A STUDY OF ADDISON'S DISEASE.

An Investigation of the Chemical Pathology,

Diagnosis and Treatment of Adrenal Insufficiency.

A Thesis for the Degree of Doctor of Medicine

submitted by

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INTRODUCTION.

ADDISON'S DISEASE.

In 1855 Thomas Addison published his monograph "On the Constitutional and Local Effects of Disease of the Supra-Renal Capsules".

Though some doubt was expressed concerning the significance of disease of the suprarenal glands in several of the eleven cases described by Addison (Wilks (1862), Habershon (1864), Greenhow (1866)), the work nevertheless stimulated experimental research and further clinical observations. Addison undoubtedly realised that he had only touched the fringe of the subject for he expressed the hope that"by attracting the attention and enlisting the co-operation of the profession at large, they (the facts) may lead to the subject being properly examined and sifted, and the enquiry so extended as to suggest, at least, some interesting physiological speculations, if not still more practical indications".

In describing the cases reported by Addison and by others, Trousseau(1856) first used the term Addison's disease and in 1869 the more elaborate if academic title of "Morbus Addisoni, idem Valent Cutis aerea, melasma Addisoni" appeared in the first edition of the "Nomenclature of Diseases"published by the Royal College of Physicians of London.

CLINICAL MANIFESTATIONS.

In his original communication Addison(1855) described the disease as characterised by "Anaemia, general languor or

debility, remarkable feebleness of the heart's action, irritability of the stomach, and a peculiar change of colour in the skin."

While the disease may arise suddenly, the onset is as a rule very gradual. The usual history is of asthenia and anorexia, with or without restlessness and depression. Most patients report hypersensitivity to cold. This stage may be described as the latent phase for very often no signs accompany these symptoms and the diagnosis is difficult to establish without special biochemical investigations.

As the disease progresses typical gastro-intestinal and cerebral disturbances develop. The former are characterised by anorexia, nausea and vomiting or diarrhoea; there is usually some complaint of abdominal pain or lumbar tenderness and loss of weight. The chief cerebral manifestations are faintness, insomnia, depression, yawning, hiccough and photophobia. The chief signs are pigmentation of the skin and mucous membranes, low blood pressure, weak rapid pulse and faint heart sounds.

The stage of acute adrenal insufficiency or crisis may arise suddenly in a patient already known to have Addison's disease, or it may herald the disease for the first time. The crisis is characterised by acute attacks of vomiting or diarrhoea, hiccough, abdominal pain so severe as to simulate an acute abdomen. Generalised muscle tremors and convulsions are common and the blood pressure may be so reduced that it may not be possible to record it. If the patient is not promptly treated he will pass into coma and death will ensue within 12 hours.

PATHOLOGY.

Till 1930 tuberculosis of the adrenal glands was considered to be responsible for 80 - 90% of the cases of Addison's disease (Snell (1934)). Analysis of the more recent post-mortem reports indicate that in 57% of the cases atrophy of the suprarenals was found (Wells (1930)), Cragg (1940)). The cause of the atrophy is not at present known. In contrast with tuberculosis, the atrophy begins in the cortex and compresses the medulla. It is possible that some of these cases may follow primary atrophy of the anterior lobe of the pituitary gland. Haemorrhagic infarction, tumours and syphilis of the supra-renals have been reported; excellent résumés of the histo-pathology of Addison's disease have been published by Barker (1929) Paul (1931) and Hall and Heinken (1936).

CHEMICAL PATHOLOGY.

Many attempts have been made to correlate the clinical manifestations of Addison's disease with physical and chemical alterations in the body fluids. Extensive investigations of a similar nature have been carried out on adrenalectomised animals. Addison's disease has never been completely reproduced in experimental animals for when the suprarenal glands are removed the animals do not become pigmented. Moreover it is possible to maintain these animals in perfect health over an indefinite period by suitable replacement therapy. This cannot be said of patients with Addison's disease for in spite of the best treatment available, they still remain pigmented and do not regain their normal stamina and full physical strength.

Nevertheless very valuable information has been gained from initial studies on animals, which has been substantiated later by clinical observation. The chief direction in which the investigation of adrenal insufficiency has been pursued has centred on the study of renal, carbohydrate and electrolyte metabolism.

Renal Metabolism.

From their work on adrenalectomised animals Harrison and Darrow (1938) suggested that an alteration in renal function would account for the decreased urea clearance, increased sodium excretion and retention of potassium, urea and phosphate in the blood. To a certain extent this is confirmed by the results of Willson and Sunderman (1939) on patients with Addison's disease. While there is no doubt that there is some disturbance of renal function in both experimental and clinical adrenal insufficiency, the indications are that it represents only the renal effects of some more profound changes within the body as a whole. No histological changes in the kidney have yet been observed in either experimental animals or in patients with Addison's disease. Gersh and Grollman(1939) and Swingle (1938) have shown that the renal function is largely restored by the administration of adrenal cortical compounds. This conclusion is supported by the creatinine clearance studies made by Margitary -Becht and Gomori (1938). Whether the extra-renal cause is due to alteration in blood concentration and renal blood flow as suggested by Stahl, Kullman and Urban (1938) remains to be confirmed.

Carbohydrate Metabolism.

Many studies have been made of carbohydrate metabolism in adrenalectomised animals and the results indicate that a low blood sugar and diminished liver and muscle glycogen storage are characteristic (Britton and Silvette (1932); Long, Kazin and Fry (1940); Kendall (1940)). This derangement of carbohydrate metabolism is chiefly brought about by a decrease in the rate of formation of dextrose from endogenous protein and by an impairment in the synthesis of dextrose. (Buell, Anderson and Strauss (1936)). It is noteworthy that a normal carbohydrate metabolism is restored when the animals are injected with either cortical extract or certain steroid hormones.

Porges (1909) was the first to draw attention to the low blood sugar level in Addison's disease but according to some reports this is by no means a constant factor. In a recent analysis of a large series of cases Kepler and Wilder (1938) have shown that the blood sugar level is usually within normal limits and is only occasionally subnormal. Crooke and Russell(1935) examined a number of pituitary glands from cases of Addison's disease and reported that the number of basophil cells in the anterior lobe was greatly reduced; they suggested that this cytological abnormality was an important factor in the disturbance of carbohydrate metabolism. This conclusion cannot be wholly sustained as Rhind and Wilson (1941) have shown that where diabetes mellitus is associated with Addison's disease, there is still a decrease in the basophil cells of the anterior pituitary gland. At present it is a matter of conjecture whether

the disturbance in carbohydrate metabolism is directly attributable
to the adrenal cortex or to the anterior pituitary gland.

Electrolyte and Water Metabolism.

The experimental work of Loeb(1932) which followed that of
Baumann and Kurland(1927) and of Marine and Baumann(1927) has
been concerned with the study of the weakness, dehydration and
hypotension which occurs in adrenalectomised animals.   He found
that in these animals there is an increase in blood concentration,
a decrease in serum chloride and bicarbonate, and an appreciable
and constant low level of serum sodium.   The output of sodium in
the urine was sufficiently increased to indicate not only a loss of
sodium from the blood but also from the interstitial fluid.
Harrop(1933) confirmed these findings and showed that there was
also an increase in serum potassium.

The stage of acute adrenal insufficiency or crisis in
patients with Addison's disease is characterised by a shock-like
state which is associated with a loss in blood volume, diuresis,
and an increased output of sodium in the urine.   As he found it
difficult to attribute the peripheral circulatory collapse entirely
to a loss of fluid, Loeb(1936) suggested that the production of shock
was due to some interference with the control of the vascular bed.
Swingle and co-workers(1938) considered the picture to be very
similar to the collapse produced by histamine and concluded that
the mechanism of shock was due to loss of capillary tone.   They
considered that the beneficial effects brought about by treatment
with cortical extracts were due to restoration of capillary tone.

The recent work of Menkin (1940) supports this conclusion for he showed that extract of adrenal cortex inhibits the dilatation of capillaries which follows the subcutaneous injection of leukotoxin and tissue exudates.

The hypothesis that the symptoms of Addison's disease are due to toxaemia has been advanced on various occasions since Brown-Séquard (1856) described his experiments. He held the view that when the adrenal glands are removed the blood becomes toxic. Abelous and Langelois (1892) considered that a substance similar to curare is produced in the muscles and is normally destroyed by the adrenal glands; in the absence of any adrenal cortex the substance is free to act and produces the picture seen in adrenal insufficiency. Shortly afterwards Myers (1898) showed that by incubating cobra poison with an emulsion of guinea pig adrenal cortex, the cobra venom became inactivated and no longer produced its characteristic toxic effects; work of a similar nature has been described more recently by Perla and Marmorston (1933). Since cobra venom liberates large amounts of histamine from the lung (Feldberg and Kellaway (1939)) this is probably the mechanism of the toxic action of the venom. Not only are adrenalectomised animals more sensitive to cobra venom and to histamine (Lewis (1923), Perla and Rosen (1935)) but it has also been shown by Rose, Karady and Browne (1940) that there is an accumulation of histamine in the tissues of adrenalectomised animals and that the histaminase activity of the lung is decreased. The latter workers have observed that in

such animals the ability to inactivate histamine as well as the histaminase activity of the lungs are restored to normal by the administration of adrenal cortex extracts.

It is possible, therefore, that histamine may play an important role in the pathology of adrenal insufficiency and that the hormones of the adrenal cortex perform some specific anti-histamine function.

SCOPE OF THE PRESENT INVESTIGATION.

The work about to be described is an account of the investigations made by the author into the chemical pathology, diagnosis and treatment of Addison's disease.

The first part describes the experimental investigation of the role of histamine in the blood of adrenalectomised animals and the effect of extract of adrenal cortex on the blood histamine level of these animals.

The second part is concerned with the investigation of patients with Addison's disease with particular reference to the distribution of histamine in the plasma and blood cells of these patients. An attempt is made to correlate these findings with the hypotension and clinical condition of the patients.

The diagnosis of Addison's disease is discussed in the third part and a description is given of the various methods of assessing adrenal insufficiency.

The fourth part concerns the occurrence of other endocrine diseases in association with Addison's disease. A rare case of Addison's disease associated with diabetes

mellitus is described.

The treatment of Addison's disease is described in the fifth part and an account is given of some observations on the efficacy of desoxycorticosterone acetate.

Conclusions and Summary are followed by an Appendix containing details of some of the methods and diets employed in the investigations.

PART ONE.

THE EFFECT OF ADRENALECTOMY ON THE BLOOD HISTAMINE OF RABBITS.

THE EFFECT OF ADRENALECTOMY ON THE BLOOD HISTAMINE
OF RABBITS.

INTRODUCTION.

In 1912 Dale and Laidlaw first demonstrated the sensitivity of animals to injections of histamine. Eight years later Dale (1920) pursued his earlier studies by showing that the adrenalectomised cat was extremely sensitive to histamine, but recognised that it was impossible by his experiőmnts to determine whether the decreased resistance to histamine was due to absence of the medulla or of the cortex.

Working with perfused organs Dale and Richards(1918) suggested that one function of adrenalin might be to maintain the tone of the capillaries for such tone might depend on the relative amounts of histamine and adrenalin present. Kellaway and Cowell(1922) developed this theory of antagonism between adrenalin and histamine by showing that histamine injected into partially and completely adrenalectomised cats causes an acceleration of secretion from the medulla of the adrenal glands. They further observed that while haemo-concentration is a feature of adrenalectomised cats and of normal cats injected with histamine, there was no direct evidence that the blood concentration in adrenal insufficiency is due to histamine, as loss of cortex alone produces this condition. Perla and Gottesman(1929) regarded the protective action of adrenalin against the damage caused by inoculation of histamine as a hormonal effect and not entirely dependent on the pharmacological antagonistic action of adrenalin

to histamine.   They showed that the sensitivity of adrenalectomised animals to histamine can be lessened if adrenalin is administered, but this obtains only if adrenalin has been administered repeatedly for some time before the injection of histamine.   Experimenting on rats Wyman(1932) claimed that the decreased resistance to histamine was due to removal of the medulla.

On the other hand Ingle(1937) streased the importance of adrenal cortex in maintaining normal resistance to injections of histamine and pointed out that while removal of the medulla alone lowered resistance to histamine, a much lower resistance resulted from complete extirpation of the adrenal glands.   He further observed that adrenalin increased the resistance of rats in which the medulla had been destroyed but that cortical extract did not have a similar effect.   Scott (1928) considered that the increased susceptibility to histamine was due to lack of cortical tissue and suggested this as a basis for the assay of cortical extract.   Such an assay was later suggested by Perla and Gottesman(1931).

Rose and Browne (1938) injected histamine intravenously into normal and adrenalectomised rats and showed that the rate of disappearance from the blood was much slower in the adrenalectomised animals.

As no study appeared to have been made of the effect of the adrenal cortex on the blood histamine level of animals, it seemed important to determine whether histamine could be formed in the absence of the adrenal cortex.   All the previous workers had injected histamine into the animals.   It was further considered desirable to ascertain what influence, if any, cortical extract has on the blood histamine level of adrenalectomised animals.

In designing this investigation it was decided to study the effect of adrenalectomy on the whole blood of the rabbit. Rabbit blood is relatively rich in histamine so that small quantities suffice for histamine assay. This is of considerable importance where blood has to be withdrawn at intervals from an adrenalectomised animal. There are technical difficulties associated with the separation of plasma in rabbit blood and it was decided that histamine values of plasma should not be attempted in this study.

METHODS.

Male rabbits 3-4 months old were used in all the experiments. The anaesthetic was ether and anaesthesia was slowly induced using a gauze covered wire net conical mask. Adrenalectomy was performed in a one-stage operation details of which are described in Appendix(page 110). Immediately after the operation the animals were given 3.5 c.c. 5% sodium chloride intravenously and 1 c.c. cortical extract subcutaneously. The rabbits were maintained for 2 days after operation on a diet of cabbage, oats and bran with 2-3 c.c. cortical extract subcutaneously daily. The cortical extract used was "Cortin Organon", 1 c.c. extract equivalent to 50 gm. fresh suprarenal gland.

Samples of blood (2 - 2.5 c.c.) were taken by cardio-puncture from the left side of the heart. No anticoagulant was used; whole blood was immediately extracted by the method of Anrep, Barsoum, Talaat and Weininger (1939). The histamine was assayed on the isolated gut of the guinea-pig according to Code's (1937) modification of the method of Barsoum and Gaddum.

The arterial blood pressure in the ear vessels was determined by the capsule of Grant and Rothschild (1934). The

rabbits were kept at rest in quiet and warm surroundings before each determination of blood pressure and the mean of 10 evaluations was taken.

RESULTS.

I.   The effect of adrenalectomy on the blood histamine.

Twelve hours after withdrawal of cortical extract all the rabbits showed signs of adrenal·insufficiency, indicated by thirst, anorexia, weakness and diarrhoea.   Control animals which were subjected to similar operative technique, but without removal of the suprarenal glands, were given the same treatment.   The blood pressure recording showed considerable variation (Table I) but in each case the blood pressure fell after adrenalectomy, and the histamine content of the blood was raised.

TABLE I.   The effect of adrenalectomy on the blood histamine and blood pressure (rabbit).

Animal No.	Blood histamine. (ug./cc.)		% Difference.	Blood pressure (mm./Hg.)	
	Before adrenalectomy.	After adrenalectomy.		Before adrenalectomy.	After adrenalectomy.
41	4.6	16.7	263	76-78	70-72
51	4.7	6.2	32	82-84	70-74
501	5.0	6.4	28	64	45
231	1.9	2.3	21	66-68	46-48
251	0.9	2.2	145	82-84	16-20
401	2.7	11.5	326	--	--
Controls	Before operation	After operation		Before operation	After operation
483	3.3	2.9	- 12	68-70	68-70
493	2.9	2.5	- 14	70	68
473	3.8	3.8	--	70	70

II. The effect of cortical extract on the blood
     histamine level of adrenalectomised rabbits.

The effect of cortical extract was observed on six rabbits
which had been adrenalectomised, and had received no cortical
extract for at least 12 hrs.   Blood was withdrawn at the intervals
specified in Table II.   1 c.c. cortical extract was given
subcutaneously immediately after taking zero-hour samples of blood.
The level of blood histamine was lowered within 1 hr. in all cases
except in animal 25.   The duration of fall varied, in the
observations made, from 3 to 24 hr.   The histamine level at death
was increased in four animals, while in animal 05 there was a
marked decrease, though the animal died in typical convulsions.
Animal 52 remained alive for 7 days, but at autopsy no accessory
glands were apparent.   The effect of cortical extract on the blood
pressure varied considerably.   In all animals there was an increase
in blood pressure after cortical extract had been given, but in no
animal did the blood pressure attain the value recorded before
adrenalectomy.

TABLE II.   The effect of cortical extract on the blood histamine
            level of adrenalectomised rabbits.

Time (hr.) Animal No.	0	1	3	5	8	12	15	24	Death
			Blood histamine (ug./cc.)						
52	6.2	3.4	-		4.5				5.9
23	2.3	1.4	-				1.6		4.3
25	2.2	2.3	-				2.7		4.5
03	6.0	-	4.3	6.9					6.1
05	8.6	-	6.5					6.8	4.2
06	3.4	3.0	-		2.8				4.1

A further series of experiments was made to determine whether cortical extract could prevent a rise in blood histamine if given immediately after adrenalectomy. Five rabbits immediately after adrenalectomy were given 3.5 c.c. 5% saline intravenously, and 1 c.c. cortical extract subcutaneously. Blood was withdrawn 6 hr. after completing the operation. This period was considered sufficient to allow the animals to recover from the anaesthetic. The blood histamine in all animals which had received cortical extract after adrenalectomy was decreased 6 hr. after adrenalectomy (Table III).

TABLE III. The effect of cortical extract on blood histamine
6 hr. after adrenalectomy.

Blood histamine (ug./cc).

Animal No.	Before operation.	After operation.	% Difference.
38	4.30	2.3	−46.5
39	2.90	1.8	−37.9
41	2.23	1.7	−23.8
42	1.65	1.6	− 3.0
44	1.60	1.3	−18.8

DISCUSSION.

Much attention has been paid to the protective mechanism against the action of histamine. The inactivation of histamine by the emzyme histaminase has been studied by Best and McHenry(1930) and others. On the other hand, Anrep, Barsoum and Talaat(1936), following the observation of Weiss, Ellis and Robb (1929), and of Jacobs and Mason (1936), have shown that "when histamine is given by slow intravenous injection to dogs it does not remain in active

form in the plasma". They stated that the histamine is, in part, rendered physiologically inactive by being taken up by the red blood cells. Later, Code (1938) pointed out that, while the white blood cells carry the major part of the blood histamine during anaphylactic shock in dogs, the plasma contains the greater part of histamine at the peak of reaction. In their experiments with rats, Rose and Browne (1938) made interesting comparisons of the fate of intravenously injected histamine in normal and adrenalectomised animals. They found that at the end of 3 hr. the amount of histamine in the blood of the normal rat was 0.2 ug./cc., whereas in the adrenalectomised animal it was 7.0 ug./cc. While they stated that the initial blood histamine level in the normal rat is 0.5 ug./cc. they do not appear to have determined the corresponding level in the adrenalectomised animal. The evidence produced by the experiments here described indicates that an explanation of the decrease in the rate of disappearance of histamine injected into the adrenalectomised rats may be due in part to an increase in the initial blood histamine level. The use of cortical extract in the treatment of "shock" has been advocated by Swingle, Parkins, Taylor and Hays (1938) who stated that in circulatory collapse the cortical hormone exerts a beneficial effect on the control of capillary tone. To determine whether histamine can be inactivated by cortical extracts observations were made on the incubation in vitro of histamine with cortical extract. Solutions of pure histamine acid phosphate and extracts of blood containing histamine were incubated with cortical extract at 37°C. for 1 hour. No significant loss of histamine activity resulted. It would thus appear that the reduction of the blood

histamine level in adrenalectomised rabbits which follows the
administration of cortical extract is not directly due to a destruction
of histamine by the cortical extract.   The failure of the blood
pressure to return to a normal level after the injection of
cortical extract suggests that the capillaries have already
suffered some damage which cannot be reversed by cortical
extract.   Whether the higher blood level of histamine is
responsible for this damage is a point which it is important but
difficult to determine.

SUMMARY.

Adrenalectomy produces a rise in the blood histamine of
rabbits.   Cortical extract given subcutaneously reduces the
blood histamine level of adrenalectomised rabbits within 1 hour.
This effect is maintained for a period of from 3 to 24 hours.

P A R T   T W O.

THE   INVESTIGATION   OF   BLOOD   HISTAMINE   IN   PATIENTS   WITH   ADDISON'S   DISEASE.

THE INVESTIGATION OF BLOOD HISTAMINE IN PATIENTS WITH
ADDISON'S DISEASE, WITH PARTICULAR REFERENCE TO THE
DISTRIBUTION OF HISTAMINE IN THE PLASMA AND BLOOD CELLS
OF THESE PATIENTS.

Introduction.

This investigation was designed as a sequel to the
experimental work on rabbits, described in part 1.   The purpose
of the study was to determine whether in Addison's disease the
blood histamine level differs from that in normal adults and in
patients who have arterial hypotension but no evidence of
Addison's disease.   It was decided to determine the level of
histamine in the plasma and the distribution of histamine
between the plasma and the blood cells in these groups of
patients.   It was considered important to correlate these
findings with blood pressure readings, in order to determine
what part, if any, histamine plays in arterial hypotension.

Methods.

Observations were made on 21 subjects.   Of these 12
(Group A) were patients suffering from Addison's disease; 4
(group B) were subjects with arterial hypotension who had
symptoms suggesting Addison's disease and had in fact been
admitted to hospital with the diagnosis of ? Addison's disease.
The remaining 5 cases (Group C) were normal healthy individuals
resting in bed.

Group A. Specimens of blood were taken 60 hours after withdrawal of cortical extract or D.O.C.A. whichever treatment was being used for maintenance. The sample in Case 8 was withdrawn during a "crisis".

Group B. Two of these patients (Cases 23 and 24) were on normal diet and specimens were taken 3 hours after breakfast. The other two cases (21, 22) had undergone the Cutler, Power and Wilder Excretion Test (see Appendix p. 119.) for diagnosis of Addison's disease and samples of blood were withdrawn on the third day of the test.

Group C. These patients were maintained on a normal hospital diet and blood was collected three hours after breakfast.

Venous blood was transferred to a wide-bore glass tube containing Heparin as anticoagulant in the proportion of 0.03 cc. Heparin (500 units/cc.) per 10 cc. blood. Haematocrit readings were made after centrifuging at 3000 rev./min. for 20 mins.

Separate estimations were made of whole blood, plasma and combined red and white cells. Plasma was carefully separated from the cellular layers by a fine pipette. The different fractions of blood were transferred quantitatively to a stoppered cylinder containing 5 cc. 10% trichloroacetic acid and the subsequent technique for the extraction of histamine was carried out according to the method of Anrep, Barsoum, Talaat & Weiniger (1939)

The assay of histamine was made on strips of freshly isolated guinea pig ileum suspended in a bath of Tyrode's solution containing 0.5 ug. atropine sulphate per litre.   The activity of these solutions was verified as being due to histamine by one or other of the methods described in Appendix.

Blood pressure recordings were made from a mercury sphygmomanometer; the figure expressed being the average of six separate readings.   The patients lay behind screens at rest for 30 minutes before blood pressure readings were taken.

. Results.

I. The concentration of histamine in whole blood of patients with Addison's disease and of control subjects.

The histamine equivalents for whole blood are expressed in Table IV .   In Group A the values ranged from 4.5 ug./100 cc. to 61.0 ug./100 cc. with an average value of 15.6 ug./100 cc.   In Case 8 the blood was withdrawn during a crisis and shows a remarkably high histamine content of 61.0 ug./100 cc.   These levels are considerably greater than for normal persons (Group C) where the range was from 2.7/ ug./100 cc. to 3.9 ug./100 cc. with an average value of 3.4 ug./100 cc.   The values in Group C agree with those found in normal subjects by Barsoum and Gaddum (1935) and by Kwiatkowski (1941).   In Group B the values for cases 21 and 22 who had undergone the Cutler excretion test were 1.9ug./100cc. and 5.5 ug./100 cc. respectively while for the other two on normal diet the equivalents were 0.9 ug./100 cc. and 4.4 ug./100 cc.

The mean increase of 372% in the blood histamine of

patients with Addison's disease compared with normal subjects
supports the evidence found in the earlier experimental work on
rabbits.   Adrenalectomy of rabbits resulted in a mean increase of
136% in the blood histamine equivalent.   Further as in rabbit
blood there is a variation in the individual readings about the
mean, so too for patients with adrenal insufficiency there is a
wide scatter of the individual readings about the mean.

TABLE IV. Showing the concentration of histamine in ug./100 cc.
and in whole blood of patients with Addison's disease
and of control subjects.

Case.	Group.	Histamine in whole blood.		
		ug./100 cc.	Range ug./100 cc.	Average ug./100cc

Cases of Addison's disease.

1	A	24.0		
2	A	11.1		
3	A	14.0		
4	A	4.8		
5	A	11.8		
6	A	12.9		
7	A	4.5		
8	A	61.0		
9	A	16.3		
10	A	5.6	4.5 - 61.0	15.6
11	A	6.2		

Cases of hypotension - not suffering from Addison's disease.

21	B	1.9		
22	B	5.5		
23	B	0.9		
24	B	4.4		
			0.9 - 5.5	3.2

Normal healthy subjects.

13	C	3.4		
14	C	3.4		
15	C	3.5		
16	C	3.9		
17	C	2.7		
			2.7 - 3.9	3.4

II. The concentration of histamine in plasma of patients with Addison's disease and of control subjects.

The normal level (Group C) of histamine in plasma (Table V.) ranged from 1.9 to 3.0 ug./100 cc. with an average value of 2.6 ug./100 cc.   These figures are higher than those found by Kwiatkowski (1941) who gave a range of 0.5 to 1.5 ug./100cc In Group B the equivalents varied between 1.0 and 4.9 ug./100 cc. with an average of 2.7 ug./100 cc.   It is significant that cases 21 and 22 gave values lower than the subjects 23 and 24 who had not undergone the Cutler Excretion Test.

The figures in Group A show that the plasma histamine in Addison's disease is substantially greater than in the control groups.   The equivalents ranged from 4.3 to 23.7 ug./100 cc. with a mean value of 9.6 ug./100 cc.

TABLE V.　Showing the concentration of histamine in ug./100 cc.
in the plasma of patients with Addison's disease and
of control subjects.

Case.	Group.	Concentration of histamine in plasma.		
		ug./100 cc.	Range ug./100 cc.	Mean ug./100 cc.

Cases of Addison's Disease.

Case	Group	ug./100 cc.	Range	Mean
1	A	9.7		
2	A	8.9		
3	A	10.4		
4	A	8.6		
5	A	8.8		
6	A	12.8		
7	A	4.3		
8	A	23.7		
9	A	6.4		
10	A	6.0		
11	A	6.2	4.3 - 23.7	9.6

Cases of arterial hypotension - not suffering from Addison's disease.

Case	Group	ug./100 cc.	Range	Mean
21	B	1.0		
22	B	3.2		
23	B	1.7		
24	B	4.9	1.0 - 4.9	2.7

Normal healthy subjects.

Case	Group	ug./100 cc.	Range	Mean
13	C	2.9		
14	C	2.3		
15	C	3.0		
16	C	3.0		
17	C	1.9	1.9 - 3.0	2.6

III. The relationship between plasma histamine and blood pressure in patients with Addison's disease (Group A) and in patients with arterial hypotension (Group B.)

Observations already made from Table V. that the histamine level in plasma of Group B varies little from normal subjects indicate that there is probably no direct relationship between plasma histamine and blood pressure levels.

In Table VI. the figures for plasma histamine and B.P. recordings are tabulated for each group; these facts are expressed in a more graphic form in Fig. 1 where plasma histamine values (abscissae)are plotted against systolic and diastolic blood pressure readings (ordinates).   Even if it were assumed that the cause of hypotension in Group B was different from Group A and that the low B.P. in Group A was due to histamine, there is no definite evidence of any direct relationship between plasma histamine level and hypotension.   It is true that the diastolic B.P. readings in Group A tend to fall as the plasma histamine increases, but there is no such tendency apparent with the systolic B.P. records.   It is possible that after the initial damage to the cells has been effected by histamine, any further increase in plasma histamine would not necessarily entail a further fall in systolic blood pressure.   Such a hypothesis received some support from the observations made on the effect of D.O.C.A. on the histamine content of plasma, where it was noted that even after a substantial fall in histamine level, the hypotension remained unaltered.

TABLE VI.   The Histamine content of plasma (ug./100 cc.)
and the corresponding B.P. reading (m.m. Hg.)
of patients with Addison's disease (Group A)and
of patients with arterial hypotension (Group B).

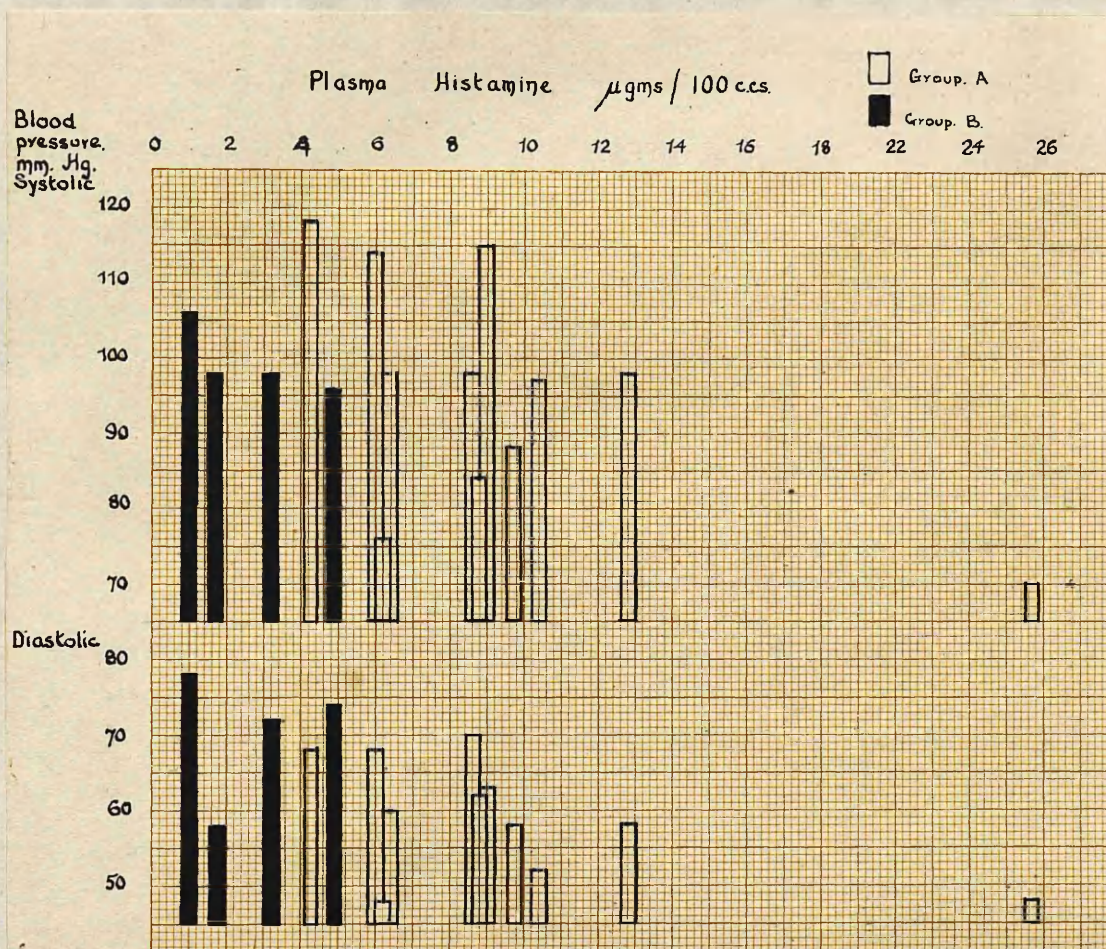| Case No.	Group.	Plasma Histamine. ug./100 cc.	B.P. m.m. Hg. Systolic	Diastolic.
1	A	9.7	88	58
2	A	8.9	115	63
3	A	10.4	97	52
4	A	8.6	98	70
5	A	8.8	84	62
6	A	12.8	98	58
7	A	4.3	118	68
8	A	23.7	70	48
9	A	6.4	98	60
10	A	6.0	114	68
11	A	6.2	76	48
21	B	1.0	106	78
22	B	3.2	98	72
23	B	1.7	98	58
24	B	4.9	96	74

FIG. 1.   The relationship between plasma histamine and
blood pressure of patients with Addison's disease
(Group A) and of patients with arterial
hypotension (Group B).

IV.   The concentration of histamine in combined red
and white blood cells of patients with Addison's disease and
of control subjects.

The histamine content of the combined red and white
cell layer of the centrifuged blood from patients in Group A
varied between 4.7 and 29.0 ug./100 cc. with a mean value of
12.2 ug./100 cc.   These figures (Table VII) represent an
increase in mean value of 183% over the corresponding value
for the control groups.   In Group C the values for normal
subjects ranged from 3.6 to 5.4 ug./100 cc. with an average
value of 4.5 ug./100 cc., while the equivalents in Group B lay
between 2.6 and 8.4 ug./100 cc. with a mean equivalent of
4.2 ug./100 cc.

TABLE VII. Showing the concentration of histamine in
ug./100 cc. in the combined red and white blood
cells of patients withAddison's disease and of
control subjects.

Case.	Group.	Concentration of histamine in red and white blood cells.		
		ug./100cc.	Range ug./100 cc.	Mean ug./100 cc.
		Cases of Addison's disease.		
1	A	4.9		
2	A	15.3		
3	A	18.5		
4	A	6.9		
5	A	16.8		
6	A	12.8		
7	A	4.7		
8	A	29.0		
9	A	20.3		
10	A	4.9		
11	A	6.2		
12	A	6.0	4.7 - 29.0	12.2
		Cases of arterial hypotension - not suffering from Addison's disease.		
21	B	3.0		
22	B	8.4		
23	B	2.6		
24	B	3.7	2.6 - 8.4	4.2
		Normal healthy subjects.		
13	C	4.2		
14	C	4.9		
15	C	4.3		
16	C	5.4		
17	C	3.6	3.6 - 5.4	4.5

V.   The distribution of histamine in the red and white
blood cells and plasma of normal subjects and of patients with
hypotension and with Addison's disease.

When histamine is added to normal dog blood either in
vitro or in vivo the histamine is distributed in a fairly constant
ratio between the cells and plasma (Anrep, Barsoum and Talaat
(1936)).   This suggests that in normal dog blood a mechanism
of partition distribution for histamine exists between cells and
plasma.

If such a partition mechanism for histamine exists in
normal human blood then the ratio of cells to plasma would tend
to be constant.   On Fig. 2 the concentration of histamine in
cells (ordinates) are plotted against the corresponding
concentration in plasma (abscissae).   It will be observed that
for normal subjects the individual values are fairly evenly
distributed about the mean and while the mean value for patients
with low B.P. (Group B) closely approximates that for normal
subjects there is a much greater variation in the individual
readings.

When corresponding values for cells and plasma histamine
of patients with Addison's disease are plotted a wide variation
about the mean is seen and there appears to be very little
relationship between the histamine in the cells and plasma.
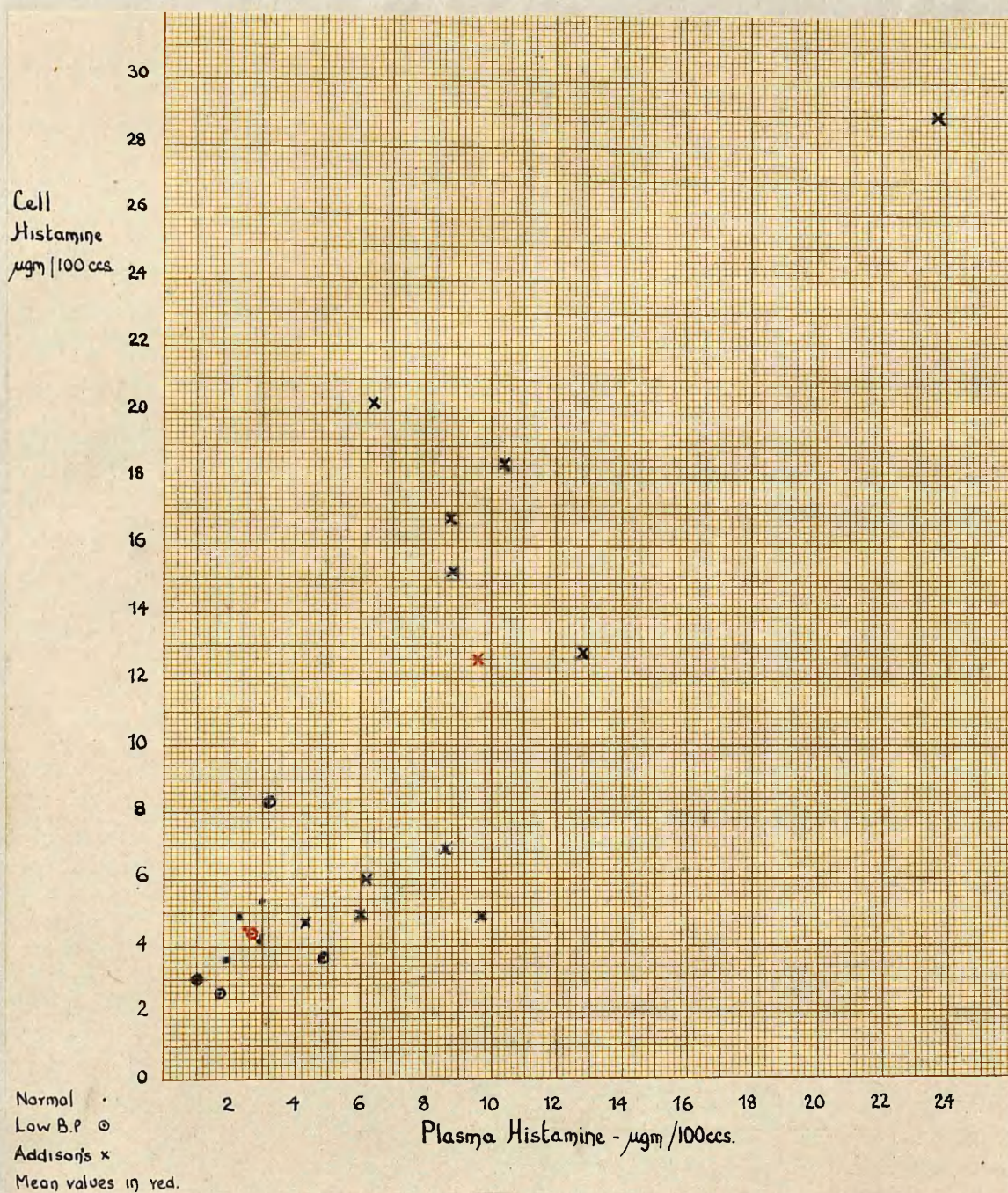
FIG. 2. The relationship between plasma and cell histamine in the blood of patients with Addison's disease, with arterial hypotension and normal persons.

VI.   The effect of D.O.C.A. on the distribution of
histamine on the red and white blood cells and plasma of
patients with Addison's disease.

Patients with Addison's disease are not ideal subjects
for purposes of investigation involving venepuncture.   Their
veins are often collapsed and where it is desirable to withdraw
their replacement therapy to the point of adrenal insufficiency
as near as possible to crisis, the trauma of venepuncture is not
without difficulty or danger.   For this reason the original plan
of investigating the effect of hormone therapy on the histamine
distribution in blood was unfortunately restricted.   Altogether 4
patients were studied in order to observe the effects of
desoxycorticosterome acetate (D.O.C.A.) on the distribution of
histamine between cells and plasma.   Specimens of blood were
taken 48 hours after withdrawal of hormone therapy and again
4 hours after intramuscular injection of 5 mg. D.O.C.A.   Blood
pressure recordings were also made.   The results are shown in
Table VIII.

TABLE VIII.   The levels of histamine in blood cells and
plasma of patients with Addison's disease
before and 4 hours after intramuscular
injection of D.O.C.A. 5 mgm.

Case No.	Plasma Histamine ug/100 cc.			Cell Histamine ug./100 cc.			Ratio cell histamine / plasma histamine	
	Before	After	% Diff.	Before	After	% Diff.	Before	After.
1	23.7	18.4	-22.3	134.0	47.0	-64.9	5.6	2.5
2	6.8	3.8	-44.1	16.9	15.7	- 7.1	2.4	4.1
3	9.4	4.2	-55.3	12.0	19.0	+58.3	1.2	4.5
7	4.3	4.0	- 7.5	4.7	7.4	+57.4	1.0	1.8

In all cases the plasma histamine level was reduced four hours after the injection of D.O.C.A. The extent of reduction varied considerably; in Case 7 the drop was of the order of 7%. the other cases showed a fall in histamine of 20-55%. It might be expected that the histamine values of the cells would correspondingly rise. This is not clearly shown for while in 2 cases there was an increase of 58-80%, in the remaining cases there was an appreciable fall in the cell histamine values. Comparison of the ratio of cell histamine to plasma histamine $(\frac{c}{p})$ indicates that after D.O.C.A. the ratio was increased in all cases except in No. 1. It is possible, however, that the amount of D.O.C.A. administered was not sufficient to produce the maximum effect or that samples of blood withdrawn at a later period would have shown a higher ratio.

There is some evidence from these results that the change in distribution of histamine between cells and plasma which results from the administration of D.O.C.A. is fairly rapid in onset. It is not yet possible to state whether the loss of histamine is due to absorption of histamine by the cells or whether histamine is destroyed by histaminase activated by D.O.C.A. The evidence of Rose, Karady and Browne (1940) that the lung histaminase activity of adrenalectomised animals is restored to normal by "adreno-cortical substances", supports the latter explanation that the anti-histamine effect produced by D.O.C.A. operated through the medium of histaminase.

Discussion.

The results of this investigation lend further support to
the evidence provided by the work on adrenalectomised rabbits.
Allowing for species variation where the histamine content of
normal rabbit blood is much greater than in normal human blood,
it is evident that in Addison's disease there is an increase of
approximately 370% in blood histamine.   It is not possible to
state at present whether the high level of blood histamine in
adrenal insufficiency is due to excessive generation or to
diminished destruction of histamine.   The experiments of Rose
and Browne(1938) indicate that the rate of destruction, at least
in rats, is greatly reduced.   Some attempts were made by the
author to settle the question more definitely by studying the
excretion of histamine in the urine of normal persons and of
patients with Addison's disease.   Extracts of urine were
electrolysed in an attempt to isolate histamine with the minimum
of hydrolysis but it was not possible to obtain consistent results.
When a suitable method is evolved for isolating histamine from
urine, it should be possible to determine the rate of destruction
and elimination of histamine in the body.

In Addison's disease there is an increase in the histamine
level of both plasma and blood cells but there is no evidence of
any definite distribution of histamine between cells and plasma,
as is the case with normal blood.   If this distribution of
histamine performs an important protective function in the normal
person, as Anrep and his colleagues(1936) suggest, then no such
protective mechanism is available to the patient with Addison's
disease.   It may well be that the absence of this protection is

the basis of the increased sensitivity of such patients to drugs and trauma.

Though there is no direct relationship between B.P. level and the histamine content of plasma, histamine may play an important role in causing the circulatory collapse in adrenal insufficiency. The increased values for blood histamine reported in both animal and human adrenal insufficiency provide some support to the conclusions of Swingle(1938) and the high levels of histamine in plasma indicate a close parallel with the picture found at the height of anaphylactic shock by Code (1938) It will only be possible to assess more accurately the significance of these abnormal values of histamine in the blood of patients with Addison's disease, when the mode of secretion and elimination of histamine in the body is more intimately known. In the meantime the evidence presented by this study indicates that one result of administering cortical hormone (D.O.C.A.) is a reduction of the histamine content of plasma and a redistribution of the histamine in the blood between cells and plasma. This may well be the basis of the clinical response of patients with Addison's disease to treatment with D.O.C.A.

P A R T   T H R E E.

THE DIAGNOSIS OF ADDISON'S DISEASE
WITH SPECIAL REFERENCE TO DIAGNOSTIC TESTS FOR
ADRENAL INSUFFICIENCY.

THE DIAGNOSIS OF ADDISON'S DISEASE.

The diagnosis of Addison's disease in the late or terminal stages as a rule presents no difficulty: the disease may be obscure in the early stages when symptoms and signs are more or less indefinite.   Where the onset is not sudden, the patient may present exacerbations and remissions over a period of years and various interpretations may be made as to the origin of the complaints, especially if the patient at the time is well nourished.   The literature contains many case reports in which the general languor and debility, which best describes the early symptoms of asthenia, served to classify the patient as neurasthenic. The importance of gastro-intestinal disturbance is difficult to assess when there are no other physical signs and no immediate significance may be attached to a complaint of anorexia and indigestion; even when the patient complains of abdominal pain and vomiting or diarrhoea, the occurrence may be so intermittent that the cause is either not investigated or the patient is referred to the surgeon for appendicectomy.

Though the classical description of the signs centres round the presence of pigmentation and low blood pressure, one or other may not be evident.   The depth of pigmentation may vary from a faint yellow tinge to an intense bronze colour; it may occur in patches or be distributed uniformly over the whole body surface; the palmar and plantar surfaces are however much less intensely coloured.   Where the pigmentation is very slight and uniform it may give the appearance of a normal sallow complexion;

in these circumstances pigmentation of the buccal mucous membrane
is a valuable sign and the margins of the lips and oral cavity
should be carefully inspected for isolated patches of
pigmentation. The vagina and anus may also be pigmented.
Biopsy of the skin may serve to exclude other causes of
pigmentation such as silver, arsenic, pellagra, haemochromatosis
and acanthosis nigricans.

Arterial hypotension is nearly always a constant feature
of Addison's disease. The blood pressure in the terminal phase
reaches a very low level but in the early stages of the disease
the systolic reading may vary from 110 to 90 mm. Hg., while the
diastolic level may lie between 75 and 60 mm.Hg., where the
blood pressure is just within the lower limit of normality and
other signs are equally indefinite it may be exceedingly difficult
to come to a definite conclusion, for it is almost as important to
exclude Addison's disease as to diagnose it.

It is unfortunate that at present there is no simple
method available, as in diabetes mellitus, whereby minor degrees
of adrenal cortex insufficiency may be measured. Nevertheless
various attempts have been made to provide clinical and biochemical
tests by which the diagnosis of Addison's disease may be confirmed.
Some of these methods have been investigated by the author in
order to assess their value as diagnostic aids. Owing to wartime
restrictions and in some instances to the condition of the patients,
it has not been possible to carry out all the tests on each
patient.

Costo-lumbar Tenderness.

When moderate pressure is made in the costo-lumbar angle Rogoff(1931) stated that the patient with Addison's disease complains of a dull pain which radiates towards the pelvis of the same side. The pain is only felt if the disease is due to extensive degeneration or inflammation of the adrenal gland and is absent when the gland is atrophied or fibrosed. Rogoff suggested costo-lumbar tenderness as a diagnostic test for Addison's disease.

The response to cost-lumbar pressure on 13 patients with Addison's disease (Group A) and 7 patients with hypotension (Group B) is recorded in Table IX. It will be seen that 10 patients of Group A gave a response varying from marked (++) bilateral tenderness to slight (+) unilateral tenderness; only 1 patient with Addison's disease did not respond. On the other hand in Group B 3 of the 7 patients had definite tenderness, though none of these patients had any evidence of an infection of the urinary tract. It is evident that costo-lumbar tenderness is not diagnostic of Addison's disease and though it may provide confirmatory evidence, where other signs of the disease are indefinite this sign is of little value in settling the diagnosis.

TABLE IX. Showing incidence of costo-lumbar tenderness
in patients with Addison's disease (Group A)
and in patients with hypotension but no
Addison's disease (Group B).

Case No.	Group.	Costo-lumbar tenderness. R.	L.
1	A	+	+
2	A	++	–
3	A	–	+
4	A	+	++
5	A	–	–
6	A	+	++
7	A	+	–
8	A	++	++
9	A	+.	+
10	A	+	–
11	A	+	+
12	A	–	–
14	A	–	–
15	B	–	–
16	B	–	–
17	B	–	–
21	B	+	+
22	B	++	+
23	B	+	–
24	B	–	–

Serum Electrolytes.

Since Loeb's (1932, 1933) observations that some
patients with Addison's disease have a low serum sodium and a
high serum potassium level, many attempts have been made to
use these biochemical findings as a basis for the diagnosis of
adrenal insufficiency.   Thus Allott(1936) found there was an
increase in serum potassium as well as urea in cases of
oncoming crisis.   Snell (1934), however, observed that
during the more chronic phase of the disease patients had
quite a normal blood chemistry.

In the present study the levels of serum sodium,
chlorides and plasma potassium were determined in 13 cases
of Addison's disease (Group A) and in 9 cases of hypotension
(Group B).   Estimations of serum sodium were made according
to the technique of McCance and Shepp(1931) and of plasma
potassium by the method of Weechelsbaum, Somogyi and Rusk(1940).
Serum chlorides were determined by Whitehorn's(1921) technique.
On Table X the values of sodium, potassium and chloride are
expressed in mgm. per 100 c.c of serum or plasma.   The
results show that in Group A only 8 cases had a serum sodium
level less than the normal lower limit of 325 mgm.   In case
4 the level was 279 mgm. while in case 8 and 11 the blood was
withdrawn during a crisis and gave values of 235 and 280 mgm.
per 100 c.c. respectively.   Although no patient in Group B
had a serum sodium level below 300 mgm., 4 patients, however,
had values less than 325 mgm.   It is evident that there is
some overlapping in serum sodium values between the groups and

TABLE X.   Showing the Values for serum, sodium, plasma potassium and serum chloride in patients with Addison's disease (Group A) and in patients with hypotension but not suffering from Addison's disease (Group B).

Case No.	Group.	Serum Na. mgm.per 100 cc.	Plasma K. mgm.per 100 cc.	Serum Cl. mgm. per 100 cc.
1	A	332	-	280
2	A	410	17.7	-
3	A	346	16.9	177
4	A	279	26.4	374
6	A	349.5	16.1	701
7	A	497	-	394
8	A	235	-	261
9	A	310	26.9	320
10	A	315	20.0	350
11	A	280	20.8	308
12	A	319	18.1	300
13	A	315	27.6	408
14	A	301	21.9	315
Range	A	235 – 497	16.9 – 27.6	177 – 701.
Mean	A	329.9	21.2	349
15	B	328	-	245
16	B	323	-	360
17	B	300	22.1	490
18	B	330	18.6	350
19	B	330	-	360
20	B	335	18.6	380
21	B	319	18.5	328
22	B	321	16.2	-
24	B	359	18.3	-
Range	B	300 – 359	16.2 – 22.1	245 – 490.
Mean	B	327	18.7	359

it would appear that unless the serum sodium level is below 300 mg m per 100 c.c.,no definite conclusion of Addison's disease is justified.

With regard to plasma potassium 4 patients in Group A had levels higher than the normal range of 16-20 mgm.but there is no clear cut distinction between the two groups for there was 1 patient in Group B with a level higher than normal, though the general trend in this group is between 16 and 20 mgm. The same conclusion may be made of the serum chloride estimations.   It is worthy of note that the remarkably high level of 701 mgm. was observed in case 6; though it was not appreciated at the time, it was later realised that the high chloride value contributed, as will be discussed later,·to the onset of oedema in this patient.

The evidence presented here is in agreement with the conclusions of Dryere(1939) that it is not possible to make a definite diagnosis of adrenal insufficiency from the blood electrolyte values alone.

Potassium Sensitivity Test.

In 1937 Zweiner and Truzkowski suggested as a result of animal experiments that intolerance to an increased intake of potassium might be used as a method of detecting adrenal insufficiency.   They stated that when patients with Addison's disease were given potassium salts, their serum potassium level rose rapidly.   This method was tried by Gordon,Severinghaus and Stark(1938) but was found by them to be unreliable; Dryere (1939) and Greene, Levine and Johnston (1940) also found it unsatisfactory.

Salt Restriction Test.

First used by Harrop, Weinstein, Soffler and Trescher(1933)
this test has been claimed to be of some diagnostic value, as
patients with Addison's disease suffer a relapse after three to
four days on a salt-free diet and their plasma shows a considerable
drop in sodium and chloride content.   Wilder, Kendall and others
(1937) found that the response to this test depended to a large
extent on the potassium intake of the patient at the time of the
salt restriction test and they concluded that unless, due
importance is attached to this aspect, the results are unreliable.
This test appears to be decidedly hazardous to the patient if he
should have Addison's disease for at least two deaths have been
reported during its use.   (Lilienfield (1938), Garvin and
Reichle(1940)).

Excretion Tests.

In 1938 Cutler, Power and Wilder described a new test
for the diagnosis of adrenal insufficiency.   The principle of
the test depends on the observations made by them that during
maintenance on a low sodium - high potassium diet for two and a
half days, patients suffering from Addison's disease continue to
excrete considerable amounts of sodium and chloride in their
urine, while normal subjects show a marked diminution in urinary
sodium and chloride excretion under similar conditions.   They
stated that values for urine chlorides on the third day greater than
225 mgm.% are diagnostic of Addison's disease, whereas
concentrations of less than 125 mgm.% are evidence of normality;

values between these limits were regarded as inconclusive.
Dryerre (1939) confirmed these observations but stated that the
urine sodium level is much more reliable than the chloride
level.   For patients with adrenal insufficiency the range of
urine sodium is 160-298 mgm.% while for normal subjects the
level is 25 - 66 mgm.%.

The Cutler, Power and Wilder Excretion Test, details of
which are given in Appendix (p. 119.), was carried out on one
patient with Addison's disease (Group A) and on three patients
in Group B, who had been admitted to hospital with a diagnosis
of ? Addison's disease because of atypical pigmentation and low
blood pressure.   This investigation could not be carried out
on more patients on account of the restriction on diet imposed
by war-time conditions; it is therefore not advisable to draw
any definite conclusions from the small number of tests
performed.   The results of the tests are shown in Table XI,
where it will be seen that the urinary excretion of sodium
and chloride in the patient with Addison's disease does not
conform to the standards set by Cutler, Power and Wilder(1938)
and by Dryerre(1939).   Indeed the only clear cut distinction
between Group A and Group B is seen in the diminished output
of urine and in the blood sodium, potassium and chloride levels.

Table XI Showing Analyses of Urine and Blood collected during the forenoon of the third day of the Cutler, Power and Wilder Test.

Group A = Patients with Addison's disease.

Group B = Patients with Arterial Hypotension but not Addison's disease.

Case No.	Group	Urine					Blood.					
		Vol. per min. c.c.	Sodium mgm%	Sodium milli equiv.	Chloride mgm%	Chloride milli equiv.	Serum Sodium mgm%	Serum Sodium milli equiv.	Plasma Potassium mgm%	Plasma Potassium milli equiv	Plasma Chloride mgm%	Plasma Chloride milli equiv.
9	A.	0.17	125.6	54.6	61	17	280	121.7	26.9	6.9	366	103.1
18	B.	0.49	134.2	58.4	146	41	320	139.1	18.6	4.8	350	98.6
21	B.	1.33	13.0	5.6	109	31	319	139	18.5	4.7	328	92.4
22	B.	2.48	20.6	9.0	109	31	321	139.5	16.2	4.1	-	-
Range	B.	0.49 - 2.48	13.0 - 134.2	5.6 - 58.4	109 - 146	31 - 41	319 - 321	139.	16.2 - 18.6	4.1 - 4.8	328 - 350	92.4 - 98.6
Mean	B.	1.43	55.9	24.3	121.3	34.3	320	139.2	17.7	4.4	339	95.5

The decrease in the volume of urine is in agreement with the findings of Maranon, Collazo and Vitoria (1935) who suggested diminished urinary output as a feature characteristic of adrenal insufficiency.   Taken individually, two cases in Group B (21,22) satisfy the criterion of both Cutler and co-workers and Dryerre for absence of Addison's disease; the results with patient 18B, who was later proved to have pernicious anaemia, serve to indicate that this test is not infallible.   In introducing the test Cutler and his colleagues(1938) stated that the patients are subjected to less risk of collapse than with the Salt Restriction Test. Dryerre (1939) however, experienced considerable difficulty in carrying out the test and concluded that it is not without risk and that it is essential to keep a strict watch over the patient in case of sudden collapse; an opinion which is shared by Willson, Robinson, Power and Wilder (1942).

A simple excretion test has recently been described by Robinson, Power and Kepler(1941) which does not involve any restriction of diet and does not subject the patient to any hazards. The principle of the test depends on the inability of patients with Addison's disease to excrete large amounts of water.   The test consists in giving a large amount of water to drink during the day and comparing the volume of urine voided during the night with the volume of the largest single hourly specimen passed during the day. If the night urine is less in amount than any specimen of day urine, the patient is not suffering from Addison's disease.   On the other hand if the volume of night urine is greater than any hourly

day specimen the patient may or may not have Addison's disease. This may be decided by determining the following ratio:

$$A = \frac{\text{urine urea mgm\%}}{\text{plasma urea mgm\%}} \times \frac{\text{Plasma chloride mgm\%}}{\text{Urine chloride mgm\%}} \times \frac{\text{Day urine cc/hr.}}{\text{Night urine cc.Total.}}$$

If A is less than 25 the patient has Addison's disease and if A is more than 30 the patient is not suffering from Addison's disease.

The test was carried out on 4 patients of whom 3 were suffering from Addison's disease (Group A); the remaining case (Group B) had arterial hypotension but no other signs or symptoms of Addison's disease. The urinary output for each patient is shown on Table XII where it will be seen that there is no evidence of a water diuresis in any patients of Group A. In the second part of the test 2 patients (12, 14) had ratios less than 25 but in case 9 the value for A was greater than the upper limit set by Robinson, Power and Kepler(1941). It is difficult to explain why this patient should give such a high value for A for she had been successfully treated for Addison's disease during the previous eighteen months. It is possible that her D.O.C.A. had not been withdrawn for a sufficient period as the test was begun 24 hours after the last dose of D.O.C.A. The patient in Group B had a prompt diuresis and a value for A well above any seen in Group A.

Not only is the Robinson, Power and Kepler test rapid and easy to carry out but it is particularly free from danger to the patient. If its accuracy is confirmed it should prove to be a very useful diagnostic test for adrenal insufficiency.

TABLE XII.   The response of 3 patients with Addison's disease (Group A) and 1 patient with arterial hypotension (Group B) to the Robinson, Power and Kepler Excretion Test.

a) Urinary Output c.c.

b) Ratio A.

Case.	Group.	Night urine.	Day urine. 9.30	10.30	11.30	12.30	Ratio A.
9	A	198	56	75	126	154	45.8
12	A	370	21	20	12	20	0.6
14	A	129	28	44	45	50	24.5
26	B	369	517	529	114	54	206.0

Therapeutic Test.

Mention might be made of the diagnostic value of the therapeutic response of patients to an injection of cortical extract. It is certainly true that patients with Addison's disease exhibit a dramatic response to an injection of 20 cc. cortical extract, but since Kline (1937) has reported that cases of asthenia respond similarly such a procedure, to be of any value as a diagnostic test, must be conducted under carefully controlled conditions. Subjective improvement in the absence of positive objective data is an unreliable diagnostic criterion.

Radiological Evidence.

In 1914 Rodleston and Boyd described the appearance of calcification of the suprarenal glands by means of X-ray photographs.

Their technique was later developed and improved by Camp, Ball and Greeve (1932) who found that of 23 patients with Addison's disease, 6 had calcified adrenals.   One of the disadvantages of this method lies in the difficulty of distinguishing calcification of adjacent lymph nodes or of the cartilages of the ribs, for normal persons occasionally present X-ray appearances corresponding with those seen in Addison's disease. Where the disease is due to atrophy of the gland, peri-renal insufflation of air has been used as a means of outlining the kidney and suprarenal gland, but the danger of producing emphysema of the tissues with accompanying pain has rendered this method undesirable and the results so far obtained have not justified the dangers incurred.   The value of radiological methods in the diagnosis of Addison's disease is therefore strictly limited.

Discussion.

In discussing the problems associated with the diagnosis of Addison's disease it is important to realise that the adrenal gland, like other endocrine glands, has a large margin of safety, for a considerable portion of the tissue may be destroyed before the function becomes insufficient to meet the normal demands of the body.   It is reasonable to expect, therefore, that the disease in its early stages will be characterised by vague symptoms and that few signs may be evident.

Exacerbations of adrenal insufficiency occasioned by mild infection or change of environment and manifested by vomiting, diarrhoea, drowsiness or atypical neurological symptoms,

constitute, therefore, very valuable information and the trouble taken to secure an accurate history from the patient will be well repaid.

Any tests for adrenal cortical function which are undertaken at this early stage may often yield indecisive results.   Indeed from the foregoing survey it is obvious that there is, as yet, no specific and accurate method of diagnosing Addison's disease.   Mention may be made of two tests which may be developed for the detection of adrenal insufficiency.

At present it is premature to suggest the histamine content of plasma or whole blood as an index of adrenal insufficiency.   So far there are no facts available about blood histamine values in conditions which simulate Addison's disease, except these cases which have been investigated in Group B.   It cannot be claimed that the estimation of histamine in blood is easy, for it entails a biological method of assay and there cannot be many hospitals where the necessary facilities are available.   Nevertheless, the clear cut distinction in plasma histamine values between Group A and Group B compares very favourably with the other diagnostic tests and taking into account that no previous restriction of diet is necessary, it is reasonable to expect that the difficulties of estimating histamine in blood may be overcome.

Recently Golla and Reiss(1943) have isolated from pregnant mare serum a substance which has corticotrophic activity.   If a sufficiently sensitive method is developed for

its detection in human urine, it may be possible to use this method for assessing adrenal insufficiency, at least in certain cases of Addison's disease.

In the meantime the Cutler, Power and Wilder Excretion Test is not without its disadvantages. The simpler method of Robinson, Power and Kepler has no apparent dangers and if it proves to be as accurate as the preliminary account suggests, it should provide a rapid and confirmatory diagnostic test for Addison's disease.

P A R T   F O U R.

THE OCCURRENCE OF OTHER ENDOCRINE DISEASES

IN ASSOCIATION WITH ADDISON'S DISEASE.

THE OCCURRENCE OF OTHER ENDOCRINE DISEASES IN
ASSOCIATION WITH ADDISON'S DISEASE.

From the knowledge which has accumulated regarding the interrelations of the ductless glands it is to be expected that disease affecting the adrenal glands may also involve other endocrine glands.

Atrophy of the adrenal glands may lead to atrophy or hypertrophy of other glands and provided the adrenal insufficiency remains within the limits compatible with life, symptoms and signs of other endocrine disease may sooner or later become evident.

The résumé which follows deals with some of the more important endocrine diseases which may be associated with Addison's disease; in some instances adrenal insufficiency followed the development of disease in another ductless gland, in other instances the sequence of events was reversed.

The relationship of the anterior lobe of the pituitary gland with the adrenal cortex is well established as a result of numerous investigations. Crooke and Russell (1935) showed that there is a reduction in the number of basophil cells and that abnormal basophil transitional cells occur in Addison's disease. They regarded these changes as the most significant of all the changes found in the ductless glands. Yet, surprisingly enough, few cases have been reported of patients who during life exhibited signs or symptoms directly referable to disease of the anterior pituitary gland. In a patient with Simmond's disease, the onset of attacks of

yomiting, abdominal pain and collapse were attributed by
Aitken and Russell (1934) to the development of Addison's
disease.   The occurrence of Addison's disease in association
with infantilism has been described by Morlat(1903) and Apert(1933).

•   Although there is considerable evidence that patients with
adrenal insufficiency may be thrown into crisis by the
administration of thyroid, the clinical association of hyper-
thyroidism and Addison's disease has only rarely been noted.
The subsequent development of Addison's disease in three patients
with a previous history of thyrotoxicosis has been reported by
Chauffard and Girot(1925), Etienne and Richard(1926) and Herman
(1933).   On the other hand the onset of toxic goitre in Addison's
disease is described and quoted by Perera and Parker(1943) who
also fully discuss the simultaneous occurrence of Addison's
disease and hyperthyroidism in a patient whose hyperthyroidism
was successfully controlled by X-ray therapy.   Two other such
cases have been noted by Etienne and Richard(1926) and Pla and
Fabregat (1932).

Hypertrophy of thymus gland has been reported, and atrophy
of the testes, seminiferous tubules and ovaries have also been
noted but no particular syndrome referable to these associated
changes have been described.   Many patients have symptoms of
amenorrhoea and of impotence, but in several instances it has
been shown that pregnancy is compatible with the development of
Addison's disease.   In view of the recent work relating enlargement
of the thymus with myasthenia gravis, it is interesting to note
that hypertrophy of this gland occurs in Addison's disease though

the type of asthenia and its distribution differs quite
markedly in each disease.   The blood-sugar in Addison's disease
is usually normal or occasionally subnormal(Kepler and Wilder
(1938)) and the occurrence of diabetes mellitus in association with
Addison's disease is extremely rare.   Of 14 reported cases,
doubt has been cast on the diagnosis of 5 (Arnett(1927)).   In
1926 Unverricht reported a case of diabetes and tuberculosis
which later developed Addison's disease.   Arnett(1927) recorded
the first well-documented case of diabetes mellitus and Addison's
disease.   A brief reference was made by Allen (1930) to 2 such
cases seen at the Mayo Clinic, and another was reported by Gowen
(1932).   The onset of diabetes mellitus during the course of
Addison's disease was described by Levy Simpson (1932), and by
Crooke and Russell (1935).   Rogoff (1936) reported a unique case
of Addison's disease resulting from bilateral adrenal denervation
in an attempt to cure diabetes mellitus.   More recently an
excellent description of the coincidence of diabetes mellitus
and Addison's disease has been given by Bloomfield(1939).

CASE RECORD.

A housewife, aged 32, was admitted to the Royal Infirmary
on July 25, 1939, complaining of dyspnoea, severe weakness and
loss of energy which had gradually increased during the previous
six months.   In the summer of 1938 she had noticed that her
skin was darkening and becoming brown.   At first she thought it
was sunburn, but the colour deepened throughout the winter.
She had lost over a stone in weight, but at no time had she any

gastro-intestinal disturbance. Menstruation had always been
normal. There was no history of any previous illness. She had
been married eight years and had a healthy daughter, six years old.
A strong hereditary tendency to diabetes mellitus is shown in the
family tree.
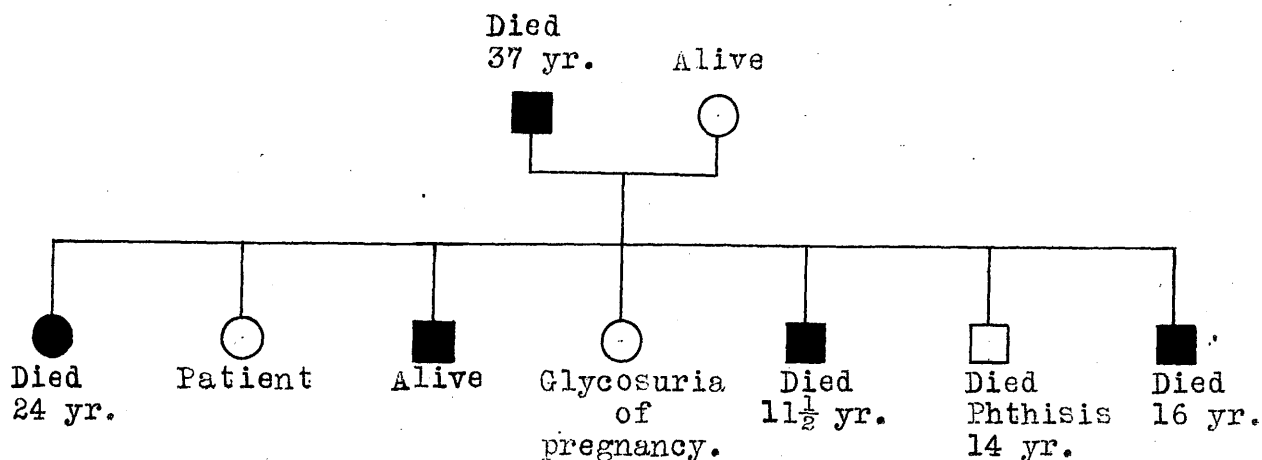
Family history of patient. Squares, male; circles,
female. Diabetics shown in black.

The patient was small, thin and very asthenic. She was
edentulous and weighed 7 st. 2 lb. There was generalised dark
brown pigmentation of her body, particularly of her face, neck,
dorsal surfaces of the forearms and hands, and the front of the
knees. The buccal mucous membrane was also pigmented. A soft
systolic murmur was heard at the apex of the heart, but there was
no cardiac enlargement. The blood pressure was 102/62; pulse
rate 88, weak but regular. There were no abnormal features in
the lungs, abdomen or nervous system and no lumbar tenderness was

elicited.   Urine: albumin faint trace; sugar and ketones nil;
deposit contained epithelial cells.   Blood Sedimentation Rate
(Cutler) 15 mm. in 1 hour; sugar 100 mg. per 100 c.cm.; non-
protein nitrogen 34.6 mg. per 100 c.cm.; chlorides (NaCl) 421 mg.
per 100 c.cm; haemoglobin 60%; red cells 4,130,000; colour index
0.73; white cells 6,600(neutrophil polymorphs 64%, eosinophils 5%,
monocytes 2%, lymphocytes 29%).   The blood picture was that of a
mild hypochromic anaemia.   Fractional test meal and histamine
test meal revealed complete achlorhydria.   Electrocardiogram and
radiograms of chest and abdomen were normal.

A diagnosis of Addison's disease was made and the patient
was given 5 mg. of D.O.C.A. intramuscularly three times a week and
ascorbic acid 100 mg. daily.   The anaemia was treated with ferr.
sulph. exsicc. gr. 3 and acid hydrochlor. dil. m. 30 three times a
day.   She improved considerably and said she felt more energetic
and less easily tired.   At the outbreak of war she was discharged
from hospital.   She attended the outpatient department for a
weekly injection of 5 mg. of D.O.C.A. and continued to take
ascorbic acid daily.   As she did not gain in weight her daily
salt intake was augmented with 15 m. of sodium chloride in cachets.
Her improvement was maintained and her weight increased, but she
complained of backache and failing vision.

On the night of June 13, 1940, she was readmitted to
hospital on account of increased asthenia and persistent vomiting.
She said she had been quite well until the recent spell of hot
weather but had been unable to attend the outpatient department
for a fortnight.   She had lost weight and the pigmentation had

become darker.   She had no diarrhoea and micturition was normal.
On admission she was extremely asthenic and markedly dehydrated.
She vomited frequently and her breath smelled strongly of acetone.
Blood pressure was 58/40, pulse rate 112, regular; respiration
rate 24; temperature 97.8°F. Urine: sugar ? , acetone , no diacetic
acid.   Within the first 24 hours she received 25 c.cm. of
Eucortone and 5 mg. of D.O.C.A. intramuscularly and a litre of 5%
glucose saline by intravenous drip.   In addition she was given
intramuscularly 100 mg. of vitamin C.   By the second day the
vomiting had ceased and the blood pressure had risen to 90/58.
Daily treatment with 10 c.cm. of eucortone was continued and on the
third day she was able to take 15 mg. NaCl daily in cachets.   On
June 20 the non-protein nitrogen was 28.6 mg.; chlorides 430 mg.;
sugar 660 mg. per 100 c.cm.   Urine: sugar ; acetone and diacetic
acid ; albumin trace.

In view of the hyperglycaemia and glycosuria she was given
10 units of insulin morning and evening.   Glucose tolerance test
gave the following result: 0 hr. 460, $\frac{1}{2}$ hr. 480, 1 hr. 575, $1\frac{1}{2}$ hr.
600, 2 hr. 565; the dose of insulin was therefore gradually raised
to 38 units in the morning and 22 units in the evening.   On June
26 her general condition had greatly improved and 5 mg. of D.O.C.A.
thrice weekly was substituted for eucortone.   The blood pressure
now varied between 85 and 88 systolic and 56 and 60 diastolic,
and the serum sodium had risen from 235 to 332 mg. per 100 c.cm.
Several attacks of hypoglycaemia ensued as a result of insulin
treatment and the dose had to be reduced repeatedly.   A further

radiogram of the chest and abdomen showed no abnormality. A piece of pigmented skin was removed for pathological examination. The report stated: "Sections of skin show an increase of melanin pigment in the basal layer of the epidermis, but no evidence of iron in appropriately stained section."

By August 26 she had gained 5 lb., did not complain of any weakness, and was able to walk about the ward. There was no change in her pigmentation and she had no recurrence of vomiting. Her blood pressure was now well maintained at 92-94 systolic and 60-70 diastolic, and her serum sodium was 340 mg. At this time her noon blood sugar level was 72 mg. on the dose of insulin 28/20, and though at times there was mild glycosuria there was no ketonuria. She was discharged on that date with instructions to take insulin 28/20 and to continue D.O.C.A. and salt treatment.

As a result of a mistake in the dose of insulin given at home she was readmitted on the night of Aug. 27 in a severe state of hypoglycaemia, and despite treatment with adrenaline and intravenous glucose she died in convulsions.

AUTOPSY FINDINGS.

Suprarenals - Both greatly reduced and each weighed 1.9 g. The cortical cells in each zone were entirely replaced by fibrous tissue, which formed a fibrous capsule round the medulla. The resultant width of the cortex was only a small fraction of the normal Round the periphery of the medulla there were some foci of lymphocytic infiltration. Within the medulla were areas of focal necrosis in which the cells were large, swollen and stained pink

and contained dark brown pigment granules in their cytoplasm.
In addition there were large focal collections of lymphocytes,
but there was no evidence of tuberculosis.   Certain parts of
the medulla were almost completely fibrosed.

Pancreas.   Slightly smaller than normal but no macroscopic
changes on section.   Many of the islets of Langerhans were
completely replaced by fibrous tissue.   Many of those still
present were remarkably reduced in size and showed various degrees
of atrophy and fibrosis.   No evidence of an inflammatory reaction.

Pituitary gland.   The anterior lobe was engorged and
contained a small chromophobe adenoma about 3 mm. in diameter.
There was obvious retention of colloid in the acini which were well
formed and lined by chromophobe cells.   There were a few basophil
cells in the acini.   Many of the chromophobe cells were larger
than normal.   There was less than the normal quota of eosinophils,
while the basophils were conspicuously few.   All three types of
cells, however, appeared to be somewhat degenerated.   In Mallory-
stained sections many of the granular cells took on both the acid
and basic stain, so that it was impossible to recognise transitional
stages of the two types.   The posterior lobe presented no
abnormal features.

Thyroid glsnd.   Smaller than normal, firm and devoid of
colloid.   The acini were lined by tall columnar epithelium and
the histological picture was indistinguishable from that of
Graves's disease.

Thymus gland weighed 17.5 g. and showed a considerable
amount of fibrosis and fatty infiltration.   The remaining

lymphoid tissue was hyperplastic.

Skin and vaginal epithelium showed increase of melanin pigment in the basal layer.

Liver.   Normal in size but flabby and pale owing to some fatty infiltration.   No haemochromatosis.

Lungs.   Terminal bronchopneumonia.   No apical scars.

The other viscera showed no significant changes.

DISCUSSION.

On her first admission the diagnosis of Addison's disease was fully confiemed by the therapeutic response to D.O.C.A.   There was no evidence to show that the carbohydrate metabolism was disturbed at this stage as the blood sugar was normal and there was no glycosuria or ketonuria, although the urine was examined on 36 occasions.   In view of her history acute adrenal insufficiency was diagnosed on her second admission.   Vomiting could be attributed to the ketosis, but the glycosuria and strong familial incidence of diabetes mellitus suggested the onset of diabetes. Since her grave condition would not permit delay for further investigation it was decided to treat the adrenal insufficiency immediately.   Some days later, when a diabetic response to glucose tolerance test was revealed, the possibility of diabetic coma or haemochromatosis with ketosis was reconsidered.   Although vomiting and dehydration, hypotension and a low serum-sodium level are factors common to both acute adrenal insufficiency and to diabetic coma, the dramatic response to treatment with cortical extract is more in favour of the former condition.   Indeed insulin was not administered until nine days later, by which time the

patient had improved considerably.   Biopsy of the skin disposed
of the possibility of haemochromatosis and further confirmation
is provided in the autopsy report.

.It is interesting to note that while the majority of the
authenticated cases of diabetes and Addison's disease developed
diabetes first, our patient, despite the strong familial tendency,
presented diabetes only after Addison's disease had been established
over a year.   A feature of several of the cases has been "insulin
hypersensitivity", which has arisen with doses as small as 5 units
insulin.   In this case a maximum of 60 units of insulin per day was
administered before the first hypoglycaemic reaction occurred.   It
seems worthy of note that, although Bloomfield and Unverricht,
whom he quotes, demonstrated a decreased need of insulin as
Addison's disease developed in their diabetic patients, it was found
that successful treatment of the suprarenal insufficiency did not
increase the insulin requirement of this patient.

Much attention has been focussed on the relationship
between the anterior lobe of the pituitary and the other endocrine
glands.   Numerous observations (Hewer(1923), Kraus (1927), Harrop
and Weinstein (1932))have been made on the diminution of the
basophil cells of the anterior lobe of the pituitary in Addison's
disease, and Crooke and Russell(1935) have suggested that this
might account for the hypoglycaemia.   The evidence presented by
this case does not seem to bear out their hypothesis; indeed, one
of their own cases (case 9) is at variance with their deduction.

Either pernicious anaemia or microcytic anaemia may occur
in association with Addison's disease.   In each instance the

anaemia can be adequately treated, provided due care is taken
in the administration of drugs, to which such patients are
abnormally sensitive.

PART FIVE.

THE TREATMENT OF ADDISON'S DISEASE.

THE TREATMENT OF ADDISON'S DISEASE.

The treatment of Addison's disease may be likened to that of diabetes mellitus. In both these diseases there is insufficient hormone secreted to meet the demands of the body and the treatment that may be employed entirely depends upon the state of the patient, that is, upon the acuteness of the insufficiency. Just as in diabetic coma the measures to be adopted are rigorous and urgent, so too in a "crisis" of acute adrenal insufficiency abundant cortical hormone and saline must be supplied immediately. On the other hand some diabetic patients are readily stabilised by dietetic measures, in a similar manner very mild cases of Addison's disease may be satisfactorily maintained without added hormone by limiting the intake of potassium salts and supplementing the diet with sodium chloride.

The different methods available for the treatment of Addison's disease may be discussed under the headings of (1) Low potassium diet and salt therapy, (2) Hormone therapy.

LOW POTASSIUM DIET AND SALT THERAPY.

Following the work of Wilder and his associates(1937) successful use of a potassium restricted diet to maintain patients suffering from mild adrenal insufficiency has been reported by Dryerre (1939) and Loeb (1941).

In the present series of cases a low potassium diet was constructed and given to several patients. Details of this diet are given in appendix (p. 121.).

The latitude allowed in this diet was sufficient to cater for the capricious appetites of most patients, but in all cases supplementary salt had to be given. The salt required varied from 10-15 gm. daily. While Loeb maintains that sodium chloride alone is sufficient, the writer agrees with Wilder that patients prefer to take a mixture of sodium chloride and sodium citrate. Indeed a mixture of sodium chloride 6 gm., sodium bicarbonate 4 gm., sodium citrate 4 gm., was found to be very beneficial. This amount was given daily in 5 subdivided doses. The best method of administering this large amount of salt mixture is to give each dose in a small glassful of milk one hour before meals. Where expense is no object the patient usually experiences no difficulty in swallowing cachets. In all instances water has been found to be unsuitable, as this vehicle produces nausea and vomiting.

This form of treatment is very limited in its application. It has no effect on the blood pressure level and has very little effect on muscular weakness. A patient may do quite well on this while resting in bed but only one case (Case 6) was successfully maintained on it for over a week. In all the other cases additional hormone therapy was required.

Particular care must be exercised in the administration of additional hormone therapy to patients on this diet. Weekly or twice weekly injections of adrenal cortex extract appear to contribute additional benefit but this is not so

with D.O.C.A.   Hampton and Kepler (1941) have recently shown
that in a group of 9 patients on this diet who were given
D.O.C.A. only 1 patient remained well.   Five patients died
within 1 month and 1 within 6 months of beginning treatment
and 2 developed diarrhoea and impending crises. Since then
it has been realised that D.O.C.A. may produce acute retention
of sodium and increased excretion of potassium.   Where it is
necessary to give hormone therapy the patient should be
maintained on a normal diet containing at least 4-5 gm. K
daily.

HORMONE   THERAPY.

The adrenal cortex does not elaborate any single
substance which can be described as the vital hormone
of the gland.   An extract of the cortex contains a large
number of closely related steroid derivatives which have
specific effects qualitatively different one from the other.
In 1928 Hartman and his colleagues suggested the name
"cortin" for the hormone of the adrenal cortex.   This they
regarded as the vital principle of the gland.   In 1934,
however, Kendall isolated a crystalline compound from the
adrenal cortex and in 1938 several more crystalline compounds
were separated which were designated as "cortin-like".   An
active extract from the adrenal cortex can now be separated
into fractions either as crystalline compounds or a purified
amorphous material, but no one compound can reproduce all
the physiological effects of the gland.

Adrenal Gland Grafts.

Animal experiments by Ingle, Higgins and Nicholson (1938) and by Loeb (1937) have emphasised the difficulties associated with the transplanting of such delicate and sensitive tissues as the adrenal glands. It is to be expected that clinical opportunities have been rare, for attempting the grafting of adrenal glands in the treatment of Addison's disease. Altogether six cases have been recorded. The most recent(Katz and Mainzer, 1941) included a review of the literature and describes the successful grafting of one adrenal gland from a subject who had recently died, into the abdominal muscle wall of a patient with Addison's disease. At the time of reporting the patient had been maintained for 15 months with only an occasional injection of adrenal cortex extract.

This type of treatment is of academic interest only.

Adrenal Cortex Extract.

A critical survey of the various methods of standardising extracts of adrenal cortex cannot be attempted in this thesis, but an excellent review of the methods at present available is given by Kendall (1941). The writer has had personal experience with two methods practiced in this country, namely (1) survival of adrenalectomised rats and (2) survival of adrenalectomised drakes. These methods are less satisfactory than the more recent "maintenance" assays now used in America.

No official extract of adrenal cortex has yet been described
but several commercial preparations are available in this
country.   Of these the following have been employed in the
course of this study:- Cortin (Organon Laboratories),
Eucortone (Allen and Hanbury,Ltd.), Eschatin (Parke Davis
and Co.).

Experimental evidence has shown that while preparations
of adrenal cortex extract are active when given by mouth
(Pfiffner (1934), Thorne (1938), Grollman (1936)) the amounts
necessary are at least 4 to 10 times greater than the
parenteral dose.   Loeb (1941) concluded from his observations
that cortical extract given by mouth is of doubtful clinical
value.   The present day attitude is that adrenal cortex has
very little clinical effect in doses of less than 5 cc. even
when administered by the parenteral route.   The extract is
usually given by intramuscular injection but in emergency
treatment the intravenous route may be used.   For maintenance
purposes the dose employed varies from 10 cc. once daily to
10 cc. twice weekly; in the treatment of a "crisis" as much
as 50-75 cc. in divided doses may be required per day.

Substitution therapy is inadequate unless the factors
which influence carbohydrate metabolism and the efficiency of
the muscles are given together with the compounds that
influence renal function and the distribution of water and
electrolytes.   Up to the present time adrenal cortex extracts
are the only preparations which produce these effects.

Steroid Compounds of the Adrenal Cortex.

In 1936 Kendall isolated from the adrenal cortex five
related steroid compounds which he named A,B,C,D and E and
in the following year Steiger and Reichstein (1937) described
one of the nine compounds which they had isolated as
corticosterone.   This compound, Kendall (1937) showed to be
similar to his compound B.   Extensive investigations on the
chemistry of the related steroid compounds followed: an
excellent review of this work is given by Mason (1939).
In America the physiological effects have been studied of four
crystalline compounds (Fig. 3.) corticosterone,
dehydrocorticosterone, desoxycorticosterone and compound E,
an amorphous fraction which is obtained in a purified form
after separation of the other compounds.   While the amorphous
fraction has more effect than any other compound on the
maintenance of life, it does not appear to influence
carbohydrate metabolism or response of muscle.   The chief
disadvantage of desoxycorticosterone is that it has very
little effect on carbohydrate metabolism and on the maintenance
of muscle efficiency.   It is, on the other hand, the most
effective compound for the maintenance of normal electrolyte
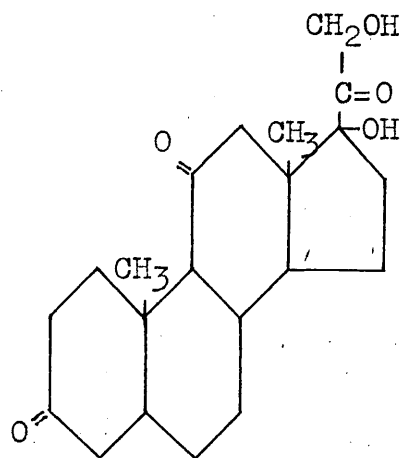balance and renal function.
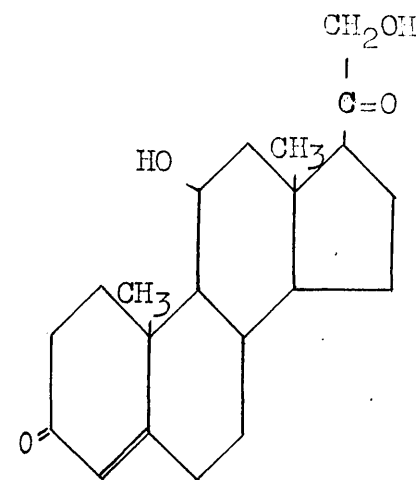
CORTICOSTERONE
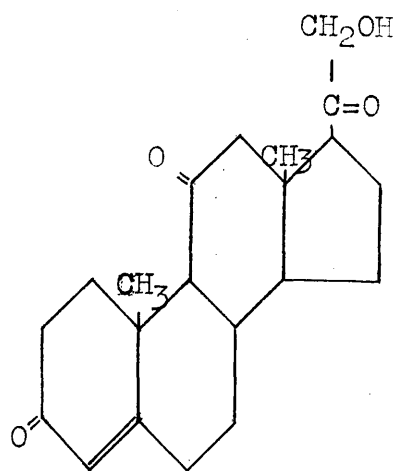
DEHYDROCORTICOSTERONE
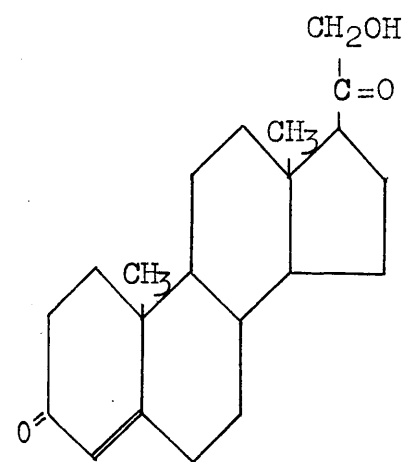
DESOXYCORTICOSTERONE

COMPOUND E.

FIG. 3.   Structural formulas and chief physiological effects
of adrenal compounds and fractions of adrenal cortex.

Physiological effect.	Compound or fraction of adrenal cortex
Gluconeogenesis)) Muscle efficiency)	Corticosterone and its derivatives with an atom of O at C_{11}
Distribution of electrolytes	Desoxycorticosterone
Renal function	Amorphous fraction.

Desoxycorticosterone acetate has been synthesised and is available commercially, it is usually referred to as D.O.C.A. The most striking action of D.O.C.A. is on electrolyte and water metabolism. As has been shown by Simpson(1938) Cleghorn (1939) and Loeb (1939), it causes marked retention of sodium salts and of water with an increase in plasma volume and a gain in body weight. That it restores renal function is demonstrated by an increased excretion of nitrogen in those cases where there is a high N.P.N. The other effects such as lowering calcium and cholesterol levels in the serum are probably due to the resultant increase in plasma volume. The effect on blood pressure may be brought about, according to Loeb, within a few hours, but in no case studied here (Table XIII) was any effect observed 4 hours after injecting D.O.C.A. On the other hand, during a period of treatment extending from 10 days to several months, both diastolic and systolic pressures were considerably raised. This is well seen in Fig. 4a, 4b. Whether the rise in blood pressure depends on factors other than the correction of abnormal electrolyte and water metabolism is difficult to decide. Certainly the blood pressure continues to rise even though the blood electrolytes have been restored to a normal level.

Pigmentation does not appear to be influenced to any extent by D.O.C.A. The colour of the skin, in some cases, appeared to be lighter after treatment but the pigment spots in the oral mucous membrane were not altered nor was the dark areola round the nipples. The effect on skin pigmentation

is probably due to rehydr tion as these patients had gained weight and appeared to be more robust.

The carbohydrate metabolism is not affected by treatment with D.O.C.A.   On the contrary, as Bloomfield(1939) has shown the blood sugar may be lowered.   Indeed Loeb (1941) has described the onset of severe hypoglycaemia in patients receiving larger doses of D.O.C.A.   It is interesting to note, on the other hand, that cortical extract raises the blood sugar level.   Whether this is due to compound E of Kendall will only be determined when sufficient amounts of this compound are available for clinical use.

There is no doubt that the subjective effect of D.C.C.A. is to increase the sense of well-being and to brighten the outlook on life.   This subjective improvement is reflected in the increased appetite and many patients gain in weight.   Of the 14 cases studied all but 3 were satisfactorily maintained on D.O.C.A. and were able to resume light work and to lead a quiet normal life.

TABLE XIII. Effect on B.P. of patients with Addison's
disease 4 hours after injection of 5 mgm.
D.O.C.A.

$\neq$ No D.O.C.A. had been given for the previous
36 hours.

Case No.	Before Injection $\neq$	After Injection.
1	92/60	88/64
2	116/78	114/68
3	96/52	98/58
4	98/70	98/70
6	98/68	98/68
7	118/78	118/78
9	106/56	106/54
12	114/60	114/62

FIG. 4a. The effect of prolonged administration of D.O.C.A.
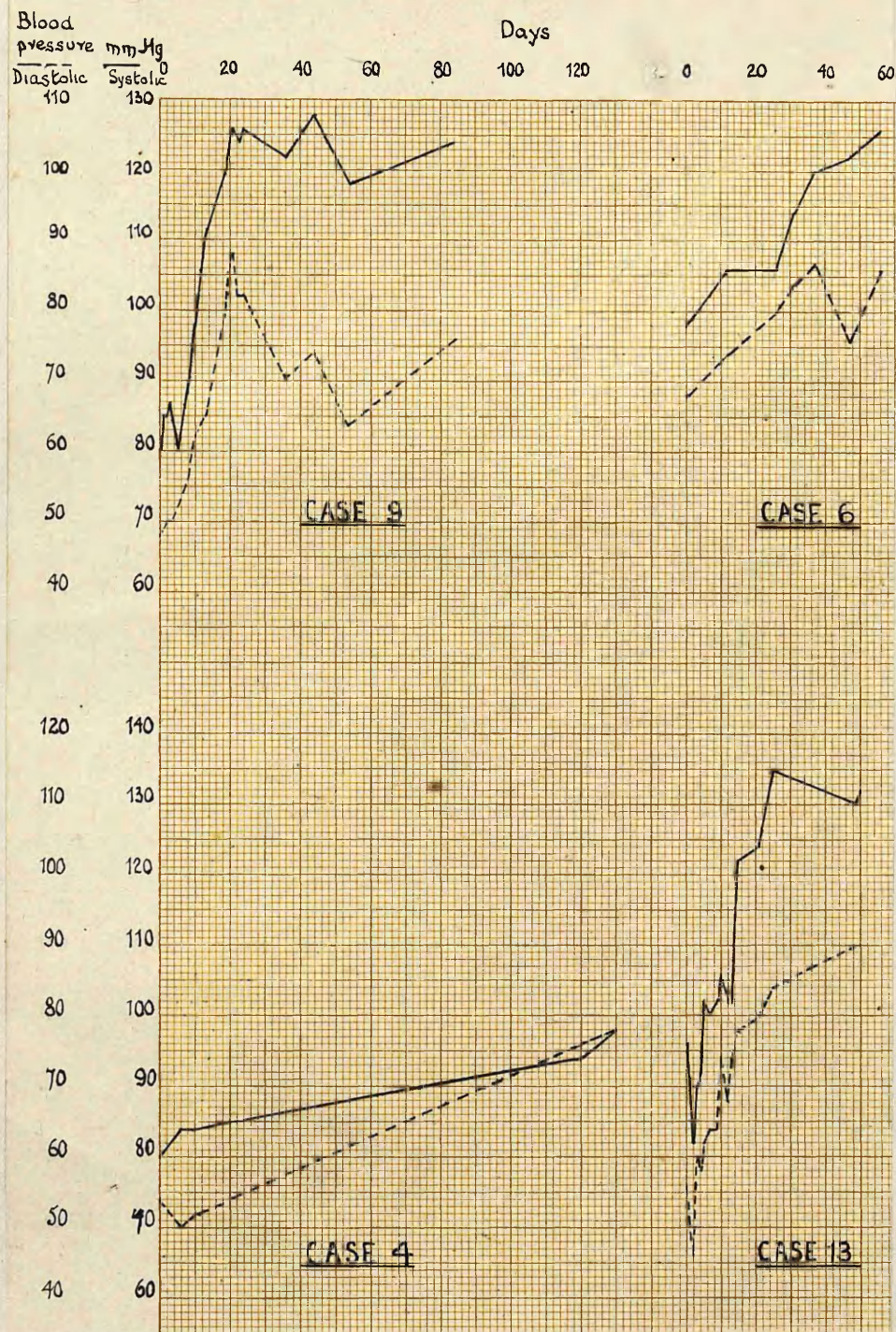on the B.P. of patients with Addison's disease.
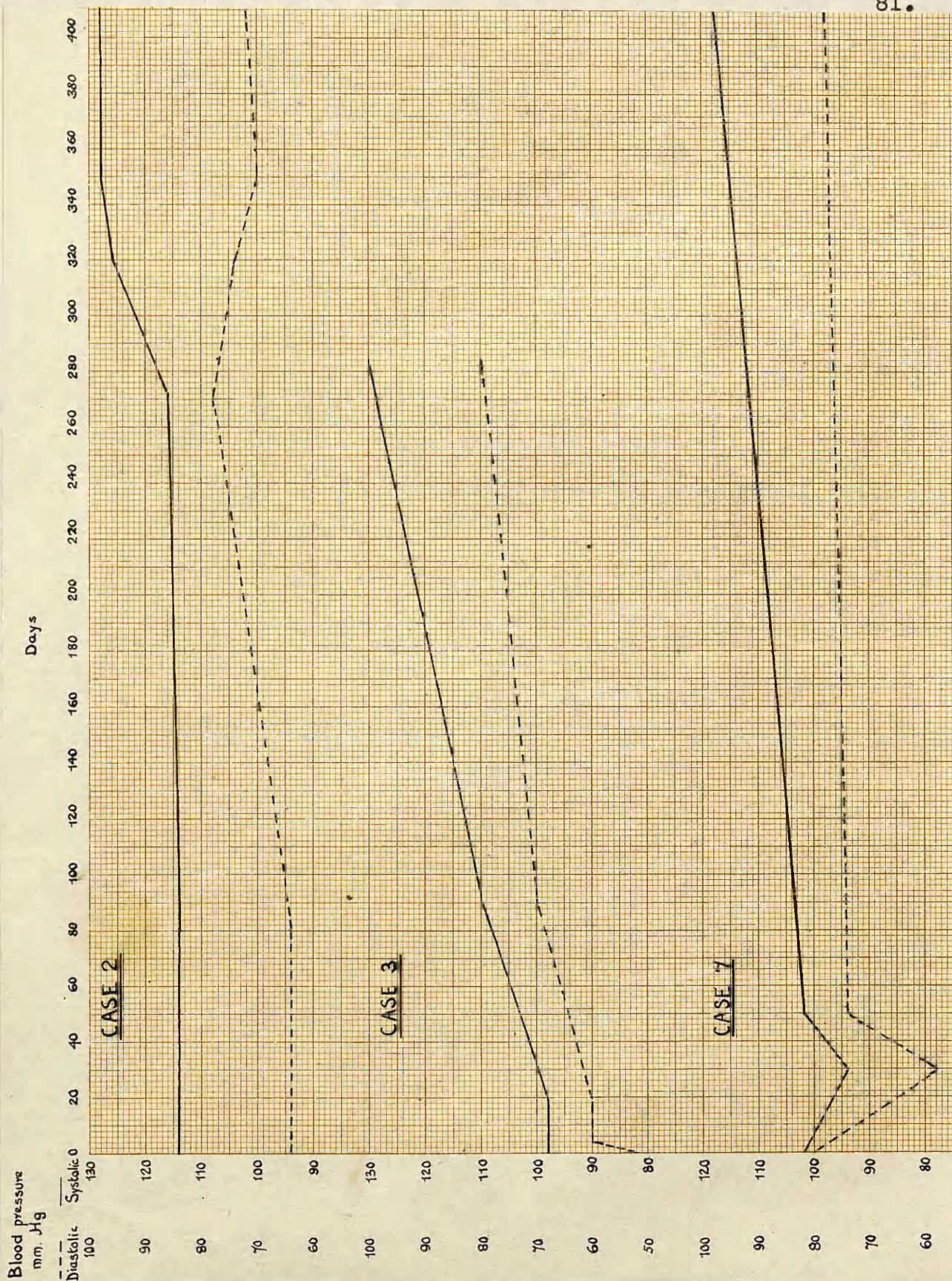
FIG. 4b. The effect of prolonged administration of D.O.C.A. on the B.P. of patients with Addison's disease.

Administration of D.O.C.A.

Three different methods are available for the treatment of Addison's disease with D.O.C.A. It may be given subcutaneously or intramuscularly in a solution of arachis or sesame oil, or sterile tablets may be implanted subcutaneously. Oral administration by sublingual absorption is a more recent mode of administration.

Parenteral Injection.

While Loeb seems to have had successful results with D.O.C.A. in the treatment of a severe crisis the use of this synthetic steroid has been reserved in the present study, for maintenance and for the treatment of on-coming crisis. Any severe crisis must in the writer's experience, be treated with adrenal cortex extract, supplemented by 20% saline and 5% dextrose drip infusions.

The maintenance requirements of D.O.C.A. vary greatly in different persons. The dose necessary to maintain the patients in the present study ranged from 5-20 mg. per week, given in subdivided doses at intervals of from once to three times a week.

During the period of assessing their requirements of D.O.C.A., patients were under close observation. Serum sodium, plasma protein and chloride estimations were made at the outset and plasma potassium determinations were in some instances also made. The diet used was a normal one of average salt content, and the patients were weighed daily. Observations

were made from time to time for signs of cardiac enlargement, increased venous pressure and oedema. The significance of these will be discussed later. After settling their maintenance requirement of D.O.C.A. , patients were usually discharged from hospital and attended regularly at the Out-Patient Department for injections of D.O.C.A. Two of the patients (3, 6) were trained in the technique of self-administration in a manner similar to that used by diabetic patients out one of them (case 3) tended to be irregular in his self administrations. The viscosity of the oily solutions adds to the difficulty of intramuscular injection and on the whole the author considers that the disadvantages of self administration outweigh any advantage of convenience.

The subcutaneous implantation of pellets.

The method of subcutaneous implantation as a means of administering hormones was first employed by Deanesley and Parkes(1937) and the following year Ingle and Mason (1938) used this method to prolong the survival period of adrenal-ectomised rats. In 1939 Thorne, Engel and Eisenberg described this method of treating adrenalectomised dogs and followed this work by successfully treating 6 patients with Addison's disease with D.O.C.A. pellets implanted subcutaneously (Thorn, Howard, Emerson and Firor (1939)). Pellets weighing from 50 to 150 mgm. and measuring 6.5 mm. in diameter by 2-6 mm. in thickness were inserted subcutaneously in the infra-scapula region. They caused no local discomfort or reaction and provided a constant supply of hormone.

This was evidenced by the gain in weight and marked increase in blood pressure and by the maintenance of a positive sodium and chloride balance.

Although the rate of absorption has been estimated as 0.25 - 0.35 mgm. per day from each tablet of 50 - 150 mgm. the amount which is absorbed varies with each individual. From the results so far obtained in the present study the average daily absorption appears to be of the order of 1 mgm. (0.74 - 1.3 mgm.)

Six patients have been treated by the subcutaneous implantation of pellets of 100 mgm. D.O.C.A.; a summary of the amounts and duration of effect is given below (Table XIV.)

The subcutaneous implantation of D.O.C.A. in the author's opinion is a successful form of maintenance therapy and in the cases so far treated no sudden crisis has yet arisen. The patients as a rule report at the out-patient department once a month and when the absorption of D.O.C.A. diminishes there is adequate warning not only by symptoms of anorexia, increasing asthenia or nausea, but also by the fall in weight and blood pressure. On the grounds of convenience and economy this method of treatment is superior to parenteral administration.

TABLE XIV.   The amount and duration of effect of
subcutaneous implantation of pellets of D.C.C.A.
in 6 patients with Addison's disease.

Case No.	Site of Implant	Total Amount mgm.	Duration of effect. weeks.	Average Requirement. mgm./day.
2	Abdomen	200	5 continuing	
3	Infra-scapula.	600	20 continuing	
9	Abdomen	100	5 continuing	
12	Abdomen	100	6 continuing	
13	Abdomen	100	18	0.74
	Abdomen	200	20	1.33
	Abdomen	100	13 continuing	
14	Abdomen	100	16	0.83
	Abdomen	200	32	0.83
	Abdomen	100	4 continuing	

Sublingual absorption of D.O.C.A.

The oral ingestion of tablets of D.O.C.A. is without value. Experimental evidence by Kuizenga, Nelson and Cart (1940) demonstrated that in the rat the parenteral route was 35 times more effective than the oral. Loeb(1940) and Anderson, Haymaker and Henderson (1940) have shown that in the treatment of Addison's disease 10 times the effective subcutaneous dose when given by mouth was unable to prevent the onset of symptoms of adrenal insufficiency.

More recently the sublingual method of absorption has been attempted. Good results were reported on this method by Anderson and co-workers (1940) who were able to maintain their patients on an average daily dose of 5 mgm. given in sub-divided doses. The period of treatment extended from 6-8 weeks and during this their patients were in excellent condition and able to carry on their usual occupations. Turnoff and Rowntree(1941) reported two cases in which the period of treatment was one month. They stated that their patients gained weight, and that the blood pressure levels were maintained.

In the present investigation 5 patients with Addison's disease were studied. They had all been previously maintained in a satisfactory condition by D.O.C.A. given intramuscularly in doses varying from 5-15 mgm. per week. At the onset of the investigation they were put on a normal diet with the addition of extra salt as indicated in the case reports. The solution used contained 10 mgm. of D.O.C.A. dissolved in 1 cc. of

propylene glycol. This was issued to the patients in dropper bottles so that 15 drops contained 10 mgm. D.O.C.A. The patients were instructed to place 5 drops under the tongue 3 times a day one hour before food and to retain the drops for at least 15 minutes and then to expectorate or swallow.

CASE RECORDS.

Case No. 2.

S.S., an engineer aged 35, had been successfully treated since Nov.1st, 1940 with 5 mgm. D.O.C.A. 4 times a week with supplementary salt, 1 teaspoonful 3 times a day. On the 12th Oct. treatment with D.O.C.A. was stopped for 3 days following which he complained of general weakness and could walk only about 200 yds., before feeling very breathless and weak in the legs. Costo-lumbar tenderness was well marked in the right side. On 16th April he began treatment with D.O.C.A. in propylene glycol and was instructed to take 5 drops 3 times a day (= 10 mgm. daily) and to continue his supplementary salt. The following day he felt stronger in the legs and in 3 days though his B.P. had fallen from 116/78 to 112/70 he reported general improvement. After a week of treatment he stated that he was no worse and that he had no sickness, but his B.P. had fallen to 108/70. At the end of the second week he complained of lack of energy, pain in the joints, and frequent headache. He had had 1 attack of abdominal pain but there was no vomiting or diarrhoea. At this time his B.P. was 108/60. After 16 days of treatment he reported numerous attacks of abdominal pain and fairly constant nausea. His appetite had diminished, and he did not feel inclined to eat

anything.   He was just as tired as he had been at the beginning
of the treatment.   Because of the weakness, he had felt it an
effort to move about, and when he did so, he felt his heart
thumping.   The following day he had a very acute attack of
epigastric pain accompanied by diarrhoea; he stumbled frequently
and was only able to walk a very short distance, during which he
felt dizzy and dragged his left foot; his B.P. was now 102/70.
By the 19th day there was evidence of impending crisis.   He had
lost $5\frac{1}{2}$ lbs. in weight, his serum sodium was 280 mg.%, plasma
potassium 23 mgm.% and his B.P. had fallen to 96/70.   Treatment
was stopped and he was given 10 mgm. D.O.C.A. i.m.i. at once.

After three daily injections of 10 mgm. D.O.C.A. he
was considerably improved, and his blood pressure rose to 110/72,
thereafter satisfactory treatment was continued with 5 mgm.D.O.C.A
4 times a week.

Case No. 4.

S.W., a farmer, aged 32, had been successfully maintained
since Dec. 1940 on a normal diet with supplementary salt mixture
15 gm. daily and 10 mgm. D.O.C.A. i.m.i. every 4 days.   He was
able to lead a normal quiet life but attempted no work as he
did not feel capable of muscular exertion.   He had no gastro-
intestinal disturbance, no headaches or dizziness, and his
weight remained steady.   There was still marked pigmentation
of skin and mucous membrane and bilateral costo-lumbar tenderness.
His B.P. was 94/76 and serum sodium 359 mg.%

On April 11th, 1941 he began trial treatment with D.O.C.A.

in propylene glycol 5 drops 3 times a day (= 10 mgm. daily)
and was maintained on a normal diet with his usual amount of
supplementary salt mixture.   During the first week of treatment
he was kept in bed.   At the end of the week he felt just as well
as when at home, had no complaint of sickness or diarrhoea and his
B.P. was 100/68.   On April 18th he was instructed to walk
about as much as he did at home and the following day he reported
that he was very well; his B.P. was 98/70.   On 20th April he
complained of sore throat and as his temperature was $101^\ominus$ he
was given 1 gm. sulphanilamide by mouth.   During the day he
vomited 3 times and the following morning his temperature was 102.4^o
his throat was inflamed and the dose of sulphonamide was repeated.
Throughout the day despite 4 hourly doses of $\frac{1}{2}$ gm. sulphanilamide
there was very little change in his condition and his temperature
remained unaltered.   By 7.15 p.m. he was very exhausted and his
B.P. had fallen to 55/40.   It was evident a crisis was imminent
and sublingual therapy was stopped.

Despite energetic treatment with cortical extract and
intravenous infusions of 10% glucose in 5% saline his condition
did not improve and at 7.0 p.m. the following evening he sank into
coma and died three hours later without regaining consciousness.

Case No. 6.

D.C., housewife, aged 37 had continued treatment since
4th Oct. 1941 on a normal diet with supplementary salt mixture
15 gm. daily, and 10 mgm. D.O.C.A. weekly.   She felt very well,
but occasionally was unsteady in her gait.   There was slight

oedema on her lower eyelids and her B.P. was 118/76. Her serum

sodium was 335 mg.%, serum chlorides 650 mg.% and plasma

potassium 18 mg.%. On 15th Oct. 1941 she began treatment on

normal diet with the usual supplementary salt mixture 15 mgm.

daily and D.O.C.A. in propylene glycol 5 drops 3 times a day

(= 10 mgm. daily). During the next 5 days she was able to

continue with her housework and felt quite normal. But on

20th Nov. she fainted while doing some housework and though she

recovered she had frequent attacks of dizziness throughout the

following day. On 22nd Nov. she continued to perform light

duties but felt very weak and unsteady on her legs and did not

feel confident about going out into the street alone. She had

no gastro-intestinal disturbances and there was no evidence of

oedema; her B.P. was 126/72, serum sodium 345.7 mg.%, chlorides

702 mg.% and plasma potassium 20.75 mg.%. She was persuaded to

continue treatment and to restrict her activities. On 29th

Nov. she complained of precordial pain and repeated attacks of

fainting. Her asthenia had greatly increased and she had been

unable to do any housework, or even to walk upstairs. There

was no evidence of any oedema in the face, abdomen or ankles.

Pigmentation was more marked on the flexor joint surfaces of

the fingers and on the back of her neck. Her B.P. was 124/72

and X-ray of the chest showed no cardiac enlargement. 3 days

later she arrived by ambulance at the Out-Patient Department feeling

very sick and faint. Since 30th Nov. she had been unable to

walk and had fainted many times; she had not even the strength

to take a bath.   There was no evidence of oedema, pigmentation
was unaltered, and her B.P. was still 124/72.   During
examination she was unable to stand and had to lie down most
of the time.   Her serum sodium was 367.5 mg.%; chlorides 640 mg.%
and plasma potassium 25.7 mg.%.   Sublingual therapy was stopped
and she was given immediately 10 cc. eucortone and 5 mg. D.O.C.A.
i.m.i.

There was considerable improvement 2 days later.   She had
had no further fainting attacks and though she was still slightly
dyspnoeic on walking she was able to do light duties.   She
was put on treatment with D.O.C.A. 5 mg. twice weekly and
reported in a week that she was able to carry out all her
household duties without any symptoms.

Case No. 7.

G.E., typist, aged 33 had been maintained since 15th July
1939 on a normal diet with no supplementary salt and with
D.O.C.A. 5 mg. i.m.i. 3 times a week.   Apart from occasional
indigestion she had no gastro-intestinal disturbances.
Pigmentation was well marked with several localised patches of
darker pigment on the left side of her face.   Though she did
not feel very strong, she was able to carry out light duties in
the house and she normally rested in bed during the morning.
Costo-lumbar tenderness was present on the right side and her
B.P. was 110/70.

On 11th April, 1941 she began treatment on a normal diet
with no supplementary salt and with D.O.C.A. in propylene glycol

5 drops 3 times a day (= 10 mgm. daily). After three days she complained of being very tired and listless though she was still able to walk about as usual; her B.P. had fallen to 108/62. She experienced slight nausea after taking the drops, but was persuaded to continue. On 16th April she was much brighter and said she felt as well as when she had D.O.C.A. by injection and her B.P. was 110/70. Two days later, however, she again complained of being very tired and was restless at night. By this time she had no difficulty in taking the drops. On 23rd April the onset of nausea and indigestion was accompanied by increased asthenia and she had to spend most of the day in bed; her B.P. was 106/70. Three days later she was unable to walk about but there was no further increased asthenia and her B.P. was 106/64. On 30th April though the nausea and indigestion persisted, there was no vomiting or diarrhoea but she complained of increasing bouts of yawning and did not sleep at all well. Her B.P. was 102/68 and she spent most of the time in bed. Two days later, after 21 days' treatment, there was increased asthenia, pigmentation was unchanged and her B.P. was 102/64. Treatment was stopped and she began her former parenteral treatment with D.O.C.A.

On 4th May she was able to go downstairs and carry out light duties; her B.P. had risen to 110/66.

Case No. 9.

A.C., a housewife, aged 40 years had been successfully maintained on D.O.C.A. 5 mgm. intramuscularly three times a week and salt mixture 15 gm. daily since October 1942. She had no gastro-intestinal disturbance and was able to walk about and do normal light household duties. Her skin was deeply pigmented and her B.P. was 110/76. On 5th December she began treatment with D.O.C.A. in propylene glycol and was instructed to take 6 drops every six hours (= 12 mgm. D.O.C.A. daily) and to continue supplementary salt as usual. After 3 days treatment there was no change in her general condition nor in her B.P., but on the 10th December she complained of nausea and weakness in the legs and arms and her B.P. was 96/60. By the 12th December the nausea persisted and she complained of being quite useless; her B.P. was 102/64. After some persuasion she continued the therapy for two days but by the 14th December she was very depressed and on account of the nausea she had eaten very little food; her legs and arms were very weak and she could only walk a few yards, her B.P. had remained at 102/64. Despite the short period of the trial, it was obvious that the dose of 12 mgm. D.O.C.A. daily was not sufficient for maintenance and the test was discontinued.

After 5 days of treatment with D.O.C.A. 5 mgm. i.m. every second day she went about as usual and her B.P. had risen to 114/70. She was bright and cheerful and by the 28th December her B.P. was 122/72. When last seen in September 1943 she was being well maintained on D.O.C.A. 5 mgm. intramuscularly twice weekly.

Comment.

Whereas the treatment of diabetes mellitus may be controlled by determining the sugar content of the blood and urine, no such simple criterion is available for assessing the results of any treatment of Addison's disease. On this account the response of patients with Addison's disease to sublingual therapy is judged chiefly by changes in well being, body weight and blood pressure. As is evident from the case reports this method of administration of D.O.C.A. has not produced the good effects reported in America. There is apparently some absorption of D.O.C.A. by this route but the amount is not sufficient for normal maintenance, in spite of the greater daily dose. Although Dunlop(1943) has recently reported much better effects with sublabial pellets of D.O.C.A., there is no doubt that from the point of view of dependability and economy sublingual therapy is much less satisfactory than either parenteral administration or implantation.

Complications of D.O.C.A. Therapy.

Although D.O.C.A. therapy has proved to be exceedingly valuable, its use is not devoid of danger and numerous reports have been published of mild and severe complications following treatment with D.O.C.A. by parenteral administration.

Hypertension.

In 1941 Loeb reported that high blood pressure ranging from 146/108 to 175/100 had arisen in some of his patients following treatment with D.O.C.A. Roth and her colleagues (1941) suggested that it might be desirable to keep in mind the possibility

of the development of hypertension on prolonged treatment with

D.O.C.A. They showed that in 10 patients with Addison's

disease, who were treated with D.O.C.A. subcutaneously during

a period up to 9 months both systolic and diastolic levels

were raised and the response to the cold pressor test was

increased. Indeed the response to this test became greater

as treatment was prolonged. It was not known, however,

whether any of these patients had hypertensive disease before

the onset of Addison's disease.

In the present study the highest B.P. records have

been 148/96(Case 3) and 130/104(Case 6).

Oedema.

In 1939 Ferrebee, Ragan, Atchley and Loeb described the

onset of oedema in 15 out of 18 patients whom they treated

with subcutaneous injections of D.O.C.A. The oedema varied

from transient puffiness of the face or ankles to massive

anasarca. Five of the patients complained of tightness of

the chest and respiratory distress. In these patients there

was an increase of venous pressure, a decrease in vital

capacity associated with X-ray evidence of pulmonary congestion

and dilatation of the heart to the right side. The authors

were unable to explain the mechanism of the development of

this cardiac insufficiency but were inclined to attribute

it to an increase in plasma volume and a decrease in the

serum potassium level. Mc.Gavack (1942) noted the occurrence

of cardiac enlargement when patients were treated with D.O.C.A.

and salt and though he did not seek an explanation of the

cardiac enlargement, he elaborated a method of controlling
treatment with D.O.C.A. according to the cardio-thoracic ratio.
In the opinion of Dunlop(1943) the development of oedema can
be regarded as an indication of the absorption of D.O.C.A..
It is interesting to note however, that of 12 patients who
received treatment with D.O.C.A. in the present study only 1
(Case 6) developed oedema.

Case 6.

D.C., housewife, aged 37, was admitted to the Royal
Infirmary, Aug. 9th, 1941 with a history of dyspnoea, fainting
attacks, asthenia, pigmentation and hypotension of 8 months'
duration. Addison's disease was diagnosed and on Aug. 15th
she was put on treatment with salt mixture 15 gm. daily. At
the onset of treatment serum sodium was 282 mg.%, chloride
701 mgm.%, haemoconc. 45%. There was no history or evidence of
oedema and the heart was not enlarged. After 6 days of salt
treatment during which the asthenia had increased and the B.P.
had fallen, her serum sodium had been raised to normal level
and plasma chlorides had increased to 860 mgm.%. She was
then given in addition D.O.C.A. 5 mgm. i.m.i. twice daily.
Three days later she developed oedema of the face and ankles
and her body weight increased by four lbs. Her B.P. was 92/64.
Five days after the beginning of D.O.C.A. treatment she
complained of tightness in the chest, attacks of dyspnoea,
she felt very thirsty and she had also some diarrhoea. Massive
oedema of the face, ankles and abdomen had developed and there

was evidence of marked respiratory distress; her B.P. was
10./68. At the time of examination it was thought that she
had an allergic reaction to D.O.C.A. and in view of the danger
of crisis D.O.C.A. was stopped, and she was given immediately
10 cc. Eucortone i.m.i. She recovered in about 5 hours and
though still dyspnoeic was very much improved. In view of the
dramatic onset of this oedema the chest was X-rayed for any
possible pulmonary cause. The radiogram showed some cardiac
enlargement to the left, but no pulmonary lesion. The salt
mixture was stopped and in view of the initial high blood chloride
it was decided to put her on a standard diet and record the
intake and output of fluid and the urinary excretion of
sodium and chloride. On Sept. 4th treatment with D.O.C.A.
5 mgm. i.m.i. twice daily was continued for 4 days without
any evidence of oedema. After 1 day of additional salt
mixture her face began to swell and there was some pitting
of the ankles on pressure. She complained also of increasing
dyspnoea. Her chest was X-rayed but showed no evidence of
cardiac enlargement or pulmonary congestion. During the next
three days there was general increase in oedema, and her B.P.
had reached the high level of 146/92. It seemed evident at
this point that D.O.C.A. and salt mixture together produced that
condition and that neither on salt alone nor on D.O.C.A. alone
did she develop oedema. On Sept. 16th she was put on salt
mixture alone and after 4 days' treatment the oedema had
disappeared from her ankles and abdomen, but she complained of
increased asthenia. On 24th Sept. treatment with D.O.C.A.

was recommended and the oedema again developed.   Again there
was no evidence of cardiac enlargement or pulmonary congestion
and on 1st Oct. this treatment was stopped.   After two daily
injections of Eucortone the oedema disappeared and her general
condition improved.

Comment.

The relationship of fluid output to the development of
oedema in the first phase of treatment is shown in Fig. 5a, 5b.
It will be seen that on salt mixture alone the output varied
between 20 and 40 ounces per day.   There was some appreciable
absorption of chloride and sodium as the blood levels increased
to 820 mg.% and 338 mg.% respectively.   With the institution
of D.O.C.A. therapy retention of water began on Aug. 23rd but
oedema was not manifest till 2 days later.   As soon as the
salt mixture was discontinued the output of fluid increased and
with it the oedema subsided so that by the 30th August there was
a normal output of 40 ounces per day and only slight residual
oedema in the ankles.

When controlled observations on urinary excretion of
chloride and sodium were commenced on Sept. 4th there was a
daily output of 1.3 gm. Na and 1.4 gm. Cl; D.O.C.A. was the
only therapy employed at the time.   With the addition of
supplementary salt on Sept. 8th, as was to be expected, there
was an immediate increase in Cl and Na output, but on 9th
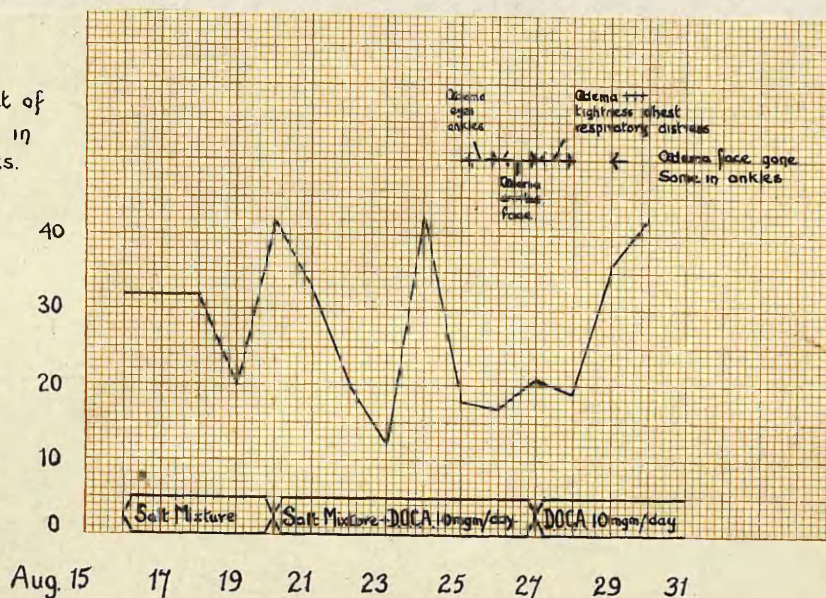Sept. evidence of oedema was noted in the ankles and by the

FIG. 5a. The effect of (a) salt mixture and (b) salt mixture and D.O.C.A. on the urinary output and the development of oedema.   Case 6.
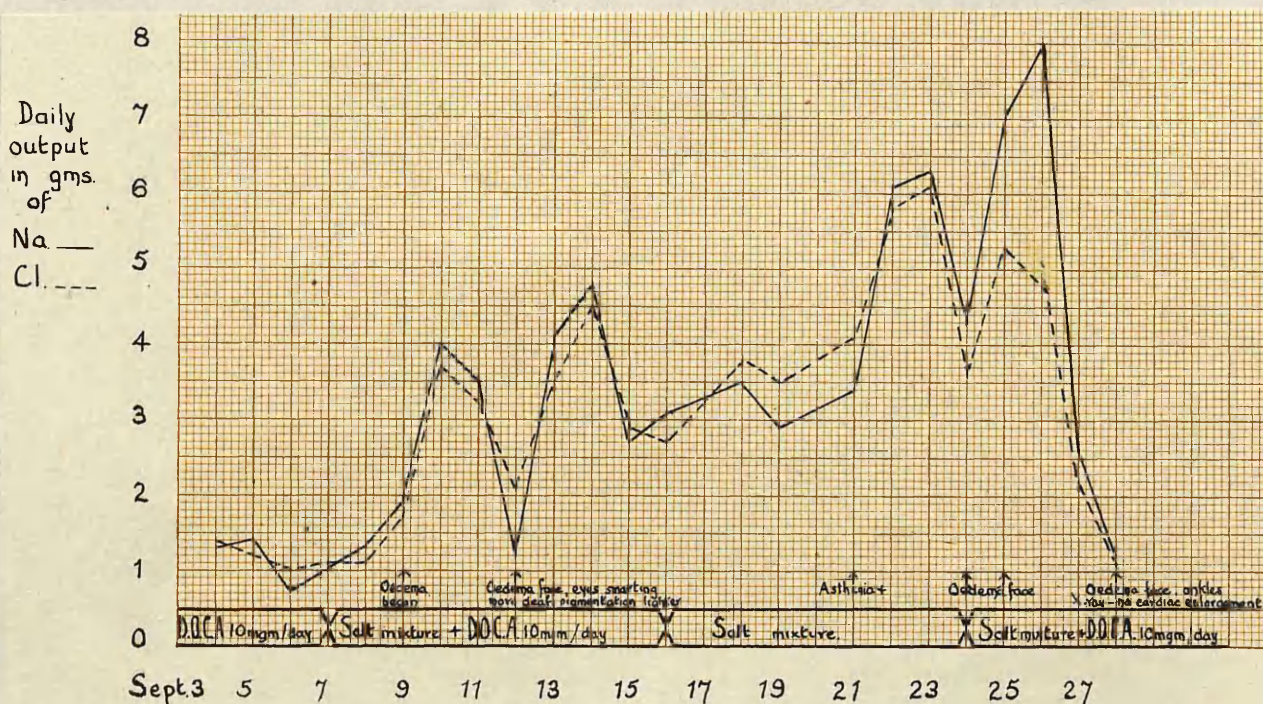

FIG. 5b. The effect of (a) D.O.C.A. (b) D.O.C.A. and salt mixture, (c) salt mixture alone on the excretion of sodium and chloride and the development of oedema Case 6.

12th massive oedema of the face had developed which coincided
with a decrease in both Cl and Na output.   For some reason not
yet apparent the output rose again and by the 14th Sept. had
reached a higher level.   The following day the ion output fell.

A further period of treatment with salt mixture alone
began on 16th Sept. and D.O.C.A. was given on 24th Sept.,
chloride retention was particularly obvious, from the 25th
onwards when oedema progressively developed reaching its maximum
on 29th Sept.   Owing to the distress of the patient further
observations had to be abandoned.

The evidence presented by this investigation suggests that
the oedema which developed when D.O.C.A. and salt mixture were
given together was due to the abnormal retention of chloride with
subsequent water retention.   It would appear that the Cl
retention in the tissues reached a maximum point after which the
output began to approach the normal level.   No other patients
reacted in this way to D.O.C.A.; Case 2 has never shown any
signs of oedema in spite of a dose of 40 mg. per week.   One
of the determining causes of the onset of oedema would appear
to be the initial blood Cl level of the patient.   There is no
evidence that D.O.C.A. has a toxic action on cardiac muscle.

Adynamia.

Loeb (1940) described the onset of periodic paralysis
associated with a decrease in blood potassium as a result of
large doses of D.O.C.A..   He stated that the paralysis was
relieved by the administration of potassium chloride and

suggested that the onset of sudden weakness in patients treated with the synthetic hormone might have a similar basis. The experience of Hampton and Kepler (1941) already mentioned lends support to this.   In their cases the disturbance was produced by smaller doses of D.O.C.A. in combination with a low potassium diet.

Plasma potassium estimations have been made in the present series of patients throughout their treatment but in no instance has there been detected any fall below the normal limits.   Even Case 2 who was receiving 10 mg. four times a week and who complained of muscular weakness in his legs, so much so that he frequently stumbled, maintained a normal plasma potassium level.

Probably the failure to relieve muscular weakness is the absence in D.O.C.A. of a factor to control adynamia; as has already been shown the synthetic hormone does not produce all the effects of adrenal cortex extract.

Conclusions.

The following scheme for the treatment of Addison's disease is recommended by the author.

1.   The patient in a state of acute adrenal insufficiency or crisis.

Treatment is urgent and must be instituted without delay. Intravenous infusion of 10% saline and 5% dextrose is commenced and continued till the patient is able to sit up and swallow fluids.   As much as 3 pints of fluids may be required in 24 hours,

cortical extract is best given in repeated doses rather than in one single dose daily. Not less than 10 cc. should be given at one time and in the early stages of treatment this should be administered intravenously. It may be necessary to use from 30-100 cc. daily. After the patient responds satisfactorily corti--cal extract may then be given intramuscularlyin doses of 10 cc. every four hours. At the end of 48 hours it should be possible to maintain the patient on salt mixture by mouth and cortical extract 10 cc. once or twice daily by intramuscular injection. Thereafter D.O.C.A. may be employed in doses of 10 mg. intramuscularly daily due precaution being taken with regard to the onset of oedema, till the maintenance level is ascertained. Any relapse should be immediately treated by intramuscular or intravenous cortical extract. It must be emphasised that in the state of crisis there is no danger of the patient being overtreated and constant nursing attention is essential.

2. The patient in a state of chronic adrenal insufficiency.

There is no hard and fast rule for the treatment of the patient in the stage of chronicity. Some patients may progress satisfactorily on a supplementary daily dose of 10-15 gms. of salt or salt mixture. As a rule there comes a time when the patient requires replacement therapy and for this purpose D.O.C.A. is the drug of choice for the effect of cortical extract is too transient. Unlike the treatment of crisis, overtreatment by D.O.C.A. in the chronic stage may be a real danger and supervision of the patient is necessary till the true maintenance dose is

reached.   The subcutaneous or intramuscular injection of
D.O.C.A. in oil is the usual method of administration in the
first place and 1.5 - 5 mgm. per day may be necessary.
Satisfactory results may be attained with 5 mgm. given once or
twice weekly.   The question of the addition of salt in diet is
a matter which can only be settled by trial.   Any evidence of
oedema should be the sign for withdrawing or reducing
supplementary salt.   The maintenance or increase in weight,
blood pressure and relief from asthenia are the criteria of
successful treatment.

Implantation of pellets of 100-200 mgm. D.O.C.A. is a very
satisfactory form of treatment and has the advantage of
eliminating the necessity of repeated injections.   Before the
implantation of pellets is undertaken, the daily requirements
of D.O.C.A. should be accurately determined and after the implants
have been administered the patient should be kept under observation
for at least 2 weeks, for the effectiveness of the amount
implanted depends on the speed at which the pellets are
absorbed.   The implants should last for from 4 to 6 months.

As with the treatment of diabetes mellitus and other
endocrine diseases, patients with Addison's disease are very
susceptible to infection and violent changes in T^{o}.   A crisis.
may be precipitated by any of these, and it is essential to
impress upon the patient the necessity of reporting immediately
any diminished response to treatment as a consequence of any
of these hazards of normal life.

With rational treatment and periodic supervision, the prognosis for the patient is increasingly more hopeful; not only may his life be prolonged but he may, within limits, take his place as a useful member of society.

SUMMARY AND CONCLUSIONS.

SUMMARY AND CONCLUSIONS.

1. In a description of the signs and symptoms of Addison's disease particular reference is made to the various chemical pathological changes which are characteristic of adrenal insufficiency in animals and in man. These changes signify a disturbance in the osmotic balance between tissues and the role of histamine as a possible cause of the altered permeability of cells is discussed. The relationship between the cortical secretion of the adrenal gland and the amount of histamine present in the blood is considered.

2. It has been shown that there is an increase of over 100% in the blood histamine of adrenalectomised rabbits, and that the blood histamine level of these animals is considerably reduced after the administration of cortical extract.

3. In patients with Addison's disease not only is the blood histamine considerably greater than in normal persons, but the distribution of histamine between plasma and blood cells also differs. This distribution of histamine is restored to a more normal ratio after the administration of D.O.C.A. These findings afford some evidence of an anti-histamine function of the adrenal cortex.

4. There is little evidence of any direct relationship between the histamine content of plasma and the blood pressure of patients with Addison's disease or other patients with arterial hypotension.

5. The difficulties of diagnosing Addison's disease in the
    early stages are discussed and various tests for adrenal
    insufficiency are described.   It is concluded that the
    Robinson, Power and Kepler excretion test is,at present,
    the most dependable method of determining adrenal
    insufficiency.

6. The occurrence of disease of the endocrine glands in
    association with Addison's disease is reviewed and a
    rare case of diabetes mellitus in a patient with
    Addison's disease is described in detail.

7. The treatment of Addison's disease in the acute and chronic
    phases is discussed and particular reference is made to the
    value of D.O.C.A. therapy.   The methods of administration
    of this hormone are considered at length and some
    complications resulting from its use are described.

8. It is concluded that Addison's disease, like diabetes
    mellitus, can be successfully treated and that the patient,
    under supervision, may pursue a life of useful activity.

A P P E N D I X.

ADRENALECTOMY OF RABBITS.

Technique of One Stage Operation.

Male rabbits 3-4 months old were used.   The abdomen was
shaved and swabbed with 0.1% aqueous iodine solution.   A left
para-median incision of 4 inches was made; skin and muscle were
retracted and protected with sterile cloths and the peritoneum
exposed.   After incising the peritoneum warm sterile cloths
were wrapped in the abdominal cavity and were used to protect
the intestines as they were moved about.

As the right adrenal gland is adherent to the inferior
vena cava and is the more difficult gland to remove it was always
extirpated first.   The right kidney was located and reflected
from its bed of fat.   The renal vein was exposed and traced to
the inferior vena cava; about 1 cm. superior to this point the
suprarenal gland was usually found.   The liver was carefully
reflected upwards with gauze pads, great care being necessary
to avoid haemorrhage.   When the suprarenal gland had been exposed
and cleared of its adventitious tissue, the superior and inferior
suprarenal veins were ligated and cut.   The adrenalectomy
clamp was carefully interposed between the gland and the inferior
vena cava and a ligature tied between the vena cava and the clamp.
A fine iridectomy knife was used to extirpate the gland.   Careful
search was made for any accessory glands and when found they were
removed.

The left adrenal gland lies just superior to the junction
of the left renal vein and the inferior vena cava and is not so
intimately associated with the inferior vena cava as is the
right adrenal gland.   Removal of this gland presents no difficulty

The wound was closed in layers and a pad of gauze was
supported over the abdomen by a linen cloth which was strapped
to the back of the animal by means of four pieces of tape.

THE DETERMINATION OF THE HISTAMINE CONTENT OF BLOOD.

Extraction of Histamine from blood.

For the quantitative estimation of histamine various
methods have been devised to extract the histamine from
biological fluids and tissues (Thorpe (1928); Gaddum and Schild
(1934)).

In 1935 Gaddum and Barsoum described a method of
extracting histamine from blood.   This method was modified
by Code (1937) and further modified by Anrep, Barsoum, Talaat
and Weininger (1939).   In principle the method consists in
deproteinising the blood with trichloracetic acid and refluxing
the filtrate with conc. hydrochloric acid for $1\frac{1}{2}$ hours.   This
destroys other depressor substances in the blood and the tri-
chloracetic acid.   The solution is evaporated in vacuo with the
aid of absolute alcohol to remove potassium and other inorganic
salts and the filtered solution evaporated under reduced pressure
to dryness.   The residue is extracted with water and the filtrate
neutralised with N/5 NaOH using brom-thymol blue as indicator.

Details are shown below:-

1.   Add known volume blood to 5 c.c. 10% trichloracetic acid and
     mix well.   Leave for $\frac{1}{2}$ to 1 hr.   Filter by suction (filtrate
     must be clear) and wash with 4 portions 5 cc. 10% trichlor-
     acetic acid.

2. Transfer to flask and add 10 cc. conc. HCl.: boil for 90 min.
    in flask with reflux air condenser. (Add water during boiling
    if necessary).   Reduce volume to less than 5 cc.

3. Add 10 cc. absolute alcohol (saturated NaCl) and dry in
    vacuo on water bath.

4. Extract dried residue with 6 cc. absolute alcohol (saturated
    NaCl) and filter.   Repeat extraction with 3 x 6 cc. absolute
    alcohol (saturated NaCl).   Bulk filtrate and evaporate to
    dryness in vacuo.

5. Add 2 cc. distilled water to dried residue, mix thoroughly
    and leave for 10 min. Filter.   Repeat extraction with
    2 x 2 cc. water.   Neutralise combined filtration with N/5
    NaOH using brom-thymol blue or thymol blue as indicator.

Assay of extracts containing histamine.

While some colorimetric methods of estimating histamine
have been described (Pauly, (1905); Knoop(1908); Zimmerman (1929))
no purely chemical method has been found sufficiently sensitive
to estimate the small amounts of histamine in tissues and
biological fluids.

The biological methods of assaying histamine may be
carried out on the intact animal or on isolated tissue.
The anaesthetised cat is usually employed in the former method
and comparative observations are made on the effect on blood
pressure of injecting alternately known amounts of a standard
solution of histamine, and of the extract to be tested.   This
method is not very sensitive and is unsuitable for the
detection of the small amounts of histamine which are present
in blood.

Barsoum and Gaddum (1935) described a specific and sensitive test for histamine which is based on the observation made by Guggenheim and Loffler(1916) that the muscle of the lower part of the guinea pig gut is very sensitive to small amounts of histamine.

Apparatus.

The apparatus(Fig. 6) consists of a small glass bath of 2 cc. capacity which is immersed in an outer brass cylinder maintained at a constant temperature of $37^{\circ}C$. The perfusion fluid is Tyrode's solution which circulates from the reservoir through a coil to the inner bath. A constant flow of oxygen is delivered to the bath through a hypodermic needle. An outlet for the bath is provided by a glass tube projecting from a small rubber stopper which serves as a base for the bath. A loop of thin platinum wire is secured to the rubber stopper and is used as an anchor for the isolated tissue.

A piece of the lower part of the ileum is carefully excised from a newly killed guinea pig. The tissue is washed with Tyrode's solution and to a portion of about 2 inches, a ligature of white cotton thread is sewn on each end. One end of the gut is anchored by means of the ligature to the platinum loop and the other is attached by the other thread to a recording lever. The lever is adjusted to record its movements on a smoked drum revolving at 0.1 mm. per second.
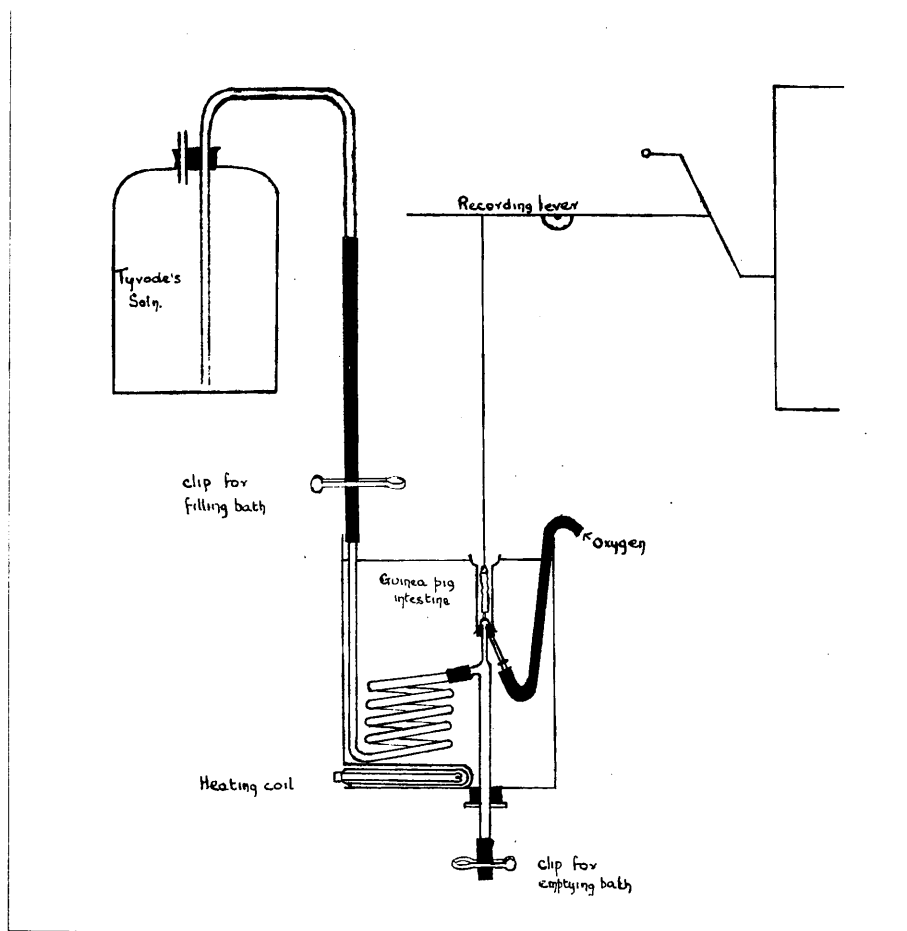
114.

FIG. 6.   Apparatus for assay of histamine
            on isolated guinea pig intestine.

Tyrode's Solution for perfusion bath.

Sodium chloride	8.00 gm.
Potassium "	0.20 gm.
Calcium "	0.20 gm.
Magnesium "	0.01 gm.
Dextrose	1.00 gm.
Sodium di-hydrogen phosphate	0.05 gm.
Sodium bicarbonate	1.00 gm.
Glass distilled water to	1000 cc.

Atropine sulphate 0.5 ug. is added before final dilution to
1 litre.

Standard Solution.

The standard solution of histamine is freshly prepared

by dissolving histamine acid phosphate in Tyrode's solution

to produce a 3 in 1 thousand solution of histamine acid phosphate.

The activity of the solution is expressed in terms of the base

histamine which is 1/3 of the salt, so that the standard

solution is 1 in 1000 histamine.   Suitable dilutions of this

solution are made varying from 1 in 10 million to 1 in 40

million (0.1 - 0.025 ug. histamine per cc.)   The test and

standard solutions are delivered from a microsyringe and the

amount of solution added to the bath should not exceed 0.2 cc.

Assay.

A definite 2 minute cycle of events is established as

follows:

The drum is run for $\frac{1}{2}$ minute to record the position of

relaxation of the gut.   With the drum still running the

histamine solution is added and a record is obtained of the

contraction of the gut during $\frac{1}{2}$ minute.   The drum is stopped

and the gut is washed by emptying and refilling the bath 3

times.   The gut is allowed to relax for the remainder of 1

minute, when the cycle is started again.

The method of comparison is carried out according to the sequence S.T.T.S. (Fig. 7) though this may be altered to the sequence S.T.S.T. Comparisons are made within 0.005 - 0.01 cc. of solution depending on the sensitivity of the gut. The activity of the extract, after making due allowance for dilution, is expressed in μg. histamine per c.c. blood.

For example: S   0.1   T   0.064

S   0.1   T   0.060

S   0.1   =   T   0.062

where S = 1 in 40 million histamine.

S O1 = T/1 0.062.   T = $\frac{.1}{.062}$ S = 1.61 S

But S = 0.025 ug. histamine/cc.

T = 1.61 x 0.025 = 0.04 ug. histamine/cc.

But T was diluted from 4.5 cc. blood to 6.0 cc. extract.

Sample contains 0.053 ug. histamine per cc. blood.

Verifying the Results.

Three biological methods are available to confirm that the substance tested is histamine:

1. Barsoum and Gaddum (1935) showed that the hen's rectal caecum is also very sensitive to histamine and its tissue can be used as an additional test to verify the results obtained by the guinea pig gut method.

2. A large dose of histamine is added to the guinea pig gut bath, the gut is allowed to relax after the contraction. Subsequent small doses of histamine fail to produce any further

contraction, though the gut responds normally to injections
of posterior pituitary extract or barium chloride (Fig. 7)

     3.   The test solution is incubated with a suspension
of histaminase for 24 hours at 37^OC.   The solution is then
assayed for histamine and compared with its activity before
incubation.   With the concentration of histamine in blood
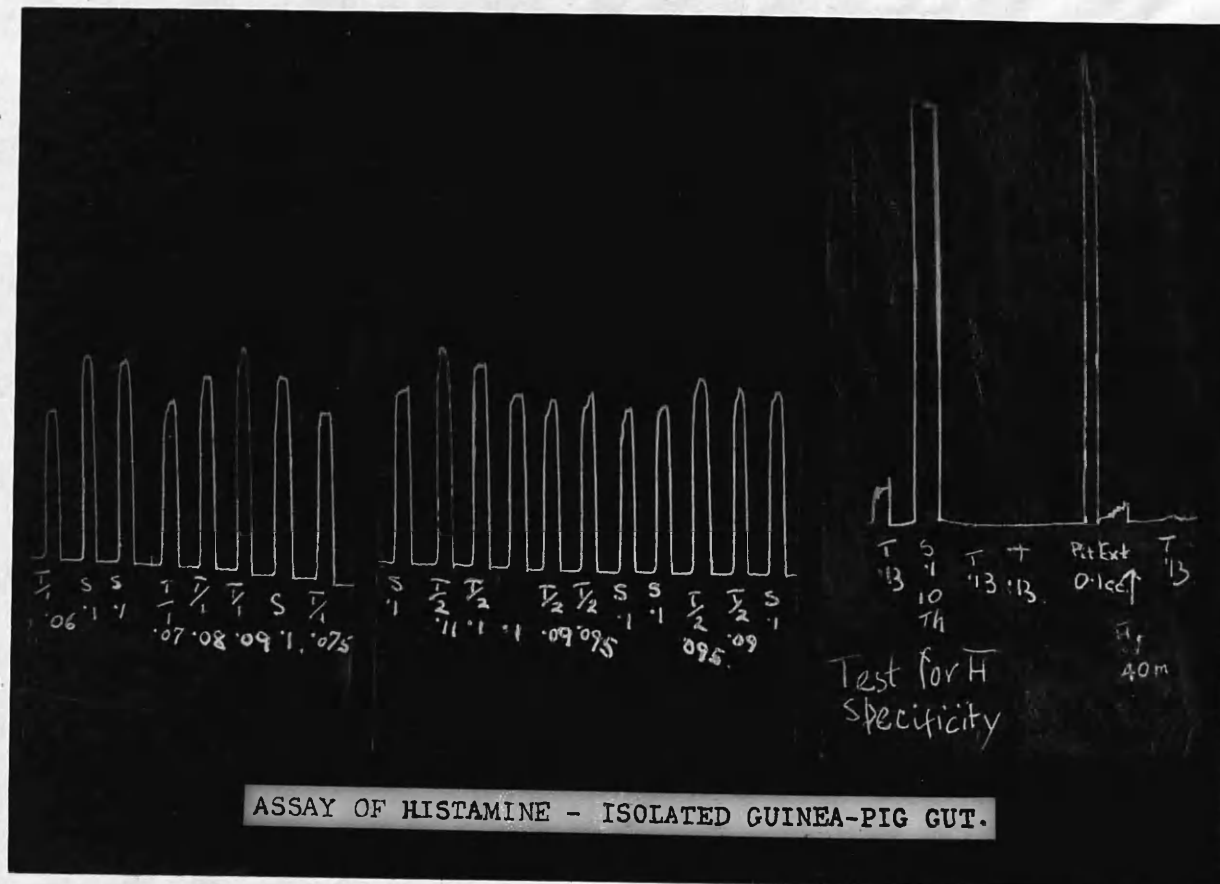no activity is found after incubation.

ASSAY OF HISTAMINE - ISOLATED GUINEA-PIG GUT.

FIG. 7. Assay of histamine.

Left: Methods of comparing Standard and Test.

Centre: -do-

Right: Test for verifying activity due to histmaine.

CUTLER, POWER AND WILDER EXCRETION TEST.

The following technique which is a replica of the method used by Dryerre(1939) was used:

No extra salt or cortical extract during the previous 24 hours.

Diet: High potassium - low sodium chloride content.

1st Day

8.0 a.m. Start diet. Preserve all urine passed.

3.0 p.m. Potassium citrate 92 mgm./kg. given in water by mouth.

8.0 p.m. Collect 1st batch of urine.

2nd Day.

8.0 a.m. Collect 2nd batch of urine.   Withdraw blood for Na, K and Cl. determination.

11.0 a.m. Potassium citrate 92 mgm./kg. in water by mouth.

8.0 p.m. Collect 3rd batch of urine.

3rd Day.

8.0 a.m. Collect 4th batch of urine.

6.30 a.m.20 cc. fluid/kg. body weight by mouth.

10.0 a.m. Withdraw blood for Na, K and Cl. determination.

12.0 a.m. Collect 5th batch of urine. Finish test. Put on low K diet and give 5 mgm. D.O.C.A. i.m.i. or 20 cc. cortical extract if necessary.

Typical high potassium-low sodium chloride diet.

Approximate contents. Carbohydrate, 203 grams. Prootein, 46 grams.

Fat, 73 grams. Calories, 1650. Potassium, 4.23 grams.

Sodium Chloride, 1.7 grams.

The quantities are given below in grams.

Breakfast.   Banana, 80.   Bread - white, 40.   Butter-unsalted, 10.
Tea - two cups (300 cc.) with cream 30 grams, and
sugar 10 grams.

Dinner.   Meat, 60.   Vegetable (carrot or cabbage or turnip),
50.   Potato, 100.   Butter- unsalted, 5.   Cereal pudding
30, (10 grams cereal, 1 grams sugar, ½ egg and 150
grams milk).

Tea.   1 egg.   Bread - white, 40.   Butter - unsalted, 10.
Tomato, 50.   Tea - two cups (300 cc) with cream 25
grams, and sugar 10 grams.

Supper.   Bread - white, toasted, 30.   Butter - unsalted, 8.
Orange, 120.   Tea - two cups (300 cc.) with milk 50
grams, and sugar 10 grams.

In addition to the above, the patient has during the day
1 pint of Imperial Drink (100 cc. orange juice, 8 grams cream of
tartar, 20 grams sugar, made up to 1 pint with water).   The
inclusion of this is essential in order that the diet may have a
sufficiently high potassium content.

All food to be cooked without salt.   No salt served with
food.

LOW POTASSIUM DIET.

A selection may be made according to the following tables:

BREAKFAST.
Kellog's All-Bran or Cornflakes. Wholemeal bread.
Bacon or Ham.   Sausage.

Avoid: Porridge, malt-bread, coffee.

DINNER.
Soups: Potato or mixed.
Vegetables: Baked beans, boiled carrots.
Fish:. Haddock, kippers, lobster, shell fish, tinned
        salmon, sardines, bloaters.
Meat: Stewed meats not fried. Yorkshire pudding.
       Salt pork, kidney, tongue.
Sweet: Stewed figs, cooked pears, jam roll, suet pudding.

Any cheese and plain biscuits.

Avoid: Mustard, curry or more than a little pepper.
        Potatoes unless cooking water changed at least
        twice.
        Herring, conger eel.
        Mutton chops.
        Apple dumpling or pudding.
        Banana, rhubarb tart.
        Rice, sago, semolina, tapioca.

TEA:
Bread: white or wholemeal. Butter or margarine.
Cakes: Cherry, ginger, and chocolate. Ginger bread or
        biscuit.
Pastry: Flaky and short.

Avoid: Currant cakes, mince-meat.

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