressure registers on the mercury scale. A rubber cuff (A) made from a surgical rubber glove is connected to tube (R) and the animal's leg is slipped through the cuff. The latter fits neatly into a metal ring (B), so that air passing into the cuff causes constriction of the animal's leg, thus

interfering with the flow of blood in the vessels. The animal's foot, with rubber cuff in position, is fixed on a slide which is placed on the stage of the microscope (Fig. 20). The web of the foot is examined by the low power of the microscope and a fairly large blood vessel found. The blood corpuscles are easily seen flowing along the tortuous route of the vessel, but as the pressure within the cuff rises, the flow of blood cells diminishes and eventually ceases. The reading on the manometer at this stage is the systolic blood pressure; unfortunately no diastolic reading can be obtained using this technique.

The average normal blood pressure of ether anaesthetised rats varies between 80 and 100 mm.Hg. When 480 µg (8 minims) 1:1000 adrenalin hydrochloride were given subcutaneously, the blood pressure rose in the first ten minutes to 140 mm.Hg. Ten to fifteen minutes later, the vessels became so constricted that no cell movements could be seen, and no blood pressure readings were available. One half hour after the injection the blood pressure had fallen to 60 mm.Hg. and ten minutes later to 50 mm.Hg. It had returned to normal one hour after the injection.

METHODS OF INVESTIGATION.

The animals used were 56 adult white rats divided into 4 groups each consisting of 7 male and 7 female. A separate group of 3 male and 3 female animals served as controls and received subcutaneous injections of normal saline only. All animals were fed on bran, oats and nut cake; supplementary cabbage was given thrice weekly.

Subcutaneous injections of adrenalin hydrochloride 1:1000 were

given into the abdomen of the test animals at intervals of two or three days, depending on the condition of the animal. The site of injection was changed on each occasion, and the initial dose injected was 30 µg (0.5 minims) adrenalin base (see Appendix I). As the animal became adapted or accustomed to this, the amount was increased gradually to 60 µg (1 minim 1:1000 adrenalin hydrochloride), then 120 µg (2 minims), 180 µg (3 minims), etc. until 300 µg (5 minims) were being administered. As a general working rule it was found advisable to maintain the animal for 15 to 20 days on any particular dose before increasing it. The rats were not greatly upset until 180 µg of adrenalin were being injected, when they became dyspnoeic, the respirations were rapid and tachycardia was present. There was erection of the hair and coldness of the extremities and tail. The animals occasionally lay on their abdomen and sometimes moved restlessly about the cage. The attack started 10 to 15 minutes after an injection and they had recovered in 1½ to 2 hours. This reaction was described as slight in character.

When the dose of adrenalin was increased further to 300 µg the reaction began as above, but later the animals moved restlessly about the cage, eventually lying down in an exhausted manner, while tachycardia and dyspnoea were more marked than previously. In very severe cases, especially those which proved fatal, a copious blood-stained frothy discharge appeared at the nose. During the stage of exhaustion the animals could be handled without any resentment on their part. The attack usually commenced 10 minutes after the injection,

dyspnoea and tachycardia were marked for 45 to 60 minutes, and prostration lasted from 2 to 4 hours. The animals had recovered in 5 hours. This type of reaction was described as severe.

It was to be expected that some of the animals would die during the development of tolerance and, in fact, it was found that most of them died when doses varying between 180 µg (3 minims) and 300 µg (5 minims) were being given and, from a total of 14 animals in each group, 8 remained for the experiment in Groups 1, 2 and 3, and 5 in Group 4.

Group 1 animals were injected with increasing amounts of 1:1000 adrenalin hydrochloride as described above and the dose maintained as long as the reactions were severe. When they became slight, the dose was increased until a maximum was reached when the reactions were always severe. This depended on the individual animals and was found to vary from 8 to 15 minims (Table 7).

Group 2 was treated initially as described until 300 µg (5 minims) were being administered, when the dose was kept constant and the reactions noted. They were mostly slight in character, especially in the later stages of the experiment (Table 8).

Groups 3 and 4 received the same treatment as Group 2, namely, increasing doses of adrenalin up to 300 µg (5 minims). This amount was maintained for a period almost similar in duration to that of the experiment in Groups 1 and 2 (147 days). The animals were then changed to a slowly absorbing form of the drug, 1:100 adrenalin ascorbate in oil, which was injected intramuscularly into the gluteal muscles, alternate sides being used with each injection. Initially

both groups received 300 µg (0.03 c.c.) of the oil, which was maintained throughout in Group 4 but was gradually increased by 100 µg (0.01 c.c.) in Group 3 so that severe reactions, similar to those seen in Group 1, were obtained. The maximum dose of adrenalin administered in this way varied from 600 µg (0.06 c.c.) to 1500 µg (0.15 c.c.) in Group 3 (Table 9), while all animals in Group 4 received 300 µg. although the last one was given 1000 µg two hours before it was killed (Table 10). The experiments lasted 160 and 155 days in Groups 1 and 2 respectively and 288 days in Groups 3 and 4.

Throughout the investigation the animals were dissected and examined as soon as possible after death, but there was a delay of a few hours if death occurred at night. After weighing the animals, the liver, spleen, pancreas, kidneys, lungs and suprarenals were removed and placed in the refrigerator for 15 minutes before being fixed in 10% neutral formalin. The microscopic appearances after this technique were superior to those obtained by immediate fixation of the organs, which produced a blurred histological picture.

The auricles were separated from the heart and the ventricles opened in the usual manner, blood was removed and the heart weighed accurately to two decimals, and placed with the other organs in 10% neutral formalin. The suprarenals from Groups 1 and 2 were fixed in equal quantities of 5% potassium dichromate and 10% neutral formalin as described for chromaffin tissue (page 7), but a few adrenals in Groups 3 and 4 were fixed in 10% neutral formalin and examined for cortical lipoids.

Paraffin sections were made of spleen, liver, kidneys, pancreas and heart. They were stained by a variety of methods including haematoxylin and eosin, Gallego's modification of Mallory's stain and Weigert-van Gieson for elastic tissue. The chrome-fixed suprarenal glands were stained by the modified Giemsa method; suprarenals fixed in 10% neutral formalin received subsequent treatment with 1% osmic acid (further details of this method are given in part 3, page 54). A modified Mallory's stain was specially adapted for pancreatic acinar tissue by reducing the orange G content of the stain by 50%. Details of the stain are as follows:-

Mallory (1) as in the usual stain.

Acid fuchsin 0.5 g.

Water to 100 c.c.

Mallory (2) 1% phosphomolybdic acid.

Mallory (3) Aniline blue 0.5 g.

Orange G 1 g.

Water 100 c.c.

The sections were stained in Mallory (1) until they were deep red in colour, then differentiated in Mallory (2) until the acinar tissue became discrete and then slightly overstained in Mallory (3). Subsequent differentiation was carried out using methylated spirit until the granular acinar tissue stained bright red and the non-granular tissue blue.

R E S U L T S.

THE AMOUNT OF ADRENALIN BASE ADMINISTERED AND THE TYPE OF REACTIONS PRODUCED.

The amount of adrenalin base administered to Group 1 animals (Table 7) varied from 12.1 mg. (No. 1) in 77 days to 28.6 mg. in 160 days (No. 8). One animal (No. 6) was able to take very large doses of adrenalin and 32.6 mg. were administered to it in 140 days. The reactions produced were mainly severe and varied from 12 (No. 1) to 40 (No. 8) in number.

The total amount of adrenalin injected in Group 2 was smaller and varied from 3.1 mg. in 66 days to 15 mg. in 155 days. The reactions were generally slight and varied from 3 to 30 in number (Table 8). There was a preponderance of severe reactions in two cases only (Nos. 4 and 5).

Large doses of adrenalin base were administered in Group 3, partly as a solution of 1:1000 adrenalin hydrochloride and partly as an oily suspension of 1:100 adrenalin ascorbate. The dose of drug varied from 22.6 mg. in 179 days (No. 1) to 73.7 mg. in 288 days (No. 8). (Table 9). The reactions produced in the first four animals were generally slight, the ratio of severe to slight reactions was about equal in the next two (Nos. 5 and 6) but severe reactions predominated in the final two animals.

Although the total amount of adrenalin given in Group 4 (Table 10) was greater than in Group 2, maximum injections were similar (5 minims and 0.03 c.c. or 300 µg), but they extended over a longer period. The first animal received 22.7 mg. in 187 days

and the last 42.9 mg. in 288 days. Apart from one instance (No. 3), the reactions were mainly slight in every case.

ALTERATIONS IN BODY WEIGHT.

The animals were weighed at the beginning, during and end of the experiment. None of the control animals in Group 0 (Table 11) lost weight and all but one (No. 2) showed a gain. Half the animals in Group 1 (Table 7) (Nos. 1, 2, 3 and 6) lost weight and the remainder showed a very slight increase. Apart from one animal in Group 2 (No. 4) all animals in Groups 2, 3 and 4 showed an increase in weight.

CARDIAC INDEX.

The cardiac index is the ratio of the weight of the animal's heart to its body weight both expressed in grams. Thus -

$$\text{Cardiac Index} = \frac{\text{Heart weight in g.}}{\text{Body weight in g.}}$$

The heart weight was standardised in all cases by removing the auricles and blood as described previously (page 31), and separate indices were calculated using terminal and maximum body weights. If the animal did not lose weight during the experiment the indices were the same; on the other hand if it lost weight the indices were different. The maximum index in such circumstances was accepted and gave a significant though more conservative result.

The cardiac index in the control group of animals varied between 0.0037 and 0.0027 and was quite independent of the sex of the animal (Table 11). The index in Group 1 (Table 7) diminished

proportionately with the increase in severe reactions. Apart from one animal (No. 2), the indices were above normal in the first four, were on the upper limit of normality in the next two (Nos. 3 and 6), and low in the remainder. It should be noted that the second rat showed a considerable loss of weight and the terminal cardiac index was very high. A similar decline in cardiac index occurred in Group 3 (Table 9), but it was high in the first three animals. This fall in cardiac index was associated once more with an increase in severity of the reactions. When the latter were slight, as in Groups 2 and 4, the cardiac index showed a progressive rise far in excess of normal. The initial indices in Group 2 were normal (0.0034 in rats 1 and 2), but when a considerable degree of tolerance had been slowly developed as in Groups 3 and 4, the initial indices were high (Tables 9 and 10) (See Figs. 41 to 44).

POST-MORTEM APPEARANCES.

The external appearance of the animals varied according to the severity of the reactions. When slight, nothing abnormal was noticed, but when severe a blood-stained frothy discharge exuded from the nose. The haemorrhagic discharge was similar to that noted in some cases of phaeochromocytoma (Brunschwig and Humphreys, 1940). Notable changes were seen in the lungs. When animals died 5 to 45 minutes after a severe reaction, the lungs were extremely congested and haemorrhagic; patchy haemorrhages were present on the pleural surfaces and 1 or 2 c.c. of clear fluid were present in both pleural cavities. If the animals survived 1½ to 3 hours, the lungs were

either colourless, or patchy haemorrhagic areas were present. They were haemorrhagic in those dying 4 or 5 hours after the injection; the parts now affected were the bases on both sides. No significant changes were seen in the other organs apart from the kidneys. Sometimes they were brown in colour and at other times yellowish-white.

MICROSCOPIC CHANGES IN THE ORGANS.

Kidneys: Most of the kidneys of all groups appeared normal but a few showed marked patency of the glomerular capillaries (Fig. 22) and an exudate in the sub-capsular space (Fig. 23). In some cases the exudate was so marked as to cause partial disappearance of the glomerular tufts. Sometimes the tubules were slightly dilated, the lining epithelium flattened and pink-staining casts present in the lumen (Fig. 24). Those changes were seen in all groups and occurred after severe and slight reactions. They were found in animals dying at all times after injection but were more frequent between 3 and 5 hours. The urine contained albumen in all cases where casts were present in the tubules. There was no evidence of vascular changes in any of the groups, the arcuate and interlobular arteries showed no splitting of the internal elastic lamina (Fig. 25) and hyalinisation of the afferent arterioles was never seen.

Heart: When the cardiac index was greater than normal the muscle fibres appeared hypertrophied. Cardiac lesions were seen in some cases in Groups 1 and 3, where there was a fall in cardiac index. Many muscle fibres were hypertrophied, but at places some fibrocellular areas were present between, and were associated with atrophy

of, the muscle cells (Figs. 26a, b and c). The cellular infiltration consisted of fibroblasts and mononuclear cells but in one or two cases the reaction was more fibrous in character (Fig. 27). The lesions were seen mainly in the endocardial and pericardial aspects of the heart. The coronary vessels appeared patent and serial section failed to show any evidence of vascular lesions.

Suprarenals: The cortical lipoids were examined in a few cases in Groups 3 and 4. One of the animals (Table 9, No. 1) died 5 hours after a severe reaction and when the suprarenals, fixed in osmic acid, were examined it was noticed that the sinusoids of the medulla were widely dilated and contained a dense black osmophil-positive secretion in addition to red blood cells (Fig. 28). Examination showed that the secretion was cortical, not medullary in origin, since the material could be seen coming from the suprarenal cortex Fig. 29). Further proof of its cortical origin was obtained when sections of the gland, fixed and stained especially for chromaffin tissue, gave a negative reaction. This method also showed that the suprarenal cortex had four distinct zones. Abundant osmophil-positive granules were present in the cells of Zone 1 on the outer aspect of the gland but a few cells showed vacuolated spaces in their cytoplasm (Fig. 28). Zone 2 was densely black, rather irregular in outline and extended inwards to involve more than half the diameter of the cortex. The third zone was lighter in colour and again rather irregular in outline. It was separated from the medulla by a rather small, but densely osmophil-positive zone (Zone 4). The

cells in this region were tightly packed together in groups which were separated by dilated blood channels. Thin cord-like capillaries ran from the vascular plexus on the surface of the glands, between the cells of Zones 1, 2 and 3, and opened out into the vascular channels of Zone 4, which drained into the sinusoids of the medulla and thence to the adreno-lumbar vein. The cortex was seen to thin out considerably in the region of this vein.

In ordinary haematoxylin and eosin preparations of the suprarenal cortex, for the control animals, the three customary zones (glomerulosa, fasciculata and reticularis) were easily recognised. When osmic acid fixation was used four definite zones could be distinguished. Zone 1 was clear and very few osmophil-positive granules were seen in the cells, but occasionally it showed great irregularity in outline, being broad and prominent in some parts and very thin in others (Fig. 30). It was sharply defined from Zone 2 where most of the cells were densely osmophil-positive, although a few clear vacuoles were seen in some of them. The second zone extended irregularly inwards and Zones 1 and 2 occupied the outer third of the gland. The third zone was paler than the previous one and only slightly osmophil-positive, but the last one was again densely osmophil-positive and small in size. There was no evidence of osmophil-positive material in the medulla.

In view of the difference in appearance between the normal glands and the one described previously, it was felt that changes, worthy of investigation, occurred in the suprarenal cortex following

injections of adrenalin. Those changes, together with the relevant data, are shown in Table 12. One male and 3 female animals were studied. The first animal (Group 3, No. 2) died in a severe convulsion 20 minutes after the injection of 0.6 mg. of adrenalin base. It had received 6 previous injections of this amount and on each occasion the reaction was severe. Irregular osmophilisation occurred in the outer zone where many of the cells had intense osmophil granules in their cytoplasm but others had none or only a few. Clear vacuoles were seen in most cells in the second zone and only a few were osmophil-positive. The third zone was not well defined, it was slightly osmophil-positive and three small dilated spaces were seen near the inner border. Each space contained osmophil-positive material, which was not so prominent as in the previous section.

The last zone was once more densely osmophil-positive and formed only a narrow rim adjacent to medulla. An interesting feature of this gland was the prominent dilated capillaries seen as small clefts between the cortical cells in Zones 2 and 3. They became very prominent in the inner half of Zone 3 and Zone 4 where they were widely dilated and congested. Brown osmophil-positive secretion was present in the sinusoids of the medulla. **(Fig. 31).**

The next animal (Group 4, No. 5) received 1 mg. of adrenalin base and died 2 hours after a very severe reaction. It had been accustomed to 0.3 mg. adrenalin which caused slight reactions. A prominent capsule was seen on the surface of the gland, and Zones 1 and 2 appeared fused into a single slightly osmophil-positive

zone, in which most of the cells were vacuolated. Zone 3 was pale and had no osmophil granules; it was sharply defined from Zone 2 above and Zone 4 below. The latter was very prominent, increased in size and/strongly osmophil more in character. Brownish-black secretions were again present in the medulla (Fig. 32). The third animal (Group 3, No. 7) was accustomed to a 1 mg. dose of adrenalin and the reactions caused were slight. 0.8 mg. were injected, a slight reaction produced, and the animal killed 3½ hours later. The outer zone was osmophil-positive, and although most cells of the second stained similarly, many of them had clear vacuoles in the cytoplasm. Zone 3 was sharply defined and only showed occasional lipoid granules in the cells. There was a slight increase in size of the last zone which had many osmophil granules in the cytoplasm of the cells (Fig. 33). No chromaffin granules were seen in the cells of the medulla in any of the cases.

Pancreas: In all animals the pancreas was examined microscopically and attention paid to such factors as time of death following the injection, previous injections and reactions, and the nature of the last reaction. When sections were stained with haematoxylin and eosin no obvious abnormalities were seen, but when Gallego or a modified Mallory's method was used interesting changes were noticed in the acinar tissue in the region of the islets. The granules in the pancreatic acini appeared golden-yellow with Gallego and red or fuchsinophil-positive with the modified Mallory's method. The first animal (Table 13, Group 2, No. 1) went into a

convulsion 5 minutes after an injection of 0.3 mg. (5 minims) 1:1000 adrenalin hydrochloride; the previous injection of 0.24 mg. (4 minims) had produced no reaction. The acinar granules were scanty in amount and always more prominent around the islet tissue (Fig. 34). The next animal (Group 4, No. 1) died 30 minutes after a severe reaction with 0.3 mg. (0.03 c.c.) adrenalin-in-oil, and the pancreatic acini were all fuchsinophil-positive but those around the islets were again slightly more prominent (Fig. 35).

The third animal (Group 1, No. 3) died 45 minutes after a very severe reaction with 0.48 mg. (8 minims) of 1:1000 adrenalin solution. It had received 16 previous injections of this amount and all reactions were severe. The acini around the islet tissue were enlarged, intensely granular in appearance, and the nuclei pushed to the periphery of the cell. The interislet tissue again was slightly less fuchsinophil-positive (Fig. 36).

Some interesting features were noticed in the fourth animal (Group 4, No. 5). It was adapted to 0.3 mg. (0.03 c.c.) adrenalin-in-oil, received 60 previous injections of this amount and slight reactions were produced. 1 mg. (0.1 c.c.) of adrenalin was administered and caused a very severe reaction from which the animal died 2 hours later. The acinar tissue again was more fuchsinophil-positive around the islets, but the reaction was less marked then in the two previous cases (Figs. 37 and 38).

Control animals were killed by coal gas and when the pancreas was examined, the acinar tissue around the islets was prominently

fuchsinophil-positive and formed a halo around them. The reaction was similar to that seen in the first animal but there was not the same depletion of granules in the interislet acinar tissue. The changes occurred both in male and female animals (Figs. 39 and 40).

DISCUSSION.

The paroxysmal type of hypertension in the human subject has been shown to be due to the liberation of adrenalin directly into the blood (Part 1, Group 1A, page 17), and this condition was easily reproduced experimentally by the administration of adrenalin to the animals at two or three day intervals. It was difficult to reproduce the paroxysmal type of hypertension with raised interval blood pressure (Part 1, Group 1B, page 18), since its cause was not known. However, it was known that the paroxysmal reactions of this group did occur many times each day (Table 5), and so attempts were made to simulate these reactions by giving a slowly absorbing form of the drug (adrenalin ascorbate in oil). Unfortunately the blood pressure technique was not suitable for prolonged measurements, thus it was not possible to determine the action on blood pressure of this slowly absorbing form of adrenalin.

The reactions caused by the injections were described as severe or slight in character. When severe, the animal appeared to be in a state of clinical shock, comparable to that seen in some cases of chromaffin tumour (Engel, Mencher and Engel; Oberlung and Jung, 1927). In some patients (Brunschwig and Humphreys) and in most animals a frothy bloody exudate poured from the nose, while dyspnoea,

restlessness, pallor of the ears, coldness of extremities and marked prostration were common to both. This apparently shocked condition of the animal was associated with an initial rise followed by a fall in blood pressure, and was in agreement with the results obtained by Freeman, Freedman and Millar (1940) and Trueta et al (1947).

Many animals died early in the experiment, a point which has been noted previously (Stanton and Pearce, 1906). It was interesting to note that those animals which survived the initial injections developed a tolerance to the same amount but not to continually increasing doses of the drug.

The microscopic appearance of the organs was interesting and in none were the changes so significant as in the suprarenals.

Suprarenals: Normal glands treated with osmic acid had a histological picture quite different from that seen when they were stained by haematoxylin and eosin. In place of the three customary zones (glomerulosa, fasciculata and reticularis), there were four distinct ones, Zone 1, 2, 3 and 4, which have been referred to by the terms pre-secretory, secretory, post-secretory and senescent zones (Bennett, 1940).

The appearance of the suprarenals following adrenalin therapy provided microscopic evidence of distinct phases in the histological response of the gland to this substance. The outer zone was sometimes clear, at other times osmophilised, and similar variations were seen in Zone 2, which was densely osmophil-positive in the control animals. In one case (Table 12, Group 4, No. 5) there was

complete depletion of osmophil material from this zone following a large dose of adrenalin to which the animal was not accustomed. Occasionally the first and second zones were fused. Few changes were observed in the third zone, but the fourth was usually increased in size and intensely osmophil-positive in character. Vogt (1945) noted variations in the zonal arrangement of the suprarenal cortex of young rats when injected with adrenalin. When these glands were treated with Sudan III there was an "extension of the sudanophil substance through the whole zona fasciculata and reticularis, spreading centrally as far as the border of the medulla and peripherally frequently invading the clear zone" (Zone 1). The changes seen in the present investigation cannot be fully assessed at this stage owing to variables, sex, dosage, tolerance to adrenalin and time of death following the injection, but they form the basis of part of the experimental work described in Part 3.

The nature of the lipoids in the medullary sinusoids and cortical cells of Zones 2 and 4 and occasionally Zone 1, has been the subject of much discussion. Cramer (1926) believed that the accumulation of osmophil-positive material in the cortical cells, adjacent to the medulla, represented a means of "self-control" of the gland against the so-called avalanche phenomena described previously by himself. The present investigation proves conclusively that the cortical secretion is not related in any way to adrenalin and is not a means of self-control of the gland against exhaustion. Bennett states that, "biologically active sterones are in all probability

present in the osmophil lipoid vacuoles of the cells in the secretory zone (Zone 2)". He believes that the cells of the cortex form under the capsule of the gland and move centrally to Zone 2 where the biological sterones are formed. The method of discharge of these sterones into the cortical capillaries is unknown but after discharging their contents the cells move centrally into Zone 3 where they are osmophil-negative. Later the cells degenerate in Zone 4 and in doing so they undergo fatty change and the zone becomes osmophil-positive again. This idea of central movement of the suprarenal cells is supported by many workers (Hoerr, 1931; Bachmann, 1937; Zwemer, Wotton and Norkus, 1938), but opposed by others (Calma and Foster, 1943). If the osmophil-positive lipoids are steroid substances then the results of the present investigation would suggest that they are capable of forming in either Zones 1 or 2. This point is also considered further in Part 3,(page 73).

Many chemical and histological methods have been used to determine the nature and source of formation of the biologically active steroids. Osmic acid, sudan black B, semi-carbazide, 2:4 di-nitro phenyl hydrazine and silver impregnation methods have all been used alone and in combination with the polarising and fluorescent microscope (Bennett, Deane and McKibbin, 1946). The results have done little to elucidate the nature of these steroids or their site of formation and it would appear that a vastly different technique is required before any advance will be made, and an attempt is made to do this in Part 3.

It is to be noted that the response of the suprarenal cortex to injections of adrenalin is not peculiar to this substance, for it has been seen and is the same after exposure to cold (Cramer, 1936; Flexner and Grollman, 1939), injections of saline suspensions of agar culture media, and Gaertner's bacillus (Graham, 1916), and thyroid feeding (Deansley, 1931). The response to different stimuli have been noted by Selye (1946) who believes them to be part of the general adaptation syndrome, which he described as "the sum of all non-specific systemic reactions of the body which ensue upon long continued exposure to stress." Exposure to adrenalin, cold, injections of agar suspensions, or cultures of Gaertner's bacillus could all be described as non-specific stimuli and the reaction of the body is the same to them all. An animal or individual subjected to such stimuli develops the characteristics of the adaptation syndrome in which three distinct phases are recognised: (1) alarm reaction, (2) stage of resistance, and (3) stage of exhaustion. Since only the first two phases apply here, they alone will be described in more detail.

(1) The alarm reaction is the "sum of all non-specific systemic phenomena elicited by sudden exposure to stimuli to which the organism is quantitatively or qualitatively not adapted." It consists of two phases (a) shock, which occurs immediately and is soon followed by (b) counter-shock which is characterised by activity of the suprarenal cortex. Such phases assume considerable importance in the present investigation since the animals appeared to suffer from clinical shock during a severe reaction. This was

associated with zonal changes in the suprarenal cortex and elimination of a dense osmophil-positive secretion into the blood vessels. In the light of Selye's work that secretion would respresent the counter-shock phase of the alarm reaction. As Vogt found that blood collected from the adrenal vein after injections of adrenalin was capable of "prolonging the mean survival time of adrenalectomised rats", it was possible that the osmophil material found in the present investigation contained one of the "corticoid" substances which, Selye believes, plays an important part in the counter-shock mechanism. The necessity of determining the nature of the osmophil secretion now became of utmost importance.

(2) The stage of resistance "represents the sum of all non-specific systemic reactions elicited by prolonged exposure to stimuli to which the organism has acquired adaptation as a result of continued exposure." It is during this phase that diseases, such as hypertension, rheumatic fever and rheumatoid arthritis are believed, by Selye (1946), to develop and they are referred to by him as diseases of adaptation. He believes that such conditions result from a chronic suprarenal cortical endocrine overdosage. If the osmophil material liberated from the cortex after adrenalin stimulation is salt-active corticoid, a continuous endogenous liberation of this substance might be expected in chromaffin tumours following the action of liberated adrenalin. As Selye showed that animals treated with salt-active corticoid (desoxycorticosterone acetate) did develop chronic hypertension it is possible that endogenous corticoids,

liberated in response to adrenalin, as well as renal ischaemia, might play a part in the aetiology of chronic hypertension associated with chromaffin tumours.

Kidneys: The angiograph and anatomical studies of Trueta, Barclay, Daniel, Franklin and Prickard (1947) demonstrated, conclusively, the presence of renal ischaemia in animals following intravenous injections of adrenalin. "After the drug was given in doses of 0.1 to 0.17 mg. per kg. body weights, the surface of the kidney was often seen to blanch very rapidly and to a remarkable degree, losing the red tinge of its normal red or perhaps reddish-brown colour and passing through stages of brown to a yellowish-white colour in cases where the effect was maximal." Similar changes were seen in the animal kidneys examined during the present investigation but their significance was not appreciated at the time, although in the light of Trueta's work, there can be little doubt that they are due to renal ischaemia. It is interesting to note that Trueta's injections of adrenalin initially caused complete ischaemia of the whole kidney. This was followed later by a cortical ischaemia when the renal circulation was reestablished by means of the medullary by-pass.

In the present investigation, although larger doses of adrenalin were used, there was no evidence of vascular lesions in the kidney in any of the groups. Most of the kidneys appeared normal but a few showed patency of the glomerular capillaries and an exudate into the sub-capsular space (Figs. 22 and 23). The

lesions were present in all groups and occurred after severe and slight reactions. They were seen at all times after injection but occurred more frequently between 3 and 5 hours.

The absence of vascular lesions was disappointing, but it was felt that, in view of the renal ischaemia and liberation of osmophil material from the suprarenals which result from adrenalin injections, the administration of adrenalin in smaller quantities at more frequent intervals, should be carried out. This was done and is considered in Part 5 (page 97).

Heart: The experiments were continued over a long period (283 days) and it was natural that individual variations in body and heart weight should be found. The cardiac index is a better indication of cardiac hypertrophy than actual heart weight, and when the maximum index is used a truer, if more conservative, result is obtained. In normal untreated animals the range was found to vary between 0.0027 and 0.0037. It was highest in adrenalin treated animals when the reactions were mainly slight in character (Figs. 41 and 42); but when severe, the cardiac index showed a progressive fall (Figs. 43 and 44). In some, but not all, small areas of cellular infiltration were seen between the muscle fibres, but no lesions were seen in any of the coronary vessels. It must be realised that the lesions were not marked and were mainly endocardial and pericardial in type. Similar but more severe cardiac lesions were produced experimentally by desoxycorticosterone acetate implants in animals (Part 4, page 79), and in view of this it was thought that the cardiac lesions in a few animals treated with adrenalin might be

due to the liberation of salt-active corticoid. Is it possible that salt-active corticoid was identical with the osmophil material already noticed? Cardiac hypertrophy and myocardial fibrosis in animals treated with adrenalin have been found by others, but their ultimate cause is far from clear (Gross and Greenberg, 1944).

Pancreas: There seems little doubt that the lesions observed in the pancreas are other manifestations of the alarm reaction. They were observed in animals after adrenalin injection and in those killed by coal gas. The fuchsinophil reaction of the acinar tissue around the islets was more obvious in the early stages following adrenalin injections and was always more marked in the peri-islet acinar tissue. When the reaction was severe or the animal died in a convulsion shortly after an injection, the inter-islet acinar tissue was usually greatly depleted of granules. Later when the animal was recovering (1 to 4 hours) the fuchsinophil reaction was again evident in the inter-islet acinar tissue. Those changes were found to occur in adrenalectomised animals and so it must be assumed that they are independent of the adrenals. Similar changes have been noted during the alarm reaction (Selye, 1946), but no explanation is given of this significance. The fact that the reaction is extremely prominent around the islet tissue suggests that it has some protective mechanism for the islet tissue. Since the duct drainage of the pancreas is of the "herring-bone" arrangement it is possible that the acinar tissue around the islets is furthest from the main duct and so is last to give off its secretion. Since

the acini actually enlarge during the reaction the latter explanation is extremely unlikely.

Spleen: In view of the marked hyalinisation of septa and vessels of the spleen in patients suffering from essential hypertension (Moritz and Oldt, 1937), it was felt that some interesting information might be found from the spleen in the experimental animals. However, the changes in control and experimental animals did not differ to any great extent, as there was thickening of the capsule and hyalinisation of septa in both cases. No vascular lesions were seen.

PART 3.

THE NATURE OF THE OSMOPHIL MATERIAL LIBERATED FROM THE SUPRARENAL CORTEX FOLLOWING ADMINISTRATION OF ADRENALIN.

PART 3.

THE NATURE OF THE OSMOPHIL MATERIAL LIBERATED FROM THE SUPRARENAL CORTEX FOLLOWING ADMINISTRATION OF ADRENALIN.

The histological and chemical methods described in Part 2 were of little value in identifying the secretions from the suprarenal cortex following adrenalin injections. However, a combined histological-bio-assay technique was devised and proved to be of valuable assistance. Four groups of adult male rats were used to obviate the variation of sex and age, and severe and slight reactions produced by injections of 1:1000 adrenalin hydrochloride. The changes in histological appearance of the suprarenals were correlated with the appearance of liver glycogen and blood sugar at different stages of the reaction. This eliminated any discrepancy in dosage and type of reaction and provided a satisfactory method of identifying the osmophil-positive secretions liberated from the suprarenal cortex following adrenalin injections.

METHODS OF INVESTIGATION.

Group 1 consisted of 14 rats which varied in weight from 255 to 270 g. They received initial injections of 1:1000 adrenalin hydrochloride, as described previously, and the dose was gradually increased over a period of 113 days until the animal had become adapted to 300 µg (5 minims) of solution. 10.2 mg. were administered during this period and 9 animals died when tolerance to the drug was being developed. The remainder were injected with 1 mg. (16

minims) adrenalin and a severe paroxysmal reaction produced. One uninjected rat served as a control and the rest were killed at intervals of ½, 1½, 3 and 5 hours (Table 14). In a preliminary investigation of a similar nature the animals received no food from 5 o'clock on the evening prior to the experiment and, when the liver was examined the following morning, a marked depletion of glycogen was found. Accordingly, all animals in this series received an abundant diet of bread and cabbage up to the morning of the test, when all food was withdrawn and the animals supplied with water only during the experiment. Satisfactory storage of liver glycogen was thus ensured at the beginning of the experiment, and subsequent changes in liver glycogen could only arise from endogenous sources.

Group 2 consisted of 14 animals which were treated for 90 days in the usual manner, until they were adapted to 240 µg (4 minims) 1:1000 adrenalin hydrochloride. 6.4 mg. of adrenalin base were administered during that period and 8 animals survived. One animal served as a control and the remainder received 240 µg (4 minims) subcutaneously on the day of the test when they were killed off at hourly intervals for a period of 7 hours. A slight reaction only was produced (Table 15).

Group 3. Eight animals were present in this series and they had received no previous injections (Table 16). During the test 240 µg (4 minims) 1:1000 adrenalin hydrochloride were injected and the animals killed at intervals of ½, 1, 1½, 2, 3, 4 and 5 hours. One animal was kept as a control; it received no injections. The

reactions produced were slight in character, even though no previous injections had been given.

Group 4 (Table 17). Bilateral adrenalectomy was successful in 9 animals in this group, both adrenals were removed by a dorsal approach under ether anaesthesia and 10 c.c. of normal saline injected intraperitoneally, immediately following the operation. The animals were placed on a diet containing abundant cabbage for 5 days and 5 c.c. normal saline administered intraperitoneally every second day. In this way it was hoped that the store of liver glycogen would be built up, while 5 days would not be long enough to allow ectopic adrenal cortical tissue to develop. On the day of the test 240 µg (4 minims) 1:1000 adrenalin hydrochloride were injected and the rats killed subsequently at intervals of ½, 1, 1½, 2, 3, 4 and 5 hours. One animal was not injected and acted as control. The reactions produced were slight in character in all cases.

All animals were stunned by a blow on the back of the head and their throat cut. Blood was collected in heparin tubes for glucose estimations, post-mortem examination carried out, and the suprarenals and liver removed for histology.

Suprarenals: The suprarenals were fixed in a solution of 10% neutral formalin for 24 hours, the periadrenal fat removed by scissors and the glands cut in half with a very sharp razor blade. They were thoroughly washed in water and placed for 6 days in a small stoppered bottle containing 1% osmic acid. The fresh osmic acid solution had a pH of 5, and after 6 days it fell to 4 when the

solution was yellowish in colour and a black deposit formed at the bottom of the bottle. When this occurred staining of the lipoid granules was unsatisfactory and a yellowish colour appeared throughout the section. If the osmic acid was renewed on the third day, the above changes were prevented and staining was satisfactory. After 6 days the sections were thoroughly washed in water for 24 hours, dehydrated and embedded as follows:-

1. 25, 50, 75, 100% methylated spirits - ½ hour each.
2. Absolute alcohol - ½ hour.
3. Alcohol/chloroform - until section sinks. This varied from 5 to 15 minutes.
4. Chloroform - 2 changes of ½ hour.
5. Paraffin - 3 changes.

Paraffin embedding was found to be an important step. Sections could be left overnight in first paraffin and changed into second for ½ to 1 hour in the morning. If the sections were left in the paraffin for 1 to 2 hours only, impregnation was ineffective and the cortex separated mainly between Zones 1 and 2. This gave a very poor histological picture.

All suprarenals treated in this way were cut serially, at 3 μ, 5 μ and 8 μ alternately. It was soon realised that 5 μ sections gave the best zoning arrangement and subsequent glands were cut at 5 μ. The sections selected for examination and photography were those showing the greatest diameter of the gland.

Liver Glycogen: Originally, it was intended to estimate the glycogen content of liver by a chemical method (Good and Somogi, 1933), but preliminary experiments showed that the glycogen content of the liver of control rats varied from 10.4 to 30.5 mg./g. of liver tissue and it was felt that the method was not satisfactory. A histological technique was devised and the glycogen in the liver lobule of a control animal taken as the standard; the disappearance and subsequent regeneration of glycogen from the lobule could thus be studied under various experimental conditions. Three histological methods (Best; Mullen, 1944; Mitchell and Wislocki, 1944) were used and all gave satisfactory results once the individual technical difficulties were overcome. The method described by Wislocki was the one chosen, mainly because of its good photographic possibilities. Like most glycogen methods it has its own peculiarities and slight modifications had to be adopted. Details of the method are as follows:-

(1) Fixation for 12-18 hours in a mixture of

Saturated alcoholic picric acid ...	90 c.c.
Neutral 10% formalin	10 c.c.

(2) Absolute alcohol several changes - 4 hours in all.

(3) Alcohol/chloroform several changes - 4 hours in all.

(4) Chloroform - 2 changes of 6 hours.

(5) Paraffin embedding as usual.

When removed from the animal, the liver was cut in thin slices and placed in the refrigerator for 15 minutes prior to fixation. After 4 hours fixation the slices were trimmed and refixed for another 8 to

14 hours, then treated as described above.

Staining of Liver for Glycogen: Paraffin sections were cut at 8 μ, floated in a dish of tepid water and mounted on a slide. When required for staining they were taken to water in the usual way and treated with a solution of 47.5 c.c. 0.5% $KMnO_4$ and 2.5 c.c. 3% H_2SO_4. They were then decolorised with 0.5% oxalic acid, washed thoroughly in running water and placed overnight in a solution of 2% silver nitrate. The following morning they were treated with ammoniacal silver nitrate solution for 15 to 30 minutes and subsequently with 4% formalin for 2 minutes, when a black deposit formed. After washing in water the sections were toned with 0.2% gold chloride solution for 5 to 10 minutes then rinsed in a solution of 3% sodium thiosulphate. They were dehydrated and mounted in the usual manner. The glycogen stains black and appears as a granular deposit in the liver cells, the nuclei of which show up as white circles demarcated by the black granules. It is interesting to note that this technique produces very satisfactory staining of reticulin fibres. When the procedure was repeated after alcohol fixation negative results were obtained for glycogen, thus picric acid and neutral formalin seem to be indispensable for proper staining.

The ammoniacal silver nitrate was prepared as follows:- 15 drops of 30% caustic soda solution were added to 10 c.c. of a 10% solution of silver nitrate when a brownish-black precipitate formed. This was carefully dissolved by adding 26% ammonium hydroxide drop by drop and the solution made up to 100 c.c. with absolute alcohol, which prevented the section being detached from

the slide (Mitchell and Wislocki). When alcohol was added to the ammoniacal silver a brownish-black deposit formed, but this settled to the bottom of the container on standing overnight. The clear supernatant fluid was filtered off and used as described.

Blood Sugar: Blood sugar estimations were carried out by the method of Hagedorn and Jensen.

RESULTS.

During the process of adaptation 5 of the 14 animals survived in Group 1 and 8 in Group 2.

REACTION OF THE ANIMALS TO TEST INJECTIONS OF ADRENALIN.

Group 1. Half-an-hour after the injection of adrenalin, the reaction appeared slight. There was tachycardia, slight dyspnoea, erection of the hair, and coldness of the tail and ears. One and a half hours later the general reaction was very severe, tachycardia and dyspnoea in particular were more marked and there was coldness of the tail and nose and erection of the hairs. The animals lay stretched out and showed marked prostration. They were showing signs of recovery in 3 hours, but tachycardia and dyspnoea were still prominent features. No prostration was present and they could now move quietly about the cage. They had recovered 5 hours after the injection.

Group 2. Animals all showed slight reactions from which they began to recover in 3 hours; recovery was complete in 4 hours.

Group 3. In spite of the fact that no tolerance had been developed in this group, the reactions produced were slight in

character and similar to those found in Group 2. Two hours after the injections they showed evidence of recovery, which was complete in 3 hours. This was rather unexpected as the adapted animals in Group 2 only showed evidence of recovery after 3 hours.

Group 4. The adrenalectomised animals also responded with a slight reaction, which became evident ½ hour after the injection. Signs of recovery were seen in 3 hours; recovery was complete 1 hour later.

HISTOLOGICAL APPEARANCE OF THE SUPRARENALS AND LIVER.

Group 1 (Severe Reaction). Suprarenals: The histological appearance of the control suprarenal was similar to that described previously when 4 zones were prominent (Fig. 45a). Half-an-hour after the injection, osmophil granules became evident in the cells of Zone 1, and small clear vacuoles began to appear in many of the cells of Zone 2 in addition to the osmophil-positive granules. The third zone was not so prominent but was slightly more osmophil-positive than in the control gland. This zone passed gradually into the last which was slightly broader than normal and densely osmophil-positive (Fig. 45b). An interesting change appeared in Zone 1 after 1½ hours (Fig. 45c). The whole zone was osmophilised and separated from Zone 2 by a thin clear line of cells which had no osmophil material in their cytoplasm. Only a few osmophil granules were seen in the cells of the second zone; it was generally pale and passed imperceptibly into the third, which had a similar appearance. A characteristic feature

of this gland was the great increase in size of the densely osmophil-positive fourth zone which now occupied fully one-third the diameter of the cortex. Three hours after the injection, when the animals were showing signs of recovery, Zones 1 and 2 were fused (Fig. 45d). Many of the cells were densely osmophil-positive but others showed the presence of clear vacuoles in their cytoplasm. The third zone was similar to that in the control animal, and the fourth, though not so broad as in the previous gland, was still much wider than normal and again densely osmophil-positive in character. When the animal had recovered in 5 hours (Fig. 45e), the first zone became prominent once more, it was slightly osmophil-positive and easily differentiated from the second, where many of the cells had osmophil granules in their cytoplasm, while others were vacuolated. The third zone had the customary appearance and the fourth once more was enlarged and the cells densely osmophil-positive.

In all cases lipoid material was seen in the sinusoids of the medulla and could be traced outwards through the adreno-lumbar vein (Figs. 45b and d).

The gradual osmophilisation of the outer zone, fusion of Zones 1 and 2, and reappearance of the two zones can be seen clearly in Figs. 46a, b, c, d and e. The medullary cells showed little or no reaction with osmic acid and the golgi bodies were invariably small.

Liver: Glycogen was present in the cells of all parts of the liver lobule of the control animal (Fig. 47a). Half-an-hour after the injection, the cells around the central vein were completely

devoid of glycogen and their nuclei could not be distinguished at all (Fig. 47b). As the reaction became more severe (1½ hours) the extent of glycogen deprivation was more extensive and the cells around the portal tract and central vein were depleted of their supply. Glycogen was present still in the mid-zone region but the liver at this stage was extremely depleted (Fig. 47c). During the onset of recovery (3 hours), the mid-zone supply became slightly increased but the picture did not vary a great deal from the previous one (Fig. 47d). There was almost complete regeneration after 5 hours, only a few cells in the immediate vicinity of the central vein and portal tract were devoid of glycogen (Fig. 47e). The disappearance of glycogen from the region of the central vein and its subsequent regeneration are shown clearly in Fig. 48A to E.

Group 2 (Slight Reaction). Suprarenals: The usual 4 zones were present in the controls and, as was expected, the first zone was poor in osmophil granules and the second was osmophil-positive (Fig. 49). The glands of animals, killed 1 to 7½ hours after injection, presented variations only in the outer and second zones. One hour after the injection lipoid granules were present in a few cells of the outer zone and clear vacuoles became obvious in many cells in the second one, but the cytoplasm of most cells in this layer was osmophil-positive (Fig. 50). The osmophil character of the cells was more marked in the outer zone after 2 hours, and vacuolation was still seen in a few cells in the second one. The remaining cells were not so intensely osmophil-positive as in the previous gland, only a few

densely osmophil-positive cells were seen (Fig. 51). The outer zone was clear once more after 3 hours, but the cells of the second one were again osmophil-positive, although a few were still vacuolated. This process of alternate pallor and osmophilisation of Zone 1 was observed in subsequent glands (Figs. 52A to G). Zone 3 in every case appeared pale and was weakly osmophil in character: Zone 4 gave only a slight osmophil reaction and was narrow in all cases. Lipoid material was observed in the medulla in every case.

Liver: Normal glycogen deposits were present in the control and first hour specimens (Figs. 53A and B). Depletion began to occur around the central vein in 2 hours (Fig. 53C), and became more marked 1 hour later. At this stage the store remained mainly in the mid-zone region (Fig. 53D), but it had returned to normal 4 hours after the injection (Fig. 53E).

Group 3 (Slight Reaction - No Previous Injections).

Suprarenals: The histological appearance of the suprarenals in this group was similar to that described in Group 2, and the changes seen already in Zones 1 and 2 were present.

Liver: Glycogen examinations were not carried out.

Group 4 (Slight Reaction - Adrenalectomised).

Suprarenals: There was no evidence of ectopic cortical tissue when the animals were examined at post-mortem.

Liver: The normal glycogen content of the control animal began to disappear from the cells around the central vein $\frac{1}{2}$ hour after the

injection. Depletion was more marked after 1 and 1½ hours when glycogen was present only in the mid-zone region (Fig. 54). This appearance remained in subsequent sections at 2, 3, 4 and 5 hours, when no evidence of glycogen regeneration was found.

BLOOD SUGAR CHANGES FOLLOWING ADRENALIN INJECTIONS.

Group 1. The blood sugar level of the untreated animal in this group was 133 mg.% and in the test animal killed ½ hour after the injection it had risen to 200 mg.%. There was a considerable rise, during the next hour, to 348 mg.% when the highest blood sugar reading was obtained. It fell rapidly to 234 mg.% in 3 hours after which the fall was much slower. Five hours after the injection it was 182 mg.% but still higher than in the control (Fig. 55). The sugar curve of Group 3 animals was similar in appearance to that described above (Fig. 55). The control level was lower (94 mg.%) and the rise during the first ½ hour slightly more marked (228 mg.%). The highest level (315 mg.%) was reached in 2 hours, after which there was a gradual fall to 285, 211 and 206 mg.% in 3, 4 and 5 hours respectively. The final reading (206 mg.%) was again considerably higher than in the control. A rather interesting blood sugar curve was obtained in the adrenalectomised group (Group 4). The control blood sugar was 137 mg.% and it rose to 210 mg.% in ½ hour and was at its maximum (250 mg.%) in 1 hour. It fell gradually to 221 mg.% in 1½ hours and returned to normal (121 mg.%) in 3 hours (Fig. 55). The blood sugar of the control animal in Group 2 was 88 mg.%.

Following the injection the rise was slower than in previous groups and the maximum (264 mg.%) was reached in 3 hours. This level was maintained during the next hour, but fell subsequently to 128 mg.% in 5 hours and had returned to the normal level in 6 hours (Fig. 56).

DISCUSSION.

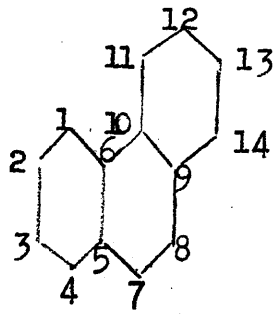
Corticosterone substances present in suprarenal cortex: Within recent years great advances have been made in the isolation of a large number of chemical substances from the suprarenal cortex. Many of them are related to the male and female sex hormones while others have assumed importance because of the part they play in the regulation of salt and sugar metabolism. It is the latter group which is of interest in the present investigation. They are referred to as "corticoid" substances and are all derived from the basic substance corticosterone. Corticosterone has the phenanthrene nucleus with a hydroxyl (OH) group attached to C11, and a hydrogen (H) group to C17. A number of compounds are formed by varying the groupings at C11 and C17. The addition of a hydroxyl group (OH) to C17 produces 17-hydroxycorticosterone. Removal of the hydrogen ion $(H)^+$ from C11 results in the compound 11-dehydrocorticosterone, while addition of a hydroxyl group to C17 produces 11-dehydro-17-hydroxycorticosterone. As both substances have been associated with sugar metabolism they are called sugar-active corticoids.

When the oxygen atom is removed from C11 of corticosterone, 11-deoxycorticosterone is formed and by adding a hydroxyl group to

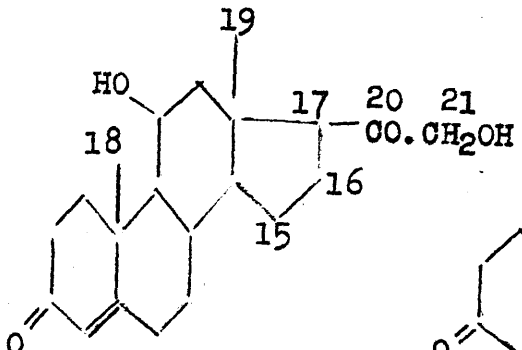
C17, 11-deoxy-17-hydroxycorticosterone is produced. As these substances are engaged in the control of electrolyte balance, they are called the salt-active corticoids.

In this thesis the descriptive terms sugar-active and salt-active corticoids will be employed.

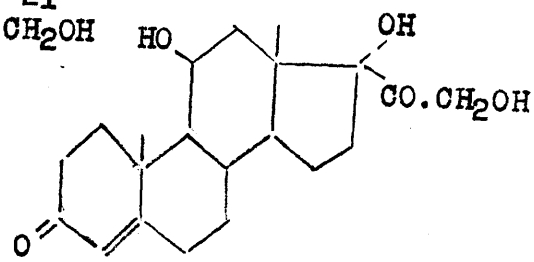
The compounds are shown in the following table (see page 66).



Phenanthrene.

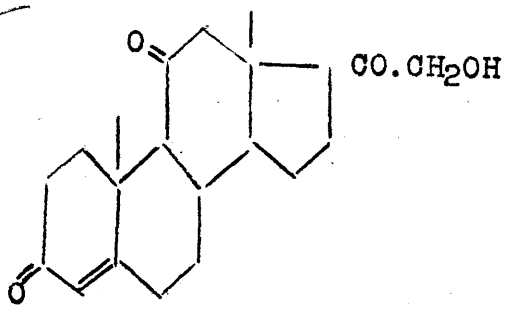


CORTICOSTERONE.

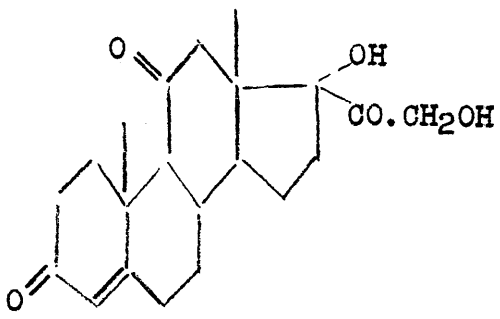


17-Hydroxycorticosterone.

Sugar-active Corticoids.

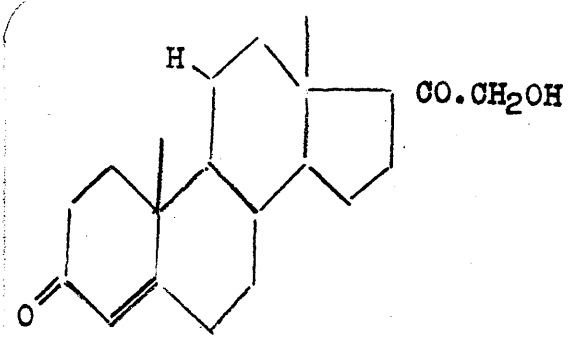


11-Dehydrocorticosterone.

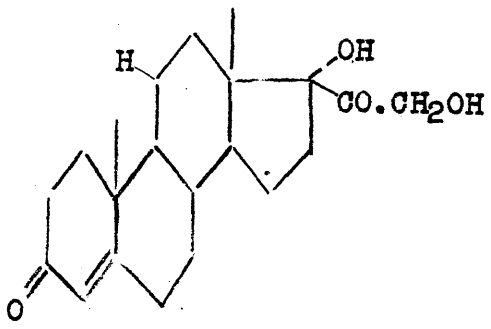


11-Dehydro 17-Hydroxy-Corticosterone.

Salt-activé Corticoids.



11-Deoxycorticosterone



11-Deoxy 17-Hydroxy-Corticosterone.

Methods of Distinguishing the Corticoid Substances: A number of biological tests have been devised to demonstrate the activity of salt- and sugar-active corticoids, but only a few will be considered.

(1) "Ingle" work test (Ingle, 1936, 1944) for sugar-active corticoid is a measurement of the total work yielded by the muscle of an adrenalectomised rat treated with the substance.

(2) The life-preservation test is a measure of the ability of salt-active corticoid to preserve the life of adrenalectomised mice or rats.

(3) The insulin insensitivity reaction depends on the ability of sugar-active corticoid to prevent the hypoglycaemic action of insulin when administered to normal or adrenalectomised starving animals (Selye and Dosne, 1939; Grattan and Jensen, 1940; Grattan and Ingle, 1941).

(4) This test depends on the ability of sugar-active corticoid to raise the level of liver glycogen in adrenalectomised or normal animals (Reinecke and Kendall, 1942, 1943; Bergman and Klein, 1943; Long, Katzin and Fry, 1940; Long, 1942; Tepperman, Engel and Long, 1943).

Although most of these reactions are used as tests for sugar-active corticoid efficiency, they seemed to present means of investigating the nature of the lipoid material which escaped from the suprarenal

cortex following adrenalin injections. The application of one or other of those methods for this purpose has been attempted by other writers. Vogt (1945) showed that plasma obtained from the suprarenal vein of a rat, after adrenalin injection, would prolong the life of adrenalectomised rats, and Venning and Kazmin (1946) used the deposition of liver glycogen as an index of the sugar-active corticoid content of urine. For my work, I selected the deposition of liver glycogen and correlated the results with variations in the blood sugar and suprarenal changes. The technique adopted was thus a histological bio-assay method.

The lipoid granules in the suprarenal cortex were examined again by the osmic acid method. Double perfusion of the gland was the method originally used by Bennett and, although it would normally be the method of choice, it was not possible here since it was necessary to examine the secretions in the medullary sinusoids. Fixation in 10% neutral formalin followed by treatment with 1% osmic acid was found quite satisfactory provided the pH of the solution was maintained by renewing the osmic acid after the third day, although it is claimed that better results are obtained by fixation in Champy, Formol-zenker or Regaud's solution prior to osmic acid (Bennett; Hoerr, 1936). The suprarenal glands must be handled as little as possible and it was found advisable to section them in two, with a sharp razor blade, prior to treatment with osmic acid. In order that uniformity of results may be obtained the glands must be cut serially at 5 μ.

Liver glycogen determinations raised many problems. Normal rat livers varied greatly in their glycogen content when estimated chemically and thus standardisation was difficult. This was overcome by adopting a histological technique using the glycogen content of the normal liver lobule as the standard. The disappearance and regeneration of glycogen in the lobule could then be compared at different intervals. Although the method was originally qualitative it yielded more than a rough quantitative result and proved very satisfactory in practice.

The silver impregnation method of Mitchell and Wislocki was chosen mainly because of its good photographic properties and the "rim-staining" effect, commonly seen in many glycogen sections, was avoided except in a few instances only. The histological results obtained were excellent but it should be noted that the organs were placed in the refrigerator for 15 minutes prior to fixation. Fixation at 0°C. seems to prevent the rim formation and the technique is adopted in the latest modification of the method (Deane, Nesbett and Hastings, 1946). Unless proper fixation with alcoholic picric acid and neutral 10% formalin is carried out the method can give rise to endless trouble and so the technique described by Mitchell and Wislocki must be followed carefully. The liver of the control adrenalectomised animals was well supplied with glycogen and this is in agreement with the finding of Long, Katzin and Fry. In both instances the animals were maintained in normal electrolytic balance; the method of Williams, Diaz, Burch and Harrison (1939), was

used in the present investigation.

Correlation of changes in the suprarenals, liver and blood sugar following a severe adrenalin reaction:

Group 1. By using the regeneration of liver glycogen as an example of sugar-active corticoid activity and correlating it with variations in blood sugar and zonal changes in the suprarenals it was possible to decide the nature of the osmophil-positive lipoid secreted from suprarenal cortex.

The changes seen in the suprarenals proved beyond doubt that zonal variations occurred in the lipoid content of the cortical cells in response to adrenalin shock or reaction. The lipoids normally appeared in Zone 2, but when the reaction was severe, premature osmophilisation occurred in the outer zone. If the reaction was maintained, premature osmophilisation persisted and the normal arrangement between Zones 1 and 2 was lost; later as the animal recovered, normal zonal relationship was reestablished (Figs. 45 and 46). The series also showed that there was a definite variation in osmophilisation of Zone 2; the clear vacuoles repeatedly seen no doubt represented the cell after it had liberated its secretion. The increase in size, and osmophilisation of Zone 4, was a constant feature after $1\frac{1}{2}$ hours and has been noted previously in response to different agents (Deansley; Graham; Cramer). At present, it is not possible to ascribe this appearance definitely to the normal fatty change in the dying cells of the zone, but subsequent work (Part 5, page 108) would tend to refute the view of Zwemer and Lowenstein (1940) that water

soluble corticoids are found in Zone 4, and does suggest that the osmophil lipoids in this zone are purely fatty materials of a degenerative nature.

It is of interest that lipoid changes in the cortical cells were seen within ½ hour of the injection. This is the more remarkable in view of Selye's (1946) statement that "cortical changes take several hours to develop." Nevertheless, my results are in agreement with those of Vogt who found the response of the cortex to injections of adrenalin was immediate and lasting.

The suprarenal changes were associated with alterations in liver glycogen and blood sugar. The glycolytic action of adrenalin was evident 3 hours after the injection, but the liver glycogen had returned almost to normal in 5 hours (Figs. 47 and 48). A rise of blood sugar was also seen and it was still elevated 5 hours after the injection (Fig. 55). Those experiments showed that zonal variations occurred in the suprarenals in response to shock or severe adrenalin reactions and osmophil lipoid material was liberated immediately and continued to be liberated for many hours. Lipoid liberation was associated with prolonged elevation of blood sugar and regeneration of liver glycogen. Since sugar-active corticoid possessed those properties it was extremely likely that the osmophil-positive substance was sugar-active corticoid. Conclusive proof of this was afforded by the failure of liver glycogen to regenerate in animals after adrenalectomy. Moreover, the blood sugar was never sustained in these animals but fell rapidly to normal levels (Figs. 55 and 56).

It has been stated that liberation of corticoid material occurs in the counter-shock phase of the alarm reaction (Part 2). The present investigation supports this view, but suggests in addition that the counter-shock phase occurs immediately and in fact may coexist with the earlier or shock phase. Further, it would appear that the corticoid liberated is sugar-active in type.

In summary, I submit that the present histological bio-assay method demonstrates that sugar-active corticoid is liberated immediately from the suprarenal cortex in response to severe adrenalin shock or reaction, and plays an important part in the counter-shock mechanism. There seems little doubt that a similar mechanism occurs in patients with chromaffin tumours after a severe paroxysmal reaction. Since corticoid material may be formed in the cells of the outer zone of the gland, it is highly probable that the small rim of cortex covering the tumour is capable of forming this substance which can be secreted with ease during a paroxysmal reaction into the dilated sinusoidal spaces of the tumour. Thus, the patient, when subjected to repeated reactions which may be severe in type, has a counter-shock mechanism available, but when the tumour is removed at operation, the cortical cells are removed with it. If the patient has a severe paroxysmal reaction during the operation, as happens frequently (Case 1, Appendix), the counter-shock mechanism, following removal of the tumour, is insufficient and a fatal issue is highly probable. The present investigation thus explains why cortical extract therapy is of value in supplementing adrenalin in

the post-operative treatment of chromaffin medullary tumours. If the operation is performed without setting up a paroxysm the necessity for cortical extract is minimised, as was seen in Case 2 (Appendix).

The present investigation also has drawn attention to the zonal variations which occur in the suprarenals in response to adrenalin shock and shows that it is necessary to examine the glands at intervals so that an idea of their activity may be found. A single examination is entirely useless. In addition the results of the present series would seem to refute the view expressed by Deane et al (1947) that sugar-active corticoid is formed in Zone 2 and salt-active corticoid in the outer zone. The present investigation conclusively shows that sugar-active corticoid normally forms in Zone 2 but, as the demand for it increases, as in shock, it can form in the outer zone of the gland. So far as I am aware, no other worker has demonstrated this point by histological methods. At present the site of formation of the salt-active corticoid cannot even be surmised, nor is the mechanism known by which it is liberated from the gland.

Correlation of suprarenal and liver changes following a slight adrenalin reaction:

Group 2. Apart from alternate osmophilisation of Zone 1 (Fig. 52A to G), the suprarenal cortex showed little variation from normal, but in spite of the apparently minimal suprarenal changes there was a well marked elevation and maintenance of blood sugar (Fig. 56), and

rapid regeneration of liver glycogen (Fig. 53E). Although the glands showed changes in the outer zones only, they were still very active and capable of producing considerable amounts of sugar-active corticoid. Those observations are important in emphasising again that single histological examinations (page 73) give no indication of the activity of the gland, and prove further the value of the combined histological bio-assay method.

The results from this series of experiments suggest that there is a specificity in the response of the suprarenal to injections of adrenalin. This response seems to be devoted to the liberation of sugar-active corticoid. Unfortunately no simple test is as yet available for a similar investigation of salt-active corticoid which can produce vascular lesions when injected or implanted into experimental animals (Selye, 1946). As no vascular lesions were found in any of the animals (Part 2) after adrenalin injections, it seems very probable that the corticoid liberated from the suprarenal cortex after adrenalin injection contained none or very little of the salt-active type. However, it should be remembered that in the series investigated by Selye and Pentz (1943) and Selye and Hall (1944), very large doses of desoxycorticosterone acetate were administered. If large doses of this substance are required to produce vascular lesions, it was possible that the absence of these lesions in adrenalin treated animals was due to insufficient amounts of salt-active corticoid being liberated to cause vascular lesions. In order to explore this possibility it was decided to test the effects of graded amounts

of desoxycorticosterone acetate implants as to their ability to produce hypertensive vascular lesions.

PART 4.

THE VASCULAR RESPONSE OF THE RAT TO GRADED DOSES OF DESOXYCORTICOSTERONE ACETATE (DOCA).

PART 4.

THE VASCULAR RESPONSE OF THE RAT TO GRADED DOSES OF DESOXYCORTICOSTERONE ACETATE (DOCA).

METHODS AND MATERIALS.

The desoxycorticosterone acetate used was compressed pellets of 10, 20 and 60 mg. weight which were implanted subcutaneously into the abdominal wall of adult male rats varying in weight from 200 to 250 g.

The animals were divided into four groups. Groups 1 and 2 each consisted of 6 rats which received 60 and 30 mg. implants respectively. Five were present in Group 3 and received 10 mg. and further doses of 10 mg. were implanted at approximately 4 to 5 week intervals.

Group 4 animals, 6 in number, were kept as controls, and received no implants. All groups were given the ordinary diet of bran, oats and nut-cake, while cabbage was given 2 or 3 times weekly. A solution of 1% sodium chloride was given to all groups as drinking water.

PERIOD OF SURVIVAL OF ANIMALS.

One animal from both Groups 1 and 2 died 7 days after the tablets were implanted, and pneumonia was found to be the cause of death in both cases (Tables 18 and 19). Two in Group 1 died 63 and 67 days after implanting but the remainder of Group 1 and all Group 2 were killed at intervals varying from 7 to 160 days. The 10 mg. implants could be palpated in the abdominal wall and their rate of

absorption gauged roughly. They were usually present after 4 or 5 weeks when further 10 mg. implants were inserted. The animals in this group were killed at intervals varying from 57 to 192 days.

When a rat died or was killed, the body was weighed, the anterior abdominal wall carefully opened, and the unabsorbed desoxycorticosterone acetate implant removed and weighed. Since the original weight of the implant was known the amount of desoxycorticosterone acetate absorbed could be calculated. Post-mortem examination was carried out in the usual manner and the cardiac index found as described previously. The heart, kidneys, liver, spleen, pancreas and suprarenals were examined histologically by the usual methods.

R E S U L T S.

RATE OF ABSORPTION OF DESOXYCORTICOSTERONE ACETATE IMPLANTS.

The absorption rate was found to depend on the size of the tablets. At the beginning of the experiment 0.33 mg. per day were absorbed in the 60 mg. group (Table 18). This rose to 0.42 mg. per day in 63 and 67 days, but fell subsequently to the original level in 160 days. The rate of absorption of the 30 mg. tablets was less and varied between 0.19 and 0.28 mg. per day (Table 19). Individual animal variations were seen clearly in this group, and 30 mg. were completely absorbed in one animal (No. 4) in 107 days and in 156 days in a second (No. 6). The 10 mg. implants were absorbed at the rate

of 0.10 mg. per day in early cases where one tablet only had been implanted. Later, with additional implants, the rate of absorption rose; 0.24 mg. per day was absorbed in No. 5, when a total of 60 mg. were administered and 45.9 mg. absorbed (Table 20).

CARDIAC INDEX.

The index of control animals varied between 0.0023 and 0.0031. There was a slight rise in the maximum index above the upper limit of normality in all but one animal (No. 4) of the 60 mg. group. In this case severe cardiac lesions were present and there was extreme ascites with pleural and pericardial effusion (Table 18). All animals in the 30 mg. group showed a more consistent, but still small, rise of maximum cardiac index above the upper limits of normality and none of the animals in this group showed any loss of weight (Table 19). Four animals in the 10 mg. group lost weight and the maximum cardiac index in all cases was slightly above normal (Table 20).

POST-MORTEM CHANGES.

Macroscopic changes in the organs were seen generally in the 60 mg. group and mainly affected the heart, kidneys and liver. There were effusions into the pericardial and pleural sac in one animal (No. 4) and 20 c.c. of serous fluid were present in the peritoneal cavity. The myocardium was pale and numerous yellow mottled pin-point areas were seen on the endocardial and pericardial surfaces. The kidneys were congested only, and the liver in this animal showed evidence of chronic venous congestion. Significant

changes were seen in the kidneys of three animals (Nos. 3, 5 and 6), they were slightly enlarged, the surface pale, irregular and granular in appearance, and the capsule stripped with ease; albumen was present in abundance in the urine in those cases. The heart, spleen, liver, pancreas and suprarenals appeared normal in all three. The organs from the remaining two animals (Nos. 1 and 2) were normal.

Relatively few lesions were seen in the animals in the 30 mg. group. The heart always appeared normal and it was only in the last one (No. 6) that changes were noted in the kidneys. The surface was granular and irregular, and a large cyst was present in the lower pole of the left kidney. A few changes were seen in the 10 mg. animals. The kidneys in three cases (Nos. 3, 4 and 5) showed changes similar to those described above, the rest of the organs were normal.

HISTOLOGICAL APPEARANCE OF THE ORGANS.

Heart: In the 60 mg. group many interesting lesions of varying intensity were seen in the heart in all animals, apart from the first where the heart was normal. The most extensive changes were present in No. 4 (Table 18) and consisted of mottled yellowish foci which were obvious to the eye. The muscle fibres in these areas were replaced by a very cellular tissue (Fig. 57) composed of large spindle-shaped cells, with pale cytoplasm and spherical nuclei. They resembled fibroblasts (Fig. 58). The muscle cells showed varying degrees of degeneration, the remains of degenerating muscle were seen in some places (Fig. 59), but generally all that remained was the sarcolemma sheath surrounded by spindle-shaped cells. Abundant

thin-walled capillaries, containing red blood cells, were present along with the fibroblasts and gave the appearance of young granulation tissue (Fig. 60). A few mononuclear cells were seen, but no giant cells of the Aschoff type were noticed.

When the foci were small and the lesions scanty (Table 18, Nos. 2, 3, 5 and 6), the cellular infiltration was composed of groups of mononuclear cells which could be seen lying in planes between and replacing the muscle cells (Fig. 61: Table 18, No. 6). In some instances the muscle fibres were replaced by a more mature fibrous tissue (Fig. 62).

Vascular lesions were observed in some coronary vessels. There was well marked concentric hyaline degeneration of the wall of the vessel with narrowing of the lumen (Table 18, No. 6), and perivascular infiltration of round cells (Fig. 63). In view of those findings all hearts were examined by serial section, and in one (Table 18, No. 6) local plaques of hyaline material were noticed lying internal to the internal elastic lamina; they projected into and obstructed the upper part of the lumen of the vessel. The lesions were focal in distribution, and the normal vessel soon reappeared (Figs. 64A to D). Other vessels in the same case showed complete hyaline degeneration of the whole thickness of the coronary vessel (Figs. 65A and B), with gradual narrowing and eventually obliteration of the lumen (Figs. 65C and D). An interesting feature was the appearance of a branch or anastomotic vessel with complete re-establishment of the circulation prior to complete blockage of the

parent (Figs. 65D and E). Perivascular cellular infiltration was most marked in Fig. 65A and B.

In spite of the extensive coronary lesions present in this animal, the myocardial changes were small, focal in distribution, and similar to those already described. Coronary vascular changes were never so marked in any of the other animals although they were seen (Table 18, No. 2). The small vessels to the columnae carnae showed concentric hyaline degeneration of the intima with narrowing of the lumen (Figs. 66A and B), and the myocardial lesions in relation to those changes are shown in Fig. 67. In spite of the severe myocardial changes present in case No. 4, it is interesting to note that serial section failed to discover any coronary damage.

No lesions were found in the heart of the first three animals of the 30 mg. group but a slight focal cellular reaction, similar to that described, was seen in the remainder (Nos. 4, 5 and 6, Table 19). The coronary vessels were normal in all cases.

Myocardial lesions were seen in all animals in the 10 mg. group but were minimal and focal in distribution. The appearances were similar to those seen in the less severe 60 mg. group, and were found after only 7 mg. had been absorbed in 57 days. Apart from the first animal slight coronary vascular changes were seen in the remainder. The lesions were characterised by slight intimal thickening with narrowing of the lumen.

Kidneys: No organs showed more significant changes than the kidneys. Lesions were absent in two animals (Nos. 1 and 2) of the

60 mg. group and slight in one (No. 4), in spite of the severe myocardial changes already noticed. Severe macroscopic and microscopic changes were present in the remaining animals (Nos. 3, 5 and 6). The lesions were focal in distribution and the intervening kidney tissue appeared normal (Fig. 68). In the affected areas, there was thickening of the walls of the glomerular capillaries with adhesion between the tuft and Bowman's Capsule, while an exudate was usually present in the subcapsular space (Fig. 70), which at times was completely obliterated. The glomerulus often showed necrosis with loss of lobulation of the tuft, cellular proliferation and heightening of the capsular epithelium (Fig. 69). Occasionally complete glomerular sclerosis was present (Fig. 71).

Focal areas of tubular atrophy and dilation were seen, the epithelium lining them was flattened against the basement membrane, and casts were present in the lumen of the tubules (Figs. 72 and 73). The vascular lesions consisted mainly of hyaline degeneration of the walls of the afferent arterioles (Fig. 74). Arteries, smaller in size than the interlobular, showed marked intimal hyperplasia with narrowing of the lumen (Fig. 75), but the interlobular and arcuate vessels were normal and showed no evidence of splitting of the internal elastic lamina (Figs. 76 and 77).

Kidney changes were seen in two animals (Nos. 5 and 6) only in the 30 mg. group, and were similar but not so severe as those already described. Apart from the first, the kidney was involved in all animals in the 10 mg. group, and all features already described were

seen. There was focal necrosis of the glomeruli with loss of lobulation of the tuft, heightening of the capsular epithelium and marked capsular adhesion. The tubular epithelium was flattened and casts present in the lumen. The afferent arteriole was hyalinised and the lumen almost obliterated (Fig. 78). Marked intimal proliferation, with almost complete closure of the lumen, was seen in one large arteriole and round-cell infiltration of the interstitial tissue was seen in the region around this vessel (Fig. 79). Extreme hyaline degeneration of the intima, with almost complete closure of the lumen, was observed in one interlobular artery (No. 2).

Liver: The liver was peculiarly free from lesions apart from one case (No. 4) in the 60 mg. group, when marked cardiac lesions, ascites, pericardial and pleural effusions were present. Extensive haemorrhage was present around the central vein of the lobule and to a lesser extent around the portal tract. Normal liver cells were seen only in the mid-zone region (Fig. 80), but the hepatic arteries showed no degenerative changes.

Pancreas: Vascular lesions were present in one case only (10 mg. group, No. 2) when many of the vessels showed hyaline degeneration of the intima with narrowing of the lumen (Fig. 81). A marked perivascular cellular reaction was present again, and the lesion was interesting since only 12.4 mg. of desoxycorticosterone acetate were absorbed in 123 days.

Spleen: Hyalinisation of the septa and capsule, thickening of the vessels in the Malpighian bodies, and narrowing of their lumen

were present in all animals. The changes were not significant since they were seen in the controls as well.

Suprarenals: The control animals in this experiment differed from those seen in Parts 2 and 3, since they always showed osmophilisation of Zone 1. Otherwise they did not differ from control animals in the other series, except that in the last animal there was little evidence of osmophilisation in Zone 2, while Zone 4 now appeared broad and osmophil-positive (Fig. 82). Changes were seen in the last two animals of the 60 mg. group; the outer zone was clear and only scanty osmophil-positive granules were present in the second. Haemorrhages were seen in the inner aspect of this zone in one case (No. 6, Fig. 83) and the last zone was increased in size and osmophil-positive in character. The suprarenals in the 30 mg. group were normal, except for the last animal which showed an osmophil mottling of the inner aspect of Zone 1; the second zone was only partially osmophilised and slightly increased in size (Fig. 84). A large number of cortical cells were seen in the medulla, but they were outside and quite distinct from the medullary sinusoids which contained osmophil-positive secretion (Fig. 85). All glands in the 10 mg. group were normal in appearance, apart from a slight diminution in osmophil granules in Zone 2 and a moderate increase in size of Zone 4 (Figs. 86 and 87).

DISCUSSION.

The rate of absorption of desoxycorticosterone acetate from implanted tablets depends on their surface area and consistency, and

the results found in this series are in agreement with previous reports (Soffer, Engel and Oppenheimer, 1940). Maximum absorption was seen in the 60 mg. implants and amounted to 0.42 mg. per day. Daily subcutaneous injections of 4 to 10 mg. of desoxycorticosterone acetate have been given for periods of 27 days to 8 weeks (Darrow and Millar, 1942; Selye and Pentz). No indication of its rate of absorption can be obtained by this method, but it must have been far in excess of that seen in the present series. Compressed desoxycorticosterone acetate implants were used by Selye and Hall (1944). They implanted subcutaneously four 10 mg. pellets, but gave no indication of their rate of absorption.

Severe organic lesions were found in the 60 mg. group, but there was no definite uniformity in them since 28 mg. of desoxycorticosterone acetate acting over a period of 67 days (Table 18) caused severe heart lesions in one animal (No. 4) and severe kidney lesions in a second (No. 3). This individual animal variation was seen also in the 30 mg. group (Table 19). Three different animals absorbed between 26 and 30 mg. in 106 to 156 days, the kidneys were unaffected in one, and moderately affected in the remaining two. It was noticed that, as the implants were allowed to act longer, there was a steady increase in the appearance and severity of kidney, and to a lesser extent of cardiac lesions. This point was clearly demonstrated in the 10 mg. group where the rate of absorption was maintained and then slightly increased by repeated implants. Very definite renal and to a lesser extent cardiac lesions were caused by

12.4 mg. in 123 days. As the amount absorbed was slowly increased over a long period (45.9 mg. in 185 days), naked-eye as well as microscopic lesions appeared in the kidney, but the lesions in heart, though present, were still minimal.

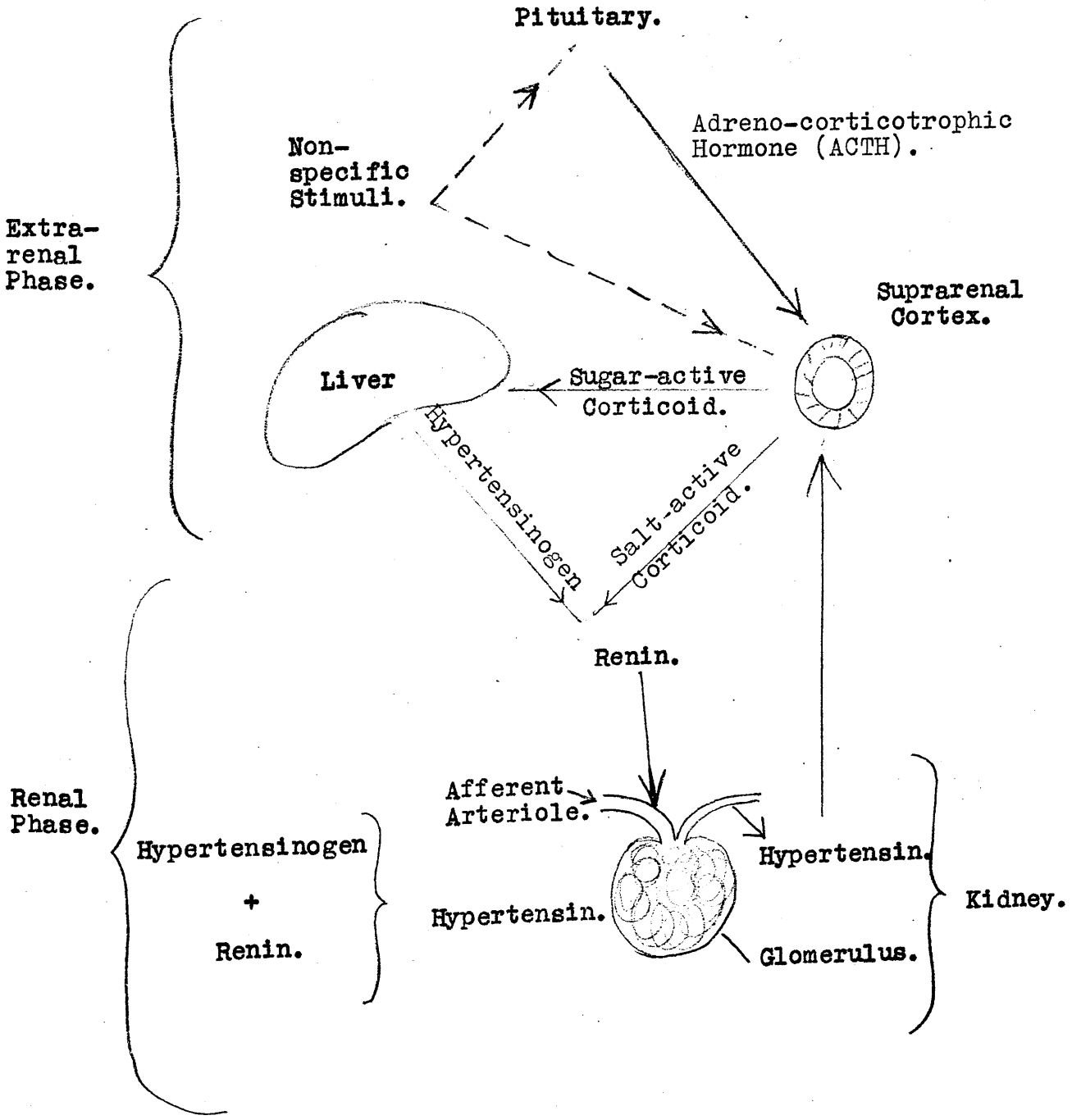
No blood pressure examinations were carried out in the present series, but Selye (1946) showed con were carried out in the present blood pressure to hypertensive levels in desoxycorticosterone acetate treated animals. However, evidence of hypertension was seen in the kidneys of all groups irrespective of the implants. There were patchy areas of sclerosis in which the glomeruli showed varying degress of loss of lobulation of the tuft, cellular proliferation, heightening of the capsular epithelium and extensive capsular adhesions. Round cell infiltration was present in the interstitial tissue and the tubules were dilated and filled with casts. Such lesions are similar to those described by Selye and Pentz, and Selye and Hall. None of the large vessels showed any evidence of splitting of the internal elastic lamina, but the interlobular artery in one case (No. 4), in the 10 mg. group, showed marked intimal thickening with narrowing of the lumen. The most important feature, common to all groups showing kidney involvement, was hyalinisation of the glomerular afferent arterioles, with narrowing of the lumen. The change was similar to that seen in the non-ischaemic kidney of some rats following the application of a modified Goldblatt clip (Byrom and Wilson, 1939). The "explosive type" of lesion, or necrotising arteriolitis described by those authors, was never seen in any of the kidneys in this series, but in all other respects the lesions were

similar and suggested a common aetiological basis.

The experimental investigations of Wilson and Pickering (1938), Verney and Vogt (1938), and Wilson & Byrom (1941) have confirmed the view put forward by Goldblatt (1937-1938) that incomplete renal ischaemia in animals produces long-sustained arterial hypertension. It is also well established that the ischaemic renal tissue is associated with a pressor substance, which acts directly on the smooth muscle of blood vessels causing constriction of the peripheral vascular bed, and subsequent rise of blood pressure. The recent investigations of Trueta et al (1947) would seem to indicate that renal ischaemia in man may be produced by a mechanism essentially similar in its effects to the Goldblatt clamp; for the "diversion of the intrarenal blood flow from the cortical pathway through the medullary by-pass causes a cortical ischaemia of sufficient degree to result in the formation and liberation of the pressor substance." In addition, Goormaghtigh (1940; 1945) believes that the arterioles of the renal cortex may be the source of the pressor substance which results from such renal ischaemia. Nevertheless, in spite of the undoubted existence of this renal humoral mechanism and the cortical ischaemia which sets it in motion, it has to be admitted that it is only one phase (renal phase) in the mechanism of arterial hypertension. It now seems more and more obvious that there exists an extra-renal phase in which the suprarenal cortex plays a part. The importance of the suprarenal cortex was observed by Goldblatt (1937), who found that a small remnant of suprarenal cortex in two of his dogs was

sufficient to cause elevation of blood pressure following renal ischaemia. Again adrenalectomy slowly decreased the sensitivity of the blood vessels to renin, although they still reacted normally to adrenalin and hypertensin (Friedman, Somkin and Oppenheimer, 1939; Houssay and Dexter, 1942). Remington, Collings, Hays and Swingle (1941) showed that the diminished sensitivity to renin was associated in many instances with a fall in the hypertensin precursor of plasma, while the vascular response to renin apparently could be restored by substitution with adrenal cortical hormones (Friedman, Somkin and Oppenheimer).

Accordingly, it would appear that a close relationship exists between renal ischaemia and the suprarenal cortex, a relationship which is strengthened by the similarity in lesions produced by Byrom and Wilson with an ischaemic kidney and my series following salt-active corticoid (desoxycorticosterone acetate) implants. As definite renal lesions were produced in this series by repeated implants of 10 mg. doses of desoxycorticosterone acetate acting over a long period, it may well be that chronic endocrine over-dosage with salt-active corticoid has a part in causing arterial hypertension (Selye, 1946). The following diagram attempts to correlate the above findings:-


Pituitary.
Non-specific Stimuli.
Adreno-corticotrophic Hormone (ACTH).
Extra-renal Phase.
Suprarenal Cortex.
Liver
Sugar-active Corticoid.
Hypertensinogen
Salt-active Corticoid.
Renin.
Renal Phase.
Afferent Arteriole.
Hypertensinogen
+
Renin.
Hypertensin.
Hypertensin.
Glomerulus.
Kidney.

The initiating mechanism is not definitely known but is presumably of the nature of a non-specific stimulus having a sympathetic-like action. This factor may act via the pituitary liberating adreno-corticotropic hormone, or directly on the suprarenal cortex, liberating corticoid materials in addition to producing renal cortical ischaemia. There is no doubt that sugar-active corticoid can be liberated by such stimuli (Part 3, page 52), but it can only be presumed that a mechanism exists which can also liberate the salt-active material as well. Sugar-active corticoid is believed to affect the globulin precursor of hypertensinogen (Selye, 1946), and the present investigation has confirmed that hyalinisation of the glomerular afferent arterioles, with subsequent narrowing of the lumen and renal ischaemia, does result from overdosage with salt-active corticoid. Thus the renal humoral mechanism is set off, and the hypertensin so formed may now act as a non-specific stimulus to initiate a vicious circle. Until the exact mechanism which governs the liberation of salt-active corticoid is known, this must remain a hypothesis, but it is one which, I submit, does explain all the facts so far observed.

Heart: Cardiac hypertrophy, as indicated by an increase in cardiac index, was not a marked feature of the series, and did not bear out the results of Selye and Hall (1944), who found actual hypertrophy of individual fibres. The maximum index was slightly above the upper limit of normality in most cases, but the rise was not extreme. In one animal (No. 4, Table 18) the index was low and

severe cardiac lesions were present (Figs. 88 and 89).

The first stage in the production of those lesions seemed to be infiltration of mononuclear cells between the muscle fibres, which later showed focal necrosis followed by various stages of myocardial degeneration. Eventually only the sarcolemma sheath remained and it was surrounded by fibroblasts accompanied by groups of thin-walled capillaries. The degenerating muscle was being replaced by granulation tissue, and there was never any evidence of a lesion resembling the Aschoff nodule as described by Selye (1946). Occasionally definite areas of fibrosis were seen between the muscle fibres and it is believed that they represent a later stage in the replacement of necrosed muscle (Figs. 57 to 62).

Various suggestions have been put forward to explain those cardiac lesions. Darrow and Miller believed that they were due to excess loss of potassium since they could be produced by diets low in this substance, but Selye disagreed, and thought the desoxycorticosterone acetate caused retention of sodium (Na^+) and chloride (Cl) ions which were responsible for the lesions. The results of this investigation throw some light on the question. There is no doubt that desoxycorticosterone acetate implants in an animal, sensitised by unilateral nephrectomy or a high salt diet, could produce coronary vascular lesions which did not appear to occur uniformly, nor did they affect all animals to the same extent, for the coronary vessels were not affected in the animals in the series which showed the severest cardiac lesions. On the other hand severe vascular lesions were

associated with minimal cardiac upset. This was explained by serial section since every occluded vessel had an adequate branch or anastomotic vessel arising before obstruction was complete. Accordingly, there was no coronary vascular insufficiency which must be considered an important cause of the cardiac lesions. If the anastomotic supply is good the cardiac lesions will be minimal, but if the supply is inadequate, coronary ischaemia results, with necrosis of cardiac muscle and subsequent replacement by granulation tissue (Figs. 63 to 66).

A feature of interest was the presence in some coronary vessels of focal areas of hyaline degeneration, which did not cause complete obstruction of the lumen. Such focal areas of necrosis must be borne in mind when attempting to explain the extensive lesions seen in one case without any apparent vascular lesion. It is very probable that vascular lesions were formed but were completely engulfed in the subsequent cellular reaction.

Those vascular changes have been reported previously and were compared to the lesions of polyarteritis nodosa (Selye and Pentz). It is not possible to comment further but there is a resemblance between the two, although it should be noted that the fibrinoid character of the polyarteritic lesion was not observed in this series. The presence of intimal thickening in the coronary vessels of all animals in the 10 mg. group is worthy of note; while the relationship between focal coronary vascular lesions in animals and coronary thrombosis in man is worthy of further investigation.

Vascular lesions were not a common feature in the other organs, apart from pancreas which showed changes in one case only. The lesion was extreme, but no explanation of the finding is possible.

Suprarenals: Osmophilisation of Zone 1 in control animals was in contrast to the appearance of this zone in the controls of previous groups (Parts 2 and 3). The change was not seen in the test animals and no explanation is at present available.

Most suprarenals in the test animals appeared normal microscopically. No variations were seen in the 10 mg. group, and it was only in the last of the 30 mg. group that irregular osmophilisation was noticed in Zone 1, with an absence of lipoids in Zone 2. Changes occurred only in the last two animals of the 60 mg. group. Scanty osmophil-positive granules, together with haemorrhagic areas, were observed in the second zone, but were not correlated at that time by the histological bio-assay method described previously. Nevertheless, observations made later (Part 5, page 104) suggest that, under the influence of large doses of desoxycorticosterone acetate, there is a tendency for the gland to become exhausted, as evidenced by depletion of lipoid from Zone 2, haemorrhages in this zone, and broadening of the fourth.

Under the influence of large doses of desoxycorticosterone acetate atrophy of the suprarenal gland has been reported, and cortical changes noted (Greep and Deane, 1947). Deane and Bergner (1947) believe that the suprarenal cortex can be depleted in two ways: (1) by atrophy after desoxycorticosterone acetate injections, and

(2) by acute hyperfunction, followed by the disappearance of secretion, when all ketosteroid reactions may disappear from the zona fasciculata in less than a day. Subsequent work (Part 5) will show that this theory of exhaustion following acute hyperfunction is correct.

"Rheumatic" joint lesions and their relation to the suprarenal gland: In 1946 Selye noted that rats, chronically treated with large doses of desoxycorticosterone acetate, developed "joint lesions which exhibit the histological characteristics of rheumatic arthritis and periarthritis." This finding has been confirmed by him in desoxy-corticosterone acetate-treated monkeys and dogs, and joint swellings have been noted in patients suffering from Addison's disease after desoxycorticosterone acetate overdosage (Dejean, 1947; Laroche, 1947). As a result of his findings Selye believes that rheumatoid arthritis, like hypertension and rheumatic fever, is one of the diseases of adaptation already discussed (Part 2, page 47).

Particular attention was paid to swelling of the joints in the animals used in the present series, but no lesions were found. This was not altogether unexpected, as Selye (1949), observed that arthritis occurred only in a certain percentage of his experimental animals, and it should be noted that much larger doses of salt-active corticoid were used by him than in the 60 mg. implants in the present investigation. When adrenalectomy was performed, Selye found that desoxycorticosterone acetate implants produced arthritis more frequently than in intact animals. The increased incidence of joint swellings following adrenalectomy, suggested that this procedure

sensitised the animal to the toxic effects of the drug because it removed the endogenous sugar-active corticoids, which are believed to exert a protective action in the body. Earlier in this section (page 84) it was remarked that although most of the suprarenal glands in the present investigation were normal, there was evidence of a tendency towards exhaustion in the last two in the 60 mg. group, while atrophy and exhaustion of the suprarenal cortex from desoxy-corticosterone treatment has been noted by Deane and Bergner (1947). This protective action of sugar-active corticoid liberated endogenously could explain the absence of joint lesions in my series and their variable appearance in Selye's animals. Recently Selye has announced the production of arthritic and periarthritic lesions in animals by injections of formalin beneath the plantar aponeurosis of the hind paws. Although the method seems unusually drastic, and the swellings produced cannot be related in any way to rheumatoid arthritis, the effects of sugar-active corticoids (cortisone), and adreno-corticotropic hormone (ACTH), in inhibiting the "formalin arthritis" are of interest. After adrenalectomy, pre-treatment with desoxy-corticosterone acetate enhanced the arthritic effects of formalin, but no swellings resulted if cortisone or adreno-corticotropic hormone (ACTH) were given. Similarly, formalin joint swellings were absent from animals subjected to alarming stimuli and as sugar-active corticoids are liberated from the suprarenal as a result of alarming stimuli (Part 3, page 52), it is probable that their endogenous liberation prevented the formalin arthritic changes.

The remarkable effects of adrenal-cortical extracts (Bassi and Bassi, 1946), cortisone and adreno-corticotropic hormone (Hench, Kendal, Slocum and Polly, 1949), in rheumatoid arthritis, may be due to the fact that the first two of these substances are rich in sugar-active corticoids and the third seems to stimulate its production. Nevertheless, Selye recognises that a definite weakness in his theory, that rheumatoid arthritis and hypertension are diseases of adaptation, lies in the inability to show an increase, in blood or urine, of salt-active corticoid in those conditions or in patients exposed to stress, and the present investigation has also drawn attention to the fact that so far we are completely unaware of the mechanism which controls its formation in and liberation from the suprarenal cortex.

PART 5.

THE EFFECTS OF FREQUENT SMALL DAILY INJECTIONS OF 1:1000 ADRENALIN HYDROCHLORIDE ON THE VASCULAR SYSTEM AND SUPRARENALS OF ADULT MALE RATS.

PART 5.

THE EFFECTS OF FREQUENT SMALL DAILY INJECTIONS OF 1:1000 ADRENALIN HYDROCHLORIDE ON THE VASCULAR SYSTEM AND SUPRARENALS OF ADULT MALE RATS.

It has been noted previously (Part 2) that subcutaneous injections of adrenalin, at 2 or 3 day intervals, failed to produce renal arterial lesions comparable with those found in human cases of phaeochromocytoma, but after each injection there was an immediate and long sustained secretion of sugar-active corticoid from the suprarenal cortex (Part 3). Although no evidence of salt activity could be found after the injections, it has been shown conclusively (Part 4) that graded amounts of the salt-active compound, implanted into animals, could induce vascular lesions similar to those seen in essential hypertension in man and identical with the lesions produced experimentally in animals by clamping the renal artery. The part played by salt-active corticoid in the extra-renal cycle of hypertension has been discussed (page 89), but it is obvious that the main weakness in the theory is the inability to demonstrate salt-active corticoids in the suprarenal secretions. In the absence of a test comparable to that used for sugar-active corticoids it is necessary to rely merely on hyalinisation of the renal arterioles as an indication of salt-active corticoid activity. When the present part of the investigation was undertaken, it occurred to me that repeated stimulation of the suprarenal with small doses of adrenalin, at more

frequent intervals, might result in the liberation of salt-active material, and it was decided to test this by injecting a series of animals with gradually increasing daily doses of adrenalin until a tolerance was developed. When this stage was reached, small injections were given twice, and later three times daily, while the animals were maintained on 1% saline drinking water, in an attempt to sensitise the kidneys to any salt-active corticoids which may be liberated from the suprarenals. During the experiment interesting changes were found in the suprarenals which led to further investigations, the results of which are included in Group 2 of this section.

INVESTIGATION.

The animals used were adult male rats and they were divided into two groups each of 10 animals, which varied in weight from 200 to 270 g.

GROUP 1: received daily subcutaneous injections of 1:1000 adrenalin hydrochloride commencing with an initial dose of 30 µg (0.5 minims). This was increased gradually at intervals of 10 days until 300 µg (5 minims) were being given. Three animals died during the preliminary adaptation phase and 7 were left and included in the test proper. They were injected with 300 µg (5 minims) daily until the reactions became slight. The first animal died 55 days and the second 75 days after commencing treatment (Table 21). The amount of adrenalin was now reduced to 180 µg (3 minims) which was given twice daily. Animal No. 3 received the initial treatment for 77 days and

180 µg twice daily for 11 days before it died. The fourth animal died the following day. It became obvious that 180 µg (3 minims) twice daily was excessive and the dose was reduced accordingly to 120 µg (2 minims) twice daily. After 45 days of this treatment the remaining animals were alive and well, so the injections were increased to 120 µg (2 minims) three times daily. They now became very apathetic, scarcely moved in the cage and it was decided to terminate the experiment. Animal No. 5 received 120 µg (2 minims) in the morning and 300 µg (5 minims) 4 hours later. It was killed one hour after the last injection. The sixth animal was rested for one week and 120 µg (2 minims) were administered; it was killed one hour after the injection. The last animal received injections of 120 µg (2 minims) 3 times a day for a further 7 days, and was killed on the eighth without having received any further injections.

Post-mortem was carried out and the heart, kidneys, liver, pancreas and suprarenals fixed and stained by the methods already described.

R E S U L T S.

The experiment lasted 150 days, the first animal had 25 severe reactions and the last 61. Four animals lost weight (Nos. 3, 5, 6 and 7) and none of the remainder showed any gain. The time elapsing between the injection and death of the animal was noted in this series; the first animal was found dead on the morning following the injection and the second died in a convulsion 10 minutes after an

injection of 300 µg (5 minims). The third had a convulsion and died 10 minutes after a second injection of 180 µg (3 minims) 1:1000 adrenalin while the fourth died 1½ hours after a similar injection. The relation between the injection and time of death in animals 5, 6, and 7 has been seen already.

CARDIAC INDEX.

The maximum cardiac index was slightly in excess of the limits of normality in all cases (Fig.90), but was not sufficiently marked to be of any significance.

POST-MORTEM CHANGES IN THE ORGANS.

The appearance of animals dying in convulsions was similar to that described in Part 2. A bloody frothy exudate came from the nose and the lungs showed mottled areas of haemorrhages throughout. Free fluid was present in both pleural cavities, and the kidneys, spleen and liver were acutely congested. No pathological abnormalities were seen in any of the organs except the suprarenals, where the cortex was studded with minute haemorrhages which were distinctly visible to the naked-eye (Nos. 3, 5 and 7).

HISTOLOGICAL CHANGES IN THE ORGANS.

The histological appearance of the kidneys, showed no evidence of hypertensive vascular lesions which might indicate salt-active corticoid activity. The only lesion present was an exudate, into the sub-capsular space, similar to that described previously (page 36, figs.23 and 24).

Suprarenals: Interesting changes were seen, however, in some suprarenals. The appearance of the glands in the first two animals differed in no way from those studied in Part 2. The first animal died about 15 hours after a severe reaction and the gland showed some osmophilisation of the outer zone, a dense osmophil-positive second, and a poorly determined third zone which fused with an enlarged densely stained fourth zone. The second animal died 10 minutes after a severe reaction and the only change noted was an increased vascularity in the region of the fourth zone. Interesting changes were seen in the suprarenals of the remaining animals. The third rat died 10 minutes after a second injection of 180 µg (3 minims) of adrenalin, and the outer zone was small, had no lipoid granules, and showed a marked contrast to the dense osmophil-positive second zone, which tailed off into an irregular third zone. The main feature here was the large number of haemorrhages present (Fig. 91). Although the next animal (No. 4) received similar treatment it died 1½ hours after the second injection. The outer zone was normal and the second large in size and densely osmophil-positive in character. An irregular haemorrhagic area was present in the middle of the zone and there was extreme congestion of the vessels at the base (Fig. 92). The fifth animal had recovered from an injection of 120 µg (2 minims) and was given 300 µg (5 minims) 4 hours later. The reaction produced was slight and the animal killed one hour later. Zone 1 was clear and Zone 2 small in size but the outer portion only was osmophil-positive. Irregular lipoid granules and numerous large irregular haemorrhages

were present in the inner portion of this zone, while the centre of the haemorrhagic area contained osmophil secretion as well as red blood cells (Fig. 93). The last zone was broken up again by dilated vascular channels. Interesting variations were seen in the last two animals. The sixth one was rested for one week, then given 120 µg (2 minims) 1:1000 adrenalin, and killed 4 hours after the injection when the suprarenal was found to differ in no way from normal, apart from a great increase in size of the fourth zone (Fig. 94). No. 7 received no injections on the day it was killed but during the previous week it had received three injections daily of 120 µg (2 minims). Multiple haemorrhages were seen once more in the inner aspect of the second zone and they extended upwards towards the surface of the gland. They were irregular in outline and remains of osmophil-positive cortical cells were present in addition to osmophil-positive secretion and red blood cells. The vessels of the inner zone again were widely dilated (Figs. 95, 96 and 97).

Pancreas: Histological changes were seen in the pancreas and they were similar in appearance to those seen in Part 2 (page 40). In all animals the fuchsinophil reaction of the acinar tissue was greater around the islets than in the inter-islet areas. This reaction was slight in 5 cases (Nos. 1, 2, 4, 5 and 6) but very marked in the remaining two (Nos. 3 and 7). Circles or halos of acinar tissue were seen around the islets, each acinus was widely dilated and packed with granules and the nucleus again pushed to the periphery.

GROUP 2: The animals in this group were injected with adrenalin in the usual manner until 240 µg (4 minims) were being given. This dose was maintained until only slight reactions were produced, and 120 µg (2 minims) administered twice daily at four-hourly intervals, for 14 days. In view of the haemorrhages caused by this treatment (page 101) and the fact that they appeared to clear up after 7 days rest, the animals were rested for 14 days to ensure that no haemorrhages were present prior to the test. Nine animals survived and, apart from the control, all were injected with 120 µg (2 minims) 1:1000 adrenalin hydrochloride, and killed at intervals of ½, 1, 2 and 3 hours as shown in Table 22. No animal was killed at 4 hours, and the 4 remaining ones received a further injection of 120 µg (2 minims) and were killed at intervals of ½, 1, 2 and 4 hours after the second injection. The animals were killed in the usual manner (Part 3), blood taken for sugar estimation and the liver fixed and stained for glycogen. The suprarenals were treated with osmic acid and cut serially at 5 µ as described previously (page 55).

R E S U L T S.

BLOOD SUGAR.

The blood sugar determination in the control animal was 104 mg.%. It rose slowly during the first ½ hour to 129 mg.% and after 1 hour it was 172 mg.%. The highest reading (207 mg.%) was reached in 2 hours and 1 hour later it had fallen to 168 mg.%. The first animal of the second series (No. 6, Table 22) had a blood sugar level of 198 mg.% which was considerably greater than that seen at

a similar period in the first series (129 mg.%). It continued to rise in the next ½ hour and reached its maximum at 225 mg.%, when it began to fall. Two hours after the injection it was 161 mg.% and had returned to normal in 4 hours (114 mg.%).

LIVER GLYCOGEN.

Using the histological technique already described (Part 3), the liver of all animals in the first series showed no evidence of any glycogen depletion. It was still normal ½ and 1 hour after the second injection (Figs. 98A and B); but a transformation occurred at 2 and 4 hours. The glycogen content of both was greatly depleted, and it was seen only in the mid-zone region (Figs. 98C and D). There was never any evidence of regeneration.

SUPRARENALS.

The changes seen in the suprarenals of the first series after an injection of 2 minims of adrenalin are of no particular significance. They showed a gradual osmophilisation of the outer zone during the first and second hours with a return to normal at 3 hours. Vacuolation of the cells of Zone 2 was present and a broadening of the fourth zone was seen. None of the glands showed haemorrhagic lesions but osmophil material was seen in the medulla of all cases. Significant changes were seen after ½ hour when the second series of animals was examined. The outer zone contained very few osmophil-positive vacuoles and a large irregular haemorrhagic area was present and occupied the region of the second and third zones. Only occasional osmophil-positive cells were seen scattered throughout those

zones (Fig. 99A). There was commencing osmophilisation of Zone 1 after one hour and Zone 2 was weakly osmophil-positive and gradually merged with a small third. The last zone was greatly widened and the cells near the medullary border separated by dilated capillaries (Fig. 99B). Two hours after the injection the gland had an appearance similar to the previous one. Osmophilisation was less marked in the outer zone but more marked in the second. The third was small and irregular and the last broad but peculiarly weak in osmophil granules (Fig. 99C). No haemorrhages were seen in those two cases. The last gland (4 hours) had a distinctive appearance. There was a dense rim of osmophil cells on the surface of the gland (Zone 1), while the second zone was almost devoid of such cells, and irregular haemorrhagic areas again occupied the inner region of the second and outer aspect of the third zone. The fourth was still broad and intensely osmophil-positive (Fig. 99D). Osmophil material was present in the medulla in all cases.

DISCUSSION.

Although the dose was much smaller than was used previously, there was still no evidence of any hypertensive kidney lesion, and the experiment showed that multiple injections of 120 µg (2 minims) 1:1000 adrenalin hydrochloride did not appear to liberate sufficient, if any, salt-active corticoid to cause vascular damage, in spite of the fact that the kidney was sensitised by 1% saline in the drinking water. Whether more minute doses, such as 30 µg (0.5 minims) or less over a longer period would produce this effect is unknown, for

circumstances did not permit this investigation to be carried out. Thus, it has to be admitted that the experiments failed to uncover the mechanism responsible for liberating the salt-active corticoid.

The effects of double or treble injections of 120 µg (2 minims) adrenalin at four-hourly intervals showed that the suprarenals were liable to develop haemorrhagic lesions between the second and third zones of the suprarenal cortex. Occasionally the lesions involved the whole of Zone 2 and extended upwards to the capsule. Single injections of this amount never produced those results and the lesions apparently were reversible as shown by the normal appearance of one gland (No. 5, Table 21), when the animal was rested for one week before it was killed. This was confirmed by the normal appearance of the glands in the first four animals of Group 2 (Table 22) when only one injection was given.

Haemorrhages into the zona fasciculata and reticularis of the suprarenals have been reported in a variety of conditions. They were found in pantothenic acid deficient rats (Deane and McKibbin, 1946) and in female rats subjected to the crush syndrome (Popjak, 1944). Minute to moderate adrenal haemorrhages are known to occur in acute infectious diseases, measles, scarlet and typhoid fever (Soffer, 1945), and have been found in acute leukaemia (Lauckner and Hebbert, 1947), haemophilia and purpura. Massive haemorrhages are found sometimes in the suprarenals of new born infants, after a difficult labour, but extensive haemorrhages into the suprarenals are generally associated with massive invasion with the meningococcus,

when the whole gland is converted into a bloody mass. Those lesions are associated with the Waterhouse-Friderichsen syndrome, which is characterised by peripheral vascular failure and shock.

Various theories have been put forward to explain the lesions. Since they occurred more commonly in infants suffering from meningococcal infection, they were believed to be due to morphological differences in the adrenal vessels in children and adults (Costa and Severi, 1936). It has also been suggested that bacterial toxins have a selective destruction on the vascular endothelium of the adrenals, and Kinsman, D'Alonso and Russi (1946) believe there is widespread injury of many tissues, including the adrenals, as the result of bacterial production, which is associated with early central movement of the suprarenal cells. A combination of those factors is responsible for the haemorrhages.

The present investigation throws some light on the mechanism. In Group 2 animals, killed at intervals of 1 to 8 hours, those receiving only one injection had a normal blood sugar curve. When the sugar curve of an adrenalectomised animal was superimposed, it was shown clearly, in spite of the differences in doses, that the adrenalectomised animals could not mobilise blood sugar in the same manner as the non-adrenalectomised (Fig. 100). The liver glycogen of those animals was normal, and although the suprarenals varied little from normal, they were very active in mobilising blood sugar, by liberating sugar-active corticoids. When the animals received the double injection, two suprarenals were haemorrhagic and there was depletion

of osmophil material from the second zone and an accumulation of it in the fourth. Those changes were associated with failure to regenerate liver glycogen, and the blood sugar curve behaved like that of an adrenalectomised animal (Fig. 100). Indeed when the suprarenal, liver glycogen and blood sugar changes were correlated, it was seen that we were, in fact, dealing with an animal, which to all intent and purpose was acting as if it had been adrenalectomised. The effect of double injections of adrenalin, though not sufficient to cause outward reactions, gave rise to a suprarenal cortical insufficiency characterised by failure to mobilise blood sugar and inability to regenerate liver glycogen. Those changes were associated with a great increase in size of the osmophil-positive fourth zone of the suprarenal, and would support the view that the suprarenal cells are constantly moving centrally and, in this case, in an exaggerated manner. It has been suggested (Zwemer and Lowenstein) that zone 4 may be the site of formation of water soluble sugar-active corticoids. The present investigation would appear to disprove this. If the osmophil-positive material in Zone 4 was sugar-active corticoid, there should have been ample evidence of mobilisation of blood sugar and regeneration of liver glycogen. Since this was not found to be the case, I believe that the large osmophil-positive fourth zone does represent degenerating cortical cells at the end phase of their aging process.

It was shown, also, that Zone 2 was depleted of osmophil-positive material and the vessels in Zones 2, 3 and 4 were loosely supported. It is my opinion that the poorly supported vessels

ruptured when the partially exhausted gland was subjected to a sudden rise of blood pressure following the second injection of adrenalin. Although two of the glands did not show haemorrhages, there was definite evidence of exhaustion in one (No. 3, 2 hours) since the blood sugar was falling and there was no evidence of regeneration of liver glycogen. In addition it must be appreciated that individual variations in reaction to haemorrhage will be present.

An explanation of the haemorrhages in the Waterhouse-Friderichsen's syndrome now becomes clearer. In most conditions giving rise to this syndrome there is usually a haemorrhagic diathesis, or a toxaemia which may effect all vascular endothelium causing increased permeability. It has been shown that the suprarenal cortex responds to and is stimulated by various non-specific stimuli such as toxins, low oxygen tension and trauma (Selye, 1946). In view of the suprarenal changes described in the present investigation it is now suggested that a combination of haemorrhagic diathesis, increased vascular permeability, toxaemia and cortical suprarenal exhaustion associated with excess central movement of the cells lead to loosely supported adrenal cortical vessels which eventually rupture. The rupture will take place between the 2nd and 3rd zones where the vessels are mostly loosely supported and extend upwards to the capsule and downwards to medulla, and the amount of haemorrhage present will vary with the intensity of these factors. In addition, any sudden rise of blood pressure will be an initiating factor.

A similar basis can be found for the haemorrhages in acute

leukaemia, haemophilia and prolonged labour. In all cases an exhausted suprarenal plays an important part in creating loosely-held cortical vessels which eventually rupture, and in addition a haemorrhagic diathesis exists in the first two.

An interesting point, arising from this investigation, is the action of injected adrenalin on the suprarenal cortex. When administered in large doses sufficient to produce a severe reaction, the cortex immediately liberates large quantities of sugar-active corticoids. The amount so liberated is far in excess of that which can ever be given therapeutically in the form of cortical extract, and supports the view of Selye (1946) that the failure of administered cortical extract to control shock is due to inability to administer sufficient amounts.

When adrenalin is given experimentally in double or treble daily doses, insufficient to cause visible reactions, the suprarenal may become exhausted and cortical insufficiency result. This action of adrenalin may have definite clinical applications. Intravenous adrenalin drips are frequently used in post-operative control of blood pressure, and the function of adrenalin in this respect has been attributed mainly to a direct action on the blood vessels. In view of this investigation it now becomes extremely probable that post-operative control of blood pressure is due to the action of adrenalin in liberating suprarenal cortical hormone. Likewise, the so-called success of massive doses of adrenalin in controlling peripheral circulatory failure (Bryant et al, 1947) is in all

probability due to liberation of cortical hormone in amounts far in excess of that which can be administered therapeutically.

The investigation also shows the possible disasters of over-administration of adrenalin, namely, suprarenal insufficiency and haemorrhage. Considerable care, therefore, should be exercised in the administration of adrenalin to cases of meningococcal septicaemia. If the suprarenal has not been overstimulated or the disease has not lasted too long, moderate adrenalin therapy will be an advantage. If the condition is far advanced and the gland already in a state of exhaustion, adrenalin will merely hasten the advent of suprarenal haemorrhages and cortical insufficiency.

The action of non-specific stimuli in causing production of sugar-active corticoid, with elevation of blood sugar, may have a practical application as well. In meningococcal meningitis with suprarenal stimulation there should be an elevation of blood sugar as long as the cortex is active, and a transitory diabetic syndrome has been noted in this condition by numerous workers (Fox, Kuzina and Washman, 1947). When insufficiency and haemorrhage results the blood sugar will fall and the patient behave like an adrenalectomised animal. High blood sugar levels were noted in one of the six cases reported by Kinsman, D'Alonso et al, but blood sugar determinations were not carried out at intervals in those cases, when they would have given some indication of the activity of the suprarenal. An elevation of blood sugar might be expected in all conditions causing the Waterhouse-Friderichsen syndrome.

SUMMARY.

S U M M A R Y.

The chrome reaction is demonstrated and an explanation given of the reaction. The in-vitro results suggest that adrenalin is held in the phaeochromocyte cell as a protein complex which breaks down with disintegration of the cell and liberation of the active pressor substance directly into the blood stream, via the sinusoidal spaces. The adrenalin so liberated is responsible for the attacks of paroxysmal hypertension which occur in the adreno-sympathetic syndrome.

There is no doubt that patients with chromaffin tumours may progress in time to a persistent type of hypertension which is associated with vascular and retinal changes. However, a study of the literature on those cases indicates that the vascular damage need not be irreversible, and surgical intervention is always indicated although it is better to operate as early as possible. The aetiology of chronic hypertension, associated with chromaffin tumours, like that of essential hypertension, is still unsolved, but new suggestions are put forward in an attempt to correlate recent experimental findings.

There is no doubt that injections of adrenalin into experimental animals result in an immediate and sustained liberation of sugar-active corticoid from the suprarenal cortex. The amount liberated appears to be considerable and represents the counter-shock phase of Selye's alarm reaction. At no time was there any evidence of the liberation of salt-active corticoid which produces vascular lesions in the kidney similar to those caused by the Goldblatt clamp. It must

be noted that the method of detecting salt-active corticoid activity is not so satisfactory as that used for sugar-active corticoid, and until the mechanism governing the liberation of salt-active corticoid is known, the part played by the suprarenal in hypertension must still remain obscure.

In a final attempt to uncover this mechanism by injecting multiple daily doses of adrenalin, haemorrhagic lesions were seen in the suprarenals. The relationship between those haemorrhages and the Waterhouse-Friderichsen syndrome is discussed and an explanation of the syndrome suggested.

ACKNOWLEDGMENTS.

The work was carried out in the Pathology Department of the Glasgow Royal Infirmary, and my thanks are due to Professor J.W.S. Blacklock for his criticism of the work on Phaeochromocytoma, and to the Clinicians mentioned in Appendix II for permission to use their case reports. The mercury manometer (Fig. 21) was built by Dr. Geo. Edwards, Ph.D., Royal Technical College, Glasgow, and the "DOCA" implants supplied by Dr. Tyndall of the Organon Laboratories. I am grateful to Mr. A. Goodall for his assistance with the adrenalectomies and implants, and to Mr. D. Hay for assistance with the photographs of Figs. 1 to 21. The paintings, Figs. 8 and 10, and the rest of the photographs were personal efforts. The suprarenals stained by osmic acid and liver glycogen sections were photographed

using a double red filter and P300 Kodak plates. Velox WSG I S paper was used for printing. The work was carried out while in receipt of a Rankin Research Grant.

REFERENCES.

1. Babes and Jonescu (1908) quoted Vogt (1945).
2. Bachmann, R. (1937), Zeitsch f Mikr-anat Forsch, 41, 433.
3. Baker, B.M. and Reinhoff, W.F. (1937), quoted by Howard and Barker (1937).
4. Bassi and Bassi (1946) quoted by Selye (1949), Brit. Med. J., Nov. 19th, 1129.
5. Bauer, J. and Leriche, R. (1934), Presse Med., 42, 1385.
6. Beer, E., King, F. and Prinzmetal, M. (1937), Ann. Surg., 106, 85.
7. Bennett, H.S. (1940), Amer. J. Anat., 67, 151.
8. Bergman, H.V. and Klein, D. (1943), Endocrinology, 33, 174.
9. Biskind, G.R., Meyer, M.A. and Beadner, S. (1941), J. clin. Endocrinology, 1, 113.
10. Blacklock, J.W.S., Ferguson, J.W., Mack, W.S., Shafar, J. and Symington, T. (1947), Brit. J. Surg., 35, 179.
11. Boman, P.G. and Wells, A.H. (1937), J. Amer. Med. Ass., 109, 1176.
12. Boyd, W. (1946), Pathology Internal Diseases.
13. Brunschwig, A.E. and Humphreys, E. (1940), J. Amer. Med. Ass., 115, 355.
14. Byrom, F.B. and Wilson, C. (1939), Lancet, 1, 139.
15. Calma, I. and Foster, C.L. (1943), Nature (London), 152, 536.

16. Christie, C.C., Bowden, T.M., Mackenzie, I.R., Naismith, J. and Bryant, J. (1947), Lancet, 2, 206.

17. Coller, F.A., Field, H.Jr., and Durant, T.M. (1934), Arch. Surg., 28, 1136.

18. Costa and Severi (1936) quoted by Stigliani (1946), Abstracts World Medicine, 1947, 1, 320.

19. Cramer, W. (1926), Brit. J. Exper. Path., 7, 88.

20. Cramer, W. (1926), Brit. J. Exper. Path., 7, 95.

21. Cramer, W. (1918), J. Physiol., 52, 8.

22. Darrow, D.C. and Millar, H.C. (1942), J. Clin. Investigation, 21, 601.

23. Deane, H.W. and Bergner, G.E. (1947), Endocrinology, 40, suppl.

24. Deane, H.W. and McKibbin, J.M. (1946), Endocrinology, 38, 385.

25. Deane, H.W., Nesbett, F.B. and Hastings, A.B. (1946), Proc. Soc. Exp. Biol. & Med., 63, 401.

26. Deane, H.W., Shaw, J.H. and Greep, R.O. (1948), Endocrinology, 43, 133.

27. Deansley, R. (1931), Am. J. Anat., 47, 475.

28. Dejean, C. (1947), Presse Med., 55, 439.

29. Dejean, C., Mahoudean, D. and Bricaire, H. (1947), Bull. Soc. med. Hop. Paris, 63, 532.

30. Edward, D.G. (1937), J. Path. Bact., 45, 391.

31. Eisenberg, A.A. and Wallerstein, H. (1932), Arch. Path., 14, 818.

32. Engel, R.L., Mencher, W.H. and Engel, G.L. (1942), J. Amer. Med. Ass., 204, 649.

33. Evans, W.F. and Stewart, H.J. (1942), Amer. Heart J., 28, 835.

34. Fishberg, A.M. and Oppenheimer, B.S. (1924), Arch. Int. Med., 34, 631.

35. Flexner, L.B. and Grollman, A. (1939), Anat. Rec., 75, 207.

36. Fox, M.J., Kuzina, J.F. and Washman, W.T. (1947), Arch. Int. Med., 79, 614.

37. Freeman, N.F., Freedman, H. and Miller, C.C. (1940), Amer. J. Physiol., 131, 545.

38. Friedman, B., Somkin, E. and Oppenheimer, E.T. (1939), Amer. J. Physiol., 128, 481.

39. Good, C.A., Kramer, H. and Somogi, M. (1933), J. Biol. Chem., 100, 483.

40. Goldblatt, H. (1937-38), Ann. Int. Med., 11, 69.

41. Goormaghtigh, N. (1940), Amer. J. Path., 16, 409-416.

42. Goormaghtigh, N. (1945), J. Path. Bact., 57, 392-393.

43. Graham , G.S. (1916), J. Med. Research, 34, 241.

44. Grattan, J.F., and Jensen, H. (1940), J. Biol. Chem., 135, 511.

45. Grattan, J.F., Jensen, H. and Ingle, D.J. (1941), Amer. J. Physiol., 134, 8.

46. Griffith, J.Q. and Farris, E.J. - The Rat in Laboratory Investigation. Lippincott Company (Philadelphia, Montreal, London). Page 275.

47. Green, D.M. (1946), J. Amer. Med. Ass., 131, 1260.

48. Greep, R.O. and Deane, H.W. (1947), Endocrinology, 40, 417.

49. Gross, H. and Greenberg, S.S. (1944), Amer. Heart J., 27, 186.

50. Grollman, A. (1936). The Adrenals. Baltimore, Williams and Wilkins Co. Page 3.

51. Gutmann, D. (1947), Brit. Med. J., April 26th, 563.

52. Hench, P.S., Kendall, E.C. Slocum, C.H. and Polley, H.P. (1949), Quoted by Selye (1949), Brit. Med. J., Nov. 19th, 1129.

53. Henle, J. (1865), Z. Rationelle Med., 25, 142.

54. Hick, F. (1933), Arch. Path., 15, 665.

55. Hoerr, N.L. (1931), Amer. J. Anat., 48, 139.

56. Hoerr, N.L. (1936), Anat. Rec., 66, 149.

57. Holst, E.J. (1938), Acta. Med. Scand., 94, 510.

58. Houssay, B.A. and Dexter, L. (1942), Ann. Int. Med., 17, 451.

59. Howard, J.E. and Barker, W.H. (1937), Bull. Johns Hopk. Hosp., 61, 371.

60. Ingle, D.J. (1936), Amer. J. Physiol., 116, 633.

61. Ingle, D.J. (1944), Endocrinology, 34, 191.

62. Kinsman, J.M. D'Alonso, C.A. and Russi, S. (1946), Arch. Int. Med., 78, 139.

63. Kirshbaum, J.D. and Balkin, R.B. (1942), Ann. Surg., 116, 54.

64. Kremer, D.N. (1936), Arch. intern. Med., 57, 999.

65. Labbé, M., Tinel, J. and Doumer (1922), Bull. Soc. Méd. Hop. Paris, 46, 882.

66. Labbé, M., Violle, P.L. and Azerad, E. (1929), Bull et mém. Soc. Méd. d'hop de Paris, 53, 952.

67. Laroche, G. (1947), Bull. et mém. Soc. méd. d'hop de Paris, 63, 616.

68. Laubrey, C. and Bernal, P. (1934), Bull et mém. Soc. d'hop de Paris, 50, 658.

69. Lauckner, J.R. and Hebbert, F.J. (1947), Glas. Med. J., 28, 366.

70. Long, C.N.H., Katzin, B. and Fry, E.G. (1940), Endocrinology, 26, 309.

71. Long, C.N.H. (1942), Endocrinology, 30, 872.

72. McCullagh, E.P. and Engel, W.J. (1942), Ann. Surg., 116, 61.

73. McKenzie, D.W. and McEachern, D. (1938), J. Urol., 40, 467.

74. Manasse, P. (1893), Virchow Arch f Path. Anat., 133, 391.

75. Mitchell, A.J. and Wislocki, G.B. (1944), Anat. Rec., 90, 261.

76. Moritz, A.R. and Oldt, M.R. (1937), Am. J. Path., 13, 679.

77. Mullen, J.P. (1944), Am. J. Clin. Path. Tech. Sect., 8, 9.

78. Neuser, E. and Wiesel, J. Quoted by Eisenberg and Wallerstein.

79. Oberlung, C. and Jung, G. (1927), Bull. Soc d'obst. et gynec., 16, 279.

80. Ogata, T. and Ogata, A. (1917), J. Exp. Med., 25, 807.

81. Palmer, R.S. and Castleman, B. (1938), New Engl. J. Med., 219, 793.

82. Paul, F. (1931), Virchow Arch., 282, 256.

83. Pearce, R.M. and Stanton, E.McD. (1906), J. Exp. Med., 8, 74.

84. Peyron, A. (1930), Bull. Assoc. franc p'l étude du cancer, 19, 618.

85. Pincoff, M.C. (1929), Trans. Ass. Amer. Physiol., 44, 295.

86. Popjak, G. (1944), J. Path. Bact., 56, 485.

87. Rabin, C.B. (1929), Arch. Path., 7, 228.

88. Reinecke, R.M. and Kendall, E.C. (1942), Endocrinology, 31. 573.

89. Reinecke, R.M. and Kendall, E.C. (1943), Endocrinology, 32, 505.

90. Remington, J.W., Collings, W.D., Hays, H.W. and Swingle, W.W. (1941), Amer. J. Physiol, 123, 622.

91. Rodin, F.H. (1945), Arch. Ophthal. N.Y., 34, 402.

92. Rogers, E. (1932), Amer. Heart Journ., 8, 269.

93. Schmorl, G. (1928), Die Pathologisch-Histologischen Untersuchungsmethoden, 355. Leipzig: F.C.W. Vogel.

94. Sevki, K. (1934), Virchows Archives, 294, 65.

95. Selye, H. (1946), J. Clin. Endocrin., Feb. 117.

96. Selye, H. and Dosne, C. (1939), Proc. Soc. Biol. Med., 42, 580.

97. Selye, H. and Hall, C.E. (1944), Am. Heart J., 27, 338.

98. Selye, H. and Pentz, E.J. (1943), Can. M.A.J., 49, 264.

99. Selye, H. (1949), Brit. Med. J., Nov. 19th, 1129.

100. Shaw, F.H. (1938), J. Biochem., 32, 19.

101. Soffer, L.J. (1945), Diseases of Adrenals. London (H. Kimpton).

102. Soffer, L.J., Engel, F.L. and Oppenheimer, B.S. (1940), J. A. M. A. 115, 1860.

103. Spalding, J.M.K. (1947), Brit. Med. J., April 26th, 564.

104. Strombeck, J.P. and Hedberg, T.P. (1939), Acta. Chir. Scand. 82, 177.

105. Suermondt, W.F. (1934), Zentral f Chir, 61, 70.

106. Tepperman, J., Engel, F.L. and Long, C.N.H. (1943), Endocrinology, 32, 373.

107. Thorn, G.W., Hindle, J.A. and Sandmeyer, J.A. (1944), Ann. intern. Med., 21, 122.

108. Trueta, J., Barclay, A.E., Daniel, P.M., Franklin, K.J. and Prichard, M.M.L. (1947), Studies of the renal circulation. Blackwell Scientific Publications.

109. Van Dellen, T.R., Scupham, G.W., de Takats, G. and Fowler, E.F. (1945), Arch. intern. med., 75, 197.

110. Van Epp, E.F., Hyndman, O.R. and Greene, J.A. (1940), Arch. intern. Med., 65, 1123.

111. Vaquez, H., Donzalot, E. and Geraudel, E. (1928), Compt. rent. cong. internat. de med. trop. et d'hyg., 2, 559.

112. Venning, E.H. and Kazmin, V. (1946), Endocrinology, 39,131.

113. Verney, E.B. and Vogt, M. (1938), Quart. J. exp. Physiol., 28, 253.

114. Vogt, M. (1945), J. Physiol., 104, 60.

115. Volhard (1931) quoted by Howard and Barker (1937).

116. Williams, J.R. Jr., Diaz, J.T., Burch, J.C. and Harrison, T.R. (1939), Am. J. Med. Science, 198, 212.

117. Wilson, C. and Pickering, G.W. (1938), Clin. Sc., 3, 343.

118. Wright, S. (1940), Text-book of Applied Physiology.

119. Zwemer, R.L. and Lowenstein, B. (1940), Anat. Rec., 76, Supplement 60.

120. Zwemer, R.L., Wotton, R.M. and Norkus, M.S. (1938), Anat. Rec., 72, 249.

B O O K 2.

T H E S U P R A R E N A L G L A N D -

ITS FUNCTION IN HYPERTENSION AND THE COUNTER-SHOCK MECHANISM

by

THOMAS SYMINGTON, B.Sc.(Hons.), M.B., Ch.B.

PHOTOGRAPHS, CHARTS AND GRAPHS.

FIG 1.

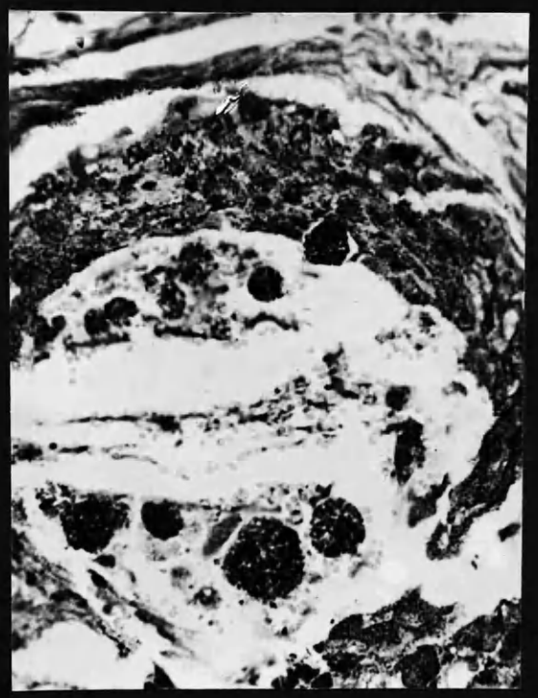


FIG 2

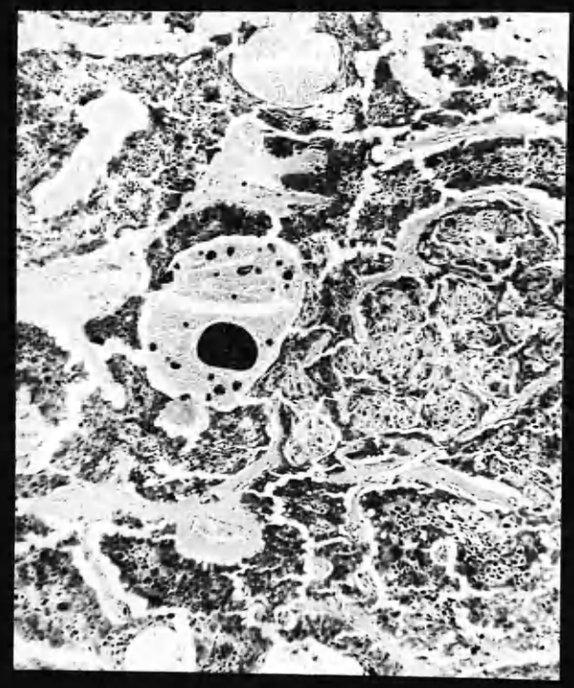


FIG 3.

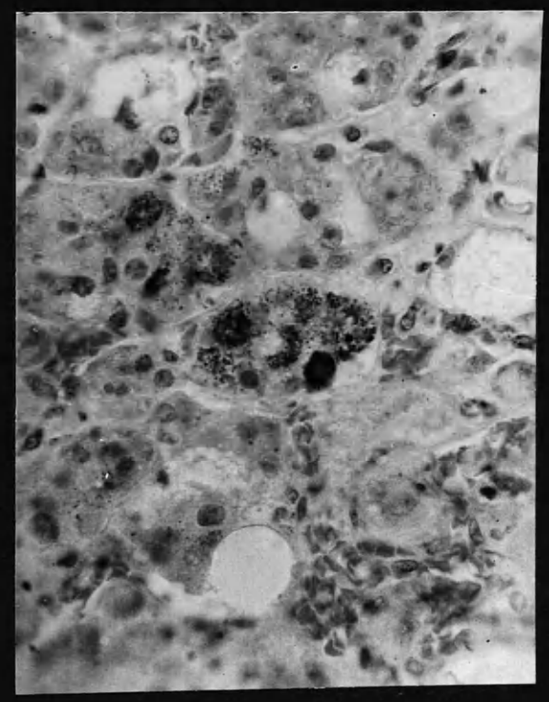


FIG. 1 - Case 2. Sinusoidal space with adrenalin granules stained black. Phaeochromocyte cells lining the space have a similar black granular deposit. Modified Bielschowsky's method. (X 250).

FIG. 2 - Case 2. Disruption of tumour cells due to poor fixation with Müller's fluid. Sevki. (X 75).

FIG. 3 - Case 4. Excellent fixation and chrome reaction using formol-dichromate. No disruption of cells. Material although 5 hours post-mortem shows adrenalin granules in cells in centre of section. Sevki. (X 500).

FIG 4

DEMONSTRATION OF THE CHROME REACTION IN VITRO USING FLANNEL.

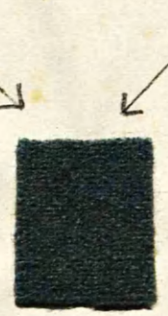
A. POSITIVE CHROME REACTION.

1. Flannel treated with 1:1000 adrenalin.

2. Treated with formol-dichromate solution. Note the 'dun' colour due to the formation of chromium dioxide.

3. Following treatment with 0.5% $KMnO_4$ and 1% oxalic acid.

4. Olive green colour when the material is stained with dilute Giemsa stain and differentiated with 95% alcohol.



5. If differentiation with 95% alcohol is continued the original 'dun' colour (No. 2) is obtained.

5a. Treatment as in No. 3 stabilises the oxide of chromium formed and prevents a reversion to the original 'dun' colour seen in No. 5.

B. NEGATIVE CHROME REACTION.

1. Flannel untreated with adrenalin.

2. Treatment with formol-dichromate solution.

3. After staining with dilute Giemsa solution a blue colour is obtained comparable to that seen in chromaffin tumours when no adrenalin is present.

FIG 5

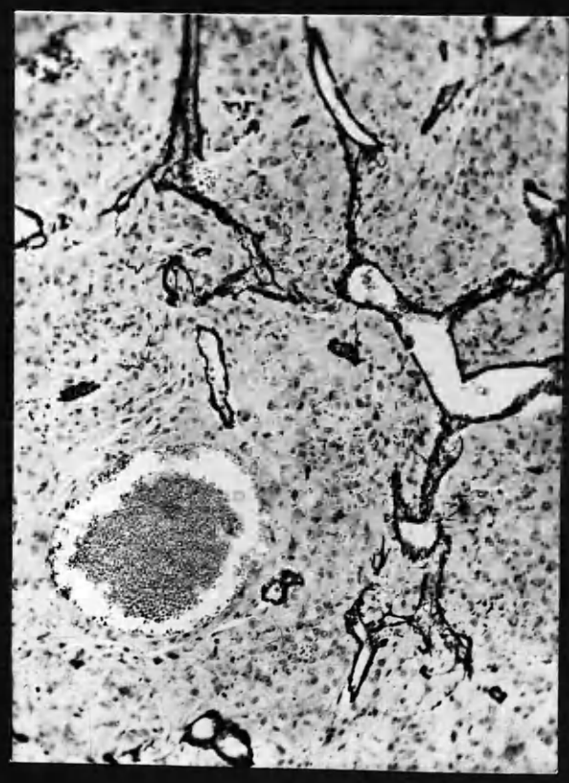


FIG 6.

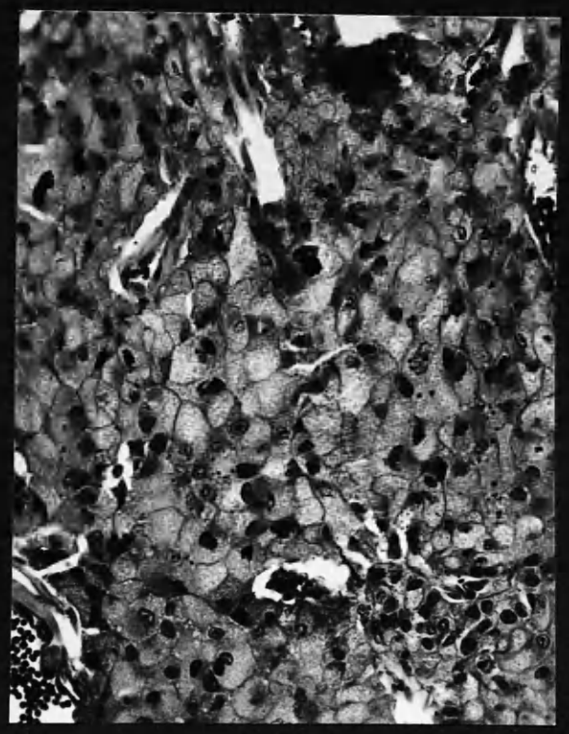


FIG 7.

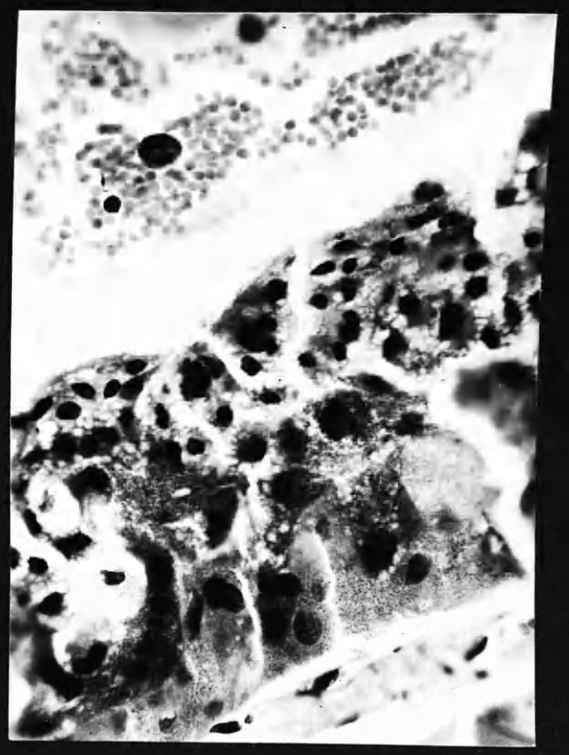


FIG. 5 - Case 2. Large sinusoidal space, lined only by tumour cells seen at bottom left. Many blood capillaries, composed of irregular spaces lined by reticular basement membrane, seen above and to right. Bielschowsky's stain. (X 150).

FIG. 6 - Case 2. Shows the finely granular appearance of the phaeochromocyte cell. Fixative formol-corrosive. Modified Masson's trichrome. (X 250).

FIG. 7 - Case 2. Part of a sinusoidal space, at top. Fine granules in cells at bottom right. Cells in neighbourhood of sinusoidal space are vacuolated and free from granules. Sevki. (X 500).

Fig 8.

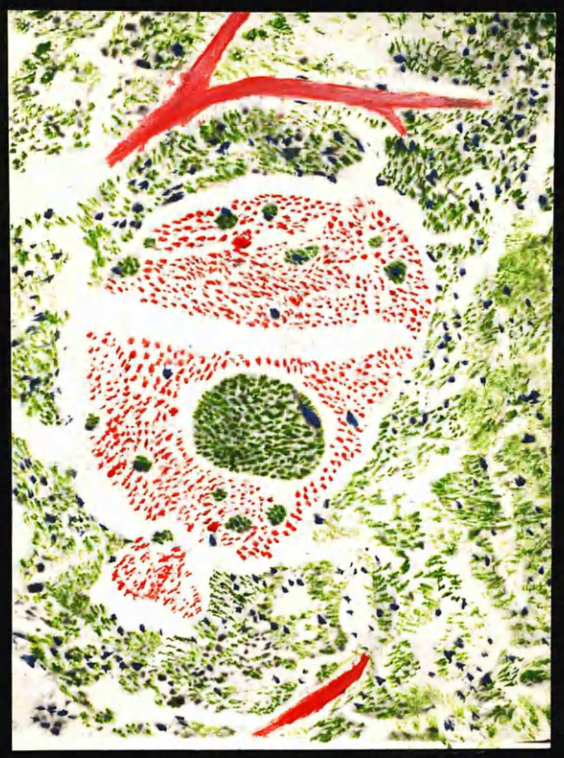


Fig 9.

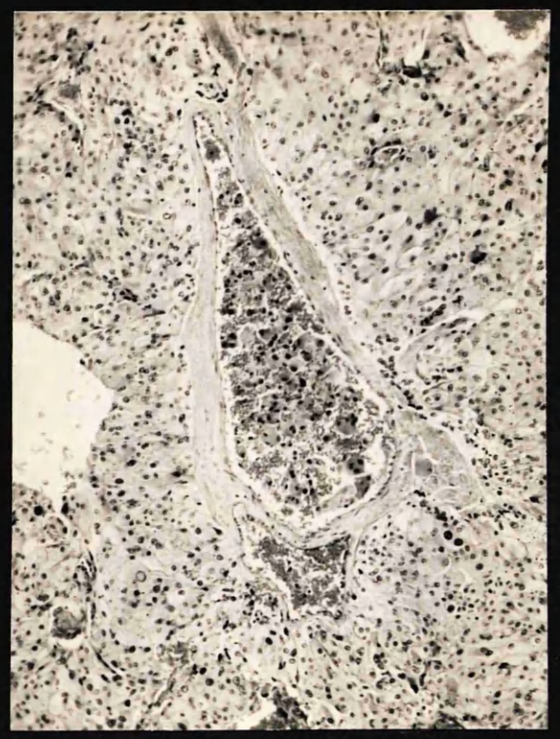


Fig 10.

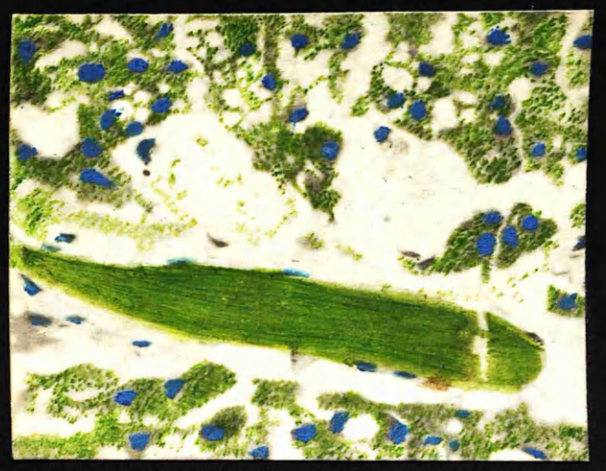


FIG. 8 - Case 2. Sinusoidal space containing red blood cells and tumour cells full of pro-adrenalin granules. Modified Sevki. (X 150).

FIG. 9 - Case 2. Tumour cells in lumen of thick-walled vein. H. & E. (X 100).

FIG.10 - Case 1. Cells mainly vacuolated, only a few are granular. Green staining fluid is seen in the efferent vein. Sevki. (X 500).

Fig 11 a.

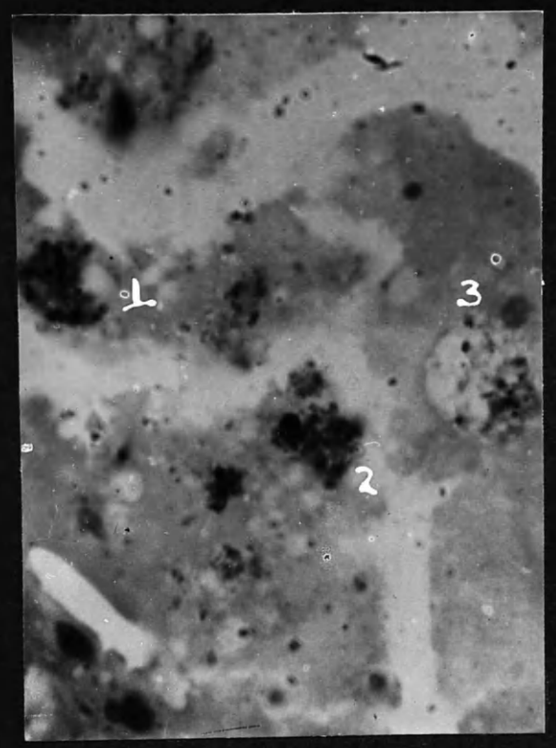


Fig 11 b.

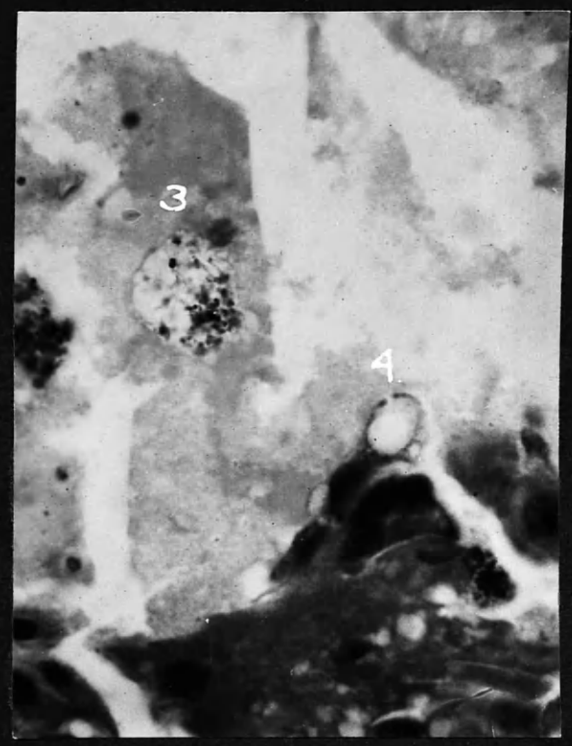


FIG. 11 (a & b) - Case 1. Vacuolated space containing disintegrating phaeochromocyte cells. Cell No. 1 is just beginning to break up while No. 2 is at a more advanced stage. The process is almost complete at No. 3. A few granules can be seen at bottom right corner of this cell. The disintegration is complete at No. 4, a non-staining vacuole only remains. Sevki. (X 900).

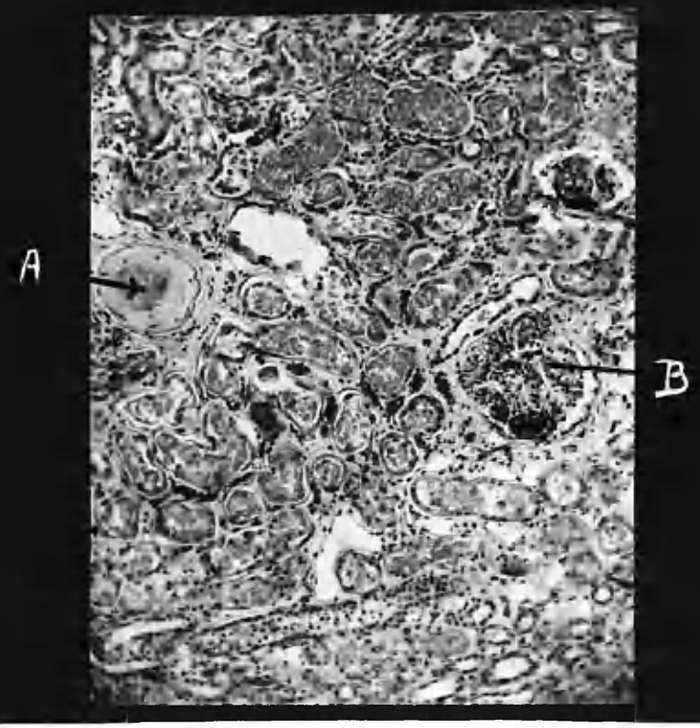


FIG.12-Case 1. Sclerosed glomerulus seen to left of field (A). One to the right is enlarged and adherent to capsule (B). H. & E. (X 150).

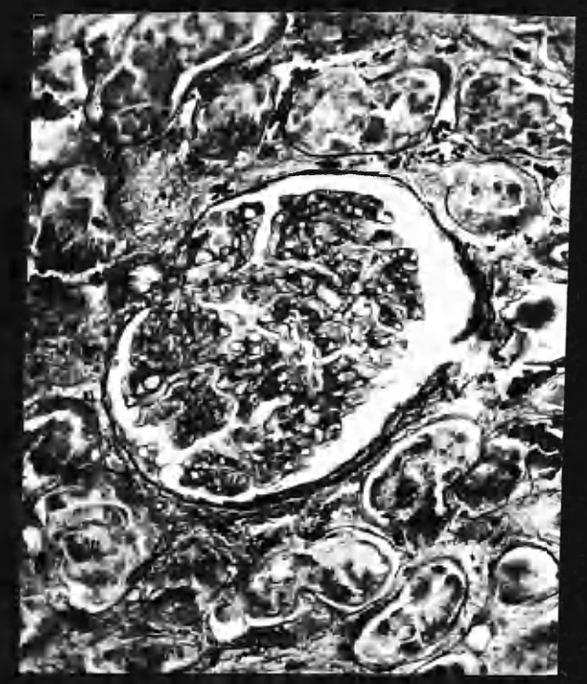


FIG.13-Case 1. Glomerulus showing thickening of wall of afferent arteriole, glomerular capillaries and Bowman's capsule. Mallory. (X 200)

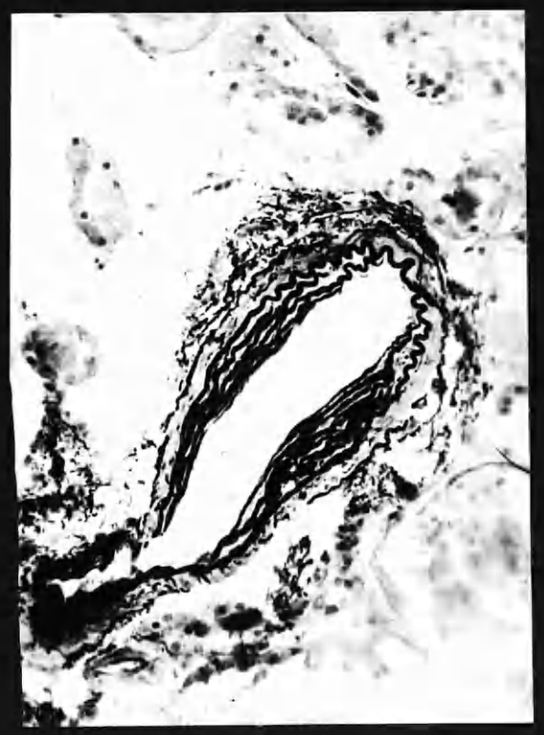


FIG.14-Case 1. Interlobular artery of kidney showing marked reduplication of internal elastic lamina. Weigert-Van Giesen. (X 150).

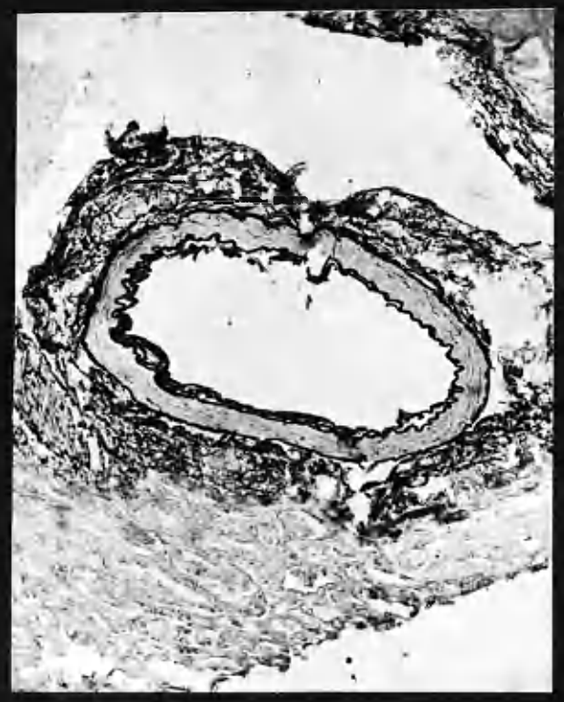


FIG.15-Case 1. Arcuate artery showing reduplication of internal elastic lamina (kidney). Weigert-VanGiesen. (X 75).

FIG 17.

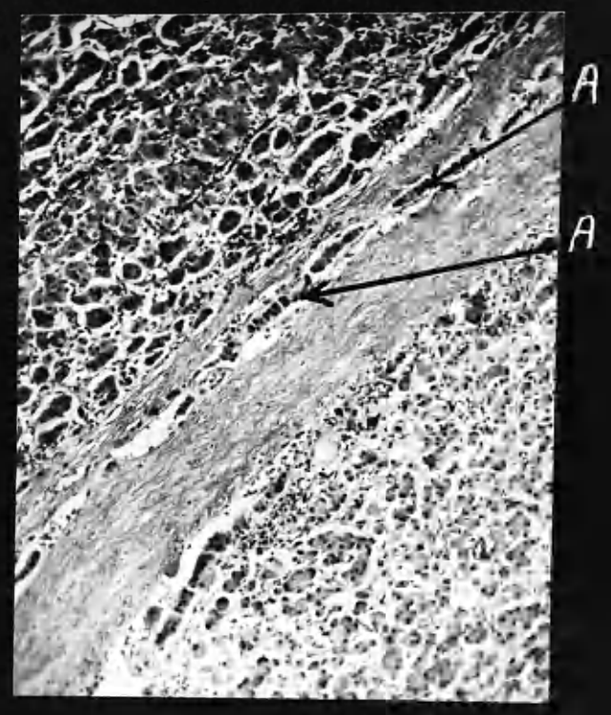


FIG 16.

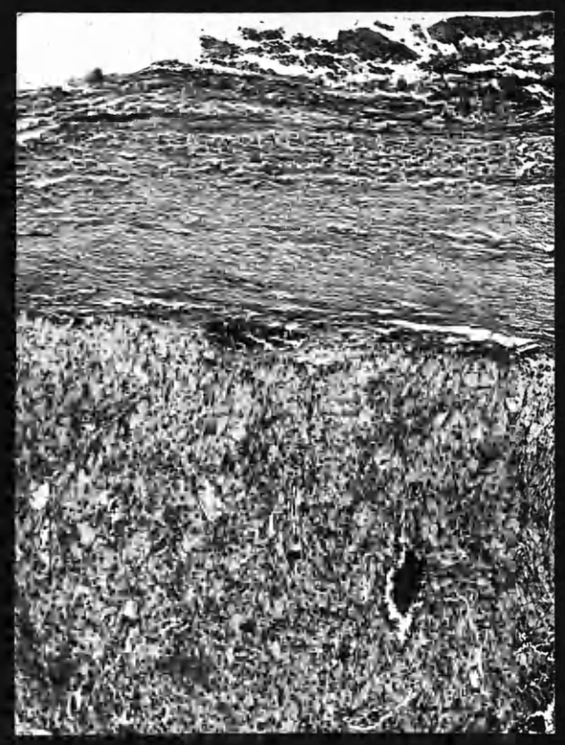


FIG 18.

FIG.16 - Case 4. Tumour to right and below. Cortex to left and above. Note group of Cortical cells (A) enclosed in fibrous capsule. H. & E. (X 100).

FIG.17 - Case 2. Suprarenal tumour, showing rim of cortical tissue, above and below. (X 2/3).

FIG.18 - Case 2. Thick fibrous capsule over the tumour. Remains of cortical tissue seen A. H. & E. (X 75).

FIG 20.

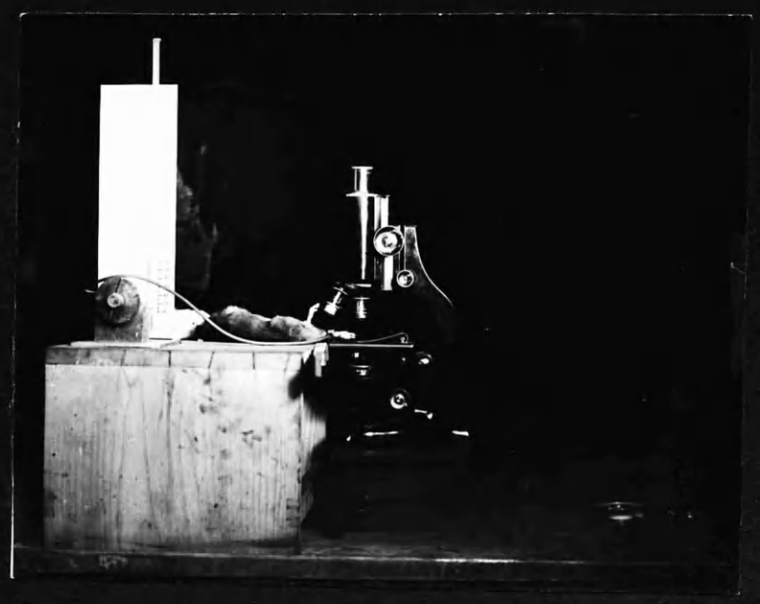


FIG 19.

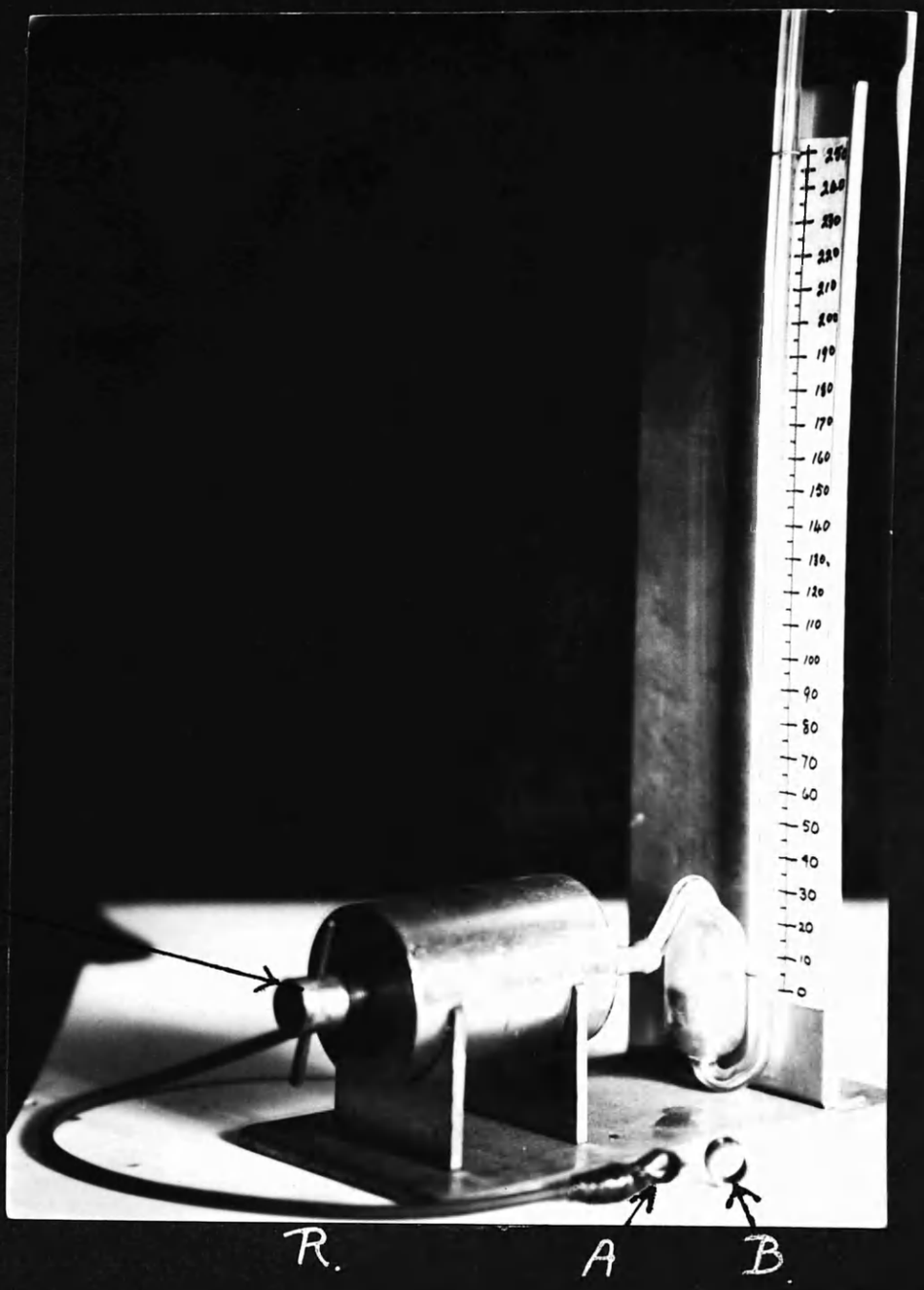


FIG.19 - Apparatus in position when used to determine the blood pressure of rats.
FIG.20 - Web of foot fixed on a glass slide under microscope (10mm ocular 16mm objective).
FIG.21 - Manometer with rubber cuff (A) which fits round the animal's leg. Outside metal cuff (B) fits round (A).

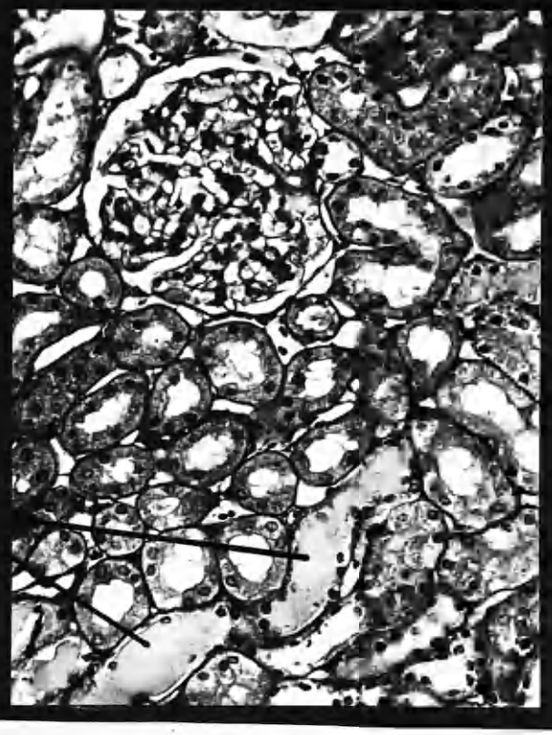


FIG.22 - Kidney showing marked patency of glomerular capillaries. Note casts in 2 tubules (A) at bottom. H. & E. (X 250).

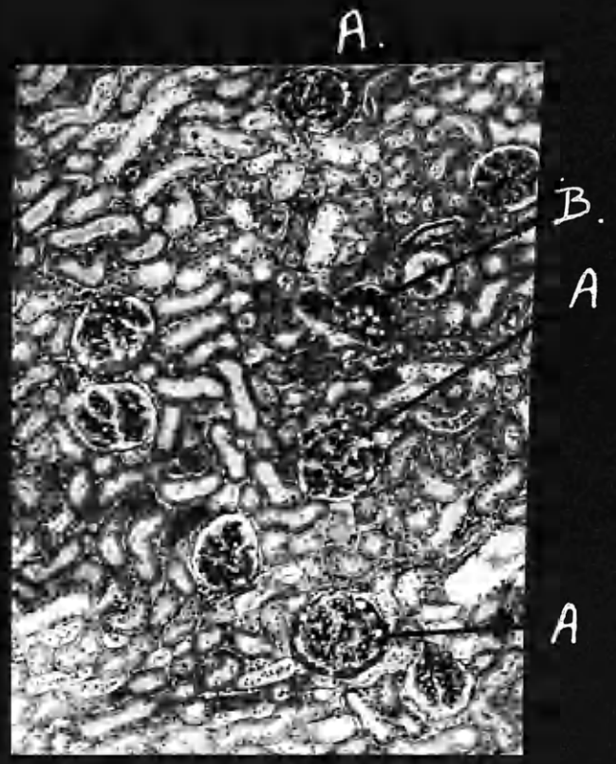


FIG.23 - Glomeruli (A) with exudate in sub-capsular space. Partial disappear-ance of tuft seen in (B). Gallego (X100).

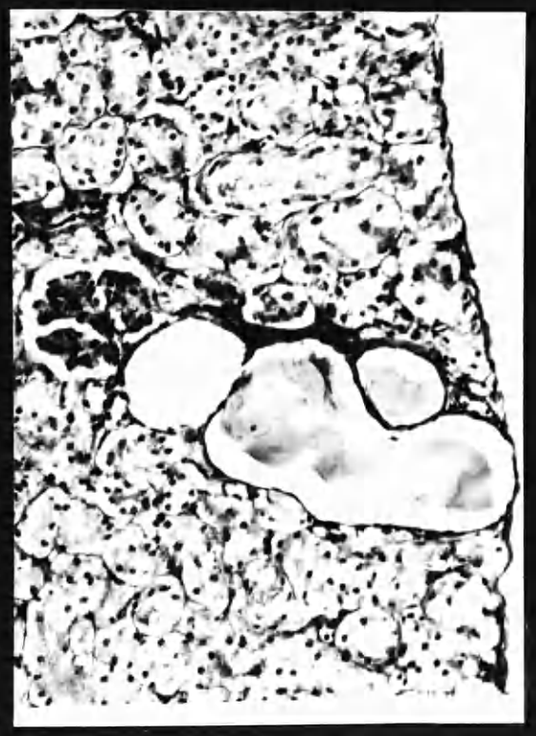


FIG.24 - Kidney. Dilated tubule showing flattening of the epithelium and pink-staining casts in the lumen. Gallego. (X200).

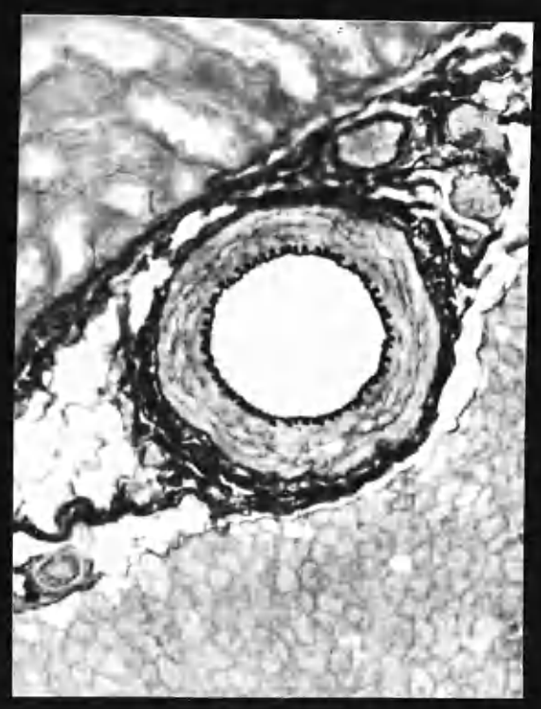


FIG.25 - Arcuate vessel, showing normal internal elastic lamina. Weigert-Van Gieson. (X 200).

Fig 26 a.

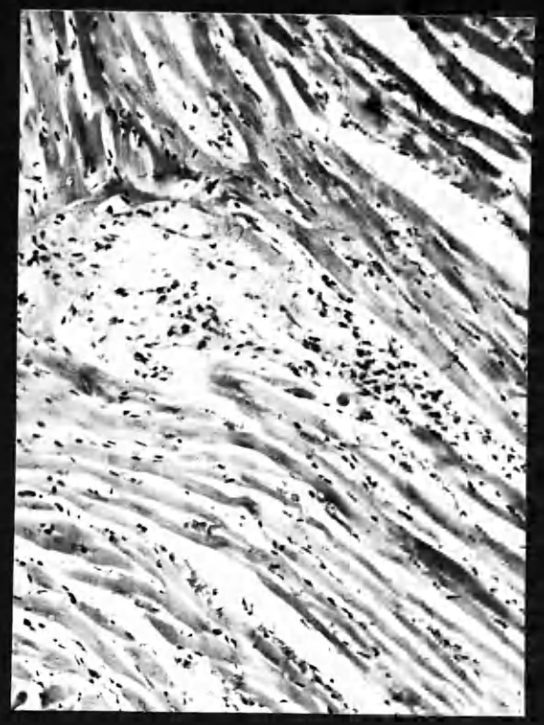


Fig 26 b.

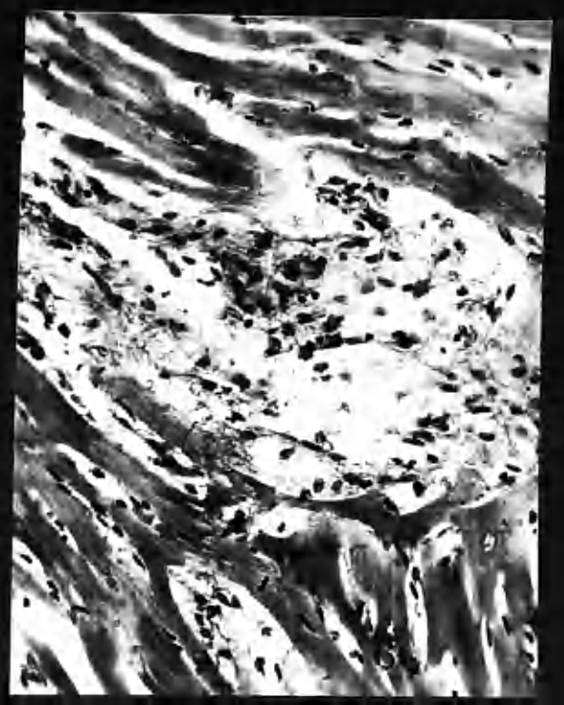


Fig 26 c.

Fig 27

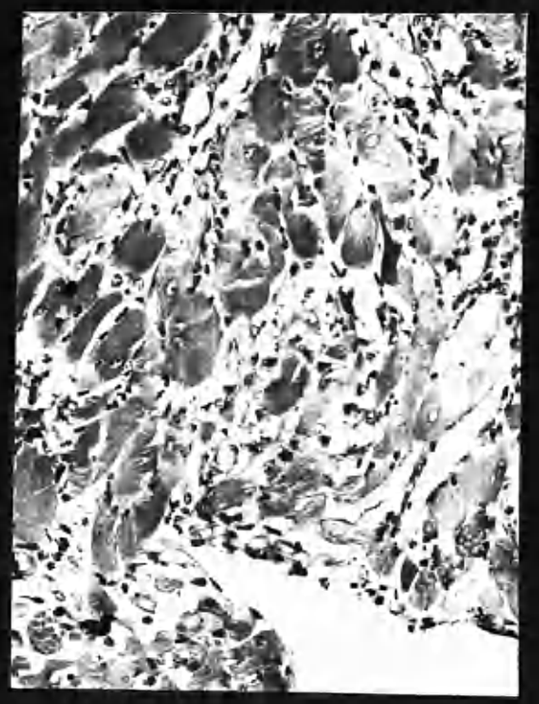


FIG.26 - (a,b & c). Heart, showing areas of mononuclear infiltration between muscle fibres. Atrophy of muscle cells seen in those areas.
(a) H.& E. (X150). (b) H.& E. (X250). (c) H.& E. (X250).
FIG.27 - Heart muscle cells replaced by a more fibrous type of tissue. H&E. (X250).

FIG 28

FIG 29.

FIG.28 - Suprarenal showing zonal arrangement of cortex. Medullary sinusoids are widely dilated and contain dense osmophil-positive material. Osmic Acid (X 75).

FIG.29 - Suprarenal. Inner zone of cortex shown above. Dense osmophil-positive secretion seen passing from cortical capillaries into medullary sinusoids. Osmic Acid. (X 250).

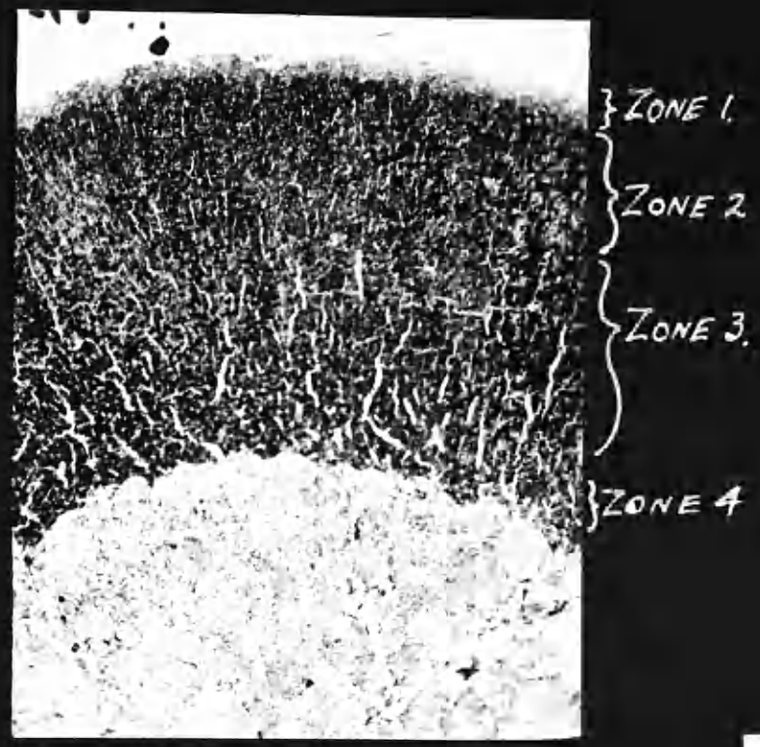


FIG.30 - Suprarenal (Control). Normal arrangement of 4 zones of cortex is shown. Osmic Acid. (X75).

FIG.31 - Suprarenal. Osmophilisation of outer zone present. Note the dilated spaces (A) containing osmophil-positive material in Zone 3. Osmic Acid. (X 75).

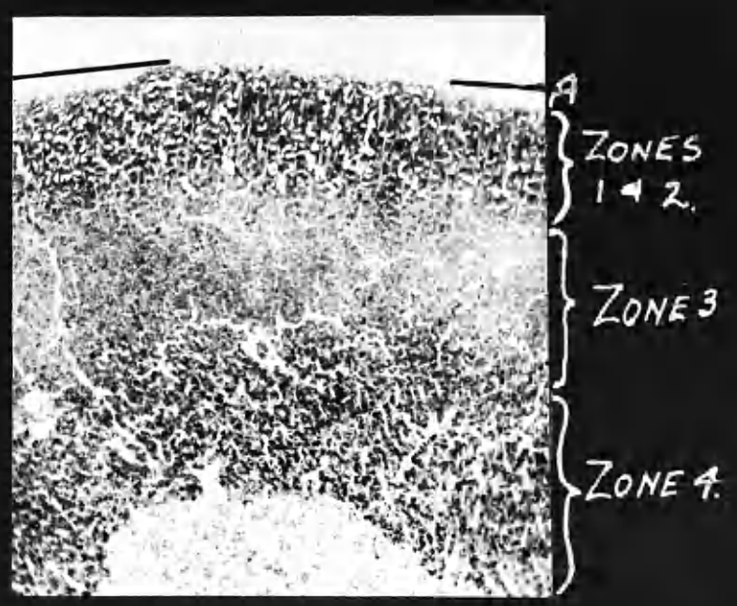


FIG.32 - Suprarenal. Prominent capsule (A) on surface. Zones 1 & 2 fused and only slightly osmophil-positive. Zone 4 greatly increased in size. Osmic Acid (X75).

FIG.33 - Suprarenal. Osmophil-positive granules in Zones 1 & 2. Note the clear vacuoles in many cells of Zone 2. Osmic Acid (X 75).

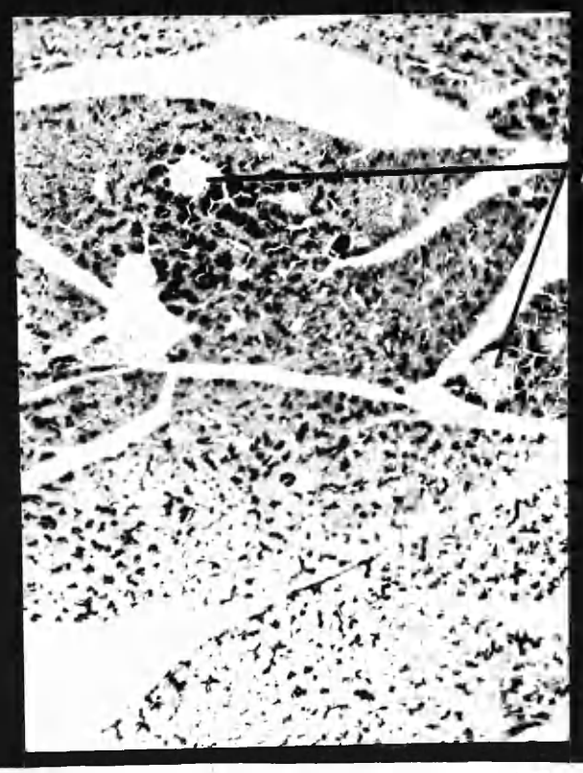


FIG.34 - Pancreas. Two small islets (A) are shown, surrounded by halos of prominent acinar tissue. Very little inter-islet acinar tissue is seen. Modified Mallory. (X 100).

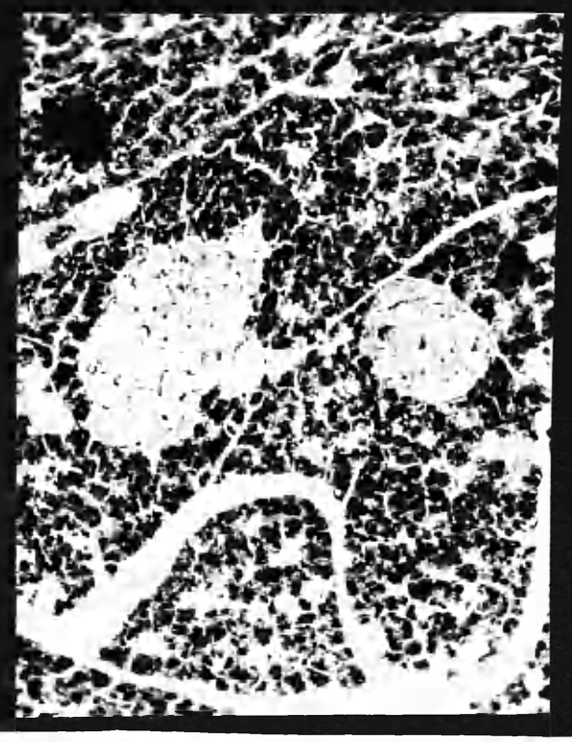


FIG.35 - Pancreas. Acinar tissue all fuchsinophil-positive. The tissue around the two islets are slightly more prominent. Modified Mallory. (X 100).

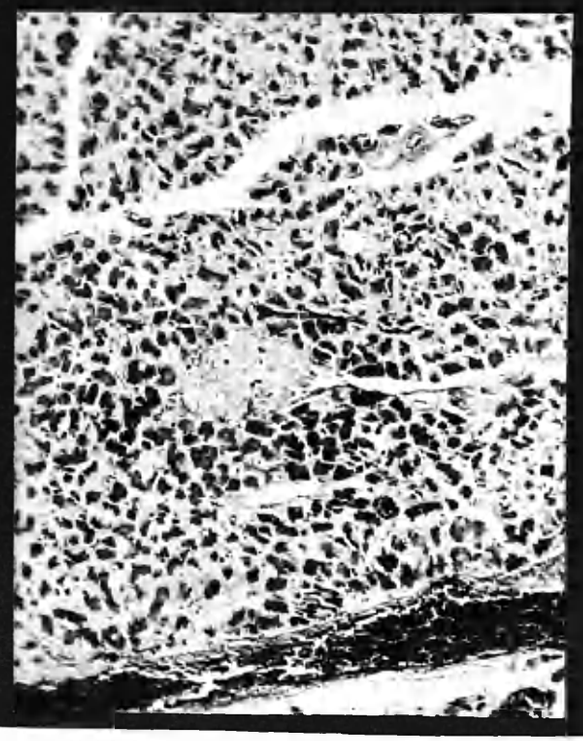


FIG.36 - Pancreas. The islet in centre of field is surrounded by a halo of fuchsinophil-positive acinar tissue. Modified Mallory. (X 100)

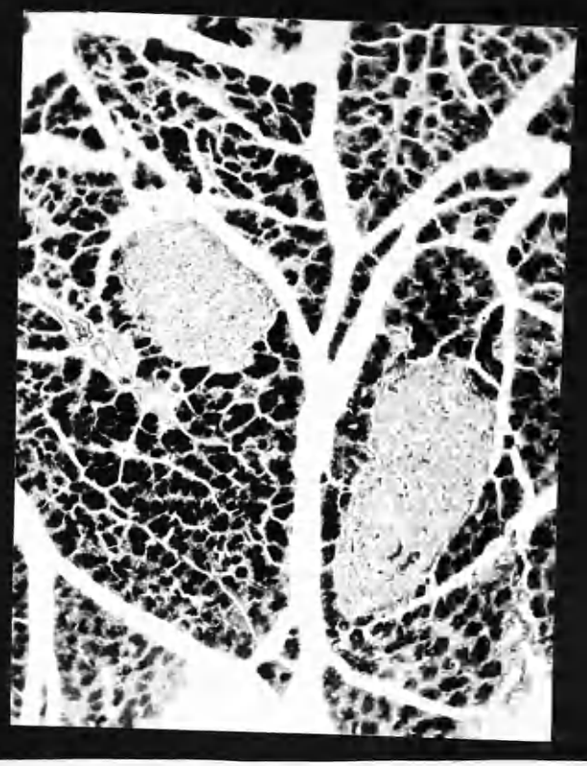


FIG.37 - Pancreas. Two islets are shown. The acinar tissue is now prominent throughout, but again more marked around the islets. Mod. Mallory. (X 100).

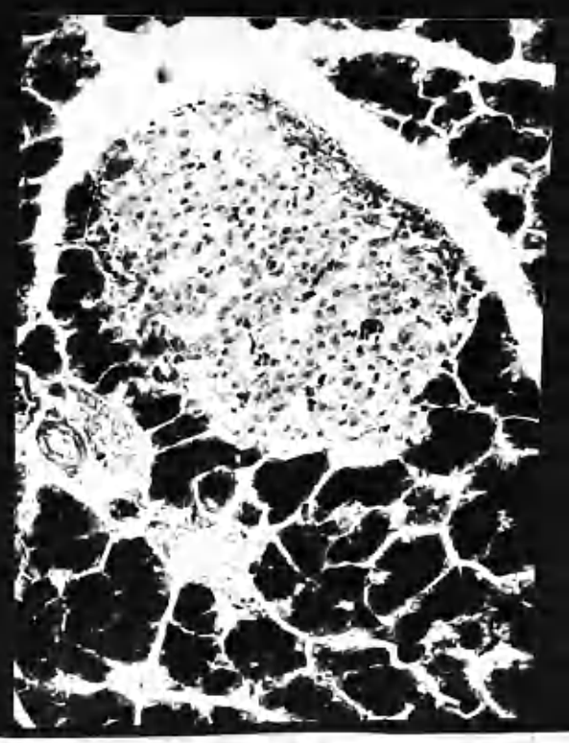


FIG.38 - Pancreas. Shows the dilated and prominent acinar tissue in relation to the islet. Modified Mallory. (X 250).

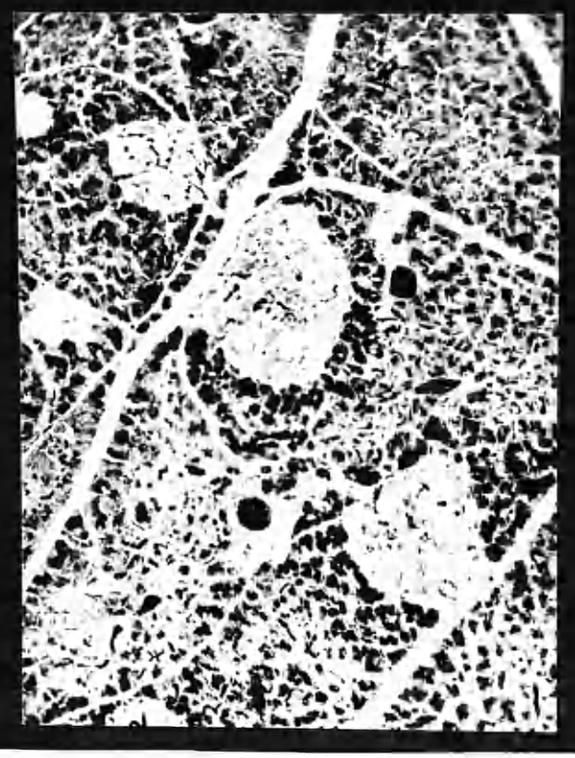


FIG.39 - (Male Control) Halos of acinar tissue are seen around all islets. Appearance similar to Fig.34. Modified Mallory. (X 100).

FIG.40 - (Female Control) Reaction around islets still present but not so prominent as in Fig.39. Modified Mallory. (X 100).

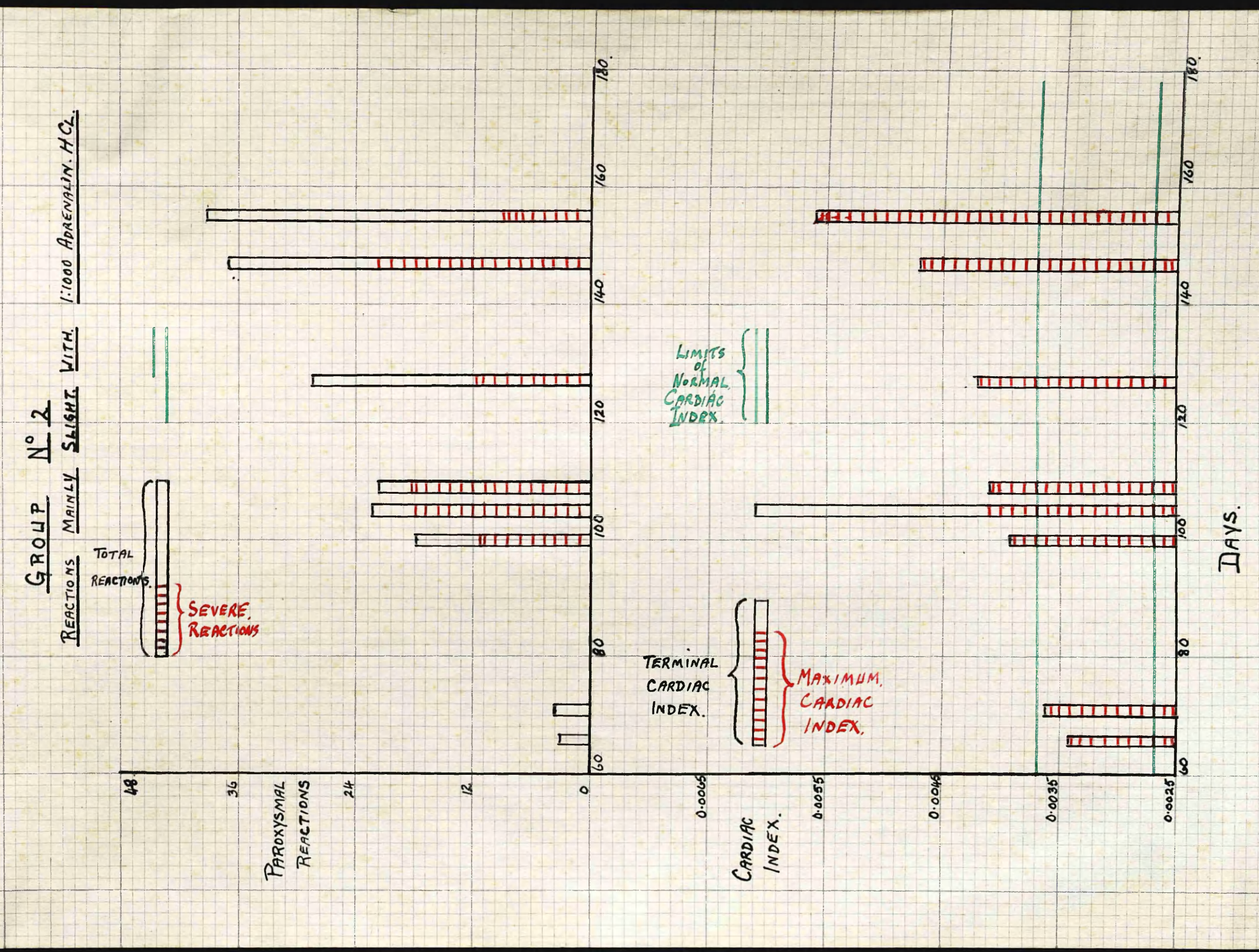


FIG.41 - The cardiac index shows a progressive increase above normal. The reactions produced mainly slight in character.

1 : 1000 Adrenalin HCl. used.

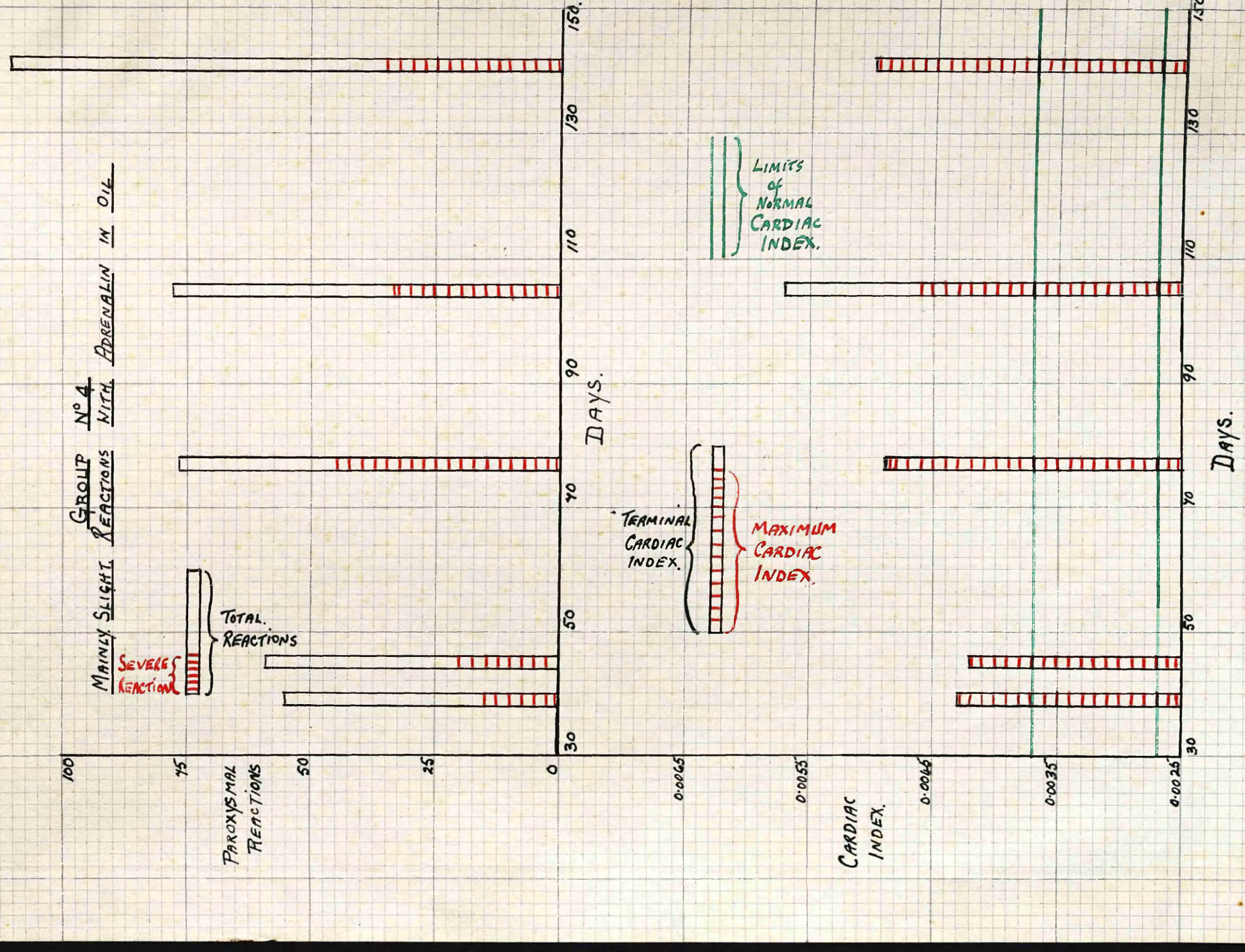


FIG. 42 - Cardiac index again is well above normal, and the reactions are mainly slight.

1 : 100 Adrenalin Ascorbate in oil used.

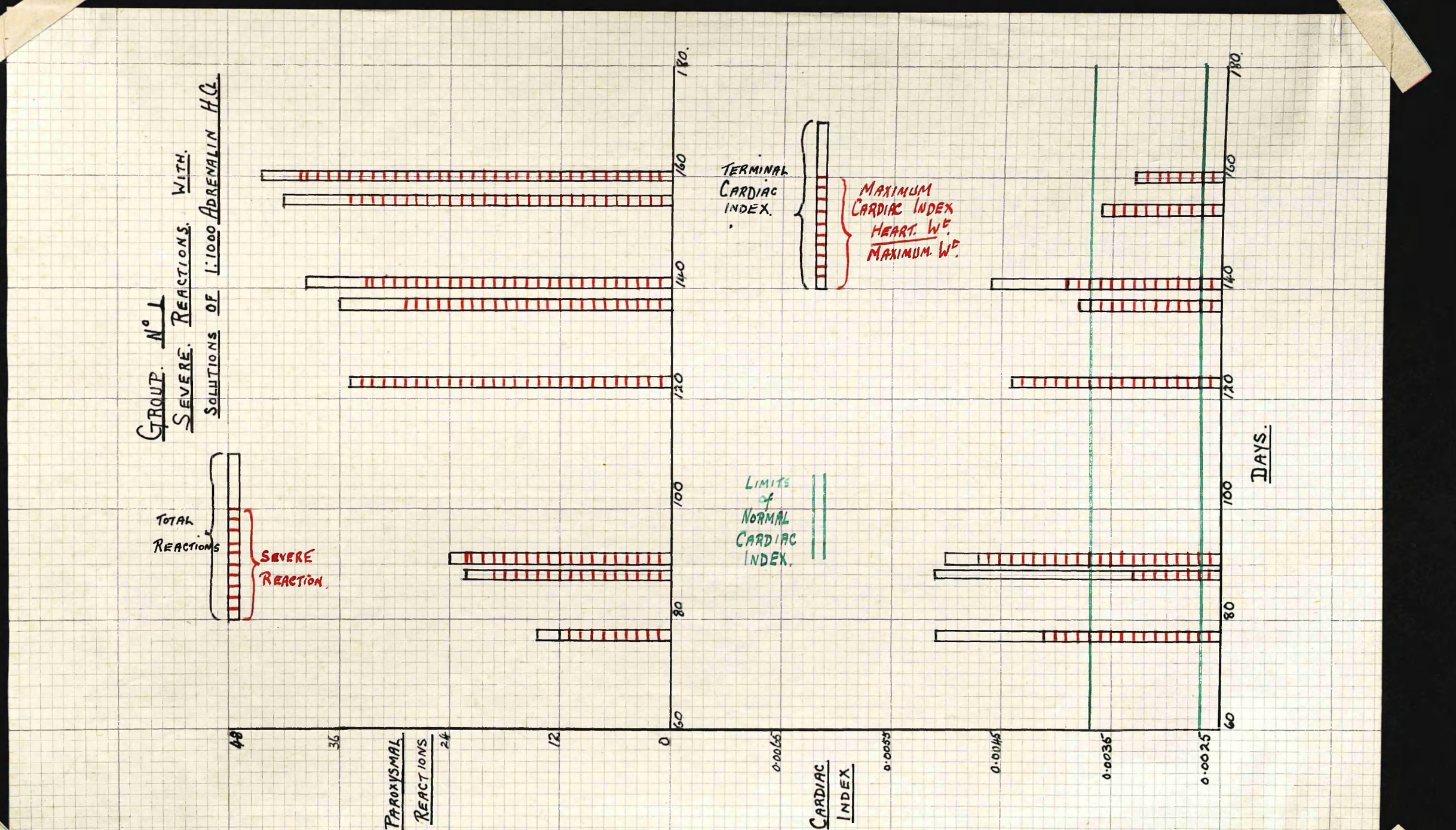


FIG. 43 - The cardiac index diminishes as the severity of reactions increase.

1 : 1000 Adrenalin HCl. used.

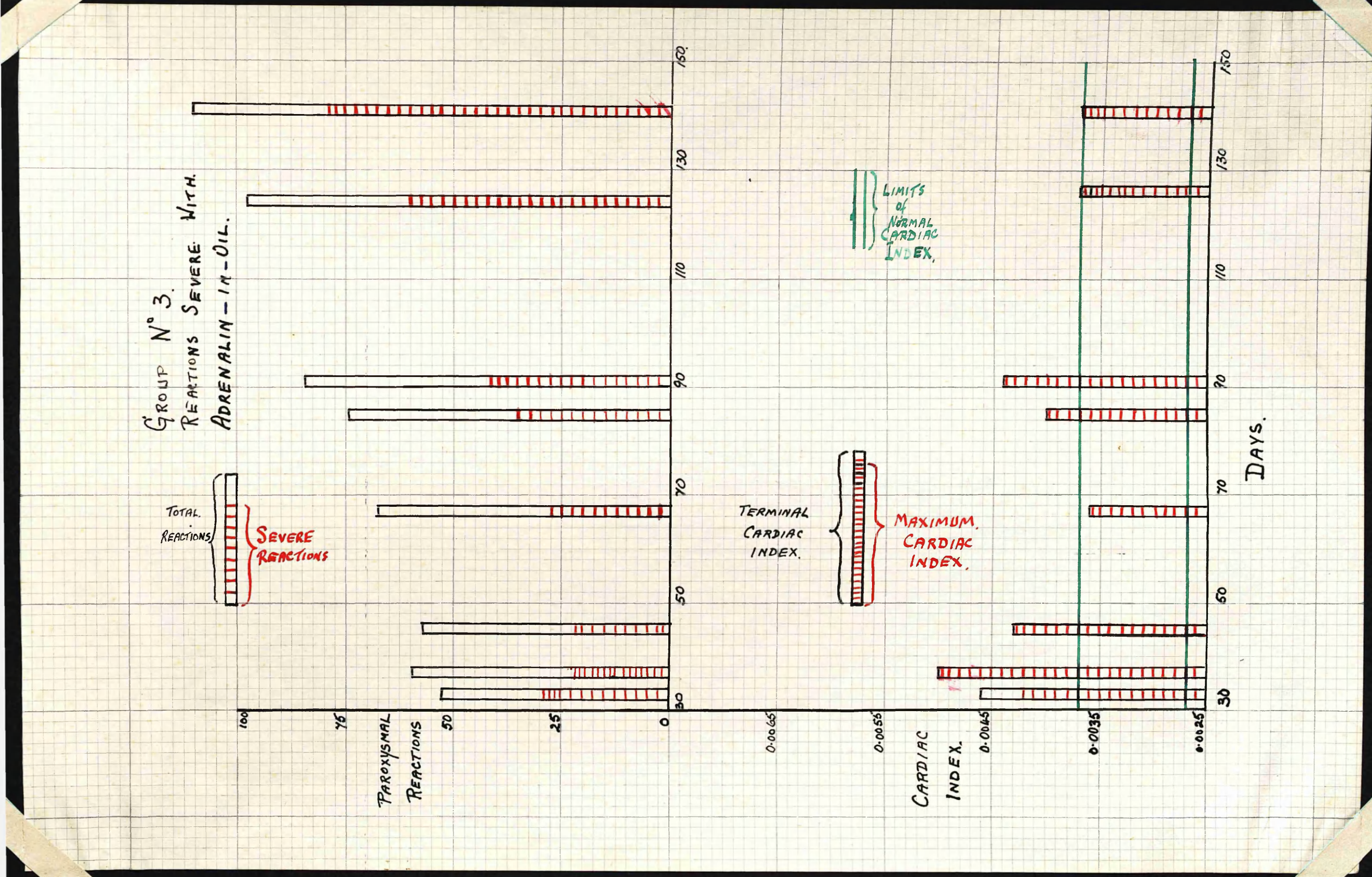


FIG. 44 - The cardiac index diminishes as the severity of reactions increase.
Note the high initial cardiac index due to previous treatment.
1 : 100 Adrenalin HCl. used.

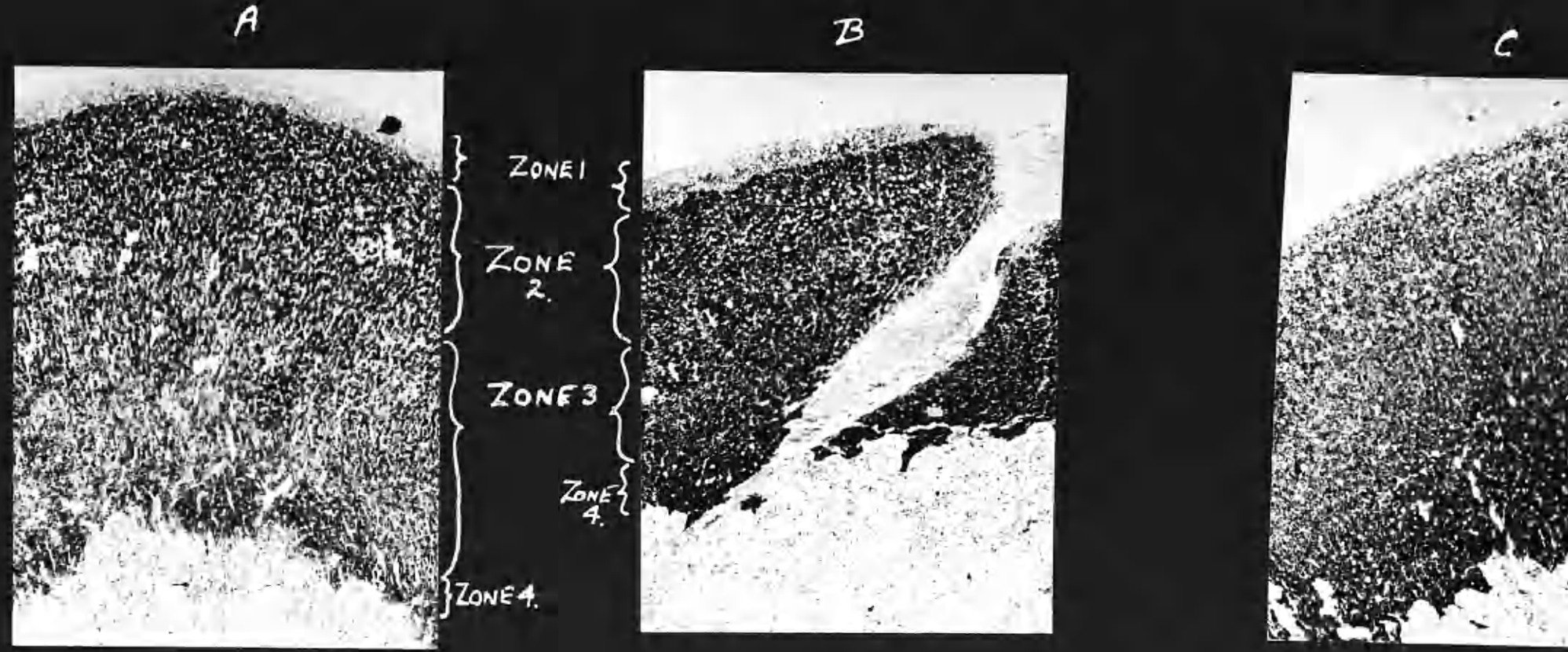


FIG. 45 - Suprarenals. Group 1. A B C D & E. show the zonal variations at different periods following a severe adrenalin reaction (1 mg. Adrenalin used). Osmic Acid. (X 75).

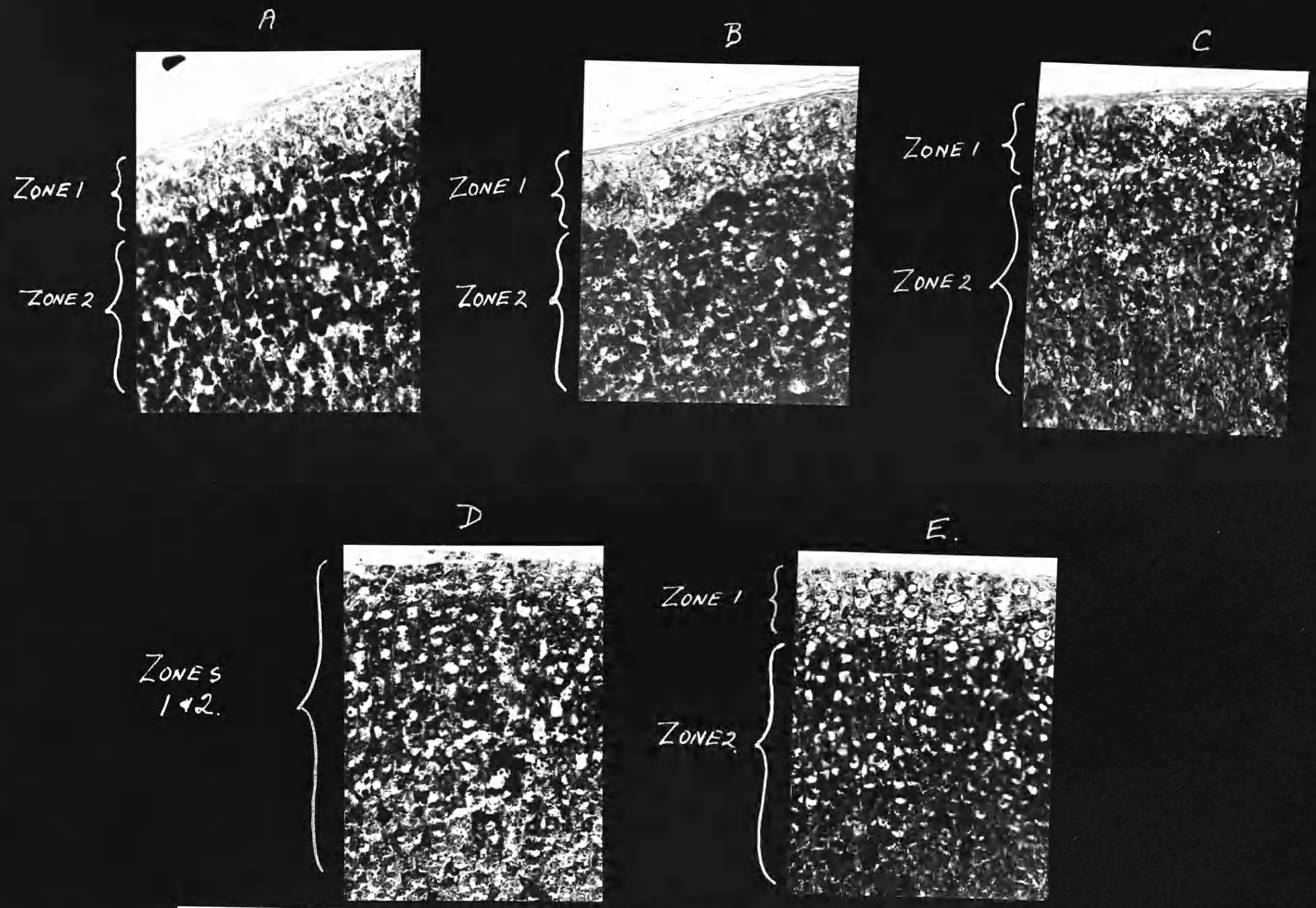
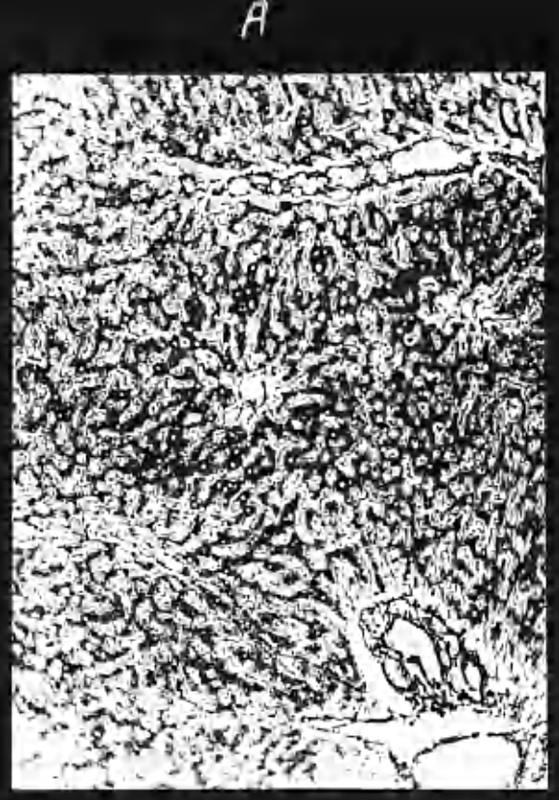
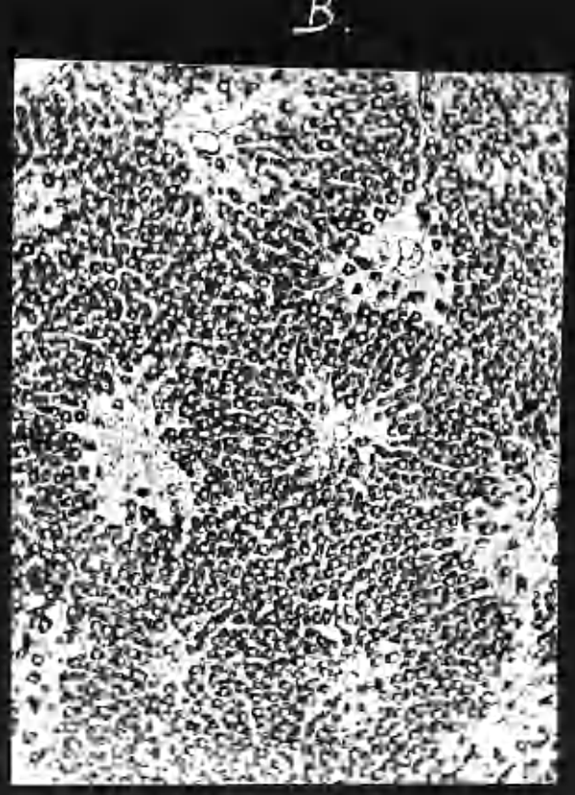
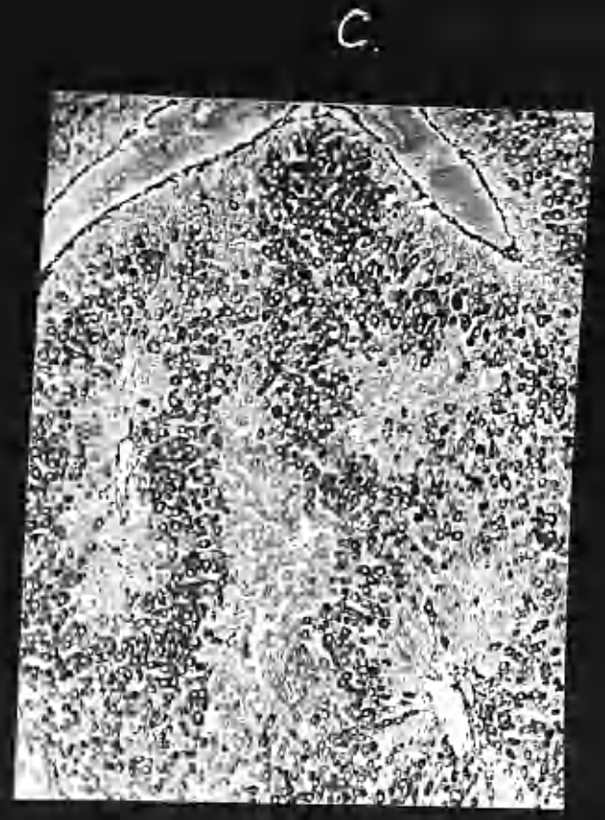
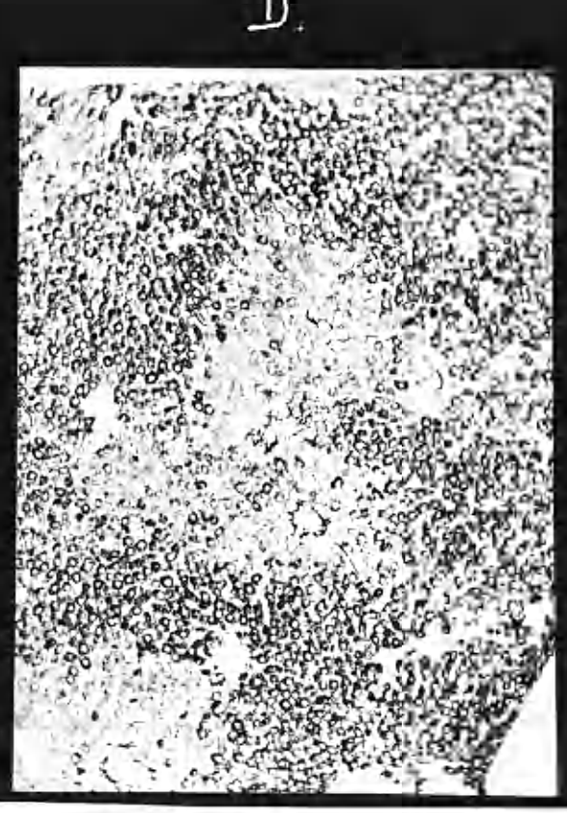


FIG. 46 - A to E. Group 1. Suprarenals. The changes in Zones 1 and 2 are shown at different stages of a severe adrenalin reaction. Osmic Acid. (X 250).









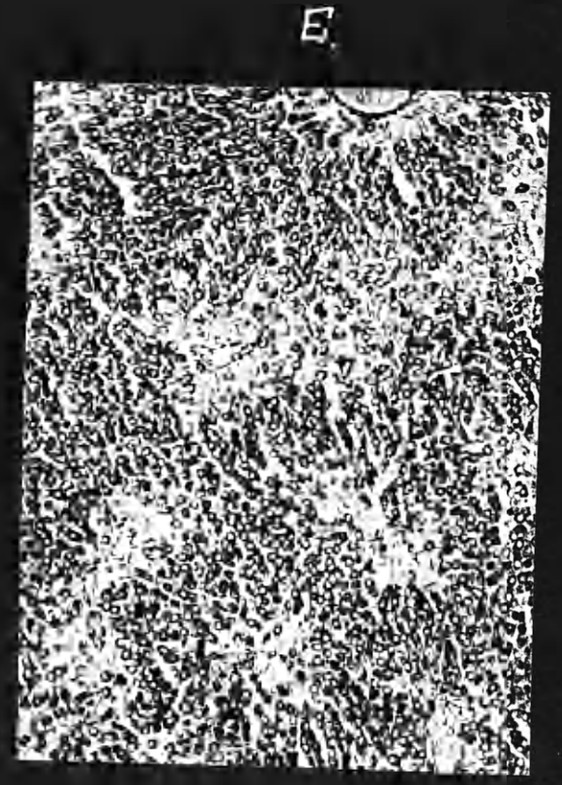
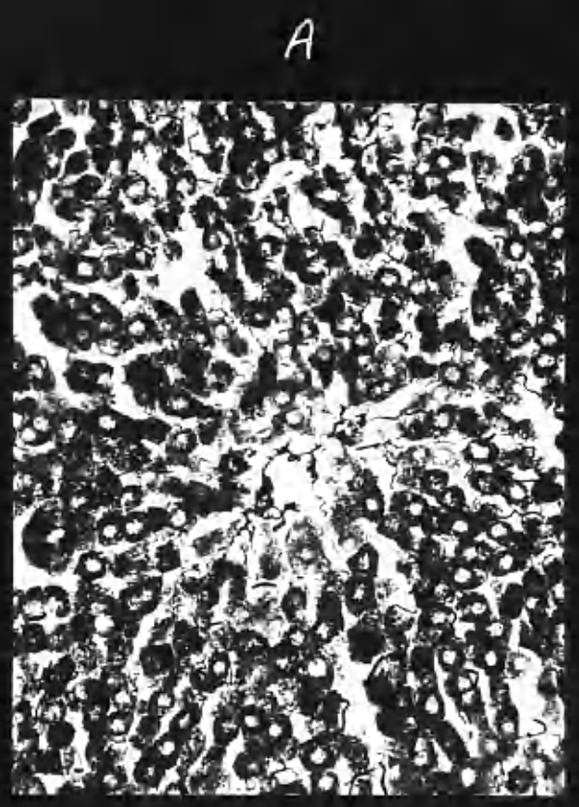
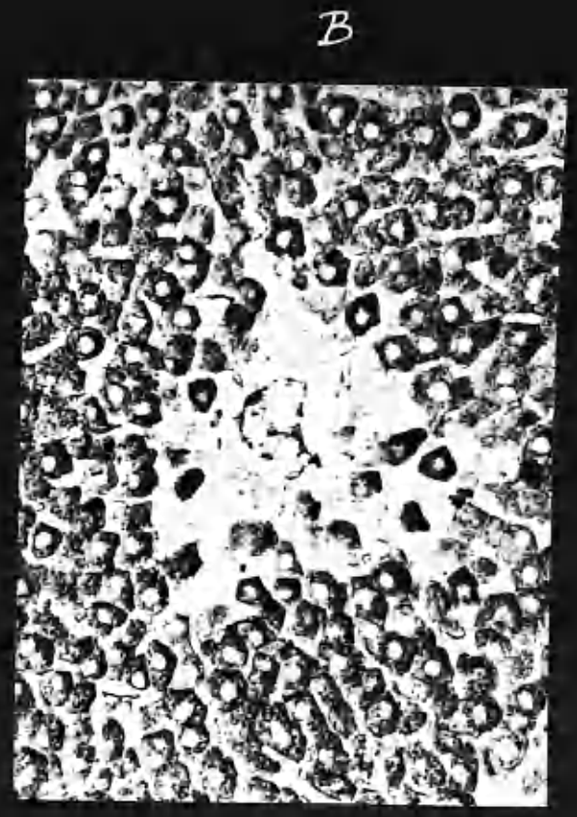
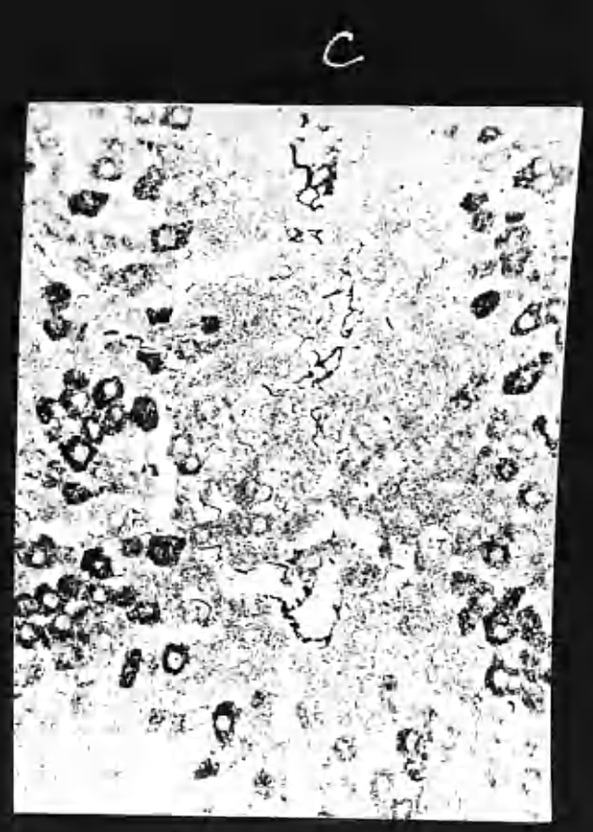
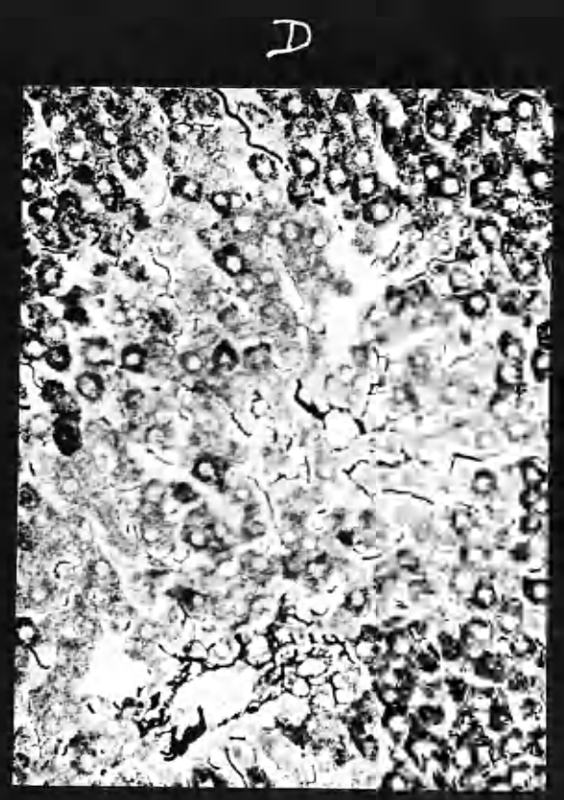


FIG. 47 - A to E. Group 1. Liver showing the disappearance and subsequent regeneration of glycogen in the liver lobule after a severe adrenalin reaction. Modified Silver method. Mitchell & Wislocki. (X 100).









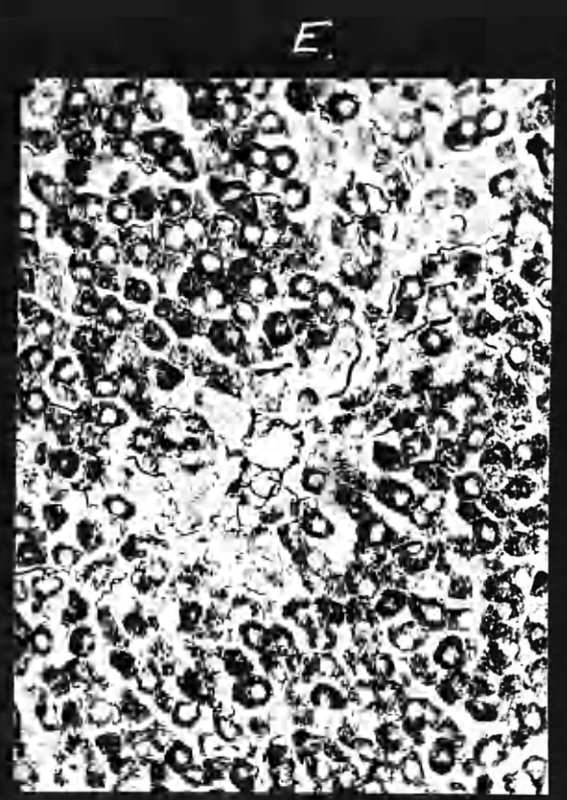


FIG. 48 - A to E. Group 1. Liver. The disappearance of glycogen from the central vein of the lobule and its subsequent regeneration is shown. Modified Silver impregnation method. (X 250).

FIG 49.

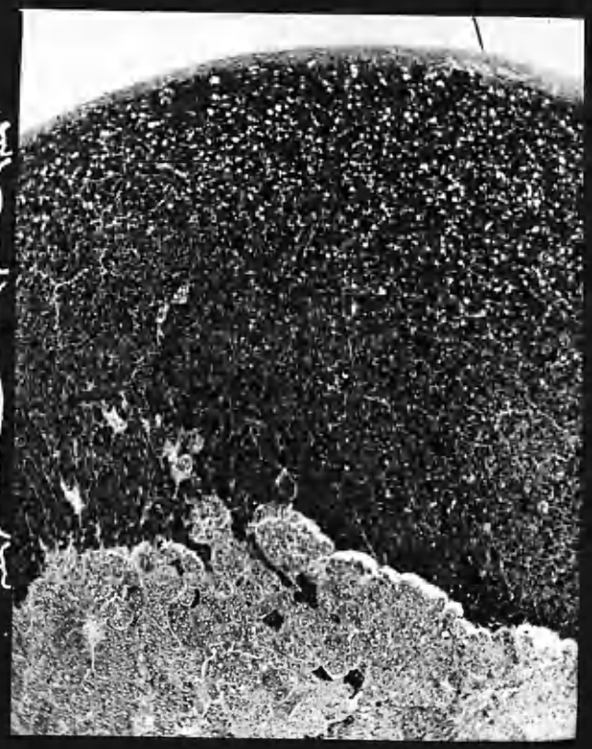


FIG 50.

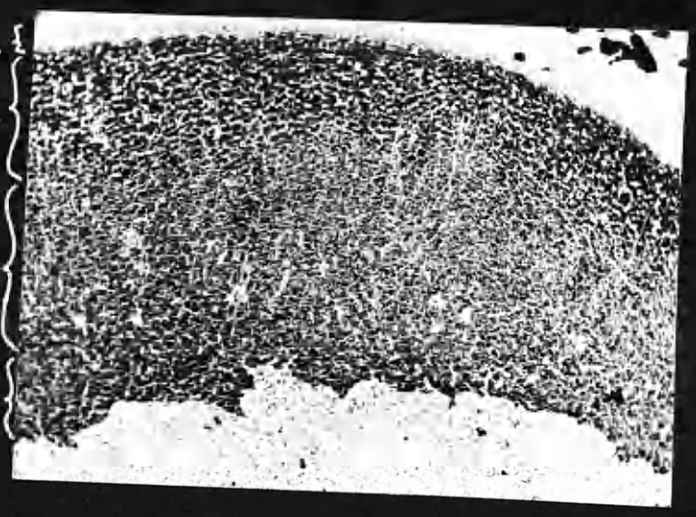


FIG 51.

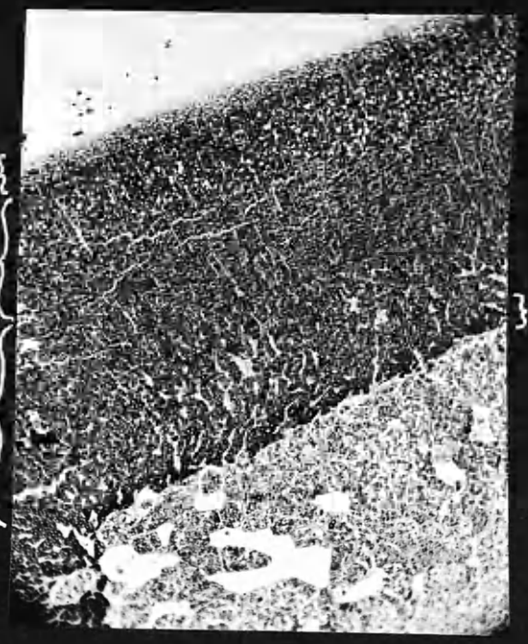
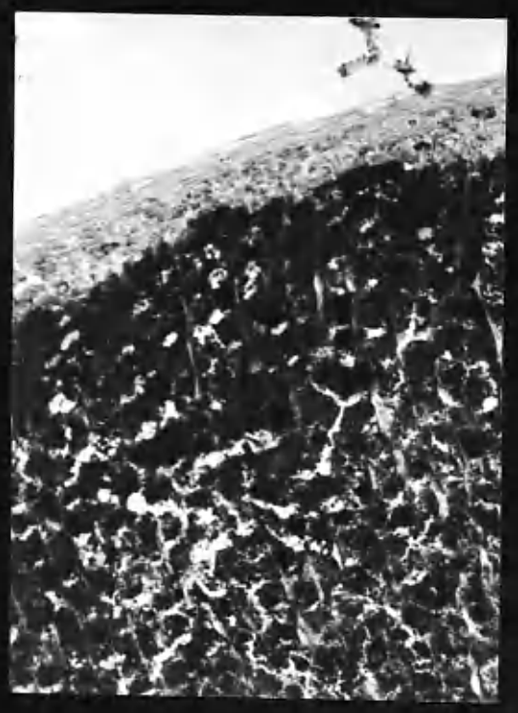


FIG. 49 - Group 2. (Control) Suprarenal, showing the variation in size of the first zone. Osmic Acid. (X 75).

FIG. 50 - Group 2. Suprarenal. The gland shows little change from normal, one hour after a slight adrenalin reaction. (4 m 1:1000 Adrenalin HCl.) Osmic Acid. (X 75).

FIG. 51 - Group 2. Suprarenal. Osmophilisation of the cells is present in the outer zone, two hours after a slight adrenalin reaction. (4 m 1:1000 Adrenalin HCl.) Osmic Acid. (X 75).



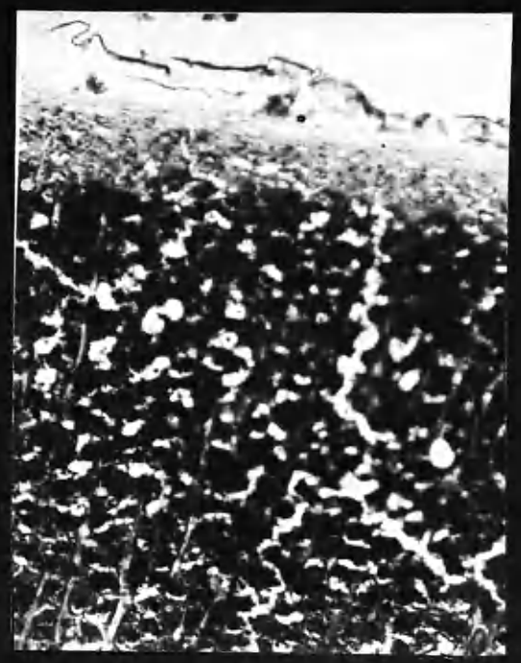
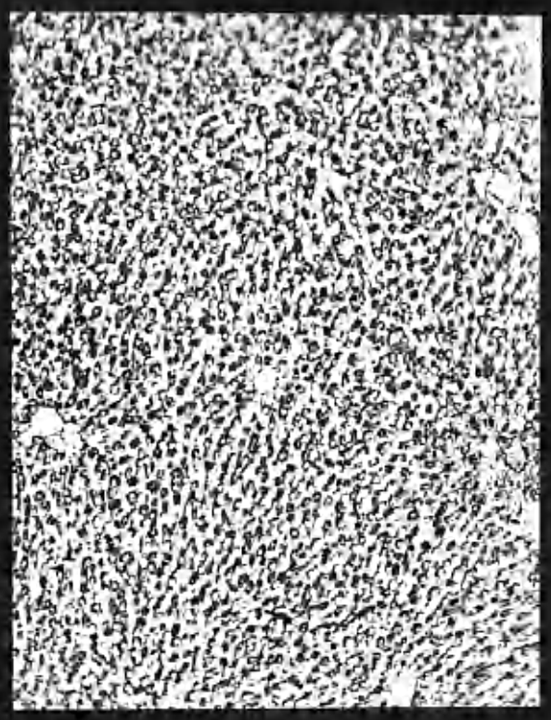
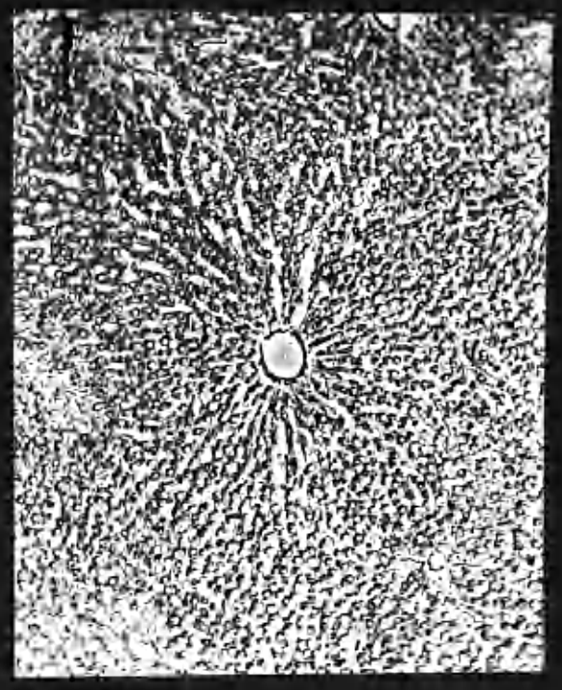
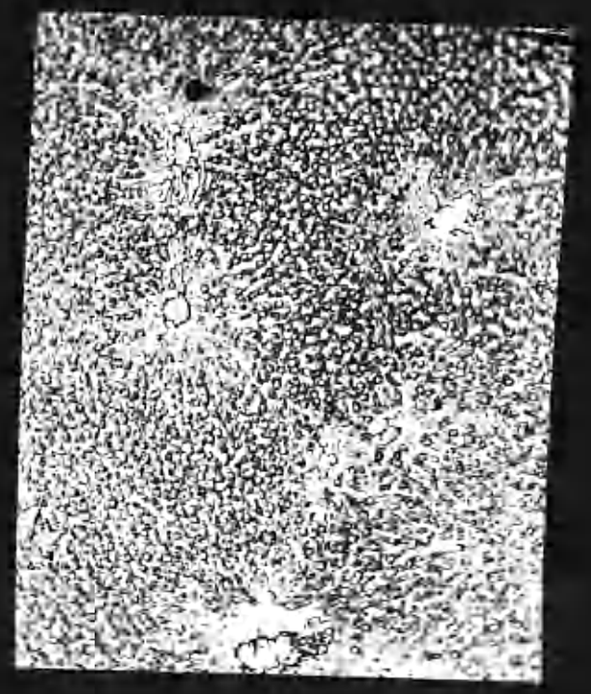
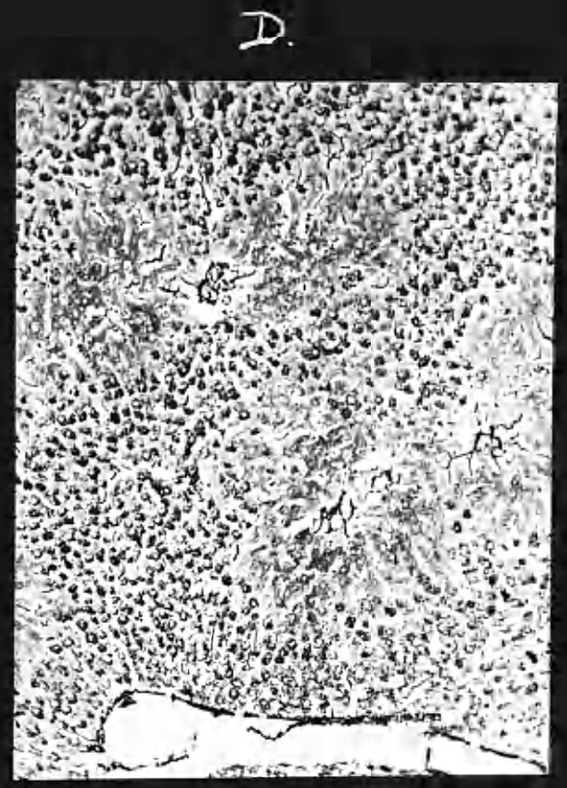


FIG. 52 - A to G. Group 2. Suprarenals, showing the appearance of zones 1 and 2 at different stages of a slight adrenalin reaction. Osmic Acid. (X 250).









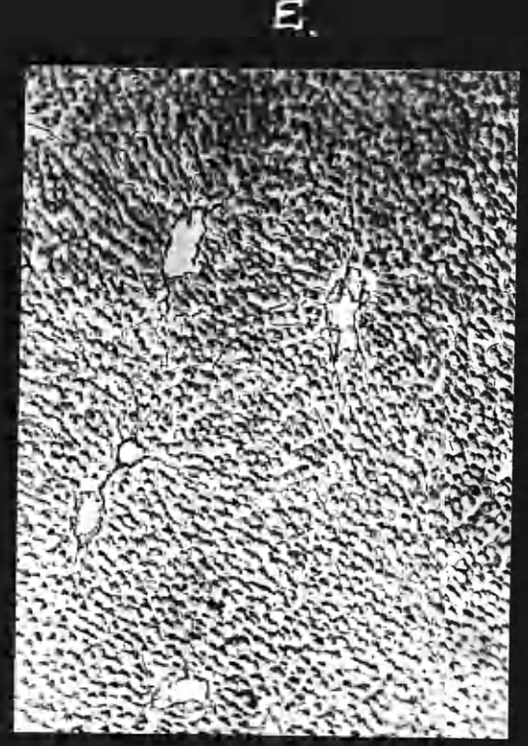


FIG. 53 - A to E. Group 2. Liver showing the changes in liver glycogen after a slight adrenalin reaction. The glycogen is normal in the control and 1 hour animals (A & B). Depletion commences at 2 hours (C) and is at its height in 3 hours (D). Regeneration is complete in 4 hours (E). Note the "Rim staining" of glycogen is prominent in E. Modified Silver Impregnation Method. (X 100).

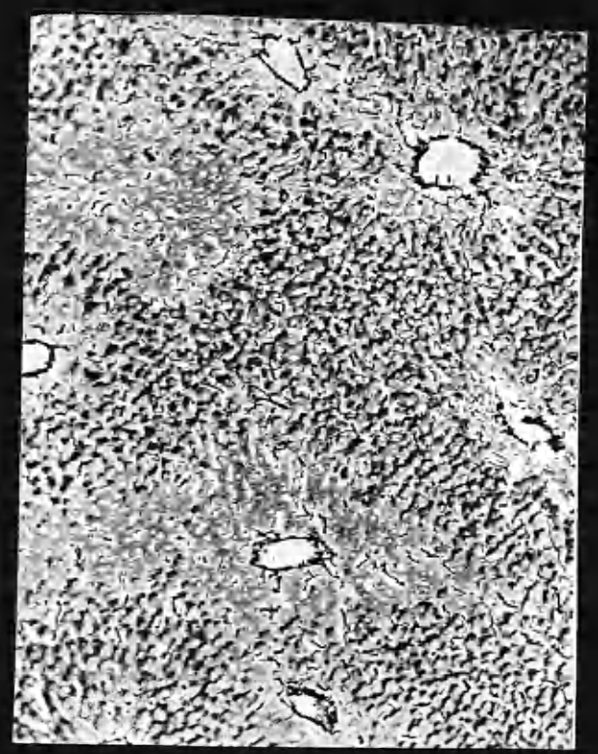


FIG.54 - Group 4. Liver. Glycogen is present only in the mid-zone region, 1½ hours after a slight reaction in adrenalectomised animals. No regeneration of glycogen is present. Modified Silver Impregnation Method. (X100).

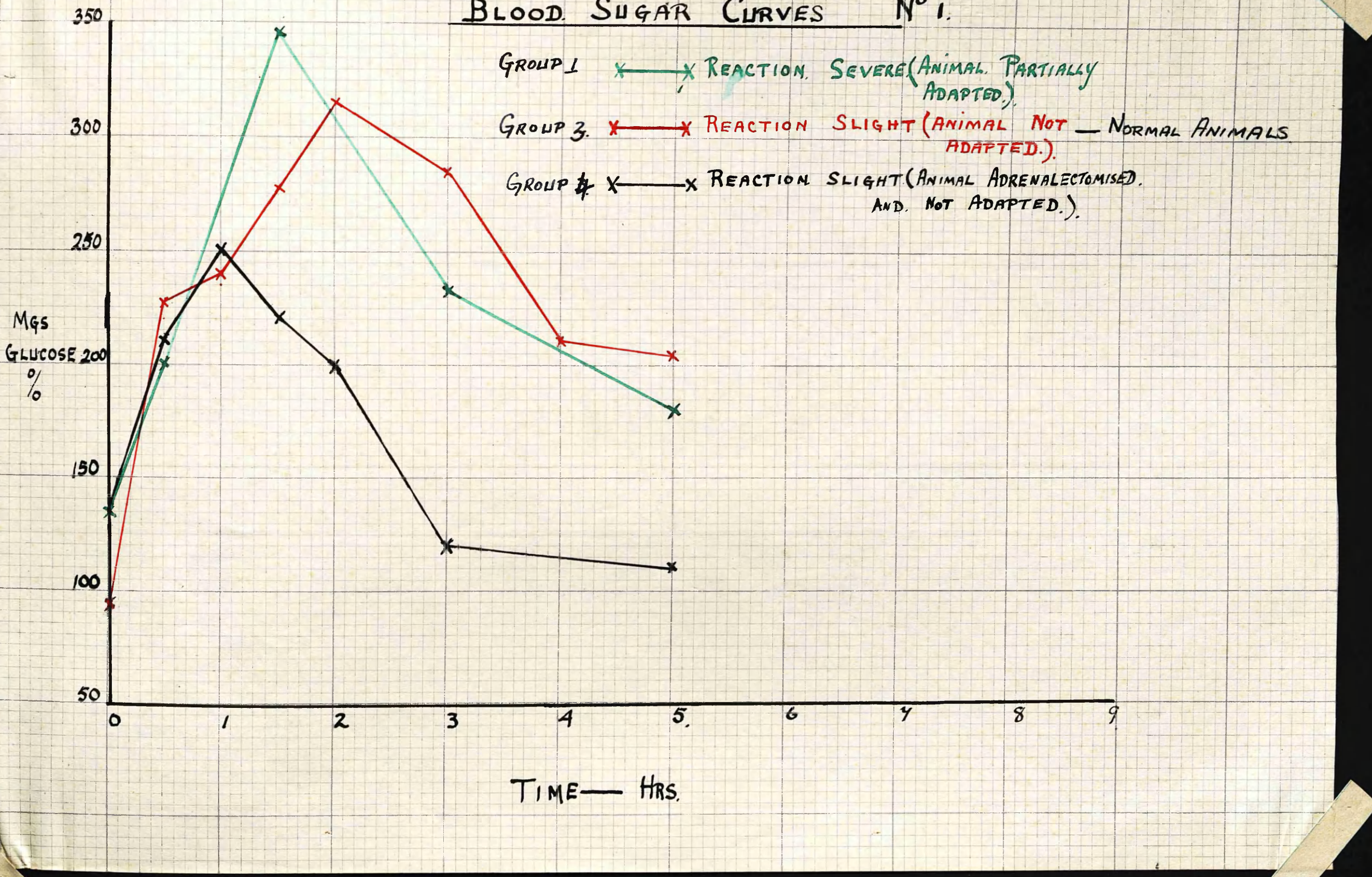


FIG. 55 - The blood sugar curves show the effect of suprarenals in mobilising blood sugar. The blood sugar in adrenalectomised animals is low in comparison with the others and returns quickly to normal.

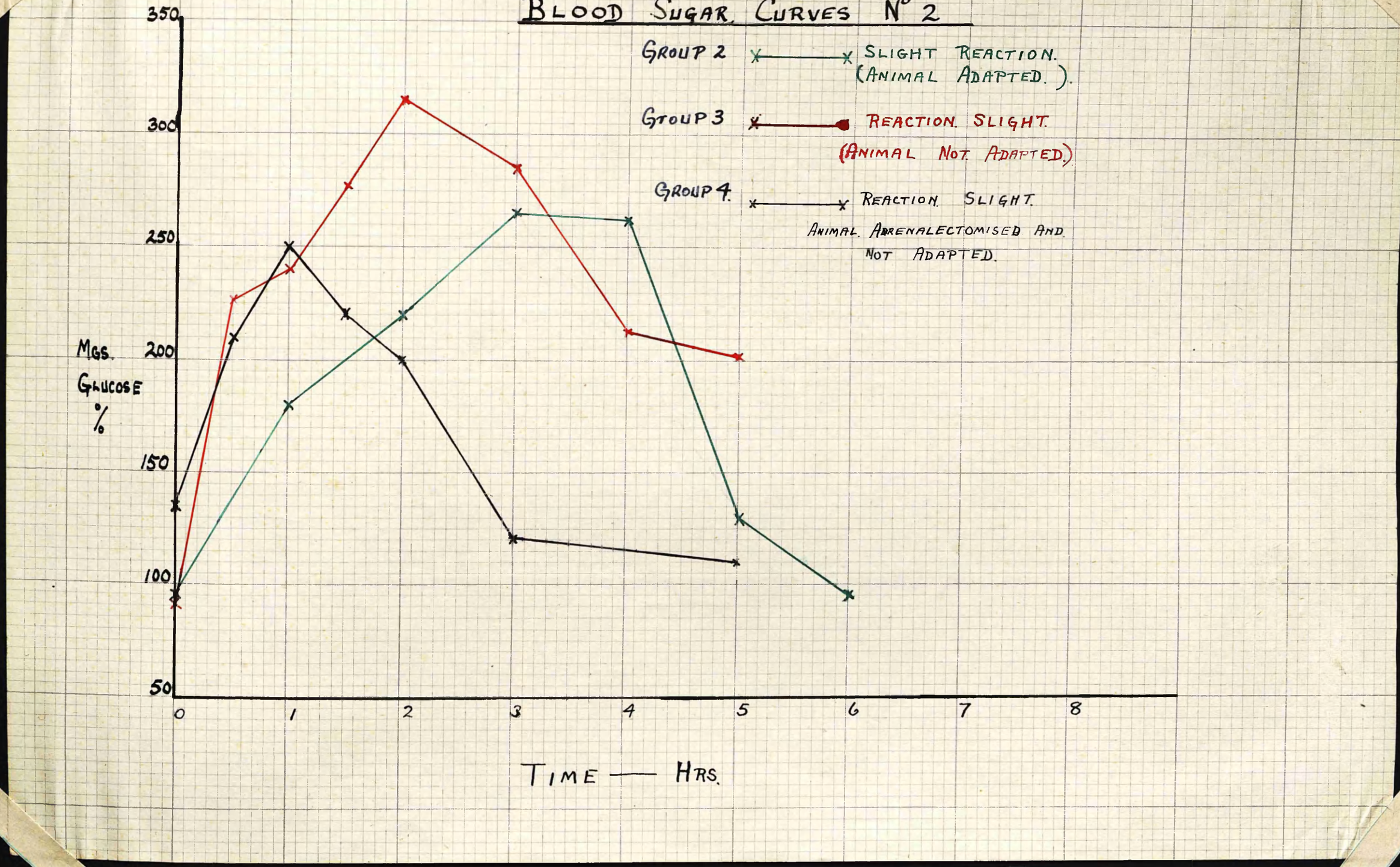


FIG. 56 - The effect of the suprarenals in mobilising blood sugar is seen again after a slight adrenalin reaction. Note the blood sugar returns to normal in 3 hours in adrenalectomised animals, but takes 5 hours in Group 2 animals.

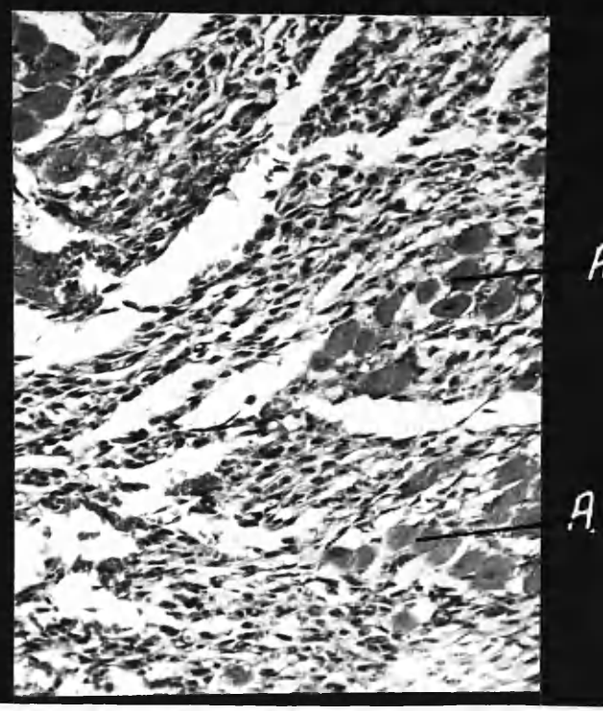


FIG.57 - HEART. A few muscle fibres only remain (A). They have been replaced by a cellular tissue. Giemsa Stain. (X 150).

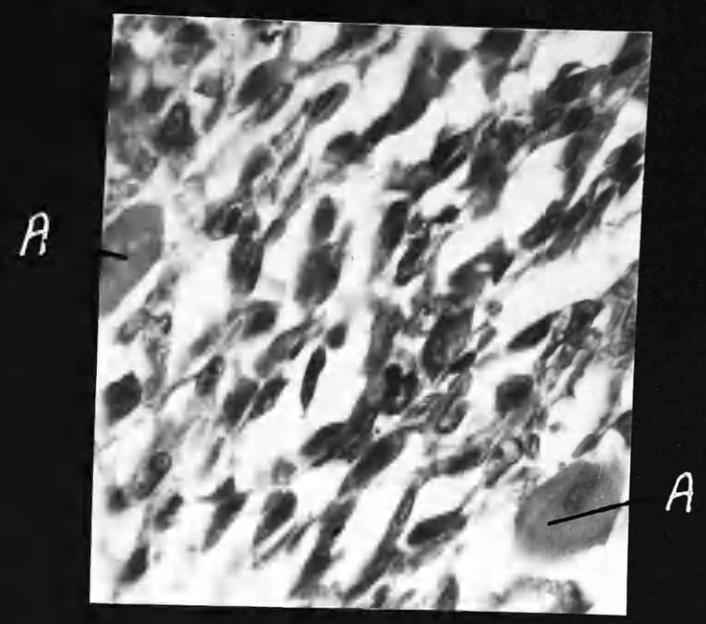


FIG.58 - HEART. Two muscle cells are shown (A). The intervening tissue consists of large spindle shaped cells with pale cytoplasm and spherical nuclei The cells resemble fibroblasts. Giemsa Stain. (X 700)

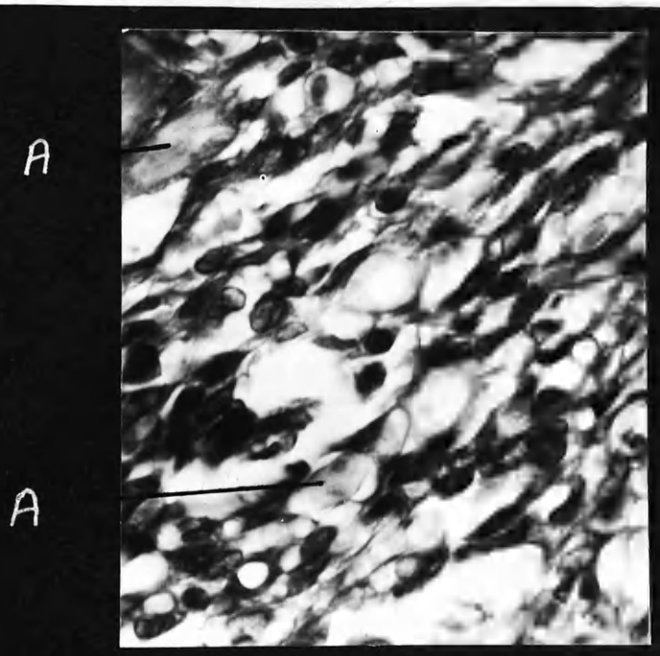


FIG.59 - HEART, showing the remains of degenerating muscle fibres (A).In most cases the muscle has completely degenerated and only the sarcolemma sheath remains. Gallego. (X 700).

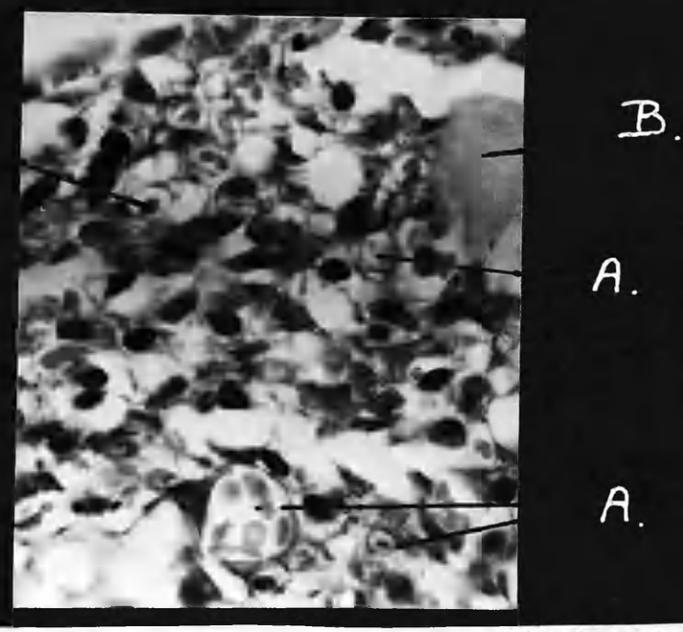


FIG.60 - HEART. The cellular tissue is composed of thin walled blood vessels containing red cells (A). It has the appearance of young granulation tissue. A degenerating muscle cell is seen above and to the right (B). Giemsa Stain. (X 700).

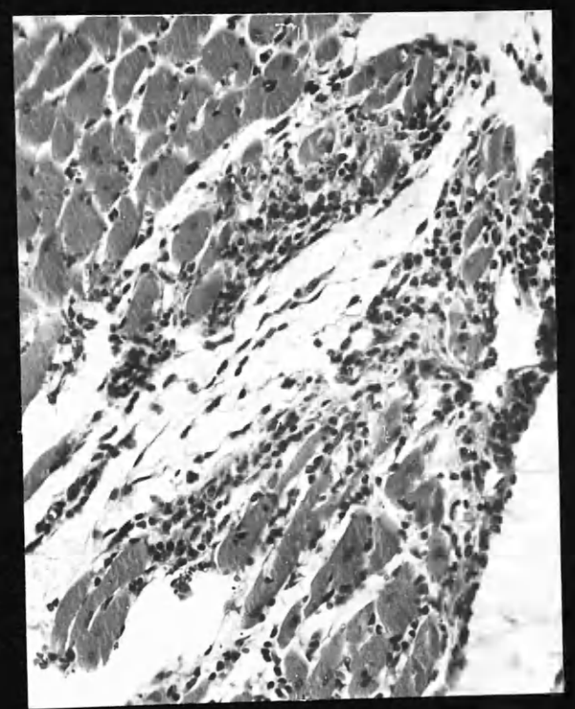


FIG.61 - HEART, showing an early stage of the lesion. The cells present between the muscle fibres are mononuclear cells. H. & E. (X 200)

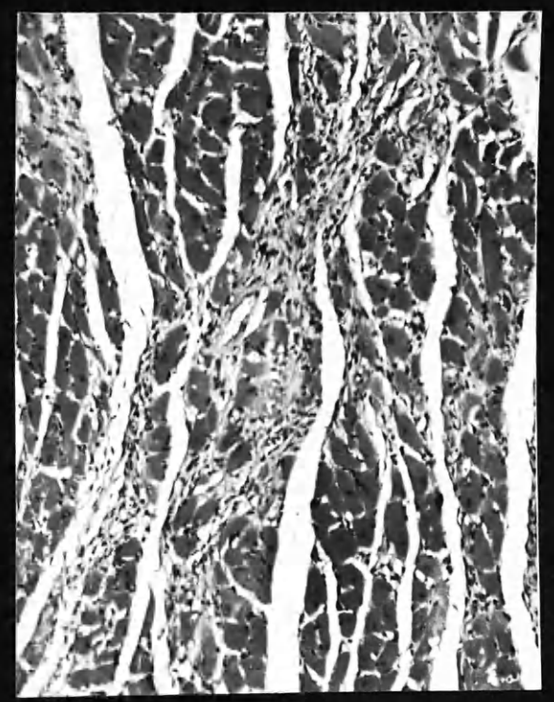


FIG.62 - HEART. The muscle fibres are replaced by a more fibrous type of tissue. H. & E. (X 200).

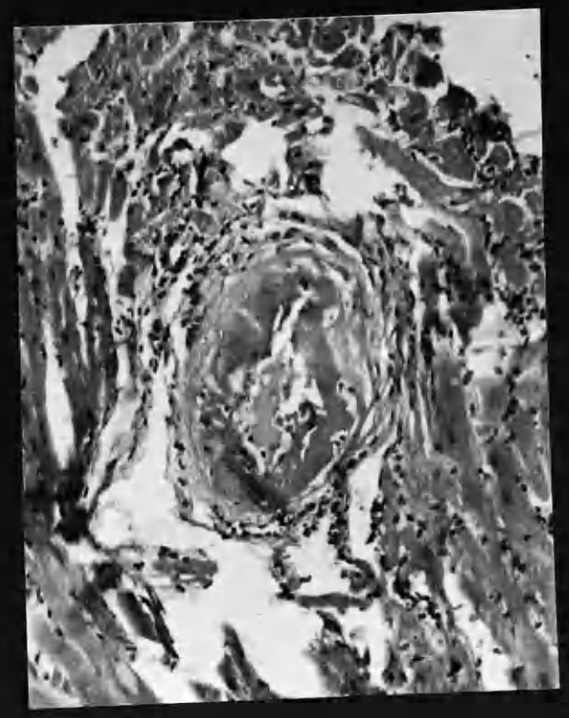
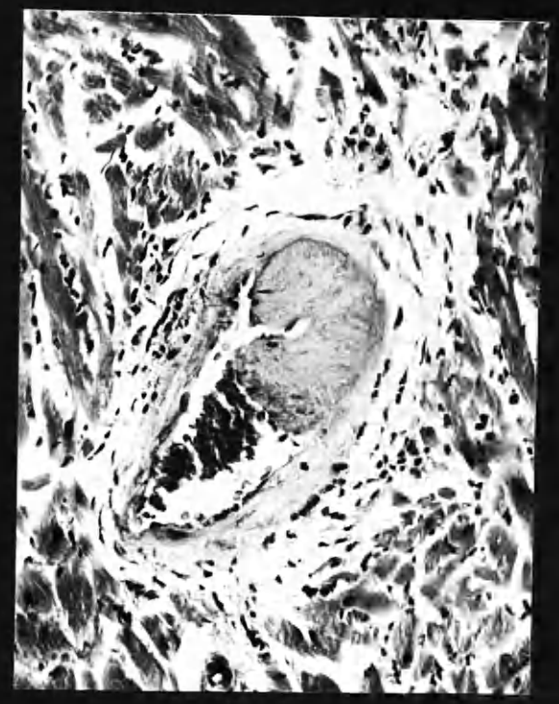
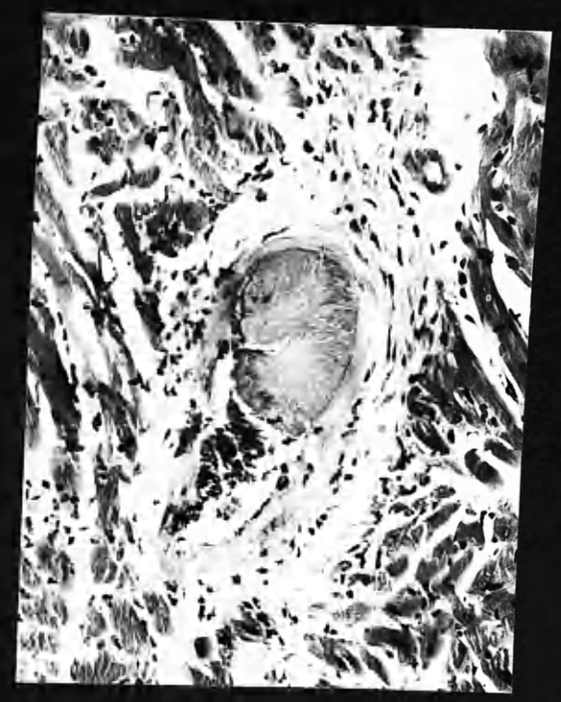


FIG.63 - HEART. A large coronary vessel showing marked concentric hyaline degeneration of the intima is present. H. & E. (X 250).

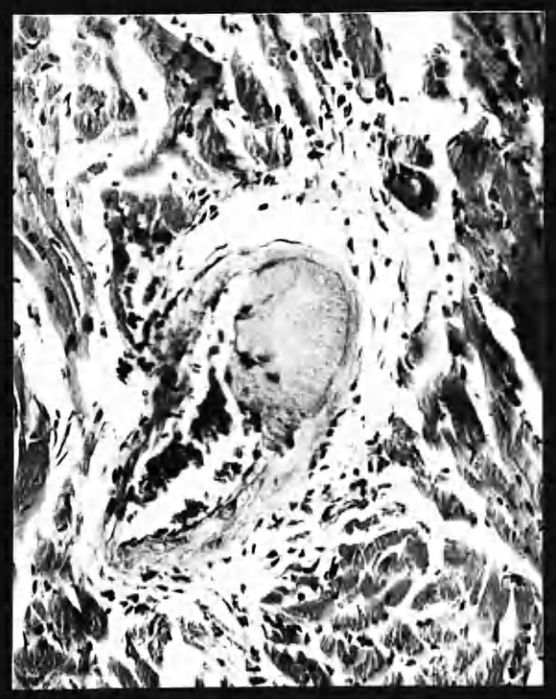
A



B.



C



D

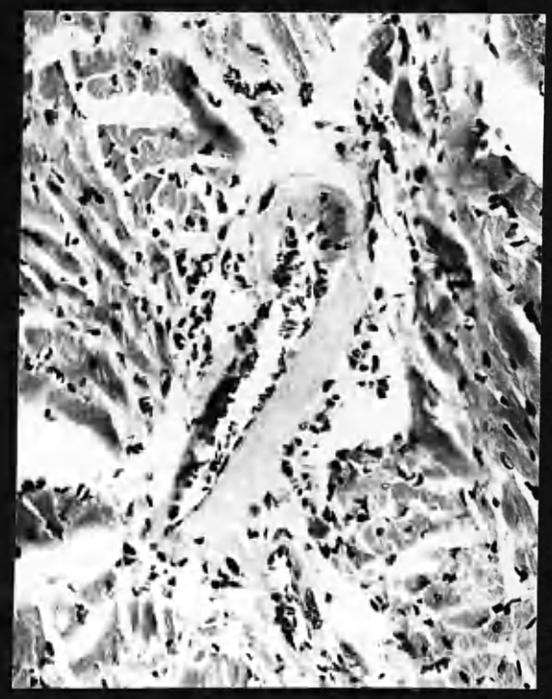
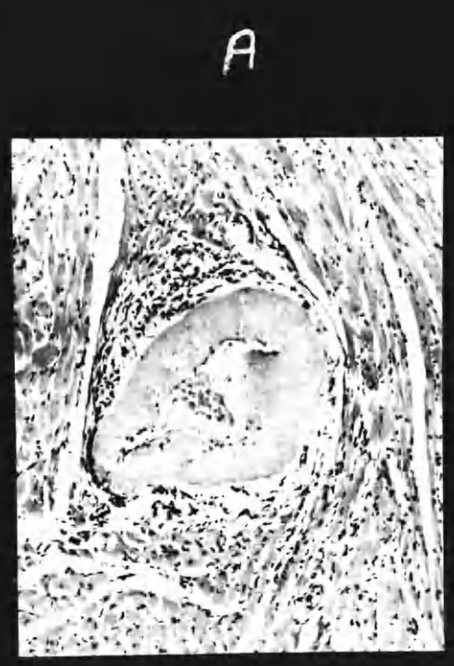
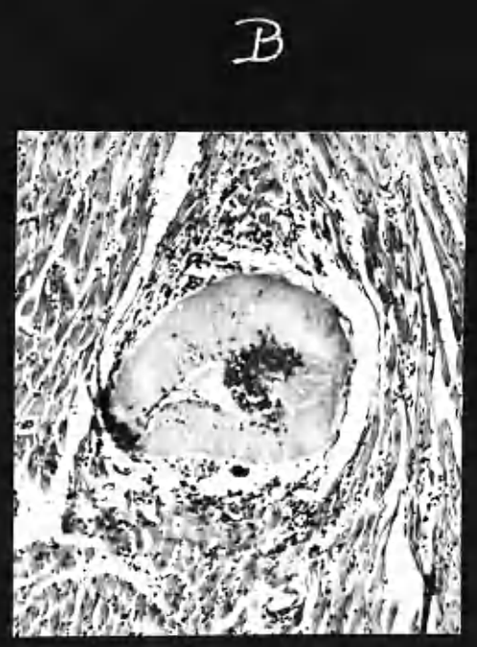
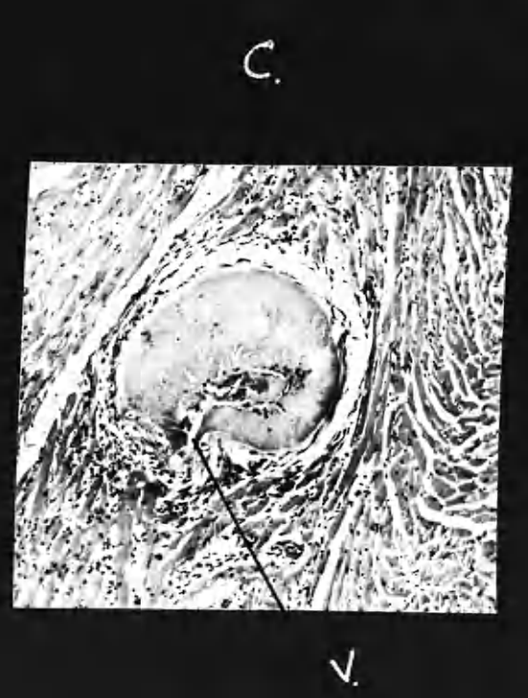
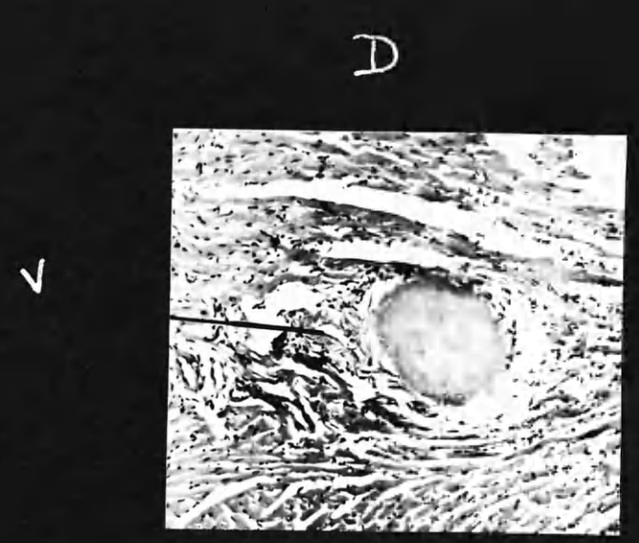


FIG.64 - A to D. HEART. Serial section of a coronary artery is shown. A large hyaline plaque projects into the upper pole of the vessel and partially obliterates the lumen. The end of the plaque is seen in Fig. D. H. & E. (X 250).









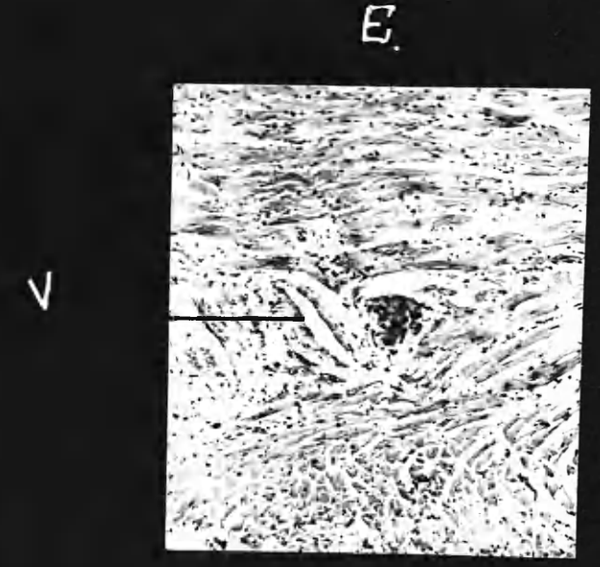


FIG.65 - A to E. HEART. Concentric hyaline degeneration of a large coronary artery is shown on serial section. The lumen becomes occluded in C D & E, but a branch or anastomotic vessel is seen taking its place (V). Note the perivascular infiltration with round cells in A and B. H. & E. (X 100).

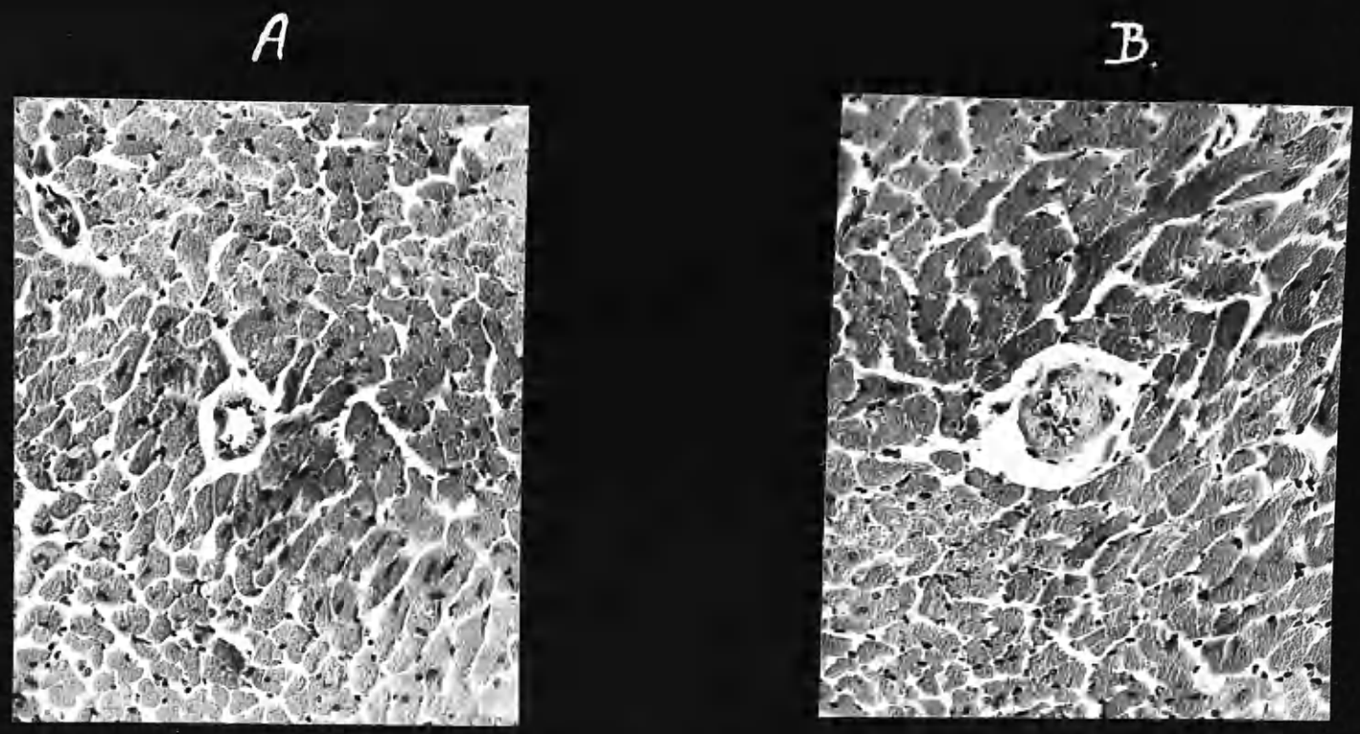


FIG.66 - A & B. HEART. Serial sections of a vessel in the columnae carnea are shown. In A the vessel is normal. Later concentric hyaline degeneration of the intima is present, and the lumen narrowed B. H. & E. (X 250).

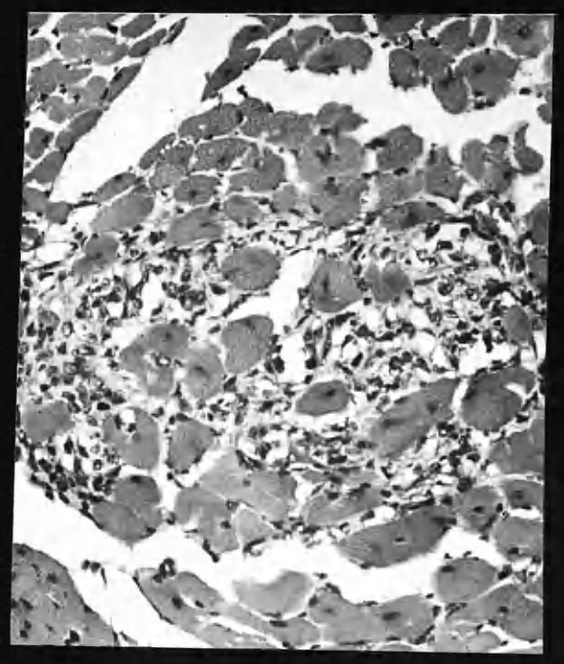


FIG.67 - HEART, showing myocardial lesions in the columnae carnea supplied by the coronary vessels seen in Figs.66 A & B. H. & E. (X 250).

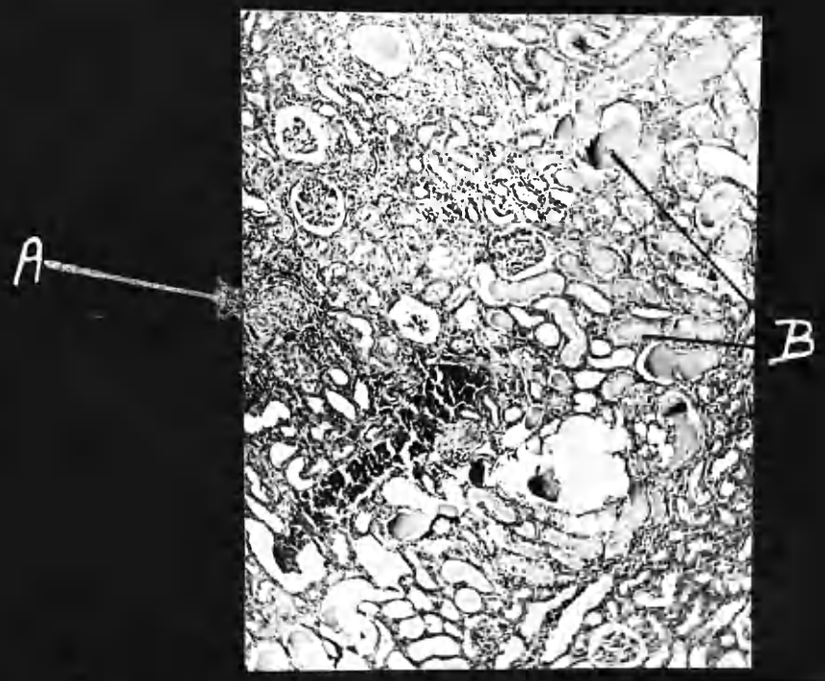


FIG.68 - KIDNEY, showing the focal nature of the lesion. An affected glomerulus is seen at A, the others are normal. Many tubules are dilated and casts present in the lumen (B) H.& E. (X 100).

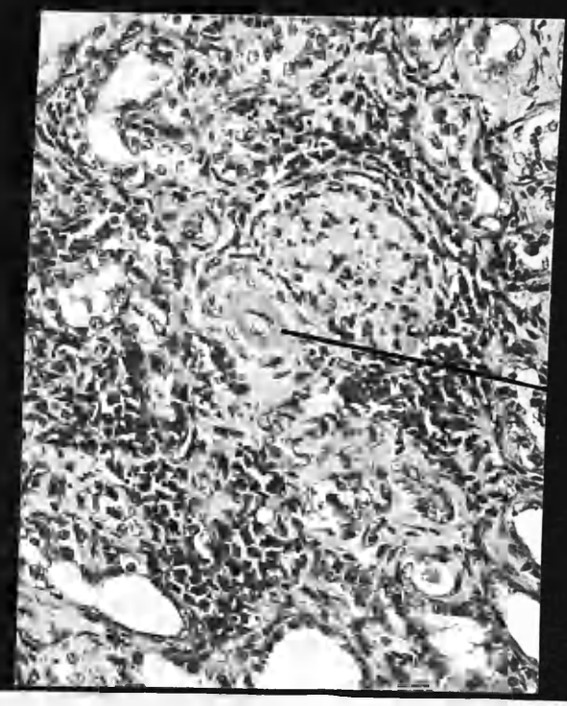


FIG.69 - KIDNEY. The glomerulus A (Fig. 68) is seen in centre of field. There is necrosis of turf, heightening of capsular epithelium, and marked hyaline degeneration of the afferent arteriole. H. & E. (X 250).

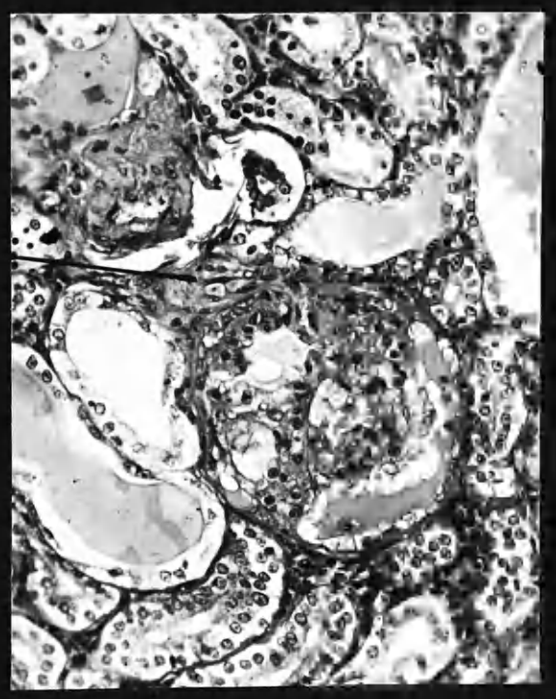


FIG.70 - KIDNEY. Glomerulus in centre of field shows adhesion between tuft & capsule. An exudate is present in the sub-capsular space. Two dilated tubules with flattened epithelium are seen on either side of glomerulus. The thickened afferent arteriole is also seen (A) H. & E. (X 250)

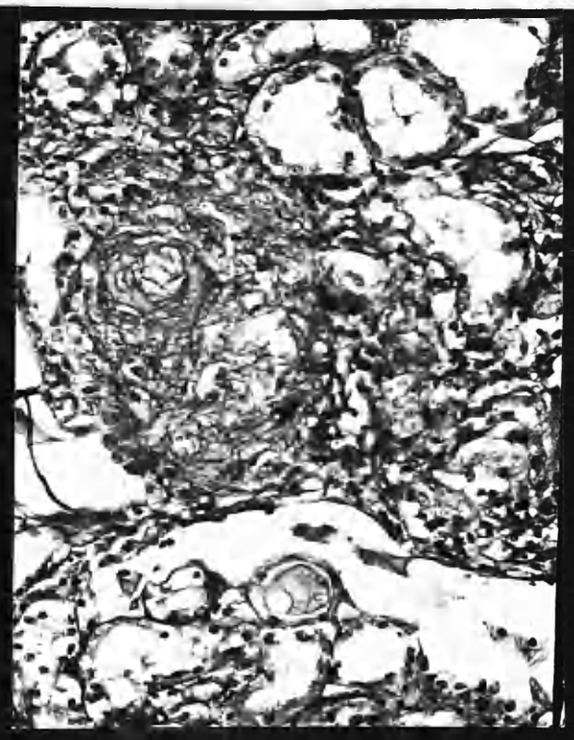


FIG.71 - KIDNEY. A glomerulus showing almost complete sclerosis is seen in the centre of the field. H. & E. (X 250).

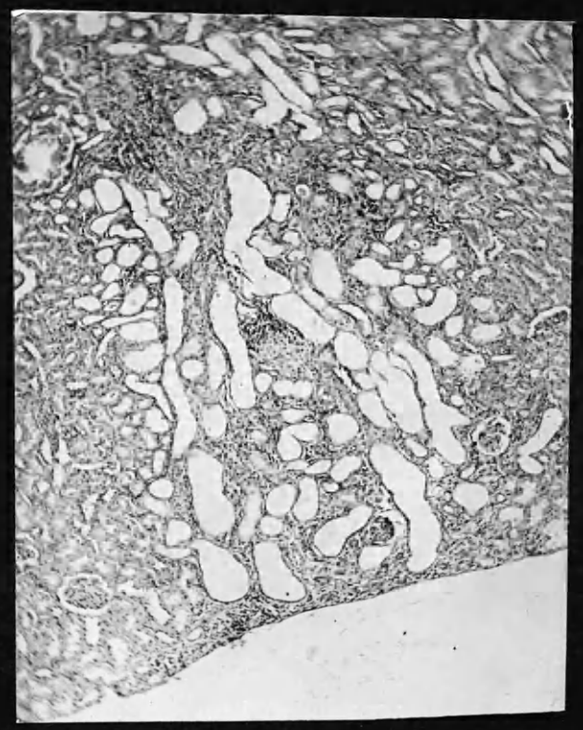


FIG.72 - KIDNEY, showing focal involvement of tubules. H & E. (X 75).

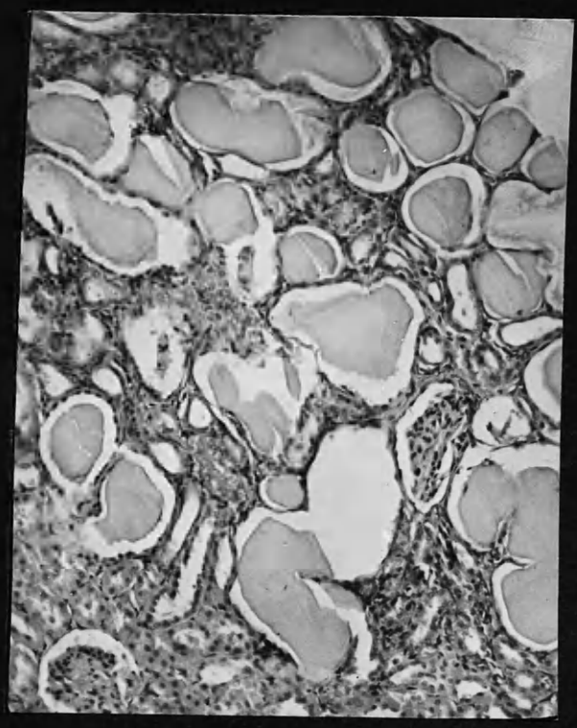


FIG.73 - KIDNEY. The tubules are dilated and irregular in appearance. The epithelium is flattened against the basement membrane and casts are abundant in the lumen. H. & E. (X 150).

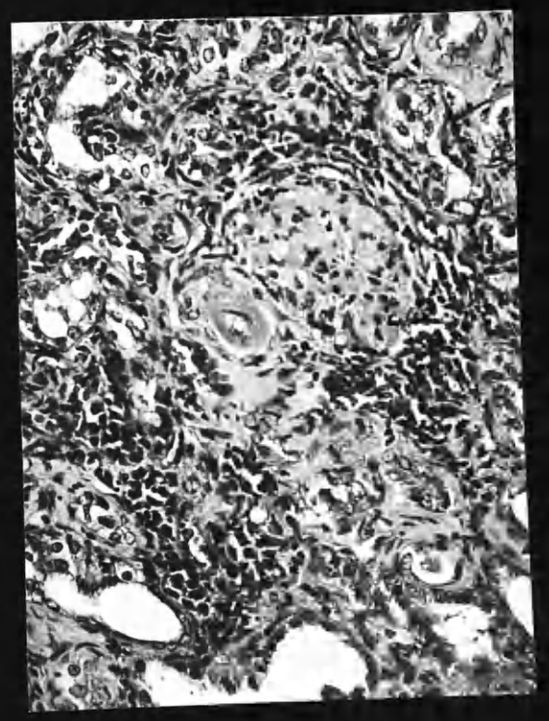


FIG.74 - KIDNEY, showing hyaline degeneration of the afferent arteriole with narrowing of lumen. H.& E.(X250).

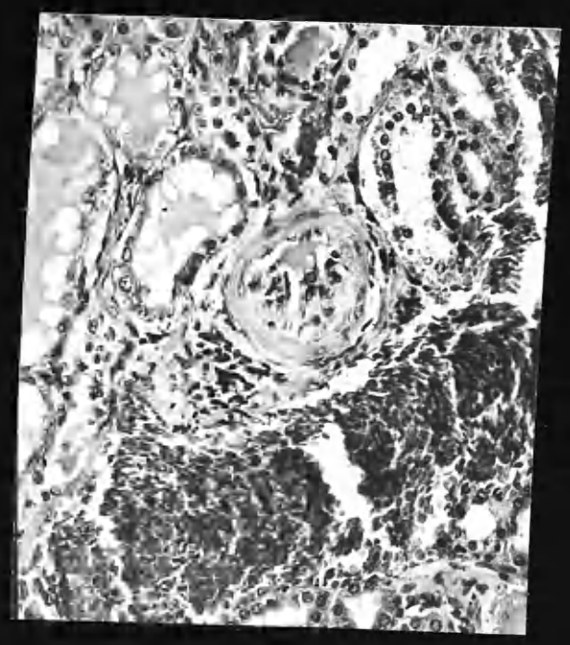


FIG. 75 - KIDNEY, shows marked intimal hyperplasia of a small renal artery. Note the narrowing of the lumen. H. & E. (X 250).

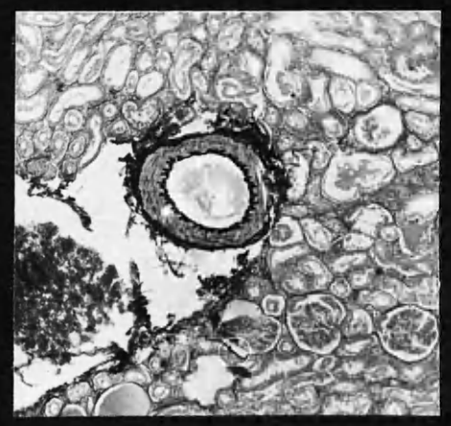


FIG.76 - KIDNEY. Interlobular artery showing no splitting of internal elastic lamina. Weigert-Van Gieson. (X 75).

FIG.77 - KIDNEY. The arcuate artery is seen on cross-section. The internal elastic lamina is normal. Weigert-Van Gieson. (X 75).

A

AFFERENT.
ARTERIOLE.

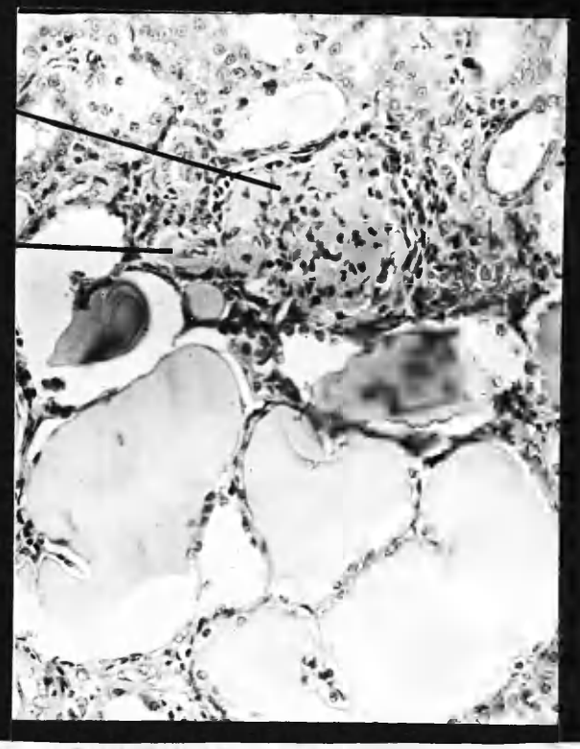
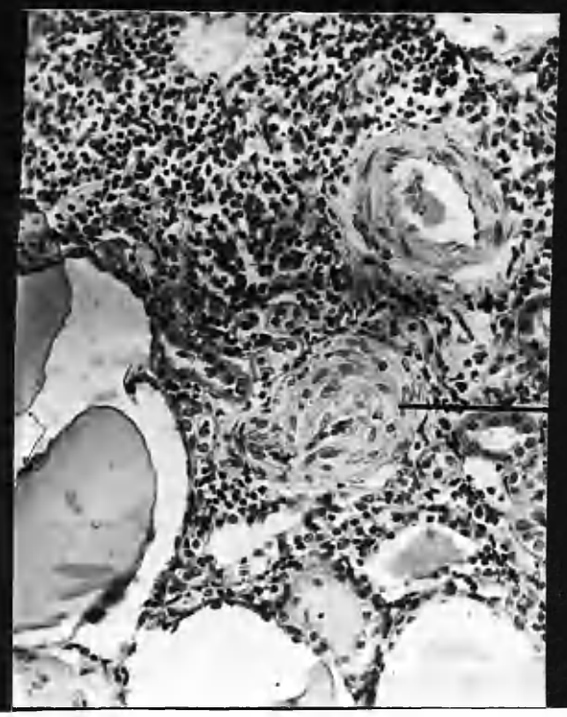


FIG.78 - KIDNEY. A glomerulus is shown in centre of field (A) above widely dilated tubules. Note the necrosis of glomerular tuft, adhesion to capsule & thickened afferent arteriole (B).H&E.(X250)



A.

FIG. 79 - KIDNEY. A large arteriole is shown (A). Marked intimal proliferation with almost complete closure of lumen is seen. Note the round cells in the interstitial tissue. H. & E. (X 250).

A.

FIG.80 - LIVER. Extensive haemorrhage present around central vein of lobule. Normal liver cells seen in mid-zone region (A). H.& E. (X 75).

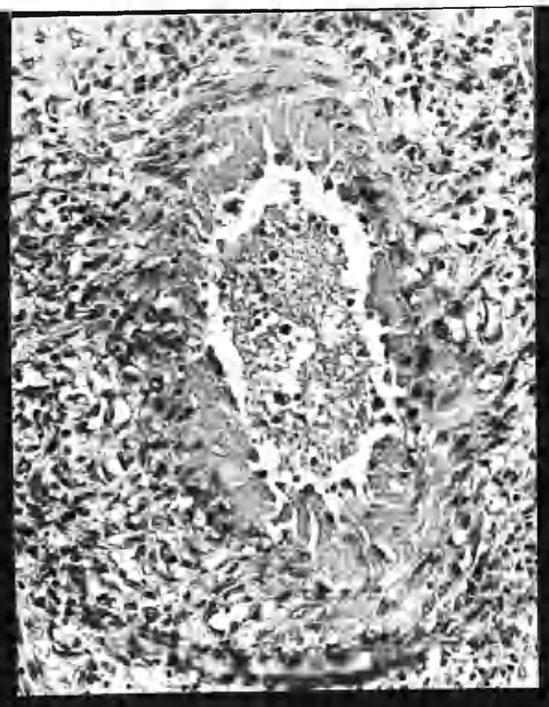


FIG.81 - PANCREAS. Large artery in centre of field showing hyalinisation of intima. H. & E. (X 150).

FIG.82 - Suprarenal (Control) showing osmophilisation in the outer zone. The second is free from osmophil granules and the last is broad and osmophil-positive. Osmic Acid. (X 75).

FIG.83 - SUPRARENAL showing haemorrhage in the inner aspect of the second zone, and a large osmophil-positive fourth zone. Osmic Acid (X75).

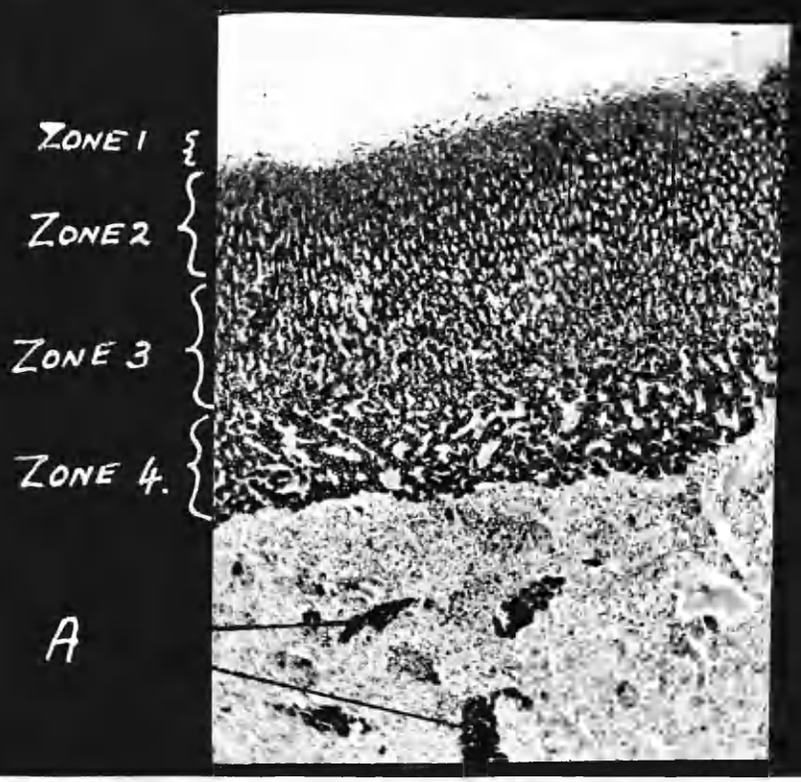


FIG.84 - SUPRARENAL showing a slight osmophil reaction in zone 1. Osmophil-positive cells are seen in medulla A. Osmic Acid. (X 75).

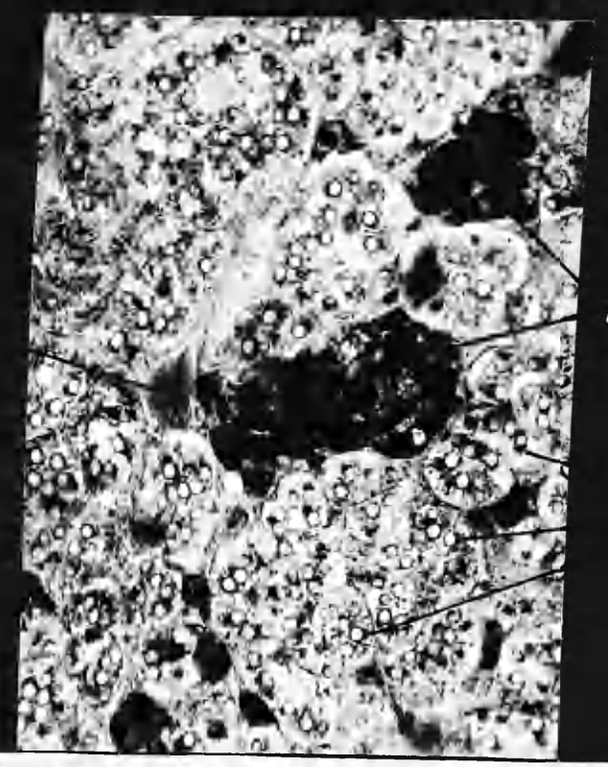


FIG.85 - SUPRARENAL. Cortical cells are shown in medulla (A) and are outside the sinusoid (B). Note the golgi apparatus in relation to the nucleus of the medullary cells and the absence of osmophil granules in the cells. Osmic Acid. (X 250).

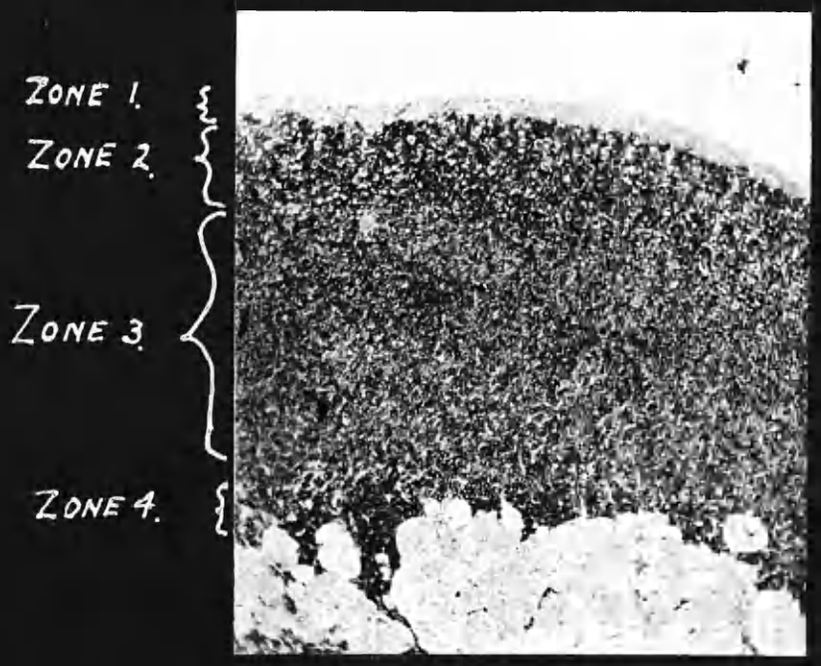


FIG 86.

FIG 87.

FIGS.86 & 87 - SUPRARENALS. Both glands show slight diminution in osmophil granules in zone 2. There is a moderate increase in size of zone 4 in one Fig.87. Osmic Acid. (X 75).

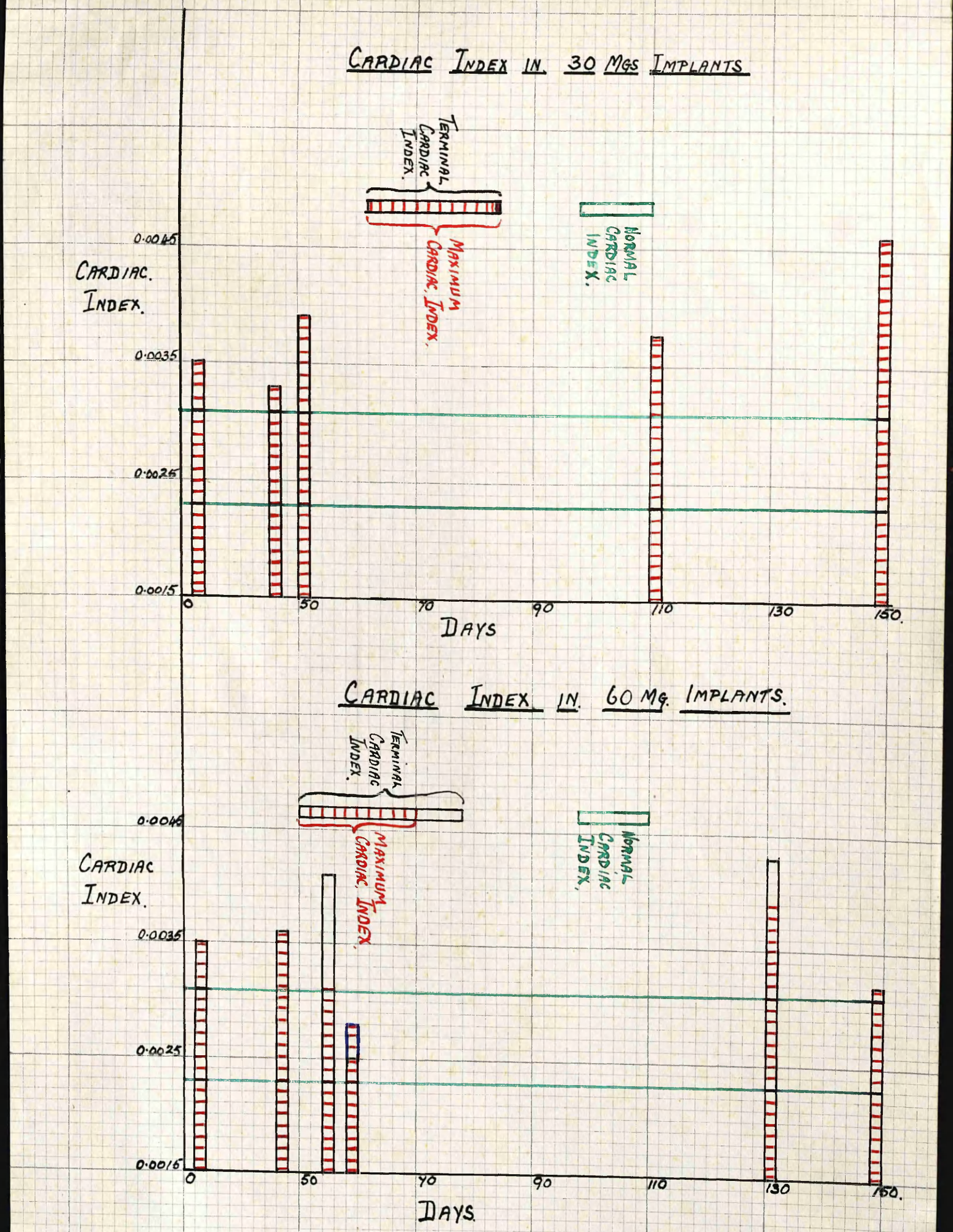


FIG. 88 - The cardiac index in 30 and 60 mgs. groups are shown and the normal range is also present.

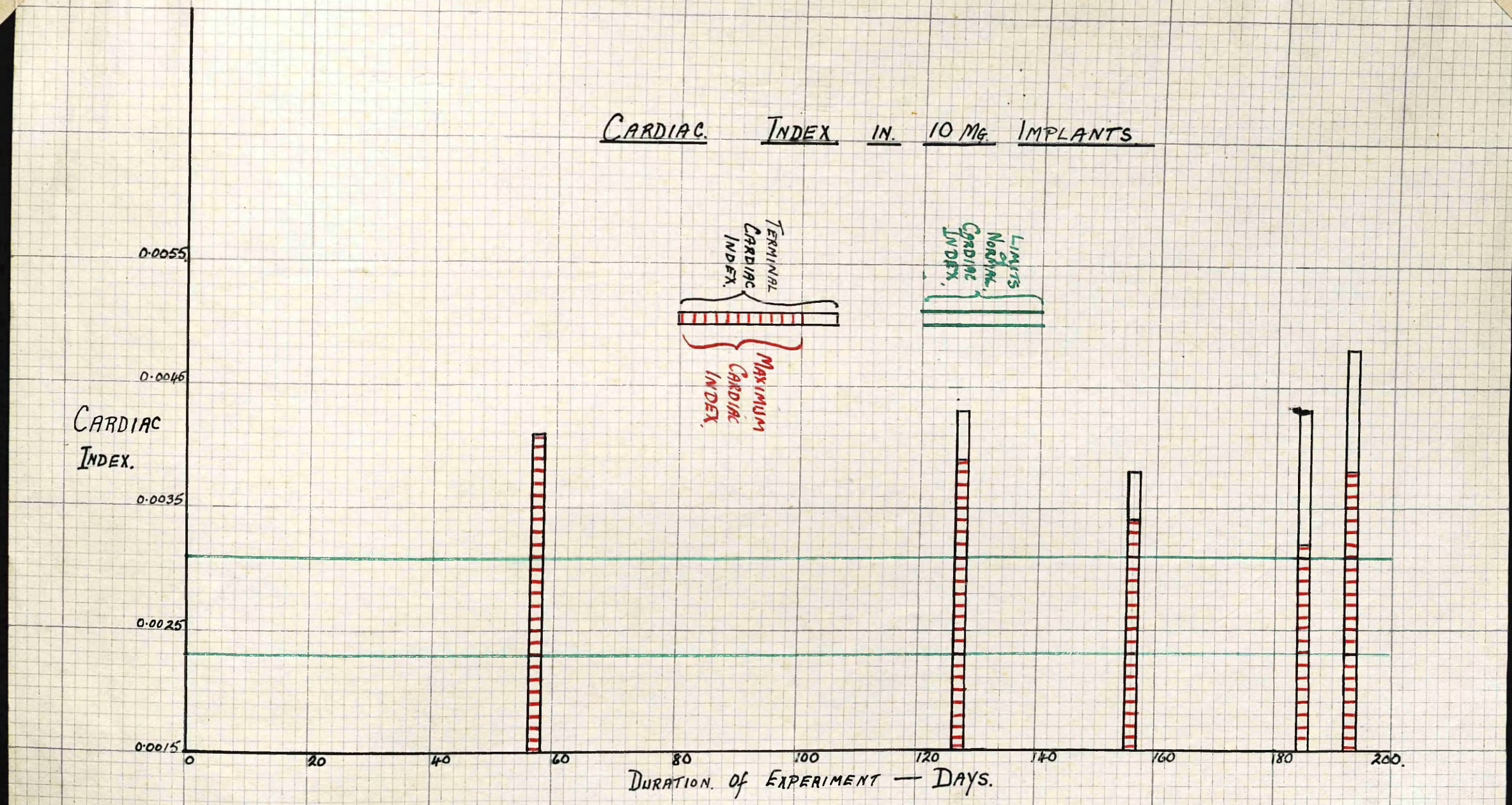


FIG.89 - Showing the cardiac index in the 10 mg. group of implants.

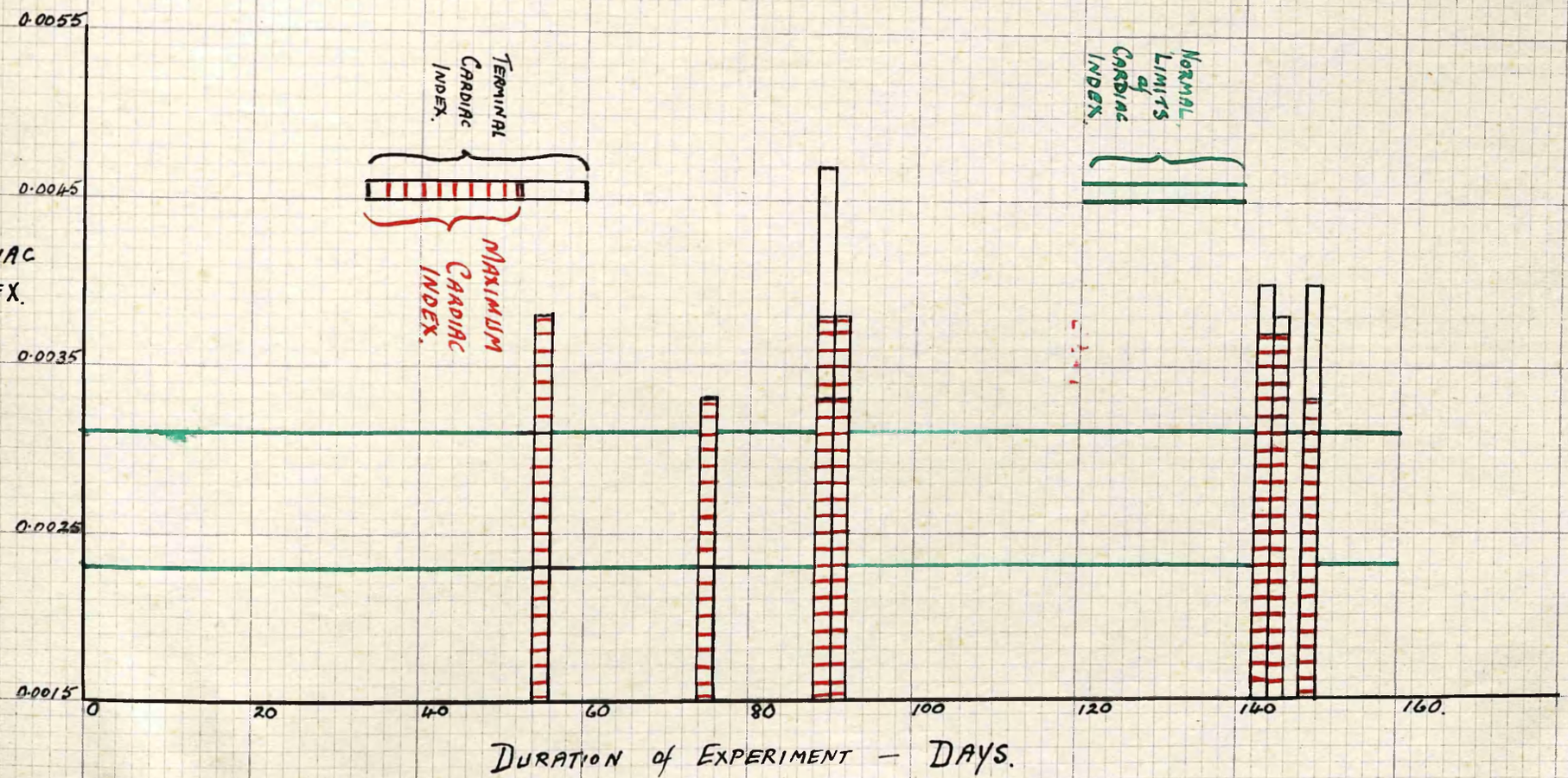


FIG. 90 - Showing the cardiac index in adrenalin-treated animals given 1% saline as drinking water.

FIG 91.

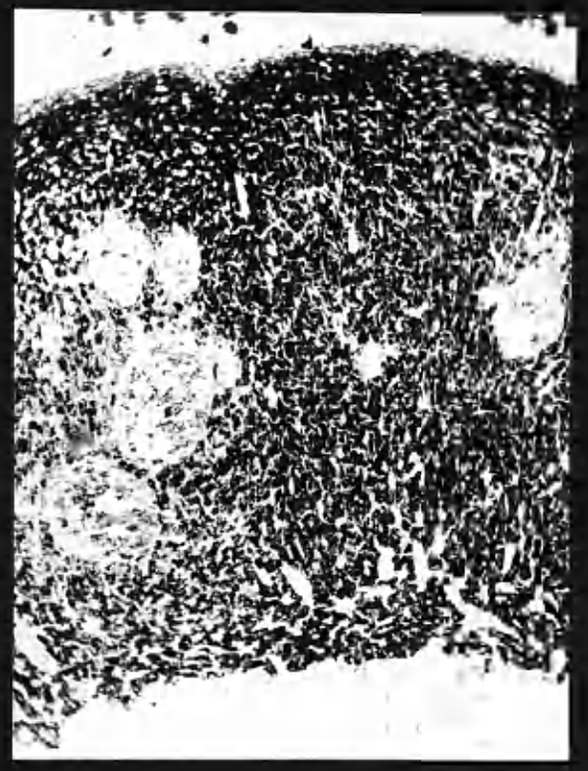


FIG 92.

FIG 93.

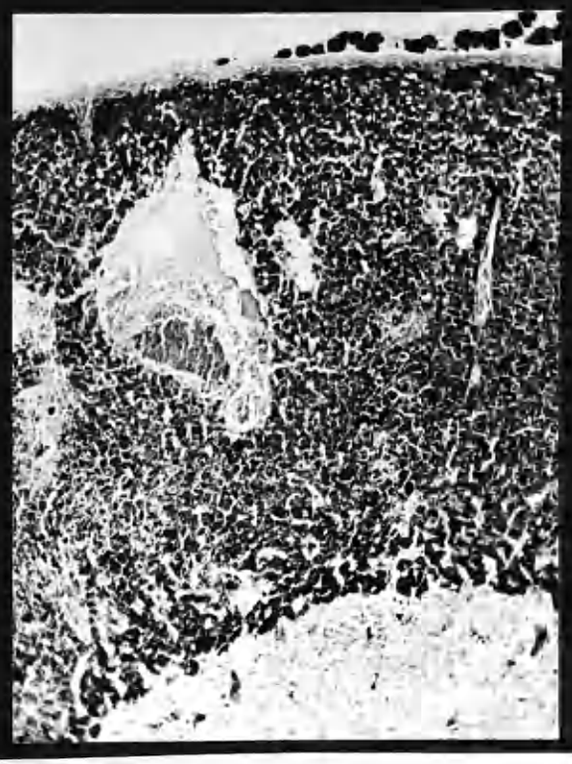
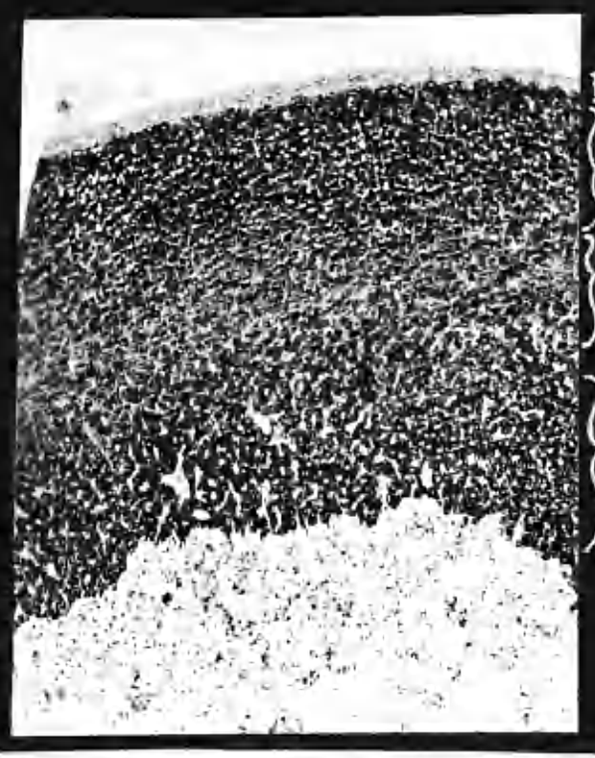


FIG 94.



FIGS.91,92 & 93 - SUPRARENALS. Show the presence of haemorrhage in zones 2 and 3.
FIG.94 - SUPRARENAL is normal in appearance, apart from an increase in size of zone 4. No haemorrhages are present.
Osmic Acid. (X 75).

FIG 95.

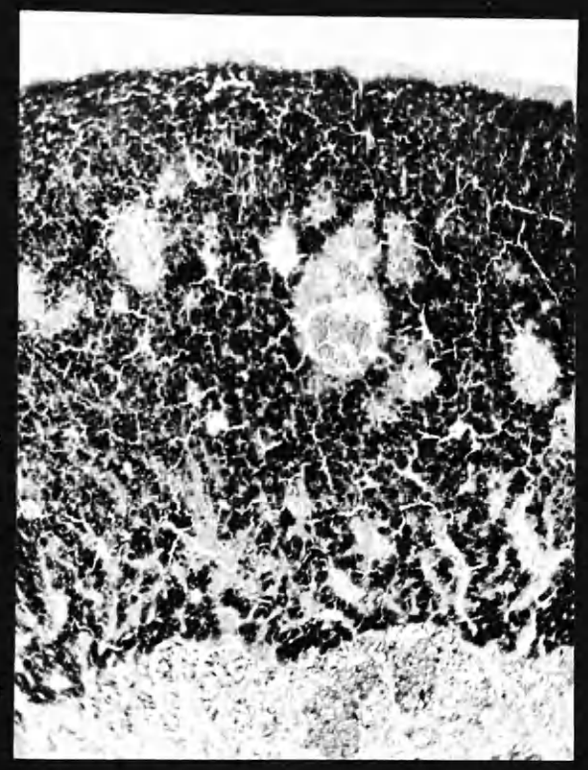


FIG 96. A

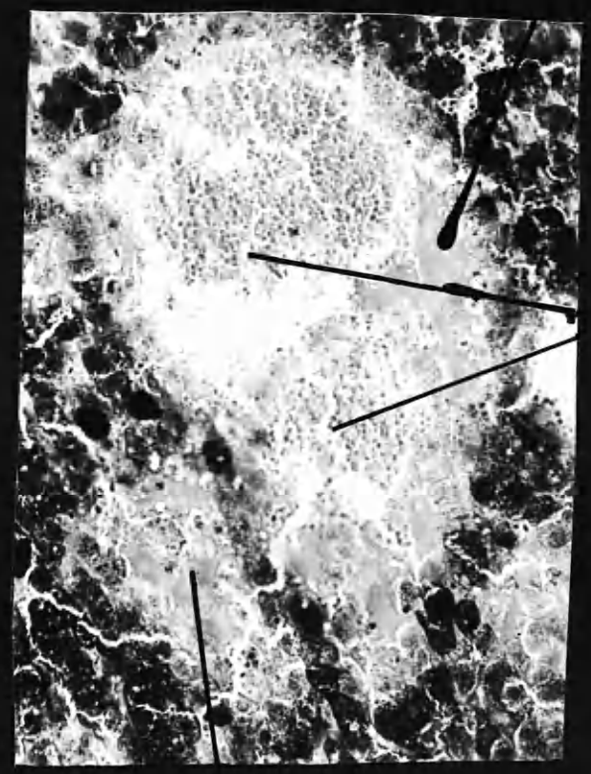


FIG 97.

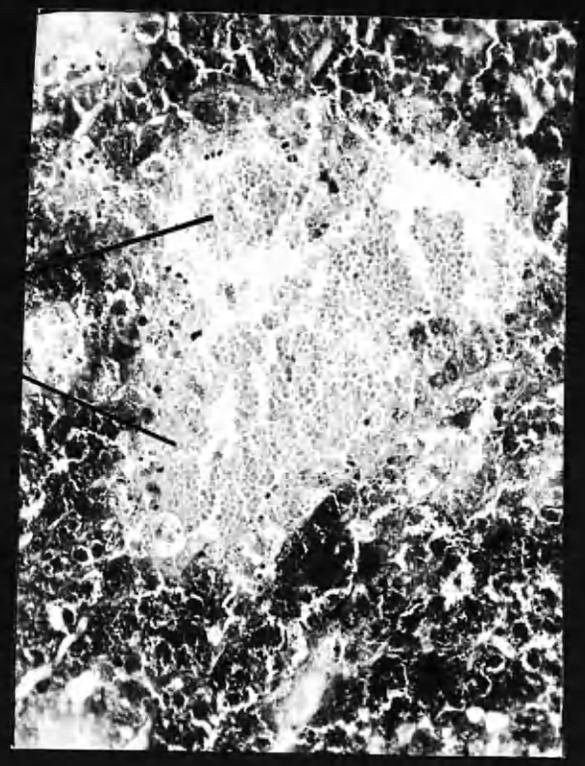


FIG.95 - SUPRARENAL. Multiple haemorrhages are seen involving the third zone of the cortex. There is extreme congestion of the vessels in zone 4. Osmic Acid. (X 75).

FIGS.96 & 97 - SUPRARENAL. High power magnification of the haemorrhages are shown. Note the irregular outline of the haemorrhages, osmophil secretion (A), and red blood cells (B). Osmic Acid. (X 250).

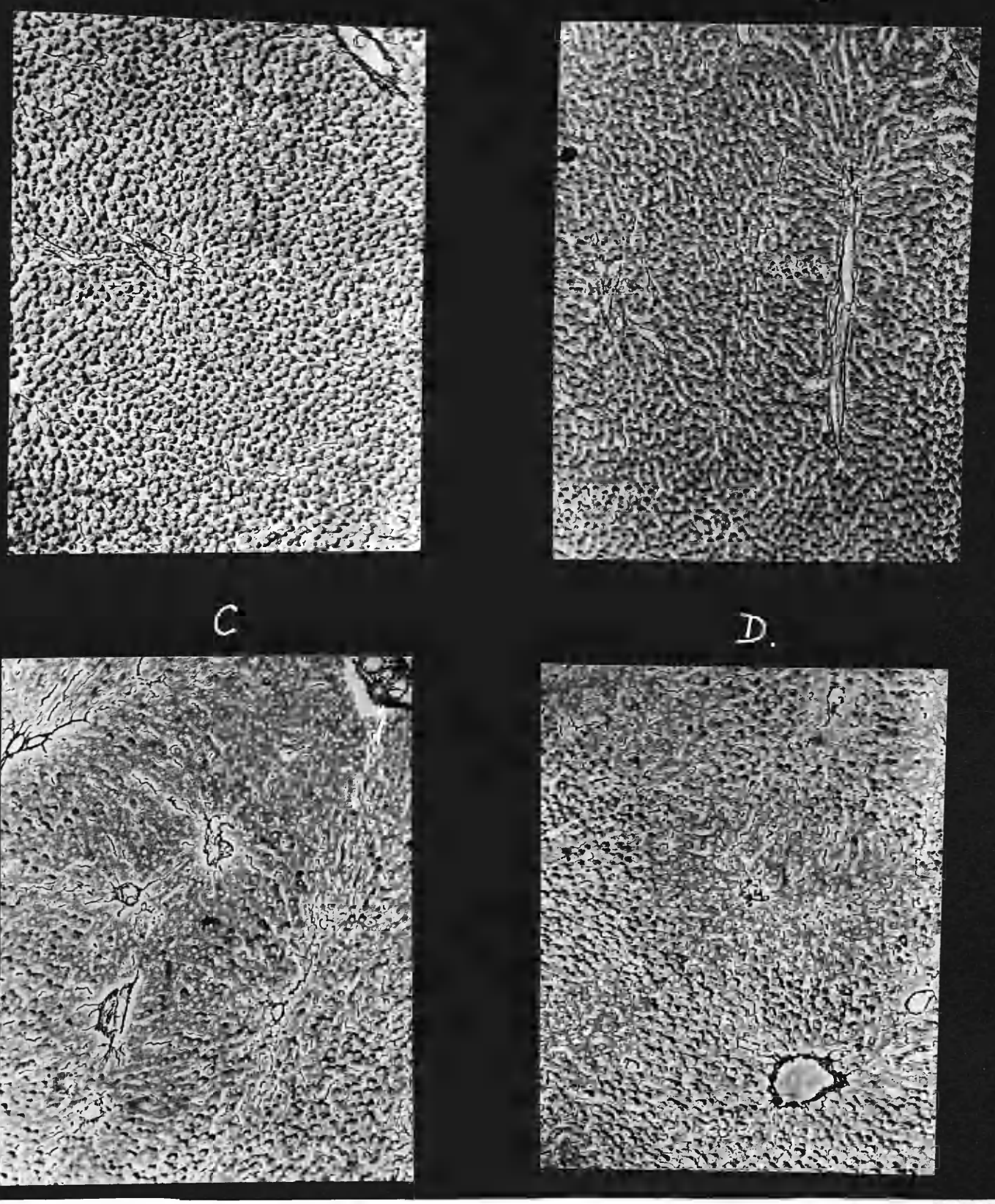


FIG.98 - A to D. LIVER, showing the appearance of liver glycogen at intervals, following a second injection of 1:1000 Adrenalin hydrochloride. A & B are normal, C & D are depleted and show no evidence of regeneration. Osmic Acid. (x 100).

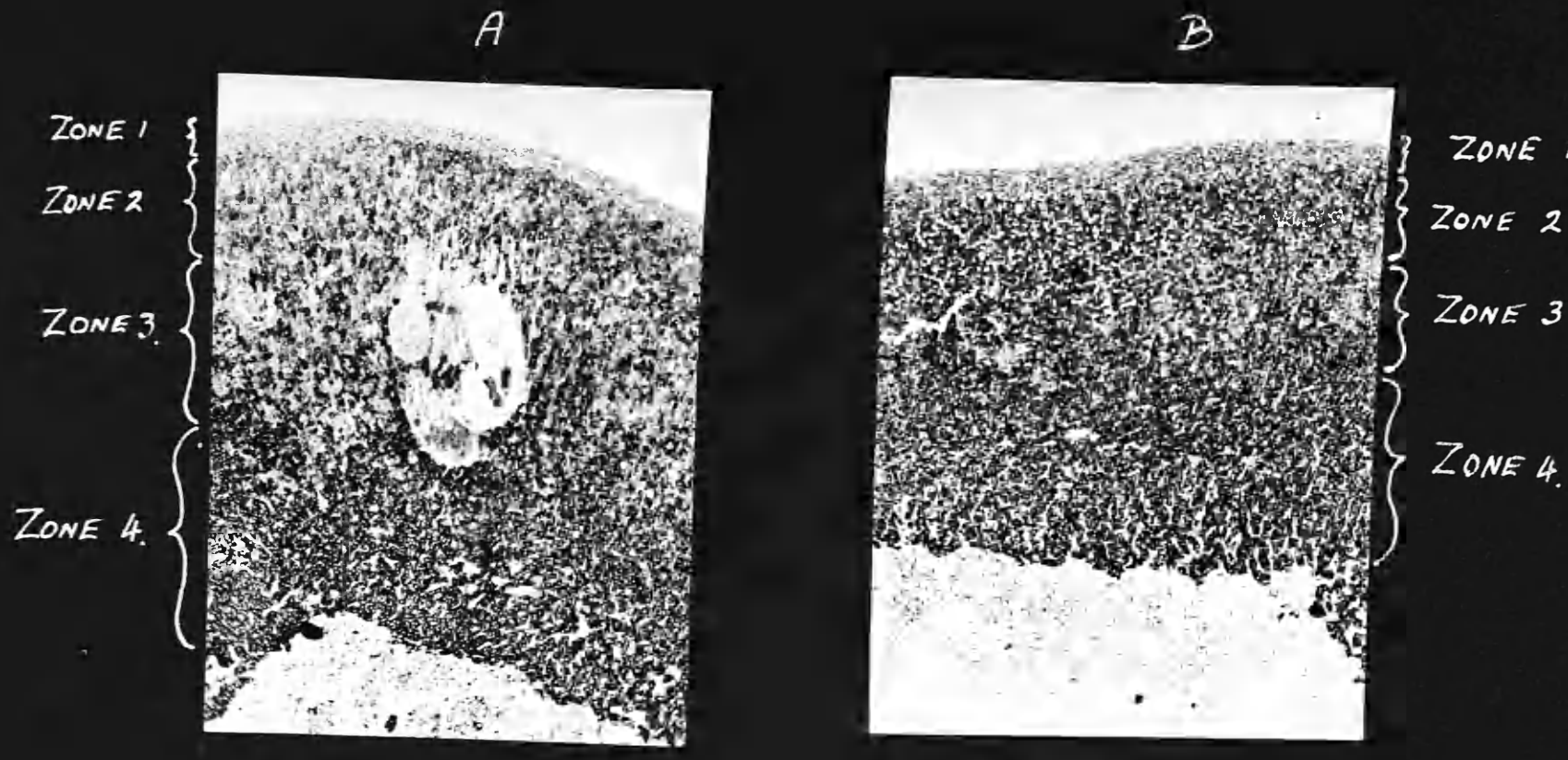


FIG.99 - A to D. SUPRARENALS showing appearance of the glands at intervals following a second injection of 1:1000 adrenalin hydrochloride. Note the haemorrhage in zone 3 (A & D), the depletion of osmophil granule from zone 2, and the increase in size of zone 4 fig. D. Osmic Acid. (X 100).

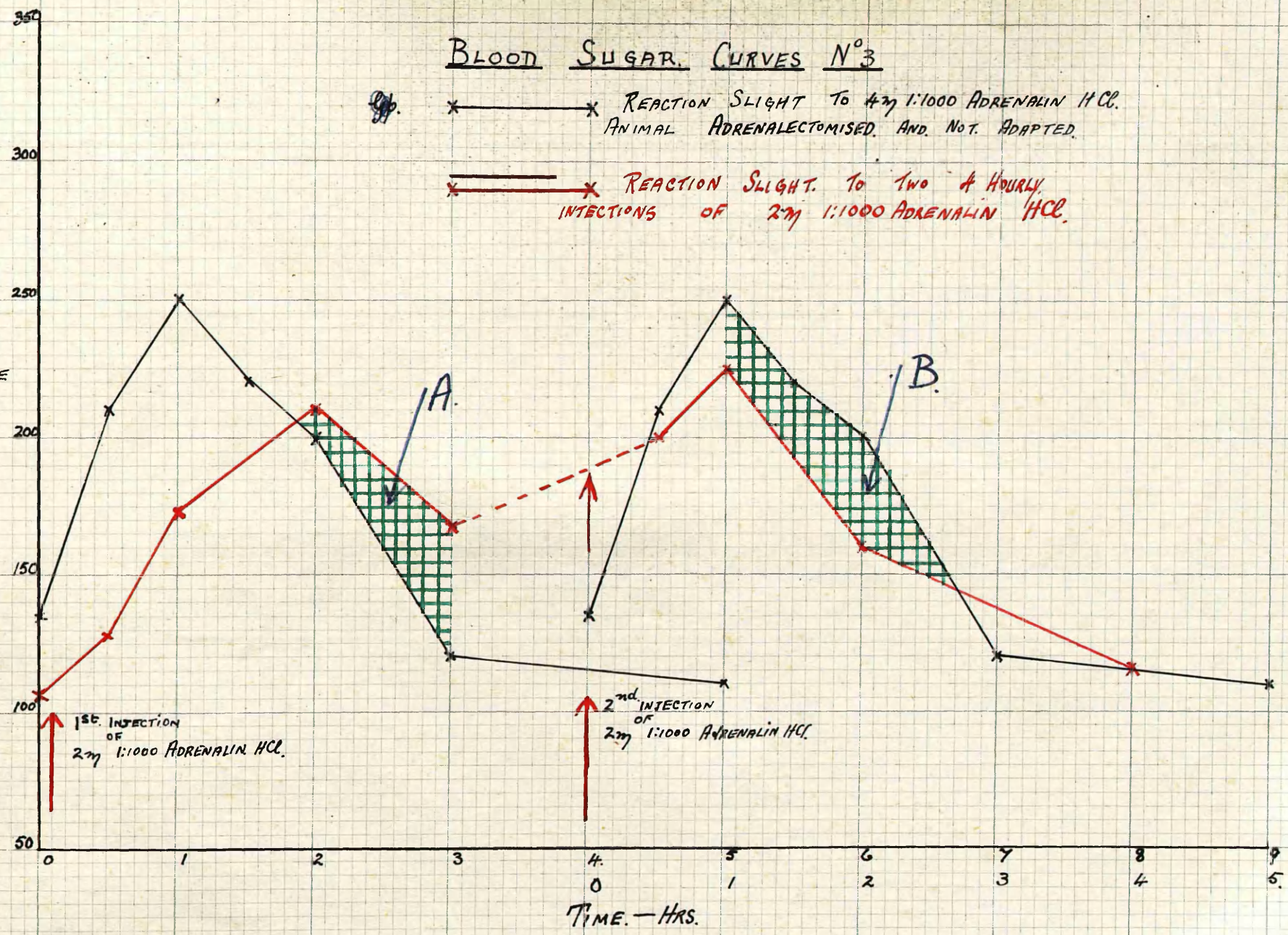


FIG.100 - The blood sugar curve is shown (red) after a slight reaction with 1:1000 adrenalin hydrochloride. The arrows denote the time of injections. The sugar curve of an adrenalectomised animal which received 4 m 1:1000 adrenalin is superimposed. The crossed area A (green) shows the ability of the suprarenals to mobilise sugar. This is lost (B) after the second injection when the suprarenals were seen to be in a state of exhaustion.

B O O K 3.

T H E S U P R A R E N A L G L A N D -

ITS FUNCTION IN HYPERTENSION AND THE COUNTER-SHOCK MECHANISM

by

THOMAS SYMINGTON, B.Sc.(Hons.), M.B., Ch.B.

TABLES 1 to 22: APPENDIX I and II.

TABLE 1. CASES REPORTED FROM THE LITERATURE BY EISENBERG AND WALLERSTEIN (1932).

Blood Pressure.	Age Groups. Not Stated	1-10	10-20	20-30	30-40	40-50	50-60	60+	No. in Series
Paroxysmal.			Fränkel.	Labbe, Azerad, and Violle; Labbe, Tinel, and Doumer; Mayo; Oberlung and Jung; Shipley.	Vaquez et al; Biebl and Wichels; Zeckwer.	Barker; Connor (3); Orth; Neusel and Wiesel.		Lascagna.	16
Persistent.						Rabin			1
Hypertension only mentioned.	Kerpola.					Bergstrand; Herde (2); Robert; Schroeder; Helly.		Thomas.	7
No mention.	Masson (2); Neusel and Wiesel (1); Bonnamour et al.	Wahl (2½ y.)	Marchetti.		Hedinger; Wagelin; Weber; Laignel-Lavastin and Aubertin.	Harbitz; Masson and Martin.	Herxheimer.	Berdez; Herde (1); Perley; Suzuki (1, 2, 3); Manasse (1, 2).	21
Normal.				King.	Kawashima.	Riemer; Gravier and Bernheim.	Lazarus and Eisenberg; Rossum and Barry; Eisenberg and Wallerstein.		7

Note:- Figure in brackets after name denotes the number of the case in the paper quoted.

TABLE 2. CASES REPORTED FROM THE LITERATURE BY EDWARD (1937).

Blood Pressure.	Age Groups. Not Stated	1-10	10-20	20-30	30-40	40-50	50-60	60+	No. in Series.
Paroxysmal.			Collier et al; Ernould and Picard; Paul (1).	Suermondt; Von Der Mühll; Paul (3).	Bauer and Leriche; Kelly et al; Nordman and Kalk; Porter and Porter; Appelmans and Van Goidsenhoven; Volhard; De Wesselow.	Belt and Powell; Kahlau; Rogers; Sachs and Russum; Sevki; Tillman.	McKenna and Hines.	Paul (2).	21
Persistent.			Kremer.	Edward.				Hick.	3
Hypertension only mentioned.					Paul (4).	Görog; Kaulback; Popken.	Paul (5).		5
No mention.						Buchner.			1
Normal.	Fingerland; Popken; Bianchedi.			Fein and Carman.					4

Note:- Figure in brackets after name denotes the number of the case in the paper quoted.

TABLE 3. CASES REPORTED IN THE LITERATURE SINCE 1937, INCLUDING PRESENT SIX CASES.

Blood Pressure.	Age Groups. Not Stated	1-10	10-20	20-30	30-40	40-50	50-60	60+	No. in Series.
Paroxysmal.		Neff et al.	McCullagh and Engel (1); Hyman and Mencher (4); Holst (1); Holst (2); Evans and Stewart.	Van Epp et al (1); Hyman and Mencher (1); Hyman and Mencher (2); Broster and McKeith; Boman and Wells; McKenzie and McEachern; Tenenbaum; Burgess et al; Binger and Craig; Holst (3); McCullagh and Engel (2); Kirshbaum and Balkin (1); Palmer and Castleman; Blacklock and Symington (1).	Nettleship; Biskind et al; Van Epp et al (2); Hyman and Mencher (3); Kenyon; Landau; Strombeck and Hedberg; Rodin; Baker and Reinhoff; Blacklock and Symington (2).	Brunschwig and Humphreys; Heath and Cahill; Borch-Johnson; Borras and Mota; Eleosser; Allen; Pincoff.	Nuzum and Dalton.	Howard and Barker.	39
Persistent.				Kirshbaum and Balkin (2); Oppenheimer and Fishberg.	Thorn et al; Blacklock and Symington (3).				4
No mention.		Wahl and Robinson.							1
Normal.					Blacklock and Symington (6). McGavack et al.		Blacklock and Symington (4); Rosenthal and Willis.	Blacklock and Symington (5).	5

Note:- Figure in brackets after name denotes the number of case in the paper quoted.

TABLE 4. GROUP 1 A. PAROXYSMAL HYPERTENSION WITH NORMAL INTERVAL BLOOD PRESSURE.

Author.	No. of Case.	Age Yrs.	Sex.	Size of Tumour.	Site of Tumour.	Short Clinical History.	Blood Pressure. Before Operation	Blood Pressure. After Operation	Eyes. Before Operation	Eyes. After Operation	Kidneys.	Heart gms.	Other Organs.	Results.
Howard & Barker 1937.	1	69	M	Grape-fruit.	Left	Typical paroxysmal attacks. Albumen - trace.	134/84 to 260/130	-	Moderate sclerotic scar of old haemorrhage.		No glomerular or vascular lesions.	420	-	Died. No operation.
Neusser & Wiesel.	1	43	M	Bilateral Tumour.		Vasomotor instability. Died 2 hrs. after dental extraction.	-	-	-	-	Kidneys and arteries not affected.	-	-	Died. No operation.
Biskind et al 1941.	1	31	F	11 x 8 x 6 cm. Wt. 300 g.	Right	Typical paroxysms. Sugar in urine.	120-280/75-150	Normal	Flame-shaped haemorrhage. Thickened retinal vessels.	Normal. 7 mths later.	-	-	-	Operation successful
Shipley & Pincoff 1929.	1	45	M	4.5 x 4 x3.5 cm. Wt. 28.5 gm.	Right	Typical paroxysms. Albumen and sugar in urine.	140-260/90-150	Normal	Arteriosclerotic changes in optic fundi.	No mention.	-	-	-	Operation Successful
Engel, Mencher & Engel 1942.	1	23	F	5.5 x 3.5 cm. Wt. 31 gm.	Right	Typical paroxysms. Albumen and sugar present.	310-140/180-190	Normal	Narrowing of fundal vessels.	No mention.	-	-	-	Operation successful
Wells & Boman 1937	1	30	F	5 cm.	Right	Typical paroxysms.	Normal to 180/140	-	Spastic retinitis.	-	No mention.	325	-	Died.
Holst 1938	1	17	F	280 gm.	Left	Typical paroxysms. Albuminuria Glycosuria.	115/70 to 260/100	-	Albuminuric retinitis.	-	P. M. refused.			Died.
Rodin 1945.	1	31	F	-	Right	Paroxysmal attacks. Hyperglycaemia Glycosuria.	130-100/95-80 to 260-240/150-180	Normal	Hypertensive retinopathy.	Normal in one year	-	-	-	Operation. Successful.
Baker & Reinhoff 1937.	1	40	F	13 x 12 x 8½ cm. Wt. 560 gm.	Right	Typical paroxysms.	B.P. rose to 290/185	-	Arterial changes and haemorrhage into retina.	-	Moderate arteriosclerosis.	Enlarged	-	Died

TABLE 5. GROUP 1 B. PAROXYSMAL HYPERTENSION WITH RAISED INTERVAL BLOOD PRESSURE.

Author.	No. of Case.	Age Yrs.	Sex.	Size of Tumour.	Short Clinical History.	Blood Pressure. Before Operation	Blood Pressure. After Operation	Eyes. Before Operation	Eyes. After Operation	Kidneys.	Heart.	Other Organs.	Result.
McCullagh & Engel 1942.	1	18	F	5.5 x 4.3 x 3.5 cm. Wt. 35 gm.	Paroxysms 4 years' duration. "Spells 15-20 per day", dizziness, fainting and later sweating. No typical proxysms when under observation. Albumen present.	B.P.150/100 rose when suprarenal was handled at operation.	130/70	Changes in retina resemble those in essential hypertension.	No mention.	-	-	-	Operation successful.
McCullagh & Engel 1942.	2	28	M	16 cm. 735 gm. Right adrenal.	Nervousness and weakness 5 years' duration. Diabetic sugar curve. Albumen and sugar in urine. (Note: Nodule 1 cm. in diameter lying on Aorta - Phaeochromocytoma).	174/104 rose to 270/150		-	-	Little or no sclerosis of blood vessels and glomeruli. Moderate arterio-sclerosis of aorta.	450 gm.	-	Died. No operation.
Beer & Prinz-metal. 1937.	1	26	F	Grape-fruit.	9 years' history of attacks. Increased in frequency until they occurred every 30 mins.	140/100 to 280/200.	-	Thinning of arteries.	Same after operation.	Kidney removed at operation shows arterio-sclerosis.	-	-	Operation. Patient well years after.
Holst 1938.	2	19	M	Walnut.	No history of paroxysms. Symptoms developed rapidly 3 months before operation. Headache, blurring of vision. Albuminuria. Diabetic sugar curve.	180-285 / 120. Rose on manipulation of tumour.	-	Extreme albumin-uric retin-itis.	-	No mention.	Left ven-tricle hyper-trophy.	-	Operation. Died 30 mins. after operation.
Holst 1938.	3	25	M	Size of a kidney.	Headache and vomiting 2½ yrs. Admitted in convulsion. Albumen and casts in urine. Diag-nosed chronic nephritis.	B.P.200/130	B.P.rose to 290/185 when he died.	Albumin-uric Retin-itis.	-	No mention.	Hyper-trophied.	-	Died. No operation.
Green 1946.	1	-	-	-	History of 12 years. Albumen and sugar in urine.	1945 B.P. 140/110 rose to 190/160. During a paroxysm 260/210.	140/108	Bilateral papill-oedema, haemorrhage & exudate into retina.	Normal.	-	-	-	Operation successful.
Labbé, Tinel & Doumer 1922.	1	28	F	Small orange.	Attacks irregular at first. Later they occurred every day. Duration of attacks 1-4 hours.	Resting B.P. 150/100. During paroxysm 280/160.		No mention.		No trace of sclerosis or glomerular lesions.	340 gms.	Lungs: Filled with blood.	No operation Died.

TABLE 5. GROUP 1 B (Contd. 2). PAROXYSMAL HYPERTENSION WITH RAISED INTERVAL BLOOD PRESSURE.

Author.	No. of Case.	Age Yrs.	Sex.	Size of Tumour.	Short Clinical History.	Blood Pressure. Before Operation	Blood Pressure. After Operation	Eyes. Before Operation	Eyes. After Operation	Kidneys.	Heart.	Other Organs.	Result.
Labbé, Violle & Azerad, 1929.	1	29	M	Small orange.	Patient seen over period of 12 yrs. Evacuated from front 1914-1918 as chronic nephritis. Paroxysmal attacks lasted 30 mins. Albumen ++	B.P.160/100 to 250/?		No mention.		Stated to have chronic nephritis with glomerular sclerosis of a mild degree.	Hypertrophy of left ventricle.	Lungs: Haemorrhagic exudate in lungs.	Died. No operation.
Paul, 1931	3	23	F	6 x 5 x 4 cm. 85 gm.	Pains in head during periods. Afterwards weakness. Developed a typical paroxysm before admission and died of marked pulmonary oedema.	No blood pressure measurements taken.		No mention.		Hyalinised glomeruli in wedge shaped areas related to a large arteriole. The wall is hyalinised.	Hypertrophy of muscle fibres.	Liver: Splitting of internal elastic lamina of vessels.	Died. No operation.
Vaquez, Donzalot and Geraudel, 1929.	1	37	M	7 x 6 x 5 cm.	Acute painful crisis, paroxysmal in nature. Attacks occurred first daily, later several times daily. Albuminuria following attacks.	$\frac{140-130}{80}$ later $\frac{210}{130}$. Patient refused operation. Later became bewildered, agitated and blind.		Haemorrhage into fundi.		No mention.	Hypertrophied.	No mention.	Died. No operation.
Palmer and Castleman, 1938.	1	23	F	5 x 4 x 3 cm. 35 gm.	Severe headache 9 years. First paroxysm 4 years ago. Later daily attacks. At first felt well between attacks. In the last year she had not felt well. Faint trace of albumen.	1936 - B.P.140/120. 1938 - B.P.190/160 atrest. 310/160 during attack.		Choking of optic disc. Many white spots in both retina. Large exudates and haemorrhage in macula more marked on left.		Normal microscopically.	250 gm.	No vascular lesions in any organs.	Died. No operation.
Evans and Stewart, 1942.	1	14	F	$5\frac{1}{2}$ x $4\frac{1}{2}$ x 4 cm. 63.5 gm.	3 years weakness and sweating. 1 year dyspnoea. 5 months severe headache. Attacks became more frequent. Moderate polyuria. Severe bitemporal headache. Sudden onset of blurring of vision. Albumen in urine. R.B.Cs. and granular casts.	150/118 to 250/160.	After operation: 18 days 120/84 2 mths. 118/80 8 mths. 98/54 1 year. 98/64	Marked retinal changes in both eyes.	Gradual improvement to normal.	-	Electro showed Lt. axial deviation, which returned to normal post-operative.	-	Operation successful.
Rogers, 1932.	1	49	M	Grapefruit.	Duration of symptoms - 9 years. Attacks occurred several times daily. After attacks usual weakness. Albumen and sugar in urine.	Resting B.P. = 160/120.		Old haemorrhages in fundi.		No mention.	Enlarged.	-	Died. No operation.

TABLE 5. GROUP 1 B (Contd. 3). PAROXYSMAL HYPERTENSION WITH RAISED INTERVAL BLOOD PRESSURE.

Author.	No. of Case.	Age Yrs.	Sex.	Size of Tumour.	Short Clinical History.	Blood Pressure. Before Operation	Blood Pressure. After Operation	Eyes. Before Operation	Eyes. After Operation	Kidneys.	Heart.	Other Organs.	Result.
Paul, 1931.	2	72	F	1. 260 g. in right adrenal. 2. Hazel nut left adrenal.	Healthy until 1 year ago. Pains in head. Died suddenly after 6 months symptoms. Albumen++ casts.	B.P. 185-192 Diastolic not given.		No mention.		Reduced in size. "Diffuse arteriosclerosis with a moderate number of hyaline glomeruli". Hyalinisation of renal arterioles.	Pronounced left ventricular hypertrophy.	-	Died. No operation.
Suermondt, 1934.	1	29	M	Size of a fist.	Attacks first started 1½ yrs. previously. At first once daily, later several times daily. Albumen present.	B.P. 150/115 to 325/200.	125/95 after operation.	No mention.		-	-	-	Operation. Well 6 months later.
Bauer & Leriche, 1934.	1	41	M	Cherry.	Attacks of 1½ yrs. duration. Mild at first, later increased in severity and occurred daily. Albumen and sugar in urine. Increased after attack.	B.P. 200/100 to 340/110.	140/100. Albumen and casts persisted.	No mention.		-	-	-	Operation. Still having reactions 8 yrs later. (Biskind).
Coller, Field & Durant, 1934.	1	16	M	8 cm. in diameter.	Attacks began 5 months previously. They increased in frequency until they were occurring daily. Albumen present. It increased after attacks.	B.P. 195/125 to 300/200.	120/80 6 months later.	Arterial changes with haemorrhages into fundi.	No mention.	-	-	-	Operation successful.
Volhard, 1931.	1	38	M	Egg.	Attacks of 17 months duration. Only occasional at first, later 3-4 times daily. Urine normal at first later contained albumen.	B.P. 145/? 1 yr. later 180/130. During attacks 330/120.	-	Haemorrhagic retinitis with exudates.	No mention.	-	-	-	Operation. Day after operation he gave a cry & died. Cause of death not found.
Gutmann, 1947.	1	38	F	2.5 cm. in diameter.	Cough with spit of several years. Usual features. Albumen and sugar.	200/120 to 300/170.		Bilateral papilloedema, multiple haemorrhages and exudates.		"Well marked hypertensive changes with arteriosclerosis and necrosis".	Marked lt. ventricular hypertrophy.	-	Died before operation.
Spalding, 1947.	1	24	F	86 gm.	Blurring of vision, headache and palpitation of 2 yrs duration. Attacks increased in frequency until they occurred daily.	120/80 to 250/170 between attacks. 300+/205 during paroxysms.	15 mths. after operation 135/100.	Albuminuric retinitis.	15 mths. after operation discs normal.	-	-	-	Operation successful.
Blacklock & Symington, 1947.	1	21	M	8 x 6 cm. Wt.86 g.	Headache, palpitations, breathlessness. Attacks occurred 5 times daily and lasted 5 minutes.	130-165/85 to 260/120.	Fell to systolic 50mm 2 hrs. after operation.	No abnormality of fundus.	-	Marked splitting of internal elastic lamina of arcuate vessels. Some hyalinisation of afferent arteriole.	400 gm.	Thickening of arterioles of spleen.	Died 62 hrs. after operation.

(Believed to be in transition stage from Group 1A to Group 1B).

TABLE 6. GROUP 2. PERSISTENT HYPERTENSION.

Author.	No. of Case.	Age. Yrs.	Sex.	Size of Tumour.	Site of Tumour.	Short Clinical History.	Blood Pressure. Before Operation	Blood Pressure. After Operation	Eyes. Before Operation	Eyes. After Operation	Kidneys.	Heart.	Other Organs.	Result.
Rabin, 1929.	1	45	F	4 cm. 40 gm.	Right.	Hypertension many years. Dyspnoea and palpitation 10 years. No paroxysms.	No operation. B.P. 226-177 / 108-122.		No mention.		Arteriosclerosis of vessels. Hyalinisation of arterioles. Glomeruli mostly normal. Hyaline material in some tufts.	515 gm.	Vascular changes in liver, spleen and pancreas.	Died. No operation.
Thorn et al, 1944.	1	40	F	9.3 x 7.6 cm. wt. 220 gm.	Left.	Hypertension 7 years. Headache and vomiting. Albuminuria, R.B.C. Changes in retina. No paroxysms noted.	1934-120 to 130mm. 1937-220/150. 1938-45 - 200-260/165-150.	240/160 to 140/100. 9 months after 140/90.	1937. Narrowing of arteries of fundus oculi. No haemorrhage or exudate appeared.	Gradual return to normal.	-	-	-	Operation successful.
Oppenheimer & Fishberg, 1924.	1	24	M	6 x 4 x 2.5 cm. Wt. 25 gm. Cortical adenomas.	Right. Left.	Dyspnoea and palpitation - 3 months.	220/160.	-	Albuminuric Retinitis.	-	Arteriosclerosis of arcuate and interlobular arteries. Glomeruli:- Mostly normal. Few are sclerosed.	880 gm.	-	Died. No operation.
Kremer, 1936.	1	14	F	4 cm. 70 gm. 3 cm. 35 gm.	Left. Right.	1928. Shortness of breath and palpitation. 1931. Admitted in coma. No paroxysms. Albumen+. No sugar.	1928-95/60. 1931-130/110. 1933-172/110. 1934-190/150.	-	Arteries small and sclerosed.	-	Arteriolar and intraglomerular hyaline thrombosis. Unilateral eccentric thickening of the intima of renal arteries.	Marked concentric hypertrophy.	Arteriosclerosis of vessels of pancreas.	Died. No operation.
Edward, 1937.	1	29	M	6 cm. 20.7 gm.	Right.	Sudden hemiplegia. Well until onset of attack.	230/140.	-	Flame shaped haemorrhage into right and left fundus.	No change in arterioles.		365 gm.	Lungs, liver, spleen - nil	Died. No. operation.
Hick, 1933.	1	64	F	4 x 2 x 2 cm.	Left.	Admitted in coma. Ruptured aneurysm anterior communicating artery.	180/105.	-	No mention.	No histology.		350 gm.	-	Died. No operation.
Kirshbaum & Balkin, 1942.	2	54	M	10 x 8 x 8 cm.	Left.	Admitted in coma. Right cerebellar haemorrhage.	230/170.	-	No mention.	No histology.		Not given.	-	Died. No operation.
Blacklock & Symington, 1947.	3	39	M	1 cm. in diameter.	Left.	In good health until 3 months previously. Severe attacks of neck pain. Loss of consciousness. Albumen & R.B.C's.	240/130	-	Blurring of margins of optic discs. Arteriosclerosis of vessels.	No histology available.		620 gm.	-	Died. No operation.

TABLE 7. PART 2. GROUP NO. 1.

No. of Animal.	Maximum Dose Adrenalin 1:1000 given. m	Days Animal Survived.	No. of Reactions			Total Adrenalin given.		Weight of Heart. Gms.	Cardiac Index		Weight of Animal (Gms.)			Time of Death after Injection.
			Total	Severe	Slight	Mins.	Mgs.		Terminal	Maximum	Beginning.	Terminal.	Maximum.	
1	10	77	15	12	3	202	12.1	1.026	0.0051	0.0041	250	200	250	45 mins.
2	10	88	22	20	2	268	16.1	0.633	0.0051	0.0033	190	124	190	90 "
3	8	91	24	22	2	247	14.8	0.936	0.0050	0.0047	200	189	200	45 "
4	10	123	35	34	1	354	21.2	0.864	0.0044	0.0044	170	196	196	Found dead about 8 hours.
5	11	137	36	29	7	442	26.5	0.950	0.0038	0.0038	240	249	249	3 hours.
6	15	140	39	33	6	543	32.6	1.027	0.0046	0.0039	260	223	260	3 "
7	10	154	42	35	7	454	27.2	0.841	0.0036	0.0036	200	233	233	Killed.
8	11	160	45	40	5	476	28.6	0.83	0.0033	0.0033	240	254	254	Killed.

TABLE 8. PART 2. GROUP NO. 2.

No. and Sex of Animal.	Maximum Dose of Adrenalin (1:1000) m.	Days Animal Survived.	No. of Reactions			Total Adrenalin given.		Weight of Heart. Gms.	Cardiac Index		Weight of Animal (Gms.)			Time of Death after Injection.
			Severe	Slight	Total	Mins.	Mgs.		Terminal	Maximum	Beginning.	Terminal.	Maximum.	
1 M	5	66	0	3	3	62	3.1	0.764	0.0034	0.0034	180	222	222	Immediately.
2 F	5	70	0	3	3	65	3.9	0.666	0.0034	0.0034	150	184	184	Immediately.
3 M	5	100	7	10	17	136	8.2	0.866	0.0039	0.0039	165	218	218	15 mins.
4 M	5	103	17	5	22	141	8.5	0.738	0.0061	0.0041	180	120	180	2 days.
5 F	5	105	17	4	21	150	9.0	0.95	0.0041	0.0041	190	231	231	5 mins. Convulsion.
6 F	5	126	11	17	28	177	10.6	0.675	0.0041	0.0040	140	165	170	Found dead. Injected previous day.
7 F	5	148	16	21	37	239	14.3	0.885	0.0047	0.0047	150	188	188	3 hours.
8 M	5	155	9	30	39	251	15.0	0.84	0.0056	0.0056	140	150	150	5 hours.

TABLE 9. PART 2. GROUP 3.

No. and Sex.	Maximum dose of Adrenalin Soln. 1:1000	Maximum dose of Adrenalin Oil. 1:100	Days of Survival under Adrenalin Total	Days of Survival under Adrenalin Soln.	Days of Survival under Adrenalin Oil	Number of Paroxysms: Severe Oil	Severe Soln.	Slight Oil	Slight Soln.	Total.	Wt. of Heart. Gms.	Total Adrenalin given: Soln. 1:1000	Total Adrenalin given: Oil 1:100	Total Mgs.	Cardiac Index. Terminal	Cardiac Index. Maximum	Weight of Animal (Gms.) Beginning.	Weight of Animal (Gms.) Terminal.	Weight of Animal (Gms.) Maximum.	Time of death after injection.
1 M	5m	0.06 c.cs.	179	147	32	14 18	4	3 34	31	52	0.59	239m or 14.3 mgs.	0.83 c.cs. 8.3 mgs.	22.6	0.0046	0.0042	110	128	140	5 hours.
2 F	5m	0.06 c.cs.	184	147	37	9 21	12	8 37	29	58	0.81	239m or 14.3 mgs.	1.02 c.cs. or 10.2 mgs.	24.5	0.0050	0.0050	150	161	161	20 mins. Convulsion.
3 M	5m	0.07 c.cs.	191	147	44	9 17	8	10 42	32	59	0.97	239m or 14.3 mgs.	1.27 c.cs. or 12.7 mgs.	27.0	0.0038	0.0038	170	255	255	4 hours.
4 M	5m	0.08 c.cs.	215	147	68	21 27	6	10 40	30	67	0.778	239m or 14.3 mgs.	2.02 c.cs. or 20.2 mgs.	34.5	0.0031	0.0031	180	251	251	2½ hours.
5 M	5m	0.10 c.cs.	233	147	86	27 35	8	7 39	32	74	0.929	239m or 14.3 mgs.	2.49 c.cs. or 24.9 mgs.	39.2	0.0035	0.0035	180	262	260	Animal killed. No injection.
6 F	5m	0.10 c.cs.	264	147	117	28 41	13	12 43	31	84	0.875	239m or 14.3 mgs.	2.58 c.cs. or 25.8 mgs.	40.1	0.0044	0.0044	150	196	196	12 hours. Found dead.
7 F	5m 0.08 c.cs. given at last injection.	0.12	273	147	126	43 58	15	11 34	23	92	0.991	239m or 14.3 mgs.	4.78 c.cs. or 47.8 mgs.	62.1	0.0037	0.0037	150	287	287	3½ hours.
8 F	5m 0.2 c.cs. given at last injection.	0.15	288	147	141	49 77	28	10 32	22	109	0.84	239m or 14.3 mgs.	5.94 c.cs. or 59.4 mgs.	73.7	0.0037	0.0037	150	225	225	4 hours.

TABLE 10. PART 2. GROUP 4.

No. and Sex.	Maximum dose of Adrenalin Soln. m	Maximum dose of Adrenalin Oil. c.cs.	Days of Survival under Adrenalin Total	Days of Survival under Adrenalin Soln.	Days of Survival under Adrenalin Oil.	Number of Paroxysms Severe Oil.	Number of Paroxysms Severe Soln.	Number of Paroxysms Slight Oil.	Number of Paroxysms Slight Soln.	Number of Paroxysms Total.	Wt. of Heart. Gms.	Total Adrenalin given Soln.	Total Adrenalin given Oil.	Total Adrenalin given Total. Mgs.	Cardiac Index. Terminal	Cardiac Index. Maximum	Weight of Animal (Gms.) Beginning.	Weight of Animal (Gms.) Terminal.	Weight of Animal (Gms.) Maximum.	Time of death after injection
1 M	5	0.03	187	147	40	8 7 15		10 40	30	55	1.03	239m or 14.3mgs.	0.84ccs. or 8.4mgs.	22.7	0.0043	0.0043	210	239	239	½ hour.
2 F	5	0.03	191	147	44	13 7 20		10 38	28	58	0.86	239m or 14.3mgs.	1 c.c. or 10 mgs.	24.3	0.0042	0.0042	170	205	205	12 hours. Found dead.
3 F	5	0.03	223	147	76	25 18 43		11 33	22	76	0.83	239m or 14.3mgs.	1.6c.cs. or 16 mgs.	30.3	0.0049	0.0049	145	168	168	Killed. No injection.
4 M	5	0.03	251	147	104	26 7 33		14 43	29	76	1.15	239m or 14.3mgs.	1.76ccs. or 17.6mgs.	31.9	0.0057	0.0046	200	198	250	Died during night. No injection.
5 F	5	0.03	288	147	141	15 20 35		51 69	18	104	1.06	239m or 14.3mgs.	2.86ccs. or 28.6mgs.	42.9	0.0050	0.0050	150	210	210	2 hours after 0.1 c.c. Adrenalin in oil 1:100.

0.1 c.c. Adrenalin in oil given for last injection in No. 5.

TABLE 11. PART 2. GROUP 0. MALE AND FEMALE CONTROL GROUP.

No. and Sex of Animal.	Weight of Heart. Gms.	Weight of Animal (Gms.)			Cardiac Index		Duration of Experiment. Days.
		Beginning.	Terminal.	Maximum.	Terminal	Maximum	
1. Male	0.923	230	250	250	0.0037	0.0037	79
2. Female	0.595	170	170	170	0.0035	0.0035	112
3. Female	0.621	200	206.5	206.5	0.0030	0.0030	130
4. Female	0.564	200	207	207	0.0027	0.0027	139
5. Male	0.965	250	334	334	0.0029	0.0029	150
6. Male	0.77	240	281	281	0.0027	0.0027	160

TABLE 12. PART 2. GROUPS 1 to 4. SUPRARENAL CHANGES AFTER ADRENALIN. (OSMIC ACID FIXATION).

No. and Sex of Animal.	Immediate Accustomed dose of Adrenalin & Reactions.	Last dose of Adrenalin.	Nature of last Reaction.	Interval between injection and death.	Histological changes in Suprarenals. Zone 1.	Zone 2.	Zone 3.	Zone 4.	Medulla.
Group 3 No. 2 Female.	0.06 c.cs. Adrenalin/ Oil or 0.6 mgs. Reactions severe.	0.6 mgs.	Very severe. (Convulsion)	20 mins.	Osmophil-positive granules seen in some of the cells.	Clear vacuoles seen in most cells. A few were osmophil-positive.	Not distinct. Dilated spaces containing osmophil material present.	Small but osmophil-positive.	Osmophil secretion seen in sinusoids.
Group 4 No. 5 Female.	0.3 mgs. Adrenalin/ Oil. Reactions slight.	1 mg.	Very severe.	2 hours.	Zones 1 and 2 fused. Only slightly osmophil-positive. Most of the cells vacuolated.		Pale. No osmophil reaction of the cells.	Prominent, enlarged and strongly osmophil-positive.	Osmophil secretions present in medulla.
Group 3 No. 7 Female.	Animal accustomed to 1-1.2 mgs. Adrenalin/oil. Slight reaction.	0.8 mg.	Very slight.	Killed 3½ hours.	Slightly osmophil-positive.	Osmophil-positive. Clear vacuoles seen in many cells.	Well defined. Faintly osmophil-positive.	Slightly increased in size. Many osmophil granules in cells.	Osmophil-positive secretion in sinusoids.
Group 3 No. 1 Male.	0.6 mgs. Reaction severe.	0.6 mg.	Severe.	5 hours.	Osmophil-positive vacuoles and clear vacuoles seen.	Osmophil-positive.	Not distinct but osmophil-positive.	Narrow. Osmophil-positive.	Marked osmophil secretion coming from cortex. Note. Medullary cells not osmophil-positive.

TABLE 13. PART 2. GROUPS 1 to 4. PANCREATIC CHANGES.

No. and Sex of Animal.	Time of Death after Injection.	Immediate previous Injections of Adrenalin.	Nature of last Reaction.	Duration of Experiment. Days.	Appearance of Islet Tissue.	Histological Appearance of Pancreas.
Group 2 No. 1 Male.	5 mins.	No reaction with 4m 1:1000. One injection 5m 1:1000.	Died in convulsion.	66	Normal.	Acini around islets slightly dilated and granular. Inter-islet acini are scarcely prominent. Only a few have granules.
Group 4 No. 1 Male.	30 mins.	19 injections with 0.03 c.cs. Adrenalin/oil. Reactions slight to severe.	Severe.	187	Normal.	All pancreatic acini are prominent and the cytoplasm granular. Those around the islets are more dilated and granular. The nucleus is pushed to the periphery.
Group 1 No. 3	45 mins.	16 injections with 8m 1:1000. All reactions severe.	Very severe.	91	Normal.	Acini around islets enlarged and intensely granular. Nuclei pushed to the periphery of the cell. Intervening tissue granular but not so marked.
Group 4 No. 5 Female.	120 mins.	60 injections with 0.03 c.cs. Adrenalin/oil. Reaction slight.	0.1 c.c. Adrenalin/ oil. Reaction severe.	288	Normal.	All acini are granular but those around the islets are slightly larger and more granular.
CONTROL Male CONTROL Female	Killed by Coal Gas.					Acinar tissue, around the islets, is prominent and forms a halo to the islets. The inter-islet acinar tissue is not marked.

TABLE 14. PART 3. GROUP 1. BLOOD SUGAR, LIVER GLYCOGEN AND SUPRARENAL CHANGES AFTER SEVERE ADRENALIN REACTION IN PARTIALLY ADAPTED ANIMALS - 1 mg. ADRENALIN BASE GIVEN.

No. of Animal and Time Killed.	Test dose of Adrenalin given. mgs.	Number of Reactions			Duration of Experiment. Days.	Total Adrenalin given. mgs.	Condition of Animal.	Blood Sugar. mgs.%.	Liver Glycogen.	Suprarenals.
		Severe	Slight	Total						
Control No. 1	Nil.	12	7	19	113	10.2	Animal struggled. Difficulty in getting blood.	133	Glycogen present in all parts of liver lobule.	Zone 1: Os.-Negative. Zone 2: Os. +ve. Zone 4. Narrow but os.+ve. Medulla: Os.+ve secretions.
½ hour No. 2.	1 (16 mins.)	12	8	20	113	11.2	Slight reaction.	200	Cells around central vein completely devoid of glycogen.	Zone 1: Os.+ve granules appear. Zone 2: Os.+ve. Clear vacuoles present. Zone 4: Broader. Os.+ve. Medulla: Os.+ve secretions.
1½ hours No. 3.	1	13	7	20	113	11.2	Severe reaction.	348	Extensive deprivation of glycogen from cells around portal tract and central vein. Glycogen present in mid-zone area.	Zone 1: Whole zone osmophilised but separate from Zone 2 by a thin clear line of cells. Only a few os.+ve granules in Zone 2. Zone 4: Great increase in size. Densely os.+ve. Medulla: Os.+ve.
3 hours No. 4.	1	13	7	20	113	11.2	Severe reaction - shows signs of recovery.	234	Similar to No. 3 but increase glycogen content in mid-zone region.	Zones 1 and 2: Fused. Some cells osmophilised. Clear vacuoles present also. Zone 4: Still broad and Os.+ve. Medulla: Os.+ve secretion present.
5 hours No. 5.	1	13	7	20	113	11.2	Severe reaction. Recovered.	182	Almost complete regeneration. Only a few cells in region of immediate vicinity of central vein and portal tract devoid of glycogen.	Zone 1: Prominent and separate from Zone 2: Os.+ve, clear vacuoles present. Zone 4: Enlarged and densely Os.+ve Medulla: Os.+ve secretion present.

Os.+ve means - Osmophil-Positive.

TABLE 15. PART 3. GROUP 2. BLOOD SUGAR, LIVER GLYCOGEN AND SUPRARENAL CHANGES AFTER SLIGHT ADRENALIN REACTION IN ADAPTED ANIMALS - 0.24 mgs. (4m) ADRENALIN GIVEN.

No. of Animal and Time Killed.	Test Dose of Adrenalin given. mgs.	Number of Reactions			Duration of Experiment. Days.	Total Adrenalin given. mgs.	Condition of Animal.	Blood Sugar. mgs.%.	Liver Glycogen.	Suprarenals. Zones 1 and 2 only.
		Severe	Slight	Total						
Control No. 1.	Nil.	-	20	20	90	6.4	Normal.	88	Normal.	Usual four zones present. Irregularity of Zone 1 seen.
1 hour No. 2.	0.24 (4 m)	-	21	21	90	6.64	Slight reaction.	180	Normal.	Zone 1: Os.+ve granules present. Zone 2: Os.+ve. Clear vacuoles present.
2 hours No. 3.	0.24	-	21	21	90	6.64	Slight reaction.	220	Depletion beginning around centre of lobule.	Zone 1: Increased osmophilisation. Zone 2: Not so densely Os.+ve. Clear vacuoles stillseen.
3 hours No. 4.	0.24	-	21	21	90	6.64	Slight reaction. Beginning to recover.	264	More marked depletion.	Zone 1: Clear. Zone 2: Os.+ve. A few clear vacuoles present.
4 hours No. 5.	0.24	-	21	21	90	6.64	Slight reaction. Recovered.	262	Normal.	Zone 1: Weakly osmophil-positive. Zone 2: Osmophil-positive. Clear vacuoles present.
5 hours No. 6.	0.24	-	21	21	90	6.64	Slight reaction. Recovered.	128	Normal.	Zone 1: Clear. Zone 2: Osmophil-positive.
6 hours No. 7.	0.24	-	21	21	90	6.64	Slight reaction. Recovered.	97	Normal.	Zone 1: Slightly osmophil-positive. Zone 2: Osmophil-positive. Clear vacuoles present.
7½ hours No. 8.	0.24	-	21	21	90	6.64	Slight reaction. Recovered.	82	Normal.	Zone 1: Clear. Zone 2: Normal.

Os.+ve means - Osmophil-Positive.

TABLE 16. PART 3. GROUP 3. LIVER GLYCOGEN AND BLOOD SUGAR CHANGES IN NORMAL NON-A[illegible]PTED ANIMALS FOLLOWING A SLIGHT REACTION WITH 0.24 MGS. (4m) ADRENALIN.

No. of Animal and Time Killed.	Test Dose of Adrenalin mgs.	Previous Adrenalin Injections.	Nature of Reactions.	Blood Sugar. mgs.%.	Liver Glycogen.	Suprarenals.
Control No. 1.	Nil.	Nil.	-	94	Not examined.	
½ hour No. 2.	0.24 (4 mins)	Nil.	Slight.	228	do.	Alternate osmophilisation of Zones 1 and 2 as seen in Group 2.
1 hour No. 3.	0.24	Nil.	Slight.	240	do.	
1½ hours No. 4.	0.24	Nil.	Slight. Almost recovered.	278	do.	
2 hours No. 5.	0.24	Nil.	do.	315	do.	
3 hours No. 6.	0.24	Nil.	Recovered.	285	do.	
4 hours No. 7.	0.24	Nil.	Recovered.	211	do.	
5 hours No. 8.	0.24	Nil.	Recovered.	206	do.	

TABLE 17. PART 3. GROUP 4. LIVER GLYCOGEN AND BLOOD SUGAR CHANGES FOLLOWING A SLIGHT REACTION WITH 0.24(4m) mg. ADRENALIN IN ADRENALECTOMISED NON-ADAPTED ANIMALS.

No. of Animal and Time Killed.	Test Dose of Adrenalin mgs.	Previous Adrenalin Injection.	Nature of Reaction.	Blood Sugar mgs.%.	Liver Glycogen.
Control No. 1.	Nil.	Nil.	Nil.	137	Normal.
½ hour No. 2.	0.24 (4 m)	Nil.	Slight.	210	Disappeared around central vein.
1 hour No. 3.	0.24	Nil.	Slight.	250	Present only in mid-zone.
1½ hours No. 4.	0.24	Nil.	Slight.	221	do.
2 hours No. 5.	0.24	Nil.	Slight.	200	do.
3 hours No. 6.	0.24	Nil.	Recovered.	121	Present only in mid-zone. No regeneration.
5 hours No. 7.	0.24	Nil.	Recovered.	111	do.

TABLE 18. PART 4.GP.1. 60 MGM. DOCA IMPLANTS.

No. of Animal.	Lesions in Heart.		Lesions in Kidney.		Liver.	Spleen.	Pancreas.	Suprarenals.	DOCA absorbed. mgs.	Duration of Experiment Days.	DOCA absorbed mgs./day.	Cardiac Index.		Weight of Animal(Gms.)			Weight of Heart. (gms.)
	Microscopic.	Macroscopic.	Microscopic.	Macroscopic.								Maximum.	Terminal.	Beginning.	Maximum.	Terminal	
1. Died.	Nil.	Nil.	Nil.	Nil.	Nil.	Nil.	Nil.	Nil.	2.3	7	0.33	0.0035	0.0035	230	230	230	0.79
2. Killed.	Focal lesions. Cellular infiltration between muscle cells. Mild coronary lesions.	Nil.	Nil.	Nil.	Nil.	Nil.	Nil.	Nil.	15.9	42	0.38	0.0036	0.0036	295	310	310	1.10
3. Died.	Lesions slight. No coronary lesions.	Nil.	Severe: vascular lesions marked.	Surface irregular and granular. Urine:- Albumen++	Nil.	Nil.	Nil.	Lost.	27.8	63	0.42	0.0031	0.0042	320	320	245	0.98
4. Died.	Lesions severe. Extensive cellular infiltration between muscle cells. Coronary vessels: Nil.	Pericardial effusion. Muscle pale and numerous mottled areas.	Slight.	Congested.	Marked mid-zone haem. and necrosis.	Nil.	Nil.	Nil.	28	67	0.42	0.0028	0.0025 Ascites present.	300	365	365	0.84
5. Killed.	Reaction slight. Similar to No. 3. No coronary lesions.	Nil.	Severe. Similar to No. 3.	Enlarged, pale, surface irregular. Urine:- Albumen++	Nil.	Nil.	Nil.	Zone 1: Slightly Os.+ve. Zone 2: only slightly Os.+ve.	46.2	132	0.35	0.0039	0.0043	290	295	265	1.13
6. Killed.	Heart lesions slight. Vascular lesions very marked.	Nil.	Severe. Similar to Nos. 3 and 5.	Similar to Nos. 3 and 5.	Nil.	Nil.	Nil.	Zone 1: Clear Zone 2: Few Os+ve granules. Haemorrhage present.	52.6	160	0.33	0.0030	0.0033	320	320	300	0.96

TABLE 19. PART 4. GP2. 30 MGM. DOCA IMPLANTS.

No. of Animal.	Lesions in Heart.		Lesions in Kidney.						DOCA absorbed mgs	Duration of Experiment. Days.	DOCA absorbed. mgs./day.	Cardiac Index.		Weight of Animal.(Gms.)			Weight of Heart (gms.)
	Microscopic.	Macroscopic.	Microscopic.	Macroscopic.	Liver.	Spleen.	Pancreas.	Suprarenals.				Maximum.	Terminal.	Beginning.	Maximum.	Terminal.	
1. Died.	Nil.	Nil.	Nil.	Nil.	Nil.	Nil.	Nil.	Nil.	1.8	7	0.26	0.0032	0.0035	220	220	220	0.708
2. Killed.	Nil.	Nil.	Nil.	Nil.	Nil.	Nil.	Nil.	Nil.	Implant ulcerated through skin.	42	-	0.0032	0.0033	270	280	280	0.90
3. Killed.	Nil.	Nil.	Nil.	Nil.	Nil.	Nil.	Nil.	Nil.	9.6	48	0.20	0.0037	0.0039	270	315	305	1.19
4. Killed.	Few areas of fibro-cellular reaction between muscle fibres. No vascular lesions.	Nil.	Nil.	Nil.	Nil.	Nil.	Nil.	Nil.	30	107	0.28	0.0036	0.0035	350	362	362	1.25
5. Killed.	Slight reaction similar to No. 4.	Nil.	Few glomeruli almost sclerosed and adherent to capsule. Tubules: some dilated. Casts present. Vessels normal.	Nil.	Nil.	Nil.	Nil.	Nil.	26	112	0.23	0.0037	0.0037	300	300	300	1.11
6. Killed.	Reaction slight. Similar to 4 and 5.	Nil.	Glomerular and tubular lesions more marked than No. 5. Interstitial tissue:- Patchy areas of wedge shaped fibrosis. Vessels:- Smaller arteries and arterioles show intimal thickening with narrowing of lumen.	Granular irregular surface. Capsule strips easily.	Nil.	Nil.	Nil.	Zone 1: Os. +ve. Zone 2: Depleted. Zone 4: Increase in size. Cortical cells in medulla.	30	156	0.19	0.0046	0.0046	190	210	205	0.95

TABLE 20. PART 4. GP.3. 10 MGM. DOCA IMPLANTS.

No. of Animal.	Lesions in Heart. Microscopic	Lesions in Heart. Macroscopic	Lesions in Kidney. Microscopic	Lesions in Kidney. Macroscopic	Liver.	Spleen.	Pancreas.	Suprarenals.	DOCA absorbed. mgs.	Duration of Experiment. Days.	DOCA absorbed. mgs./day.	Cardiac Index. Maximum.	Cardiac Index. Terminal.	Weight of Animal. (Gms.) Beginning.	Weight of Animal. (Gms.) Maximum.	Weight of Animal. (Gms.) Terminal.	Weight of Heart. (gms.)
1. Died.	Nil.	Nil.	Nil.	Nil.	Nil.	Nil.	Nil.	Nil.	7	57	0.12	0.0041	0.0041	270	285	285	1.195
2. Died.	Lesions slight. Some coronary vessels show intimal thickening. Cellular reaction outside the vessels.	Nil.	Most glomeruli normal. Some sclerosed. A few casts seen in tubules. Intimal thickening of smaller vessels.	Congested.	Nil.	Nil.	Nil.	Nil.	12.4	123	0.10	0.0039	0.0043	280	280	250	1.08
3. Killed.	Lesions minimal. Some coronary vessels show slight intimal thickening.	Nil.	Patchy areas of glomerular sclerosis. Tubules: very dilated and contain casts. Vessels: larger - normal. Arterioles hyalinised.	Surface granular and irregular. Capsule not adherent.	Nil.	Nil.	Nil.	Nil.	23	156	0.14	0.0034	0.0038	280	300	250	0.94
4. Killed.	Most muscle fibres normal. Some cellular infiltration seen between muscle fibres. Coronary Arteries show some intimal thickening.	Nil.	Similar to No. 3. Vessels: smaller arteries show intimal hyperplasia.	Similar to No. 3.	Nil.	Nil.	Nil.	Nil.	31.4	192	0.16	0.0038	0.0048	350	350	275	1.34
5. Died.	Similar to No. 4.	Nil.	Similar to Nos. 3 and 4.	Similar to No. 3.	Nil.	Nil.	Nil.	Nil.	45.9	185	0.24	0.0032	0.0043	320	360	270	1.18

TABLE 21. PART 5. GROUP 1. DAILY EFFECTS OF MULTIPLE/INJECTIONS OF 1:1000 ADRENALIN HYDROCHLORIDE.

	Nature and Duration of Experiment (Days).													
	1.	2.	3.	4.										
No. of Animal.	Injection of 1:1000 Adrenalin from 0.5 to 5m until reactions become severe.	3m twice daily at 4-hourly intervals.	2m twice daily at 4-hourly intervals.	2m three times daily at 4-hourly intervals.	Total Duration. Days.	Weight of Animal(gms.) Maximum.	Weight of Animal(gms.) Terminal.	Number of Reactions. Severe	Slight	Total	Cardiac Index. Terminal	Cardiac Index. Maximum	Weight of Heart. Gms.	Time of Death after Injection.
No. 1	55	-	-	-	55	200	200	25	-	25	0.0038	0.0038	0.72	15 hours.
No. 2	75	-	-	-	75	270	270	43	-	43	0.0033	0.0033	0.89	10 minutes. Severe reaction.
No. 3	77	11	-	-	88	250	230	42	12	54	0.0047	0.0038	0.97	Convulsion 10 minutes after second injection.
No. 4	77	12	-	-	89	245	245	46	14	60	0.0038	0.0038	0.92	1½ hours after second injection.
No. 5	77	12	45	10	144	270	260	48	11	59	0.0038	0.0037	0.96	Received 2m then 5 m four hours later. Killed 1 hour after last injection.
No. 6	77	12	45	9	143	250	230	48	15	63	0.0040	0.0037	0.92	2m only. Animal recovered. Killed 4 hours.
No. 7	77	12	45	16	150	255	210	48	13	61	0.0040	0.0033	0.85	No injection. Killed.

TABLE 22. PART 5. GROUP 2. SUPRARENAL, BLOOD SUGAR AND LIVER GLYCOGEN CHANGES IN ADAPTED ANIMALS FOLLOWING DOUBLE INJECTIONS OF 0.12 mgs.(2m) ADRENALIN HYDROCHLORIDE 1:1000 AT FOUR-HOURLY INTERVALS.

No. of Animal and time killed.	Test Dose of Adrenalin given (mgs.) 1st Injection	2nd Injection	No. of Paroxysms. Slight	Total	Duration of Experiment. Days.	Total Adrenalin given. mgs.	Nature of Reaction.	Blood Sugar. mgs.%.	Liver Glycogen.	Suprarenals.
No. 1. Control.	-	-	24	24	90	6.4	nil.	104	Normal.	Normal.
½ hour. No. 2.	0.12 (2 m)	-	25	25	90	6.52	Very slight.	129	Normal.	Normal.
1 hour. No. 3.	0.12	-	25	25	90	6.52	Very slight.	172	Normal.	Slight osmophilisation of Zone 1. Osmophilisation and vacuolation of Zone 2.
2 hours. No. 4.	0.12	-	25	25	90	6.52	Recovered.	207	Normal.	Similar to above.
3 hours. No. 5.	0.12	-	25	25	90	6.52	Recovered.	168	Normal.	Gland returned to normal.
4½ - ½ hours. No. 6.	0.12	0.12	26	26	90	6.64	Very slight.	198	Normal.	Zone 1: Clear. Zones 2 and 3: Very few osmophil vacuoles seen. Zones depleted and shows a large haemorrhagic area. Zone 4: Enlarged and osmophil-positive.
5 - 1 hours. No. 7.	0.12	0.12	26	26	90	6.64	Very slight.	225	Normal.	Zone 1: Slight osmophilisation. Zone 2: Still depleted but slightly osmophil-positive. Zones 3 and 4: Fused and osmophil-positive.
6 - 2 hours. No. 8.	0.12	0.12	26	26	90	6.64	Recovered.	161	Depleted. Present in mid-zone only.	Zone 1: Less marked osmophilisation. Zone 2: Osmophil-positive. Zone 3: Small. Zone 4: Broad and osmophil-positive.
8 - 4 hours. No. 9.	0.12	0.12	26	26	90	6.64	Recovered.	114	Depleted. Present in mid-zone only.	Zone 1: Osmophil-positive rim. Zone 2: Depleted. Haemorrhages present Zone 3: Indefinite but fuses with Zone 4: Densely osmophil-positive.

APPENDIX I.

TABLE USED IN CONVERTING SOLUTIONS OF 1:1000 ADRENALIN HYDROCHLORIDE AND 1:100 ADRENALIN ASCORBATE INTO MICROGRAMMES ADRENALIN BASE.

1:1000 Adrenalin Hydrochloride Solution

means

	1	grain	Adrenalin	base	in 1000	minims.
	60	mgs.	"	"	" 1000	"
or	0.6	"	"	"	" 10	"
	0.3	"	"	"	" 5	"

Thus 5 minims of a solution of 1:1000 adrenalin hydrochloride contains 0.3 mg. or 300 µg adrenalin base.

1:100 Adrenalin Ascorbate in Oil

means

	1	grain	Adrenalin	base	in 100	minims.
	60	mgs.	"	"	" 100	"
i.e.	6	"	"	"	" 10	"
or	0.3	"	"	"	" 0.5	"

but 1 c.c. = 15 minims
0.03 " = 0.5 "

Thus 0.03 c.c. of a solution of 1:100 adrenalin ascorbate contains 0.3 mg. or 300 µg adrenalin base.

Accordingly -

5 minims 1:1000 Adrenalin Hydrochloride
and
0.03 c.c. 1:100 Adrenalin Ascorbate

contain 0.3 mg. or 300 µg Adrenalin base.

APPENDIX II.

C A S E R E P O R T S.

Case 1 (Dr. J.W. Ferguson and Mr. W.S. Mack).

Clinical Findings: The patient, a soldier, aged 21, was admitted to hospital from abroad on October 6, 1943. He had enjoyed good health until ten months previously, when he was awakened from his sleep in the early morning with severe headache, palpitation, and breathlessness. These symptoms passed off in about five minutes. Thereafter he remained well for about six weeks, when a similar attack occurred. Following this, attacks became progressively more frequent, until he was having as many as five in the day. The attacks lasted one to two minutes only, after which he experienced a feeling of warmth throughout the body. Observation in a hospital abroad had revealed the association of his attacks with paroxysms of hypertension and suggested the possible presence of an adrenal medullary tumour. He was repatriated for further investigation. On his journey home and while in this country prior to admission to hospital - a period of three weeks in all - he suffered only occasional short attacks of palpitation. There were no relevant features in the past or family history.

Inspection showed a slightly-built man of average height and good musculature. The blood pressure between attacks was variable, but tended to be slightly raised, between 130/80 and 160/85. Physical examination was essentially negative, except that the lower pole of the right kidney could occasionally be palpated. There was no detectable cardiac enlargement and no palpable thickening of the peripheral

arteries. Examination of the fundi revealed no abnormality.

Description of the Attacks: At first these occurred in the early hours of the morning, but later they took place at any time. Active exercise sometimes precipitated an attack; no other precipitating causes were noted. The attacks were characterised by the sudden onset of palpitation, and of a feeling of tightness in the chest with difficulty in breathing, followed by a pounding headache. Marked facial pallor and constriction of the larger peripheral vessels were observed. Two such attacks were witnessed in hospital. At the height of the attack the blood pressure was 260/120; after five minutes it fell to 190/110 and three minutes later the reading was 175/90. Still five minutes later the blood pressure was 150/85 and in a further five minutes 140/80. Subjective symptoms lasted only three minutes in the first attack witnessed, and one minute in the second. Immediately after these short periods the patient felt well.

Laboratory Investigations: Urine, clear, chemically and microscopically. Blood: R.B.C., 4.5 million/c.mm.; Hb. 85 per cent; W.B.C. 8,400/c.mm.; Differential count - neutrophils 55 per cent, eosinophils 3 per cent, lymphocytes 36 per cent, monocytes 6 per cent. Blood-sedimentation-rate within normal limits. Blood Kahn test, negative. Blood urea, 30 mg. per cent. Urea clearance test, 60 per cent normal. Blood cholesterol, 200 mg. per cent. Serum sodium, 327 mg. per cent. Serum potassium, 18.8 mg. per cent. Glucose tolerance curve, 70, 147, 92, 70, 70 mg. per cent. Excretion of 17-ketosteroids: Oct. 17, 10.65 mg. in 24 hours, and Nov. 30, 11.8 mg. in 24 hours. All these results are related to the periods between

attacks when the patient was free from symptoms.

Radiography of the chest, pituitary fossa, straight X-ray of the abdomen, and intravenous pyelography revealed no abnormality.

Progress: The relatively severe attacks disappeared soon after his admission, so that only two such attacks were witnessed. He was, however, subject to frequent attacks of headache and dizziness lasting only a few seconds. He was never observed while in these, since they nearly always occurred while out walking. Attempts artificially to induce an attack by means of vigorous exercises were made on several occasions without result. Massage of the upper abdomen was ineffective, although on one occasion a symptomless rise of blood pressure to 180/100 was recorded. He was kept under observation for some time, but minor bouts continued to occur. Retrograde pyelography was now performed and revealed descent of the right kidney.

Operation (April 26, 1944): Great difficulty was experienced in anaesthetizing the patient, who had had a moderate amount of pre-medication, and trouble continued throughout the operation. No blood pressure estimations were made during the operation. The incision employed was a lumbar extraperitoneal one on the right side, which was, however, extended up and over the eleventh rib. Every small vessel divided bled copiously and about forty separate ligatures had to be tied during the cutting of the muscles. The patient's breathing, which had been vigorous up to this stage, suddenly stopped and he became pale and blue about the lips. This took place even before the muscles had been divided completely and thereafter his condition was most unsatisfactory. The kidney was readily palpated

and was found lying so high that even dislocation of the last rib upwards did not give a satisfactory exposure. On pulling the kidney down, however, a tumour of the adrenal was found lying in the depths of the wound above and medial to the kidney. This tumour was well encapsulated but had a number of large veins coursing over it. To improve the access and before handling the tumour the last rib was now resected, thus affording a much better exposure. A small tear in the pleura was made at this stage but was sutured. The tumour was partly freed and ligatures were applied to vessels coming to it from the region of the renal vessels and from the diaphragm. No further vessels were encountered and the tumour was removed. The cavity was lightly packed and the wound closed.

Though intravenous plasma had been given during most of the operation, when the patient left the theatre he was in very poor condition, being pale and collapsed, with shallow respirations.

Post-operative Progress: For two hours following the operation the systolic blood pressure was below the level of 55 mm.Hg in spite of repeated intravenous injections of adrenalin totalling 45 min. of 1:1000 adrenalin hydrochloride (2.7 mg.) and 10 c.c. eucortone. Thereafter rapid but transient rises to 120 mm.Hg and over followed subsequent doses of adrenalin intravenously. Between these doses the systolic pressure fell again to levels of about 55 mm.Hg. Accordingly a continuous intravenous adrenalin drip in saline was employed, the amount of adrenalin administered varying between 0.0346 and 0.0686 mg. per minute. With this procedure the blood pressure was maintained between levels of 80 and 90 mm.Hg. Whenever

the drip was discontinued a dramatic fall in the blood pressure readings occurred. In spite of this therapy, blood and plasma transfusions (2 pints of each), and a further three injections of 10 c.c. eucortone, the patient's condition steadily deteriorated. Only 3 oz. of urine were obtained by catheterization, representing the total volume excreted in 48 hours; the blood urea then was 170 mg. per cent. Eight hours later the blood urea had risen to 220 mg. per cent and estimation of the serum sodium and serum potassium gave figures of 276 and 21.4 mg. per cent respectively. At the end of the second day the amount of adrenalin given by intravenous drip was doubled. The blood pressure rose to systolic levels of 140 mm.Hg, but in the six hours preceding death, which took place 62 hours after operation, it showed a gradual decline, and registered a level of 80 mm.Hg immediately prior to death.

Post-mortem Findings: The body was that of a well-built young man. The face was congested and cyanosed and there was a slight yellowish coloration of the skin suggestive of early jaundice. A surgical wound in which packing was inserted was present in the right loin.

The pericardial sac was healthy. The heart (400 g.) was enlarged, due to a concentric hypertrophy of the left ventricle. The myocardium showed no fibrosis, the coronary arteries were healthy, and the valves all appeared normal. No lesion was present in the left lung. That on the right side showed many adhesions over the apex; the lower and middle lobes were collapsed. The lower and posterior parts of the mediastinum showed slight interstitial emphysema.

The peritoneum showed no lesion. The stomach and bowels were

much distended. The liver was very yellowish and felt distinctly greasy. Both kidneys were congested, but no gross morbid changes were present apart from some bruising around the upper pole of the right organ. Above the right kidney there was a space with ragged walls and filled with packing from which the suprarenal tumour had been removed. Above this the diaphragm had been cut, but the wound was secured with sutures. The urinary bladder was healthy. The left suprarenal appeared normal to the naked eye. The spleen was congested.

No lesion was observed in the brain, pituitary, or thyroid.

Histological Findings: The tumour, received four hours after excision, measured 8 cm. by 6 cm. and weighed 86 g. It was well encapsulated, many prominent vessels being observed in the capsular tissue. The cut surface had a pinkish-white appearance, numerous small vessels being visible in the substance of the growth, which was rather soft in consistence. No evidence of necrosis was noted. The tumour, after a few hours in formalin solution, became brownish in colour.

Microscopically the tumour was rather cellular, being composed of large rather long polyhedral cells varying in their greatest diameter from 10 to 50 microns. The growth was well encapsulated by a dense layer of fibrous tissue, outside of which the remains of the suprarenal cortex was observed. The tumour cells just under the capsule were smaller and rounder than those more centrally placed. For the most part the cells were closely packed together, though

at places there were large spaces (the sinusoidal spaces) filled with eosinophil granular material and a few red blood cells. In the haematoxylin and eosin preparations the cell cytoplasm, which was fairly abundant, was slightly acidophil and in some cells there were small accumulations of bluish granules. The cytoplasm of many of the cells showed numerous vacuoles. The nuclei, with one to three nucleoli, varied both in size and in shape, usually being eccentric in position. A very few cells showed mitosis. The cells were usually arranged in irregular alveoli which were separated from one another by strands of fine fibrous tissue. Towards the centre of many of these alveoli were large thin-walled sinusoidal spaces, the tumour cells lying immediately adjacent to the blood cells. The tumour was very vascular, many large capillaries being present in each low-power field. Around the capillaries the cells were rather closely packed: farther out the alveolar arrangement was more apparent.

In the tissue fixed in Müller's solution for some weeks, fine brownish granules were noted in sections scattered throughout the cytoplasm of some of the cells due to the reduction of the bichromate. In sections fixed by this method and stained by the modification of Sevki's method, fine granules which had an olive-green colour were observed in the cytoplasm of a few of the cells. These greenish granules corresponded with the yellowish-brown granules seen in the sections treated only with Müller's fluid. The remaining cells showed a diffuse olive-green homogeneous cytoplasm rather than a granular appearance. Fairly numerous rather cleft-like irregular spaces (the vacuolated spaces) were seen in the section; the fluid in these also

showed a similar but rather fainter greenish colour. This faint greenish tint was also evident in the serum in the lumen of the capillaries and also in that in the veins. Sections stained by Mallory's method showed a rather indistinct finely granular cytoplasm in some of the tumour cells, the granules staining orange-brown. No fat was demonstrated in frozen sections stained by Sudan III, or glycogen in tissue fixed in alcohol or Bouin and stained by Best's carmine. In sections stained by a modification of Bielschowsky's method no reticulin was found lining the sinusoidal channels or the vacuolated spaces, the lining consisting only of tumour cells. It is possible that the vacuolated spaces are an artefact resulting from the drastic fixation in the Müller's fluid causing separation of the tumour cells in the region of the blood sinuses. They were only present in tissue fixed by this method. These vacuolated spaces, however, contained cells with granules and various stages of disintegration of the cells were noted allowing the escape of the granules. This may be the method by which the pressor substance is liberated and may explain the green-coloured fluid in the spaces. An interesting finding, and one already noted by Howard and Barker, was the occurrence of large vacuoles in some of the cells lining the spaces, but the material in the vacuoles could not be stained by any of the methods used. Detailed microscopical study of the organs and tissues taken post mortem revealed some interesting changes. The arterioles in the spleen, both in the pulp and in the Malpighian bodies, showed hyaline thickening. The arcuate vessels of the kidneys exhibited distinct fibrosis of the middle coats in addition to some increase in thickness of the intima

and splitting of the internal elastic lamina. The walls of the afferent arterioles to the glomeruli were partly hyalinized, the lumen being narrowed. The glomerular capillaries were thickened, staining deep blue with Mallory: a few were completely fibrosed, and in others there was some adhesion of the tuft to the capsule. The interstitial tissue of the kidney was slightly increased, particularly around the glomeruli, causing thickening of the basement membrane of Bowman's capsule.

Case 2 (Dr. A. Muir Crawford, Dr. J. Shafar and Mr. W.S. Mack).

Clinical Findings: The patient, a housewife, aged 37, was first seen on May 5, 1944. She complained of frequent attacks of weakness and faintness accompanied by many other distressing symptoms such as severe headaches, giddiness, buzzing in the ears, pain over the front of the chest and down both arms, palpitation, a choking sensation and nausea. These attacks, which had troubled her for at least eighteen months, were usually brought on by exertion or excitement. They steadily increased in frequency and severity, ultimately occurring as often as six times a day, even while she was resting in bed. The duration of each attack varied from five minutes to one hour. She stated that she often felt giddy when lying on her left side. There were no relevant features in the past or family histories.

On examination it was noted that she was a woman of average nutrition but of rather pale complexion. The heart, lungs, abdomen, and nervous system, including the ocular fundi, revealed no abnormality, but the blood pressure readings were found to be abnormally high

(220/115 mm.Hg). It was decided to send her to hospital for further investigation, and she was admitted four days later (May 9).

On the day of admission the urine was found to contain albumin (1 part Esbach), but the blood pressure reading was now 148/78 mm.Hg. On the following day blood urea estimation was found to be 101 mg. per cent. While in hospital she had frequent attacks of precordial pain, choking, and severe headaches, and it was noted that these attacks were accompanied by a considerable increase in the blood pressure; the reading was on an average 265/155 mm.Hg, but at times the systolic could not be estimated with the ordinary sphygmomanometer registering a maximum of 300 mm.Hg. On June 9 the albuminuria was reduced to a mere trace, and blood urea was 42 mg. per cent.

Investigations: Intravenous pyelography showed ptosis of the right kidney, but neither kidney nor urinary tract was abnormal in size or form. During a hypertensive attack the serum potassium was 33.5 mg. per cent (B.P. 265/155 mm.Hg.); during a normal period 24.6 mg. per cent (B.P. 120/70 mm.Hg.). Electrocardiogram: during a normal period, in Leads II and III, S wave increased, large P wave, and elevation of R-T segment. During a hypertensive attack, similar changes were present in S and F waves, but no elevation of R-T.

Operation (July 20): No trouble was encountered in anaesthetizing this patient, who had been heavily premedicated. Pentothal intravenously was followed by endotracheal gas and ether.

A lumbar extraperitoneal incision was made, extending up to the eleventh rib. On opening Gerota's fascia a rounded tumour was seen above and somewhat medial to the upper pole of the right kidney.

Before attempting to free this tumour the last rib was resected, thus affording an excellent exposure. The growth was cleared by gentle gauze dissection and ligatures were applied to two groups of small vessels, one of which ascended from the region of the renal pedicle and the other which descended from the diaphragm. The tumour was now drawn up gently into the wound and a third group of vessels which entered its medial aspect was clamped and ligated. The bed left by the removal of the tumour showed slight oozing, which was easily controlled by light packing.

Throughout the operation the patient's general condition was excellent, though her pulse rate rose from 100 to 120 near the end. The systolic blood pressure prior to operation was 160 mm.Hg. It rose to 180 soon after the incision had been made and remained at this level until the tumour was handled, when it reached 220 only to fall sharply to 120 when the main pedicle had been clamped. On the patient's return to the ward the pressure fell still further to 80, but responded to 5 minims of adrenalin given every half-hour for 24 hours, followed by 5 minims hourly for a further 24 hours. In the 48 hours, a total of 180 minims of adrenalin was administered. Thereafter 1½ gr. of ephedrine was given at intervals of 12 hours for two days. Eucortone (1 c.c.) was also administered six-hourly for two days as a precaution. Four hours after operation the systolic pressure was 100 and by the evening it had reached 120-130, at which level it remained throughout her smooth convalescence, which was thereafter uneventful.

Some six months later, when the patient reported, the blood

pressure was normal and she had had no further paroxysms. She was very well and was able to perform all her household duties.

Pathological Findings: The tumour in this case, received one hour after removal, when fresh measured 6.5 cm. long, 5.5 cm. broad, and 3.5 cm. thick, and weighed 70 g. It was roughly oval in shape and had a thick fibrous capsule in which blood vessels were prominent. The cut surface had a pinkish-white colour, with a few small areas of recent haemorrhage. Portions were fixed as in the first case and a similar brown colour developed after fixation. In haematoxylin and eosin preparations the cells were much better preserved than in the first case owing to the specimen having been fixed an hour after excision. Small remnants of suprarenal cortex were still present outside the thick fibrous capsule of the tumour. The tumour was very vascular and there were many small haemorrhages in relation to the sinusoids and capillaries. The cells were similar to those in the first case, though more irregular in size and in shape and much better defined. The cytoplasm was, however, more granular and had a distinct foamy appearance. The fine bluish cytoplasmic granules noted in the first case were more numerous and clumps of small dark-brownish granules which did not give an iron reaction were present in some cells and also lying free in the centre of masses of cells. Some of the cells had more than one nucleus, three being the maximum; some of the nuclei were very irregular in shape and rather large and pyknotic. Mitosis was also more frequent than in the first case. A rather unusual finding was the presence of fairly large numbers of the tumour cells in the lumen of thick-walled veins, though, as in the

first case, some tumour cells were seen lying free in the sinusoids. The staining reaction with Mallory corresponded with that in the first case, the outlines of the cells being sharply defined, giving the appearance of an irregular mosaic. In sections stained by the modification of Sevki's method fine abundant olive-green granules were observed in most of the cells, though those next the sinusoids were more vacuolated and less granular. The staining of the granules varied with the degree of differentiation, as when this was overdone the granules stained a yellowish-brown colour. The tumour cells lying free in the blood vessels and in the sinusoids also showed granules. On staining the reticulin the large sinusoids were noted to have no reticular lining, though their continuity with the capillary vessels which had such a lining could sometimes be traced.

Case 3 (Professor Harrington).

Clinical Findings: The patient, a male aged 39, was admitted to hospital on Oct. 1, 1938. Apart from having to rise at night for almost a year to pass large quantities of pale urine, he had been in good health until three months prior to admission. At that time he became subject to attacks of giddiness when assuming the erect posture after stooping. In some of these attacks there was a temporary loss of power in the legs. Insomnia was a troublesome feature, and his powers of concentration were reduced. Two months after the onset of symptoms he began to complain of severe attacks of pain in the neck radiating to the occipital region; in such an attack "everything went black" and he was forced to grasp some support to prevent himself from falling. The intensity and frequency of these latter attacks

increased and were associated with loss of consciousness.

On admission, the patient, a florid type of individual, was restless and fine generalised muscular twitchings were noted. The cardiac dullness was increased to the left and the second aortic sound was accentuated. His eyes were rather prominent and the pupils widely dilated, but they reacted well to light and accommodation. Ophthalmoscopical examination showed some blurring of the margins of the optic discs and arteriosclerotic changes in the vessels. The blood pressure was 240/130 mg. Hg and the pulse rate 120 per minute. A trace of albumin was found in the urine and a few red cells were evident in the sediment on microscopical examination. The blood urea was 43 mg. per cent.

Progress: A week after admission to hospital the patient died. During this period mental confusion appeared and progressively worsened. He was disorientated, difficult to control, and frequently screamed. Two days before death on Oct. 6 the blood pressure was 86/56 mm.Hg. and 24 hours later 100/30 mm.Hg.

Post-mortem Findings: The post-mortem was performed 12 hours after death. The heart (620 g.) showed great hypertrophy and enlargement of the left ventricle: no valvular lesion was present. The coronary vessels showed slight hypertensive sclerosis, as also did the main branches of the aorta. No lesion was noted in the lungs, liver, or spleen apart from some recent venous congestion. The kidneys (right 270 g.; left 105 g.) showed irregularity of the cortical markings and some yellowish and reddish mottling of the cut surface. No reason was observed for the greater size of the right

kidney. The brain showed no lesion: the arteries at the base were slightly sclerotic.

The right suprarenal was normal: in the upper pole of the left a small, fairly sharply defined greyish-white tumour, 1 cm. in diameter, was present in the medulla.

Histological Findings: In haematoxylin-stained sections the tumour cells showed much post-mortem autolysis, the outlines of the cells being indistinct and the nuclei showing pyknosis and chromatolysis. This marked autolysis such a short time after death was an interesting and noteworthy feature. The cells had a more foamy and vacuolated cytoplasm than in either of the two preceding cases. The same alveolar arrangement was present but the alveoli were smaller, containing ten to twenty cells. The cells were more uniform in size and the nuclei more regular in appearance than in the first two cases. A rather thin fibrous capsule was present and this ran through and included part of the zona reticularis at places. On staining by Schmorl's modification of Giemsa and by Sevki's method, only some of the cells showed the olive-green granules, most of the cells having a diffuse greenish tint in their cytoplasm, suggesting that on account of post-mortem autolysis the granules had dissolved in the cytoplasm.

Case 4 (Dr. Kennie).

Clinical Findings: The patient, a housewife, 57 years of age, was admitted to hospital on Dec. 6, 1945. She had been well until November, 1945, when she developed a cough and gastro-intestinal symptoms - nausea, vomiting and diarrhoea. The latter symptoms

persisted for three weeks. Her complaints on admission were those of general exhaustion and fatigue. There were no relevant features in the past or family history.

Examination showed a well-built woman with clinical evidence of loss of weight. Glossitis was present and the liver was enlarged to 2½ in. below the costal margin. Otherwise no mass or tenderness was detected in the abdomen. Diffuse rhonchi were audible in the chest. The other systems did not reveal any abnormal physical signs. The urine contained a cloud of albumin. Hb 74 per cent, R.B.C. 4.12 million, W.B.C. 11,000.

The patient died nine days after admission, during which time pyrexia, tachycardia and increased respiration rate were marked. No further localizing features were obtained beyond the presence of an unduly high position of the right diaphragm on radiological examination of the chest. The W.B.C. rose to 28,000 per c.mm. and the blood urea (Dec. 6) was 101 mg. per cent. Records of the blood pressure taken on six separate days showed a systolic varying between 105 and 85 mm.Hg and a diastolic between 65 and 50 mm.Hg.

Post-mortem Findings: The autopsy was performed five hours after death. The heart weighed 250 g. and appeared normal. A large chronic abscess occupied most of the right lobe of the liver: from the abscess were isolated B. coli, Staph. pyogenes, and enterococcus. The gall-bladder was thick, fibrous, and contracted around a faceted gall-stone. The kidneys (right 200 g.; left 205 g.) appeared normal. A chronic periostitis was present in the neck of the right femur.

In the right suprarenal a well-encapsulated tumour, 3 cm. long, 2 cm. broad, and 0.5 cm. in thickness was found. The growth, which was in the medulla of the gland, was encircled with cortical tissue, had a light-brownish colour, and was rather soft in consistence. The left suprarenal appeared normal.

Histological Findings: Tissues were fixed as in the previous cases, but in addition some were preserved in a solution composed of equal parts of 5 per cent potassium bichromate and 10 per cent neutral formalin. As will be observed later, this fixative gave much better results with stains used to demonstrate the chromaffin granules. In the various fixatives the portions of the growth fairly quickly became brownish in colour.

Tissue stained by haematoxylin and eosin showed fairly well-defined cells, some being rounded or oval, others more elongated and polyhedral and arranged in irregular alveoli. Most of the cells had a granular cytoplasm, but in some this was foamy and in others vacuolated. The last may have been due to post-mortem autolysis. The nucleus again was vesicular, with one or more nucleoli. Some cells had two or three nuclei. Sometimes the nucleus was distended by a large central vacuole, giving a fairly typical signet-ring appearance. Some of the cells closely resembled ganglion cells similar to those seen in a ganglio-neuroma. Most cells, including those resembling ganglion cells, contained fine brownish granules: these did not give a reaction for iron. Numerous fine capillaries were present in the growth as well as a few sinusoidal spaces. A fairly thick fibrous capsule in which small nests of cortical cells

were present separated the tumour from the encircling cortical tissue. Mallory's stain demonstrated more clearly the granular cytoplasm and the alveolar nature of the growth. The alveoli were small, containing from one to five cells. In this case only did silver staining show reticulin lining the sinusoidal spaces. In sections stained by the modification of Sevki's stain after the formol-bichromate fixation described above, abundant rather dark-green granules were seen in cells, particularly those in the vicinity of the blood vessels. These granules were much coarser and more sharply defined than in any of the previous cases: this may have been due to the different method of fixation adopted in this case or to the fact that the tumour was not physiologically active.

In the kidneys the walls of the glomerular capillaries were slightly thickened, particularly the afferent arteriole, as it entered the tuft. There was a slight increase in the interstitial tissue, particularly around the glomeruli. A few of the glomeruli were completely fibrosed and the interlobular arteries showed some fibrosis of the media, thickening of the intima, and splitting of the internal elastic lamina. On account of the patient's age it is doubtful if these changes were due to the effects of the tumour. In the spleen the walls of the capillaries, both in the pulp and in the Malpighian bodies, showed slight hyalinization, as also did the branches of the hepatic artery within the liver.

Case 5 (Dr. J. W. Ferguson).

Clinical History: The patient, a male aged 73, was admitted to a surgical ward with a history of incontinence of urine of six

months' duration. He was well built, had evidently lost some weight recently, and appeared extremely ill. He was mentally confused and no details of his illness could be obtained. Lips and ears were cyanosed and breathing was rapid, shallow and distressed, so that he had to be propped up in bed. His tongue was very dry, cracked and heavily coated. There was a moderate amount of oedema over the sacrum. Investigation of bladder function revealed no retention, and only a few cubic centimetres of residual urine were obtained on catheterization. Urine contained albumin (0.5 parts Esbach). Rectal examination revealed the prostate to be enlarged and hard and to contain one large nodule in the left lobe. He had a markedly raised blood pressure, 220/110; the peripheral arteries were thickened and tortuous. Percussion note was impaired at the base of the right lung, where abundant fine and medium crepitations were audible. Blood urea 71 mg. per cent.

He was treated with abundant fluids, sulphadiazine, and penicillin, but his condition steadily deteriorated. On his transfer to a medical ward six days after admission, temperature was 100°F., pulse 160 per minute, respirations 50 per minute; more widespread consolidation was evident in the lungs. An electrocardiograph showed changes of nodal tachycardia and bundle-branch block. The high pulse rate was maintained until death occurred three days later. Blood pressure readings taken on each of the two days prior to death gave readings of 170/110 and 180/110 mm.Hg.

Post-mortem Findings: The post-mortem examination was performed 12 hours after death. The heart, 490 g., was enlarged, chiefly on

account of hypertrophy of the left ventricle. The aortic valve was slightly stenosed (8.5 cm. in circumference), due to calcification and adhesion of the cusps. The main pulmonary arteries to the left and right lower lobes were blocked by portions of embolus which had originated from a thrombotic condition in the veins of the legs (posterior tibial and lower part of femoral vein on both sides). Massive infarcts were present in the lower lobes of both lungs. The kidneys (right 120 g.; left 140 g.) showed some adhesion of the capsule to fairly widely scattered depressed areas on their surfaces. The cortex of both organs was slightly irregular in thickness, the markings were fairly regular, and the main branches of the renal arteries in the kidney substance were thickened and there were, in addition, patches of atheroma in their intima. The prostate was slightly enlarged, irregular and hard, and microscopically a scirrhous cancer was found in its substance. In the middle of the right suprarenal there was a spherical tumour (1.5 cm. in diameter and 8 g. in weight) surrounded by a narrow zone of cortical tissue. The tumour was fleshy in consistence and pale red in colour. The left suprarenal appeared normal.

Histological Findings: The tumour in the suprarenal had similar characters to those already described, but showed a more marked alveolar arrangement than in any of the other cases, each alveolus being outlined by a well-marked layer of reticulum. The growth was only partly encapsulated by a narrow zone of fibrous tissue which ran through the zona reticularis of the gland. There was a well-developed vascularity but no sinusoidal spaces. No

granules were found in the cells in sections stained by Sevki's method, but this was probably due to the length of time elapsing between death and the time of fixation (12 hours). It had been noted that during fixation in Müller's fluid there was only slight darkening of the tumour, in contrast to the very dark colour developing in Cases 1-4.

The kidneys showed some splitting of the internal elastic lamina of the arcuate and interlobar arteries. This, however, may have been due to the age of the patient (73 years) rather than a result of hypertension. Some of the glomeruli were fibrosed, others partly so, but the number thus affected was not large. There was slight patchy irregular overgrowth of fibrous tissue throughout the cortex of the organ.

In the spleen the smaller vessels showed hyaline thickening. A fairly diffuse interlobar fibrosis involved the substance of the pancreas and hyaline change was noted in the walls of the smaller arteries and arterioles. The islets appear larger than normal and more numerous; the latter, however, was possibly due to the interlobar fibrosis causing some contraction of the organ. The liver showed passive congestion only.

Case 6 (Dr. Ralston, Kilmarnock and Mr. Norman Davidson, Victoria Infirmary).

Clinical Findings: The patient, a woman aged 34, was first admitted to hospital on October 17, 1944. Her complaints were those of progressive weakness and breathlessness, which first appeared six months before. In addition she suffered from pain of a dragging

nature in the left abdomen; relief was usually obtained by lying down, though at times its severity interfered with her sleep. A month after the onset of these symptoms menstruation, hitherto regular, ceased.

Inspection showed a thin, rather pale woman. The abdomen was somewhat prominent in the left hypochondrium and a firm elastic non-tender rounded mass was palpable in this area. No abnormalities were found on examination of the other systems. The blood pressure was 128/74 mm.Hg.

A barium enema was performed and the descending colon was shown to be displaced towards the midline. Intravenous pyelography demonstrated deformity of the calyces of the left kidney.

Operation was suggested to the patient, but at her request was postponed till January, 1945. Under gas, oxygen and ether, Mr. Norman Davidson, through a lumbar incision, exposed the tumour, which with considerable difficulty was delivered into the wound. In places it was adherent to the posterior abdominal wall and the adhesions were tied and sectioned. The kidney was found at the lower pole of the tumour. The kidney pedicle was secured and the kidney and the tumour mass removed. The wound was closed with drainage. After the operation, one pint of plasma and two pints of blood were administered. Convalescence was uneventful. Menstruation returned in February, 1945.

She was examined at frequent intervals, a steady improvement in her physical capacity taking place, until Oct. 6, when she developed marked dyspnoea and physical and radiological examination

revealed a large left-sided pleural effusion. Paracentesis thoracis showed the fluid to be blood-stained. On Nov. 30. an artificial pneumothorax was induced, but although the collapse was satisfactory no further information was obtained. Radiographs of the thoracic and lumbar spine showed no evidence of secondary deposits. In January, 1946, she began to complain of pain in the left hypogastrium and examination showed the upper part of the left rectus muscle to be thickened and firm. The induration extended and by the middle of January a mass in the abdominal wall was palpable. The patient's general condition steadily deteriorated and she died on Feb. 22, 1946. At the time of death the area covered by the tumour included almost the whole of the left side of the abdomen and the medial portion of the right hypochondrium. On the surface of the mass many discrete nodules were evident and were of varied size up to 2 in. in diameter. Post-mortem examination was refused.

From the time the nature of the growth was recognised until just before death, repeated blood pressure recordings were made but were all within normal limits.

Histological Findings: The specimen was received 24 hours after excision, and the tumour, which was above the right kidney, measured 28 cm. long, 22 cm. broad, and 14 cm. thick, and weighed 1200 g. It was roughly round in shape and portions of the suprarenal cortex could still be distinguished on the surface of the growth. The tumour, which was contained in a thick fibrous capsule, was quite distinct from the kidney attached below. The growth was much softer than any of the preceding, and the cut surface showed

large yellowish-green areas of necrosis and areas of haemorrhage. Portions of the growth were fixed as formerly, but it was observed that the brownish colour noted in the other cases did not develop in any of the fixatives, including those containing bichromate.

In sections stained with haematoxylin and eosin the cells were not as sharply defined as in the first two cases, but had for the most part the same rather elongated polyhedral character. The eosinophil cytoplasm was slightly granular, and the nuclei were smaller and darker than in any of the other cases and less vesicular in character. Mitoses were readily found in most fields examined. The alveoli were large, the fibrous tissue surrounding them being rather fine. Cells with many nuclei were present throughout the section, particularly in the neighbourhood of areas of necrosis. As in Case 2, tumour cells were found in some of the blood vessels, which for the most part were numerous fine capillaries, the tumour thus being very vascular; sinusoidal spaces were, however, scanty. Throughout the growth there were numerous areas of necrosis and haemorrhage. Tissue fixed in Müller's fluid and stained by Schmorl's, Giemsa, or Sevki's method showed no granules, nor was any greenish colour noted in the cells. This may have been due to the length of time elapsing between the operation and the fixation of the tumour. Silver staining revealed the same arrangement of the reticulin as in the other cases. No fat was observed in any of the tumour cells, though some was observed in the necrotic tissue. A fairly thick capsule surrounded the whole growth.

The kidney, which was attached to the neoplasm, showed some

thickening of the basement membrane of the glomerular capillaries and slight increase of the interstitial tissue, particularly in relation to the glomeruli in sections stained by Mallory. In tissue stained for elastic tissue some reduplication of the internal elastic lamina of the arcuate vessels was noted.

Extraction of the Tumour: Portions of the tumour were extracted with N/10 HCl according to the method described by Kirshbaum and Balkin (1942), but no adrenalin reaction was given with ferric chloride or Folin's 1 per cent phosphomolybdic acid. As these tests were negative, no biological assay was made.