STUDIES ON THE RELATIONSHIP OF THE BASOPHIL CELLS OF THE

HYPOPHYSIS AND THE ADRENAL CORTEX,

- By -

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T H E S I S

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INTRODUCTION

This thesis concerns a study of the relationship of the basophil cells of the hypophysis and the adrenal cortex as revealed by the morbid anatomy of certain disease processes which involve those structures. While many methods of investigation show that the endocrine organs are intimately related to one another and mutually interdependent for their normal activities, in terms of morbid anatomy the closest inter-endocrine relationship which can be demonstrated is that of the two tissues which I propose to discuss.

Grollman (1936, p.209) states that the "ablation of one endocrine gland will usually be reflected by obvious alterations in the structure and functional activities of the others. The alteration in activity of one gland will affect the general activity of many parts of the organism and these changes in turn will be reflected in the activities of the endocrine organs." Anyone with even a superficial knowledge of the contributions of the many investigators - biochemists,pharmacologists, embryologists, anatomists, experimental pathologists - who have studied the endocrine system will readily agree with that statement. But if all

the endocrine glands from every post-mortem examination, taking place over a period of five years in a large hospital, were preserved, I doubt if many pathologists would relish the task of attempting to demonstrate inter-endocrine relationships from the accumulated material. Although animal experimentation suggests a close relationship between the pituitary gland and the islets of Langerhans in the morbid process of diabetes mellitus (Young, 1937) this has not been substantiated by the morbid anatomist working with human material (Joslin, 1937). Although changes in the pituitary gland have been demonstrated in animals with experimental conditions simulating hypothyroidism and hyperthyroidism (Severinghaus, 1938; Griesbach, 1941) no alteration in the pituitary gland has been described in those conditions in the human subject. While the adrenal cortex has been identified with principles having an important effect on carbohydrate metabolism (Scowen, 1942) the morbid anatomist has no special description for the adrenal glands in diabetes mellitus or in islet-cell tumour of the pancreas.

In the case of acromegaly, Simmonds's disease and Addison's disease, morbid anatomists have made important contributions to our knowledge of the endocrine glands. But, if I now turn to Cushing's syndrome, the contributions of the morbid anatomist would appear to be more in terms of confusion than of construction, for here is a list of the lesions which have been described in that condition :- pathological changes in the pituitary gland - basophil adenoma, hyperplasia of the basophil elements, chromophobe adenomata, "tumours" as yet undifferentiated,

and glands said to be normal on serial sectioning; pathological

changes, other than in the pituitary gland, described as the primary

lesion - adreno-cortical hyperplasia or new growth, carcinoma of the

thymus.   A new light was thrown on the problem when, in 1939, Crooke

found a lesion common to all of those types, namely cytoplasmic

hyalinization of the basophil cells of the hypophysis.   That light was

dimmed when, in 1938, Severinghaus, a distinguished American investigator,

declared that the lesion described by Crooke was associated with nuclear

breakdown and was merely a process of cell death.   Hence Crooke's

contention, that cytoplasmic hyalinization of the basophil cells

represented an altered physiological activity, which was the essential

basis of Cushing's syndrome, was declared to be erroneous.

The reader might rightly consider that the methods of the morbid

anatomist, especially when applied to a subject many hours dead, are

too crude to reveal many changes which are there.   Indeed that is

partly borne out by what has been said already.   The fundamental

observations made by morbid anatomists mainly concern relatively gross

tissue changes - witness acromegaly and Addison's disease - the great

failure lies in their inability to investigate cellular change, for in

Cushing's syndrome it will immediately appear that some important

cellular change has been missed by many, inaccurately represented by

others.

In a review of parts of this thesis, the bulk of which is shortly

to be published, the referee of the Journal of Endocrinology states "One

of the more profitable fields of research in morbid anatomy is the study of histological changes in endocrine glands, but this has been hampered by inadequate staining methods.   This work provides a considerable advance on anything I have seen before."   It is on an advance in technique that the main contribution of this thesis rests and while admitting all the difficulties caused by post-mortem change, I hope to show that post-mortem change has no more banal effects on the pituitary gland than on any other tissue.   The new methods introduced go far to bridging the gap between the morbid anatomist and the cytologist.   Accordingly the first part of this thesis deals with the technique of examination of the pituitary gland and with an account of a control series of pituitary glands taken from routine autopsy material.

The application of these new methods has enabled me to put the morbid process of Cushing's syndrome on a rational basis and, I hope, to make Grollman's statement more real so far as morbid anatomy is concerned.   I should admit that some of my final conclusions are not new but champion Cushing's own opinion (1932), despite much evidence then to the contrary, that the syndrome which bears his name is rightly the syndrome of the basophil adenoma, though an activity equivalent to a basophil adenoma may be produced by other mechanisms.   This was also Sir Robert Muir's view (personal communication).   Cushing and Muir made their stand on judgment born of long experience.   Where in this legend the reader considers that I have inadequate proof for my statements he will have to recognise that, with the limitations of his present

armamentarium, the morbid anatomist must often rely on his own judgment, for his practice is still far from being an exact science.

From the investigation of the morbid anatomy of certain disease processes, in particular Addison's disease and Cushing's syndrome, which involve especially the basophil cells of the hypophysis and the adrenal cortex, it will be shown that those elements are so closely inter-related that, at the present stage of our knowledge, they may be conveniently considered a functional complex. There is, however, evidence that they enjoy a certain degree of autonomy. This is suggested in the condition of adrenal virilism and the second part of the thesis deals with this condition, including the description of a case. The third part deals with Cushing's syndrome, including a description of three cases. The fourth part concerns Addison's disease and some related conditions. The subject of inter-endocrine relationships is complex and relevant discussion will be included in each part of the thesis.

STUDIES ON THE RELATIONSHIP OF THE BASOPHIL CELLS OF THE

HYPOPHYSIS AND THE ADRENAL CORTEX

P A R T   O N E

ON STAINING THE BASOPHIL CELLS OF THE HUMAN HYPOPHYSIS WITH

SPECIAL REFERENCE TO THE ABNORMAL BASOPHIL CELLS OF CUSHING'S

SYNDROME.

P A R T   O N E

ON STAINING THE BASOPHIL CELLS OF THE HUMAN HYPOPHYSIS WITH SPECIAL REFERENCE TO THE ABNORMAL BASOPHIL CELLS OF CUSHING'S SYNDROME.

Pathological abnormality in the basophil cells of the human hypophysis in cases of Cushing's syndrome may be very complex. A single basophil cell may show areas of normal granularity, areas of refractile hyaline cytoplasm (of Crooke), normal granules dispersed around vacuoles, conglomerations of small vacuoles outlined by delicate refractile cytoplasmic threads, one or more nuclei pressed out as a fine rind and scalloped by the vacuoles, and cytoplasmic processes extending out and embracing surrounding cells (this thesis, part III). Though it may be that all this complexity is easily analysed in fresh material, this is not so in material 12-30 hr. post-mortem, with which I, and most morbid anatomists, have to deal.

The use of the terms acidophil, for the   granules, and basophil for the   granules, of the anterior pituitary gland is traditional but erroneous. The   granules will take up many of the acid dyes strongly (Maximow and Bloom, 1939). This has led to much confusion in the past as has been shown by Biggart (1934) in his investigation of the effects of castration on the anterior pituitary gland. I have found that if a stain is specific for   (basophil) granules then the hyaline cytoplasm of Crooke will appear unstained. While under certain conditions many dyes (iron and chrome haematoxylins,

aniline blue, methyl blue, ethyl violet) will stain β grains specifically (Gomori, 1939; Bailey,1939) ; Crooke & Russell, 1935) I have found it necessary in this study to demand that the method must permit not only of tinctorial differentiation of granules from hyaline material, but also of differential nuclear staining.   The methods described here give brilliant tinctorial distinctions between the components, nuclear, granular and cytoplasmic, of the abnormal basophil cells of Cushing's syndrome.   Acid fuchsin is used as the main vehicle of distinction for these elements, even when depicting the basophil cells finally in their traditional colouring with aniline blue.   Methods have been introduced for clarity of differentiation to eliminate, alter, or reduce the affinity of the acidophil (α) granules for acid fuchsin.

One occasionally finds references in the literature to cells "not definitely basophilic."   It would, therefore, be of value to have simple staining techniques which are highly specific for β granules.   Two of my methods are simple, but highly specific, methods in which β granules are the only granular elements of the hypophysis which are stained. Photographic records of abnormal basophil cells have been made using the new methods.   This has never been accomplished before.

MATERIALS.

"Nuclear staining with haemalum." Overstain with haemalum (Mayer's or Harris's), differentiate with acid alcohol. Any common haematoxylin nuclear stain may be used, but should be differentiated as ⌐ granules take up some nuclear stains.

"Acid fuchsin" A 2% aqueous solution of acid fuchsin containing 1% of glacial acetic acid.

"Acid fuchsin (10% acetic)" - the above, but with 10% glacial acetic acid.

"Aniline blue" A 2% aqueous solution of aniline blue (soluble blue, C.I.706 or 707), containing 1% of glacial acetic acid.

"Aniline Blue (10% acetic)" - the above, but with 10% glacial acetic acid.

"Pyrrol blue." Where this blue is mentioned after aniline blue it refers to the use of this dye, in $\frac{1}{2}$% aqueous solution, as being preferable to aniline blue. Pyrrol blue (soluble blue, C.I.710 - Vector) overlays acid-fuchsin-stained granules at a slower rate than aniline blue. If pyrrol blue is not obtainable, a $\frac{1}{2}$% aqueous solution of aniline blue should be used.

"Alcoholic phosphotungstic acid." 95% ethyl alcohol, containing 2% of phosphotungstic (or phosphomolybdic) acid. The slide should be rinsed in 95% ethyl alcohol before use.

"Strong differentiator." Phosphotungstic acid, 2 g.; saturated solution of picric acid in 95% ethyl alcohol, 70 ml.; water, 30 ml.

"Weak differentiator." Phosphotungstic acid, 2 g.; saturated solution of picric acid in 95% ethyl alcohol, 40 ml.; water, 60 ml.

"Picro-orange." A 0.2% solution of orange G (C.I.27) in 80% ethyl alcohol, saturated with picric acid (Lendrum & McFarlane, 1940).

"Crystal violet." A 0.5% aqueous solution of crystal violet.

"Aniline oil/xylol." Aniline oil, 1 part; pure xylol, 4 parts.

"Eosin" A 5% aqueous solution of eosin with five drops of formaldehyde per 100 mils.

FIXATION.

The methods described work with all common Zenker and formalin fixatives. I have usually employed, and find preferable, the following methods.

(1)   Fix the whole gland in 10% formalin or Formol-Zenker (equal parts of 10% formalin and Zenker's solution without acetic acid) for 1 hr.

(2)   Bisect in the horizontal plane, fix in Formol-Zenker for 5-6 hr.

(3)   Transfer gland to saturated aqueous solution of mercuric chloride for 48-72 hr.

Dehydration is carried out in a butyl alcohol series (see Muir & Ritchie, 1937); clearing in butyl alcohol/chloroform – chloroform series; embedding in paraffin for 24 hr. If the gland proves to be of exceptional interest, the two blocks are bisected in the vertical plane and re-embedded; the smaller blocks are more suitable for cutting thin sections. If 10% formalin is used alone, fixation should be continued for 72 hr. and the sections require mordanting in Muller's fluid (24 hr.) followed by washing in tap water (10 min.).

In my experience, basophil-cell hyalinization is so conspicuous in the hypophysis from cases of Cushing's syndrome that visual inspection of the hyaline cytoplasm and β granules, at various stages of differentiation in the staining methods, can be carried out with the low power of the microscope.   In the hypophyses from all other conditions even a single hyaline basophil cell is rarely encountered.   The criterion of differentiation is, in those cases, not that of maximal contrast between β granules and hyaline, but of maximum discreteness and intensity of staining of β granules.   Using this criterion any hyaline cell encountered in glands from routine autopsy cases has shown good tinctorial contrast between β granules and hyaline cytoplasm.

STAINING METHODS

METHOD IA.   A specific acid-fuchsin stain for β granules, with differentiation of hyaline cytoplasm.

Principle.

If a section is mordanted in alcoholic phosphotungstic acid and subsequently stained with acid fuchsin, only the β granules and stroma are stained.   All other elements, including α granules and hyaline cytoplasm, remain uncoloured.   On staining with aniline blue, the connective tissue and hyaline cytoplasm rapidly become blue.   Thus, in the hyaline basophil cell a brilliant contrast is obtained between

/β granules (red) and hyaline (blue) material, while inspection of these elements is made easy since the cytoplasm of acidophil cells and chromophobe cells appears practically unstained.

Technique.

(1)   Mordant section in alcoholic phosphotungstic acid for 2-5 min.

(2)   Rise briefly in tap water.

(3)   Stain in "acid fuchsin (10% acetic)" inspecting at intervals. In a short time (2-6 min.) the /β granules and stroma become bright red; no other elements in the anterior pituitary are stained, apart from a faint red staining of the nuclei.   In hyaline basophil cells the hyaline cytoplasm remains unstained and appears as clear refractile zones contrasting with the brightly stained /β granules.   Stop staining with "acid fuchsin 10% acetic" when the β granules are bright red, but still as discrete as possible; rinse briefly in tap water. (Overstaining can be reduced by washing in tap water.).

(4)   Stain in aniline blue (pyrrol blue), inspecting at 1-min intervals.   The stroma rapidly takes on blue, later (2-5 min.) the hyaline cytoplasm becomes stained a deep blue.   Stop staining when maximum contrast is obtained between hyaline cytoplasm (blue) and β granules (red).

(5)   Rinse in 1% aqueous acetic acid, dehydrate rapidly and mount.

Result.

Basophil cells: /β granules, bright red; hyaline cytoplasm, blue; nuclei, faint red.   Cytoplasm of the acidophil and chromophobe cells,

very faint diffuse blue or red; acidophil-cell nuclei take on the
aniline blue if the duration of the staining is sufficient (Fig. 1.   ).

Modifications of Method 1A

Nuclear reinforcement. Sharp nuclear detail (dark brown) is obtained
by staining with haemalum, with or without blueing.   The nuclear
staining is introduced before the method described.

Iodine. Iodine has a very powerful mordanting effect in increasing
the acid-fuchsin specificity of β granules. It is used in the above
method by mordanting the section in Gram's iodine for 2-5 mins. before
mordanting in alcoholic phosphotungstic acid.   The iodine coloration
is rapidly removed from the section in the latter solution.   Iodine
cannot be used where deep nuclear staining is required.

Picration. The principle of "picration" is dealt with under Method II.
Staining with picro-orange (5 mins.) can be introduced in this method,
after nuclear staining, but before iodine treatment or mordanting with
alcoholic phosphotungstic acid.   This will impart a bright yellow
coloration to the acidophil cells, chromophobe cells and red blood
cells, and thus provide a good background for inspection of the basophil
cells.

Notes

If aniline blue staining is continued beyond the limits stated, the
acid-fuchsin-stained β granules are eventually overlaid with aniline
blue to become almost black; the granules of immature cells (feebly
stained) are overlaid before the granules of mature cells).   The tinctorial

contrast between β granules and hyaline cytoplasm in terms of blue

can be increased, and staining of other elements eliminated by

subsequent treatment with the weak differentiator.

METHOD 1B.   An eosin-aniline-blue staining technique

for the anterior pituitary gland.

Principle.

If a section is stained with aniline blue, though the β granules

take on the dye more intensely than other cellular elements, no specific

staining results can be obtained.   After mordanting with aqueous solutions

of phosphomolybdic acid (or phosphotungstic acid), aniline blue will stain

the basophil cells exclusively, but there is no tinctorial distinction

between β granules and hyaline cytoplasm and the stain tends to be

diffuse.   If, however, the section is mordanted with alcoholic

phosphotungstic acid and the acetic acid content of the aniline blue

solution is raised to 10% the β granules are strongly stained while

all other cellular elements, including hyaline cytoplasm become

resistant to staining with aniline blue.   Alcoholic phosphotungstic

acid has no effect on staining with eosin.

Technique :

(1)   Nuclear staining with haemalum.

(2)   Stain in eosin, 10 min.

(3)   Wash in tap-water until the acidophil cells are/strongly alone

       stained,   5 min.

(4)   Mordant in alcoholic phosphotungstic acid, 2 min.

(5) Rinse briefly in tap-water and stain in "aniline blue
(10% acetic)," 5 min.

(6) Rinse in 1% aqueous acetic acid.

Dehydrate and mount.

Result :   Nuclei, brown; basophil cells: granules, deep blue;
hyaline material, almost colourless; acidophil
granules, red; chromophobe cytoplasm, grey.

Note :   Differentiation between basophil granules and hyaline
cytoplasm in terms of aniline blue is so clear cut
that with strong eosin staining, introduced before, or
after, staining with aniline blue, hyaline cytoplasm
can be stained bright red.   Thus the contrast between
basophil granules (deep blue) and hyaline cytoplasm (red)
can be obtained.

METHOD II.   Differential staining of all the elements in the

anterior pituitary with special reference to the

pituitary gland of Cushing's syndrome.

This method is described because it allows depiction of the
anterior pituitary in terms of Mallory's original method.   It is
essentially a modification of the Mallory technique.

Principles.

   Picration.   Lendrum & McFarlane (1940) have shown that the introduction
of an alcoholic solution of picric acid containing orange G would ensure
a uniform yellow coloration of the red blood cells when using Mallory's
stain, no matter what fixative was used.   This solution also prevents
the full staining of the acidophil cells with acid fuchsin.   The degree
of staining of the acidophil cells may be controlled by adjustment of
the duration of immersion in picro-orange and acid fuchsin and of the
intermediate washing in tap water (ef. Table 1).   If it is desired to
have the ᵅ granules stained red

TABLE I

	Staining procedure (min.)			RESULTS		
	Picro-orange	Washing in tap water.	Acid fuchsin	R.B.C.	α granules	β granules
1.	2	20	10	Yellow	Red	Red
2.	2	7	10	Yellow	Deep orange-red.	Red.
3.	2	5	10	Yellow	Light orange	Red
4.	10	1	5	Yellow	Yellow	Red

rather than orange in subsequent stages then it is only necessary to
stain for a longer time in acid fuchsin;   the picric acid is dissolved

out of the cells into the staining solution and further acid-fuchsin
staining occurs. The practical advantages of picration are: the
yellow coloration of the red blood cells; facilitation of the control
of subsequent acid-fuchsin staining since the orange-red acidophil
cells are easily differentiated from the deep red basophil cells; the
acidophil cells can be depicted orange or orange-red when acid-fuchsin
staining is used for β granules.

Differentiation. In sections stained with acid fuchsin all elements,
including α and β granules, are stained and removal of the stain from
the different elements by alcoholic phosphotungstic acid is insufficiently
controllable to be of practical use. Decolorization of the stroma and
hyaline cytoplasm may be obtained by using aqueous phosphomolybdic acid
or better by using alcoholic phosphotungstic acid containing picric acid
(strong differentiator). When Formol-Zenker/corrosive sublimate –
fixed sections are stained with the standard acid fuchsin, subsequent
washing in tap water decolorizes the β granules but not the α granules.
If acid fuchsin containing 10% of acetic acid is used the conditions are
reversed, the β granules being strongly stained and the α granules only
feebly; this may account for the action of the picro-orange described
above since the latter solution is acid. Standard acid fuchsin
containing 1% of acetic acid shows little distinction between β granules
and hyaline cytoplasm, whereas the solution with the higher concentration
of acetic acid only allows feeble staining of the hyaline material. This
may explain the advantages of using acid-containing differentiators rather

than aqueous phosphomolybdic acid solution, though the alcohol content
of the weak and strong differentiators used here may be partly
responsible for the better action (cf. method I, where mordanting with
alcoholic phosphotungstic acid confined the acid-fuchsin staining to
the β granules).

Counter staining. Two methods are available. When β granules are
stained with acid fuchsin and stroma and hyaline cytoplasm decolorized;
either subsequent aniline-blue staining is stopped when the stroma and
hyaline cytoplasm are stained (method IIA), or the aniline-blue staining
is continued until the acid-fuchsin-stained β granules are overlaid with
blue to become almost black while the hyaline cytoplasm remains blue and
the   granules remain unaffected (method IIB)

Technique: method IIA

   (1)   Nuclear staining with haemalum.

   (2)   Stain in picro-orange and wash in tap water to a degree which
will allow orange staining of the acidophil cells in stage (3).

   (3)   Stain in acid fuchsin until basophil cells are deep red (10 min.);
the acidophil cells will be orange-red.

   (4)   Differentiate with strong differentiator, inspect at frequent
intervals, and stop when the hyaline cytoplasm is decolorized - the
β granules will still be a deep red.

   (5)   Rinse in tap water.

   (6) Stain in aniline blue (pyrrol blue); stop staining when the
hyaline material becomes blue, but before the β granules are overlaid
with blue.

(7) Rinse in 1% acetic acid; dehydrate rapidly and mount.

Result

Basophil cells: granules, bright red; hyaline material, bright blue; nucleus, brownish red.

Acidophil cells: granules, orange red; nuclei, blue - some are red.

Chromophobe cells: grey.

Stroma, deep blue; red blood corpuscles, yellow (Fig. 2.   )

Technique: method IIB

The same method of staining and differentiation is carried out as for method IIA until stage (5), except that only a very slight degree of picration, sufficient to distinguish the acidophil cells (deep orange-red) from the basophil cells (deep red),is used.   In the subsequent aniline-blue staining, picric acid will dissolve out of the acidophil cells, leaving them red.   At stage (6) proceed as follows:

(6) Overstain in "aniline blue (10% acetic),10-15 min.; the aniline blue will overlay the acid-fuchsin-stained β granules.   Stop staining when the basophil cells are a deep blue-black.   Rinse in 1% acetic acid.

(7) Differentiate in weak differentiator (5 - 15 min.); it will be found that hyaline cytoplasm loses aniline blue much faster than the β granules; stop differentiating when maximum contrast between hyaline cytoplasm and β granules is attained.   Dehydrate and mount.

Result

Basophil cells; granules, deep blue; hyaline cytoplasm, light grey; nuclei, brownish red.

Acidophil cells: granules, bright red; nuclei, blue - some are red.

Chromophobe cells: very light grey.

Stroma: deep blue; red blood corpuscles, yellow (Fig. 3.).

Discussion

Method IIA gives similar results, in the hyaline basophil cells,
to method lA, but the tinctorial distinctions in the former method are
not nearly so brilliant, and it requires long practice to achieve good
result. I no longer employ this method, but have described it because
it is only by going through the stage of differentiation necessary in
method IIA that the fine distinction between β granules and hyaline
cytoplasm can be obtained in method IIB. I consider that a criterion
of differentiation similar to that which I have described will be
required at some stage of differentiation before any trichromic staining
method will give good differentiation of the various elements in the
abnormal basophil cells of Cushing's syndrome. Method IIB gives the
tinctorial distinctions between granules and nuclei on which Severinghaus
bases his chromophobe / chromophil pituitary cycles (Severinghaus, 1938).
In staining normal glands, I use the following criteria of differentiation:

Stage (3), stop staining with acid fuchsin when β granules are as
deeply stained as possible without allowing acid fuchsin staining of the
red blood corpuscles.

Stage (4) differentiate for 1 min.

Stage (7), differentiate until β granules are as discrete as
possible.

Methods lA and lB are much simpler, and easier to carry out,
than Method II. In Method II considerable experience is required
to obtain the finer stages of differentiation. Method II was, however,

the original technique from which Methods 1A and 1B were elaborated. The writer considers that Method II gives the most brilliant tinctorial differentiation between nucleus, granules, and hyaline cytoplasm in the basophil cells.

METHOD III.   The modified Gram's stain for β granules of the anterior pituitary.

Principle

If we use Gram's stain for bacteria, the basophil granules are strongly Gram-positive relative to all other structures in the anterior pituitary gland.

Technique

(1)   Section to water

(2)   Stain with crystal violet, 5 min.

(3)   Mordant in 10% aqueous solution of sodium chloride, 10 min.

(4)   Mordant in Gram's iodine, 10 min.

(5)   Rinse in water.

(6)   Blot dry.

(7)   Differentiate in aniline oil/xylol, rinsing in pure xylol and

inspecting at intervals. All the elements except the β granules rapidly decolorize (1-3 min.), leaving the β granules deep blue, almost black, as the only stained elements. The reaction is highly specific and it may be as long as 20 min. after the other elements are decolorized before excess crystal violet is removed sufficiently from the β granules for them to appear discrete. This latter decolorization, if too slow, should be hastened by using a greater proportion of aniline oil in the xylol or by breathing on the slide. Saline was introduced for Gram's stain by Kirkpatrick (see Muir & Ritchie, 1937). It is unnecessary to use saline in the staining of fresh glands, but I have found that its use makes the reaction more specific in older material. In fresh material (4-6 hr. post-mortem) differentiation can also be carried out by acetone-xylol mixtures. The Gram reaction is so highly specific that I have never failed to stain β granules discretely in material up to 24 hr post-mortem.

In this method, hyaline cytoplasm is very rapidly decolorized (1 min.) and stands out as granule-free refractile areas, contrasting with the heavily stained β granules. As a counterstain, I usually employ neutral red (10 min.), followed by carmalum (10 min.). Counterstaining is introduced before the general method. With this method hyaline cytoplasm is coloured reddish yellow, contrasting with the red nucleus and the almost black β granules, while all other granular elements in the anterior pituitary are only feebly stained with the counterstain.

T A B L E II

Principle	Method	BASOPHILS Granules	BASOPHILS Hyaline	BASOPHILS Nucleus	ACIDOPHILS	CHROMOPHOBES	STROMA	R.B.C.
Method III	Gram	Black,	all other elements unstained:					
	+Gram with Carmalun	Black	Yellow-red.	Red.	Light red	Uncoloured		Red.
METHOD 1A	Acid fuchsin specific staining.	Red	all other elements unstained:					
	+with aniline blue (1% acetic) and picro-orange.	Red	Blue	Light Brown	Yellow	Yellow	Blue	Yellow
	+with iodine and aniline blue (1% acetic)	Red	Blue	Brown	Neutral	Neutral	Blue	Orange
METHOD 1B	Aniline blue (10% acetic) specific staining	Blue	all other elements unstained					
	+with light eosin and haemalum	Dark blue	Un-coloured	Brown	Red.	Grey	Blue	Red.
	+with heavy initial eosin	Dark blue almost black	Red	Brown	Red	Grey RED	Blue RED	Red
METHOD IIB		Dark blue	Dove grey.	Red	Red	Light Grey	Blue	Yellow

DEMONSTRATION SLIDES.

SLIDE I.   Pituitary gland in Addison's disease.
NOTE the absence of areas rich in basophil cells.
Compare with slide 2 showing large (red) areas rich
in basophil cells. The stromal stain (aniline blue)
has been carried to slight excess for photographic
purposes.

        METHOD IA   Naked eye

SLIDES 3 to 10, from pituitary gland of case of Cushing's
        syndrome (Case 3, part III).

        Method 1A; 3 granules, red; hyaline cytoplasm, blue.

SLIDE 3.   without haemalum or picro-orange.

    4.   with iodine and picro-orange.

    5.   with heavy nuclear staining.

        Method 1B; 3 granules, blue; hyaline cytoplasm — very light grey / red

SLIDE 6.   with light differential eosin

    7.   with heavy eosin.

        METHOD IIB; 3 granules, deep blue; hyaline cytoplasm,
                light grey; nucleus - bright red, (this
                tinctorial distinction has never been
                accomplished previously by any worker)

SLIDE 8.   Acid fuchsin has been used to excess so that there is no
distinction between acidophils and chromophobes.

        Method III. 3 granules, blue-black
                hyaline, light reddish yellow or uncoloured.

SLIDE 9,   with heavy counterstain.

SLIDE 10,   with light counterstain.

Comparison of the different methods.

The results obtained, using the different methods, are shown in table 2. A set of slides is provided to illustrate the methods(see page 23).

Relative proportion of the various cell types in

the anterior pituitary.

Rasmussen (1929, 1933) has described a method of differential counting of the cells of the anterior pituitary gland, and has set normal variations. Rasmussen's tables of the minimal, maximal, median and mean percentages of the different types of cells in normal males and females are given in table 3. . The method involves the counting of approximately 20,000 cells in each gland and is time consuming. By method 1A it is possible to stain the basophil cells a bright red while all other cellular elements are stained bright yellow. The basophil cells of the anterior pituitary gland are commonly distributed in zones rich in those cells. Using the above method zones rich in basophil cells are easily recognised by the naked eye; basophil cells in areas of sparser distribution can be seen easily with the low power of the microscope. Similar results are obtained using the modified Gram's stain. In my control series of pituitary glands from routine autopsies, referred to in part III of this thesis,

<u>T A B L E   III</u>

Percentage of different cells in normal subjects (Rasmussen 1929, 1933).

Cell types	Minimum	Maximum	Median	Mean and probable error	Standard deviation	Coefficient of variation.
A. 100 normal adult males.						
Chromophobe	34.0	65.9	54.3	52.2±0.54	7.98	15
Acidophil	22.6	58.9	34.8	36.8±0.52	7.78	21
Basophil	4.5	27.4	10.0	10.9±0.25	3.71	34
B. 94 normal non-pregnant adult females.						
Chromophobe	32.9	74.1	49.0	49.6±0.47	6.79	14
Acidophil	19.2	57.5	44.2	43.4±0.56	8.10	19
Basophil	3.0	15.6	6.6	7.0±0.20	2.94	42

there are glands from three normal young adult subjects killed in road
accidents.   In those glands areas rich in basophil cells take up
approximately $\frac{1}{8}$ to $\frac{1}{6}$ of the total area in sections selected from
levels at $\frac{1}{4}$, $\frac{1}{2}$ and $\frac{3}{4}$ of the total depth of the gland.   A similar
result applies to 70 of 80 glands of the control series examined by
the same method from subjects dying from various diseases.   Though
present conditions have not allowed of a lengthy comparison of this
method with Rasmussen's method the writer can justifiably claim to be
able to recognise glands which have a proportion of basophil cells well
within normal limits and also glands which have a proportion well outside
normal limits.   In this thesis observations concerning relative
proportions of basophil cells are confined to those two categories.
In those cases in which I have carried out Rasmussen counts the result
agreed with the impression obtained by inspection.

The acidophil cells.

It has not been found possible to evolve a method whereby the
acidophil granules are strongly stained while all other cellular
elements in the anterior pituitary remain unstained, or are lightly
stained in a single contrasting colour.   The granules of the acidophil
cells are more closely packed than the granules of the basophil cells
and, consequently, it is more difficult to obtain discrete staining of
those elements in post-mortem material.   I find that differential
staining with aqueous eosin gives best results for the acidophil

granules and, therefore, have used method 1B for their special study.

HISTOLOGICAL FINDINGS.

Apart from the easily obtained brilliant contrast which the acid-fuchsin stain and the modified Gram's stain make between β granules and hyaline, I have found these methods of value in the study of immature cells. Anyone who studies adult human anterior pituitary glands from routine autopsy material must be struck with the great variation of tinctorial affinity and granular distribution in cells which are clearly neither mature chromophil cells nor chromophobe cells. Using any modified Mallory or eosin/methylene blue stain on post-mortem material, I have found that the granules of many of these immature cells can be made to take on characters of the granules of either the acidophil or basophil line. While this is largely due to the insurmountable difficulty of post-mortem change and, in any case, care should naturally be taken in making any deduction from the appearances, I believe that the specific

acid-fuchsin stain for β granules and the modified Gram stain are so highly specific that any immature cell which shows, by these methods, some granules stained similarly to the granules of the mature basophil cells belongs to the basophil line. In addition, the staining of the granules with one dye alone obviates the complexity of having the granules stained by a combination of dyes which, as I have already pointed out, may obtain in modifications of the Mallory method,(METHOD IIB)

Some authors have referred to Crooke's hyaline change as a process of degranulation. In addition to having the very different staining properties from β granules, which I have described, the hyaline cytoplasm of Crooke is refractile, granule-free, and has a regular mode of distribution in the cell (Crooke, 1935). The earliest lesion appears as a minute subperipheral zone a few granules below the surface (see this thesis part III, Fig.11); thence hyaline material advances centripetally, leaving in more advanced hyalinization two granular areas: one on that side of the eccentric nucleus having the most abundant cytoplasm, and another on the periphery of the cell. Ultimately, the cell may be completely hyalinized though more commonly remnants of the peripheral granular rim persist. On the other hand, the term degranulation has long been applied to a process in the basophil cells which is present to some extent in every human hypophysis. The basophil cells contain areas which are not entirely granule-free but have a sparser distribution of granules than the surrounding cytoplasm. The granules in the area lie in a ground substance which is not refractile but has the almost imperceptible fine matt surface similar in appearance

to the chromophobe cytoplasm in post-mortem material and which has
the indifferent staining properties of chromophobe cytoplasm as
opposed to the brilliant coloration which hyaline cytoplasm can take
on.   Furthermore, these areas of degranulation have no systematic
mode of distribution in the cell like that of hyaline cytoplasm, though
they are, in my experience, found most commonly in a juxta-nuclear
position, which is the opposite to that of the distribution of hyaline.
It is obvious that basophil hyalinization should be clearly distinguished
from the physiological process of basophil-cell degranulation. Severinghaus
(1938) has correlated the processes of degranulation in the chromophil
cells of the normal hypophysis with nuclear changes and, on the appearances,
has based his theory of chromophil/chromophobe cycles.

Large chromophobe cells are sometimes present in₄ the anterior
pituitary which, in fresh material, can be shown to be predestined
basophil cells.   These cells are large, of indefinite outline, and
send processes between surrounding cells.   They have a large vesicular
nucleus.   They show no β granules with specific stains (methods I & III)
for β granules.   But, in method I, on introducing aniline blue and
removing excess dye with the strong differentiator, they exhibit a   .
delicate spongioplasm which stains with aniline blue at a stage when
the acid-fuchsin-stained β granules are still unaffected by the blue
and the acidophil and chromophobe cells are decolorized.   This same
spongioplasm may be seen in cells of slightly greater maturity in which
a few granules are present which stain faintly with specific stains for

β granules.   Hence, I consider that this spongioplasm represents
a stage preceding the development of $\measuredangle$ granules in immature basophil
cells.   In some cases I have recognized a process of agglutination
and shrinkage of the spongioplasm so that it stains more intensely
with aniline blue, and finally, when this process is complete, the
nucleus becomes pyknotic and the cytoplasm has usually numerous
perforations.   Though the cytoplasm will now stain a deep blue, it
does not give any of the specific reactions of basophil granules.   I
consider that this cell is the same as the 'agglutinated' basophil cell
described and illustrated by Crooke & Russell (1935), which is often
prominent in the hypophysis in Addison's disease.   Thus, I consider
that the 'agglutinated' basophil cell represents the end-product of a
degeneration of the primitive basophil cell.   Stages in the degeneration
should not be confused with hyalinization, as the agglutinated spongio-
plasm has never the refractile waxy character of hyaline cytoplasm, is
not associated with a regular mode of distribution in a cell which has
ripe β granules, and is ultimately associated with nuclear pyknosis.

I have not had the opportunity of fixing human hypophyses earlier
than 4 hr. after death.   My material is usually 8 to 30 hr. post-mortem.
It is well-known that the staining of material fixed immediately after
death may present a different problem from staining material fixed some
hours later.   Some observations I have made may be of benefit to those
who wish to apply these methods to very fresh material.   The Gram's

stain shows increasing specificity with increasing freshness, and
I consider that it should work with material fixed immediately after
death.   The prevention of acid-fuchsin staining of the acidophil
cells by picration becomes more potent the fresher the material, and
it is more marked with chromate-containing fixatives than with those
that do not contain chromate.   Hence, when acid-fuchsin staining of
acidophil cells is desired, as in method II, picration should be avoided
in fresh material as it may take hours to get slight acid-fuchsin staining
of acidophil cells even after short exposure to picro-orange.   While
the acid-fuchsin specificity of β granules after alcoholic phosphotungstic
acid mordanting (method I) becomes more marked the fresher the material,
in some cases subsequent aniline-blue staining produces a very rapid
overlaying of the acid-fuchsin stained β granules with blue;   hence,
in making observations on hyaline cytoplasm, a very dilute aniline blue
will require to be used.

The pars intermedia and the wandering cells of the
posterior lobe of the pituitary gland.

The reader will be able to follow more easily the descriptions of
abnormal pituitary glands if he has some knowledge of the pars intermedia
and the wandering cells.   They have been described in detail by Rasmussen
(1928, 1930);   the writer is in agreement with his findings.   The
para intermedia is a very variable structure in the human hypophysis.

In my control series of glands it varies from a minute cleft, incompletely
lined by flattened epithelial cells, to a complex mass of cysts, filled
with "colloid", completely separating the anterior and posterior lobes.
The cysts are lined by a single, occasionally double, layer of cubical
epithelial cells.   The intermedia cells in general are devoid of granules,
                       ciliated
are not infrequently/abated, and stain light blue with Mallory's acid-
fuchsin - aniline blue method.   The pars intermedia is, like the anterior
lobe, a derivative of Rathké's pouch.   It is so variable in structure
in mammals that the majority of writers consider it to be a purely
vestigial element.

Basophil granules may develope in the epithelium of the pars
intermedia and all transitions up to large spherical, completely
granular cells can be traced.   The latter cells are indistinguishable
from the basophil cells of the anterior lobe.   It is from these cells
that columns of basophil cells develop and project into the pars nervosa.
Distally the columns break up and the cells lie free in the tissues of
the pars nervosa - the wandering cells.   In the normal gland the
wandering cells are very variable in distribution and number.   Generally
they do not show as much variation in size, nuclear structure and degrees
of maturity as their brothers in the anterior lobe.   They commonly
conform in size to the smaller types of the anterior lobe.   With all
the staining methods for β granules described in this paper their
granules give the same coloration as the granules of the mature basophil
cells in the anterior lobe.   Indeed observation of the wandering cells
is one of the best guides in controlling the staining of the pituitary
gland.
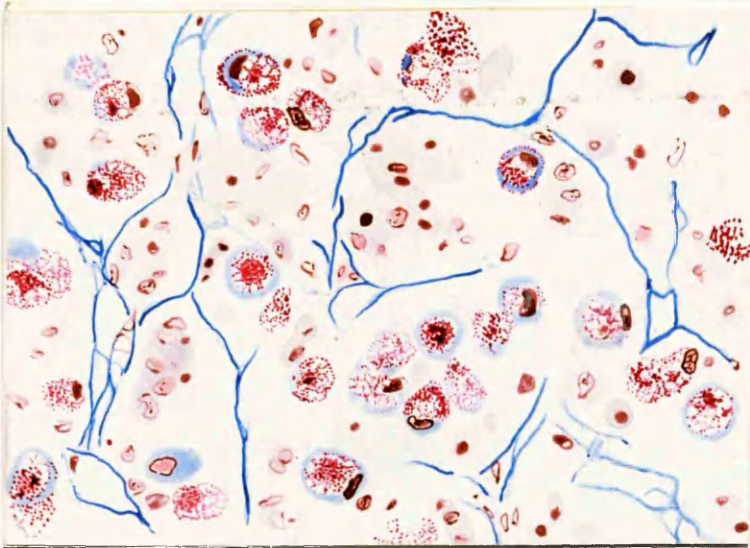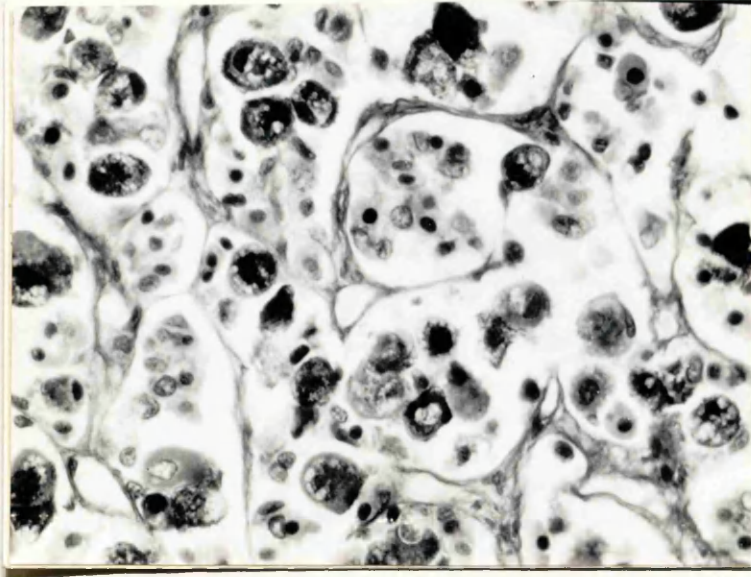
P A R T I

Figure I

Fig.1. shows a field with many basophil cells showing

hyalinization, excessive vacuolation and nuclear

displacement.   Basophil cells - granules, red;

hyaline cytoplasm, blue; nuclei, reddish brown.

Acidophil and chromophobe cells in light neutral

shade.

X 400   Method IA
                                      without picro-orange.

PART I

Fig. 1

Field of anterior pituitary gland from a case of
Cushing's syndrome (Case III, this thesis, part III)

X 400

Method IA
without picro-orange.

PART I.

Figs. 2 & 3.

Fig. 2.   Shows a hyaline basophil cell; hyaline cytoplasm,

blue (pyrrol blue); β granules, red (acid fuchsin);

two acidophil cells, orange (acid fuchsin and picro-

orange); three chromophobe cells in neutral tints.

X 800   METHOD IIA

Fig. 3.   shows two hyaline basophil cells; β granules, dark

blue (acid fuchsin overlaid with aniline blue); hyaline

cytoplasm, grey (excess aniline blue removed by weak

differentiator); three acidophil cells, red (acid

fuchsin without deviation by picro-orange; two small

chromophobe cells, pale grey.

X 800   METHOD IIB

P A R T. I.

Figs. 2 & 5

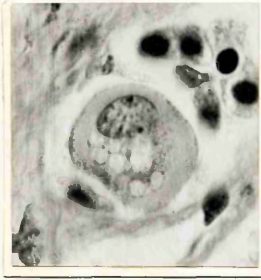Fields of anterior pituitary gland from a case of Cushing's syndrome (Case III, this thesis, part III)
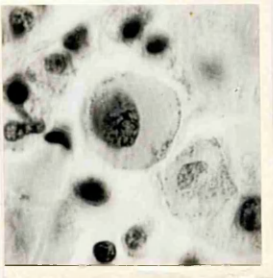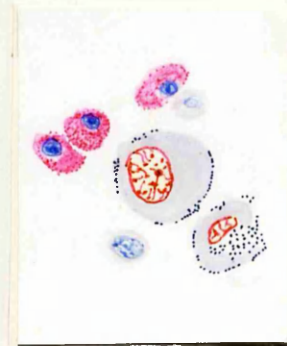

Fig. 2.

METHOD IIA

Fig. 3.

X800

METHOD IIB

STUDIES ON THE RELATIONSHIP OF THE BASOPHIL CELLS OF THE

HYPOPHYSIS AND THE ADRENAL CORTEX.

P A R T   T W O

ADRENAL   VIRILISM.

PART TWO

A D R E N A L   V I R I L I S M.

Introduction.

It is my purpose to describe the condition of adrenal virilism in order that the clinical and pathological findings may be compared with those of Cushing's syndrome. The subject of intersexuality is complex. In the infant and adolescent female abnormality of the adrenal cortex is associated with a variety of clinical conditions. I have not encountered cases of adrenal abnormality in this age group, nor has extensive pathological work been carried out on the subject. Accordingly I do not intend to discuss this group. I will confine my observations to the condition of adrenal virilism as it occurs in the female at,er near, puberty, and in the young adult. The case which I report here is unusually complex and, in the discussion, I will require to depart from the general arguement of the thesis to discuss some matters peculiar to the case.

THE REPORT OF A CASE OF ADRENAL VIRILISM

WITH UNUSUAL FEATURES.

Case History.

1903: The patient was born in 1903.   From birth until puberty the

patient appeared, behaved and was brought up as a normal female child.

There were other brothers and sisters of the family who were normal,

but cannot now be traced.

1917: At the age of 13-14 years hair began to grow on the face and the

voice broke.   Menstruation did not start, although it was noted that

the external genitalia had developed normally for the age.   The general

body habitus, voice and facies became so distinctly male in type by the

age of 16 years that the patient then changed her Christian name to

"John," donned male attire, left home and worked, first as a van-boy

and later as a lorry driver.

1925: At the age of 22 years the patient returned to his parents' home

suffering from neurasthenia.   (The masculine pronoun will now be used

when referring to the patient).   The trouble appeared to have developed

after a doctor had been called in to treat the patient for influenza

and his true condition had become known.   Later the family doctor sent

the patient to/the Western Infirmary, Glasgow, for observation in Professor

Ralph Stockman's wards.

The following are the notes from the ward journal, additional to

the family doctor's letter giving the previous history and affirming

that the patient had never menstruated.

"Condition : Neurasthenia secondary to virilism. Pyorrhoea alveolaris. The patient is 4 ft. 11 in. in height and superficially appears to be a man. He lies comfortably in bed so long as his head is supported, but whenever his head is raised from the pillow he closes his eyes and complains of light-headedness amounting almost to fainting. The breath is very foul, due to a general condition of pyorrhoea. The distribution of hair is male, the beard being abnormally strong. The patient shaves every second day. The cranial hair is receding and is of very fine texture. The hands and feet are small and suggest feminity. The external genitalia are apparently of typical female form. Figure and voice masculine; pelvis masculine; gestures and mental outlook suggest feminity. No abnormality of the cardio-vascular, renal, or nervous systems found.

5.6.25. Chloroform. Dr. Martin examined the genitalia. The clitoris has not the normal female shape but is notched like the male penis. The urethra is of female shape and position. Vagina present with rudimentary cervix, but no definite uterus. Gland palpable in position of left ovary. No signs of testes.

Complete extraction of teeth was performed at same occasion."

The patient was discharged a few weeks later, his general condition having improved. He left his parents' home shortly afterwards.

1935: The patient was again traced. He was working as a lorry driver and was apparently accepted as a male without any suspicion by his

workmates.

1941: The patient returned to the family doctor for a certificate
to support his claim as a "conscientious objector."   He had been
working since the outbreak of war as a shipyard labourer.   On being
interrogated (1943) his fellow workmen and friends at his lodging gave
no indication that they even suspected his being abnormal.   If he had
any psychological upset he managed to keep it hidden.   He had no
interest in the female sex.   The certificate given to him by his
family doctor gave the additional information that the patient now
menstruated, but the date of commencement was not given.

1943: After a history of a few months' duration of lower abdominal
discomfort, ultimately with alternate diarrhoea and constipation, sudden
acute illness supervened and the patient was admitted to the Western
Infirmary, Glasgow, as an "acute abdomen."   The doctor who sent the
patient to the infirmary had noted that there was a carcinoma of the
right breast which the patient stated had been present for two years.

Laparotomy was performed a few hours after admission to hospital
and pus was evacuated from the pelvis.   A sloughing mass of tumour in
connection with an ovarian dermoid cyst was found by the surgeon.   The
patient died a few hours after the operation.   Autopsy was performed
twelve hours later.

The only additional information obtained from the patient at
hospital was the statement that he had menstruated all his life.   This
must be taken with reserve as the patient was in extremis.   There is

post-mortem evidence that the patient did menstruate and it is known that he menstruated in 1941.   It must be presumed, therefore, that the patient started to menstruate some time after 1922, when he was examined in the Western Infirmary.

P O S T   M O R T E M   E X A M I N A T I O N.

External Appearances.

Apart from the external genitalia and breasts, the subject had the facies and bodily habitus of a male of rather slender build and of small stature (5 ft. 1 in.).   There was little subcutaneous fat and the body and limbs presented an athletic appearance.   The hair of the head was of fine texture.   There was complete baldness of the frontal area, and a heavy growth of coarse recently shaven hair on the shaving area of the face;   hair growth was not marked on the chest or limbs.   The abdominal and genital hair were of female distribution.   The breasts appeared as unusually circumscribed elevated plaques about 8 cms. in diameter and $2\frac{1}{2}$ cms. thick.   The nipples were prominent and showed no displacement.   The right breast had a uniform hard consistence.

The left breast was less hard and had the characteristic knobby

consistence of fibro-cystic mastopathy.   Both breasts were very

easily removed by blunt dissection and traction.   Section of the

right breast revealed a uniform dense white hard fibrous tissue;

section of the left breast showed similar appearances but with numerous

small cysts throughout.   No axillary glands, or other evidence of

lymphatic tumour spread from the breasts, were present.

Thorax & Abdomen.

There were large quantities of pus in the peritoneal cavity,

especially in the pelvic region.   The coils of the small intestine

were glued together by light fibrous adhesions.

The heart, 230 gms., liver, 1000 gms., spleen 90 gms., were slightly

smaller than one would expect in a male, even allowing for the build

and stature of the subject.   The kidneys weighed 120 gms. each.   No

abnormality was detected in the cardiovascular, urinary, skeletal, or

alimentary systems, except for tumour invasion of the colon, which

will be referred to later.   The lungs showed congestion and oedema.

The thyroid (15 gms) showed natural tissue.   No thymic tissue was

noted in the mediastinum.

The liver : Eight white nodules from miliary size up to 2 cms. in

diameter, having the typical appearances of secondary carcinoma, were

present in the liver.

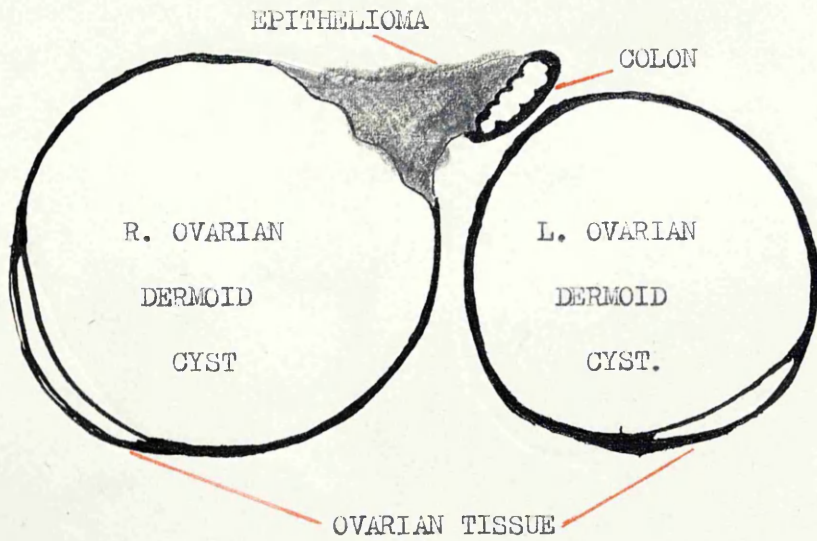The right adrenal gland (18 gms., 3 cms., x $3\frac{1}{2}$ cms.) was represented

Fig. 12.   DIAGRAM OF OVARIAN DERMOIDS.
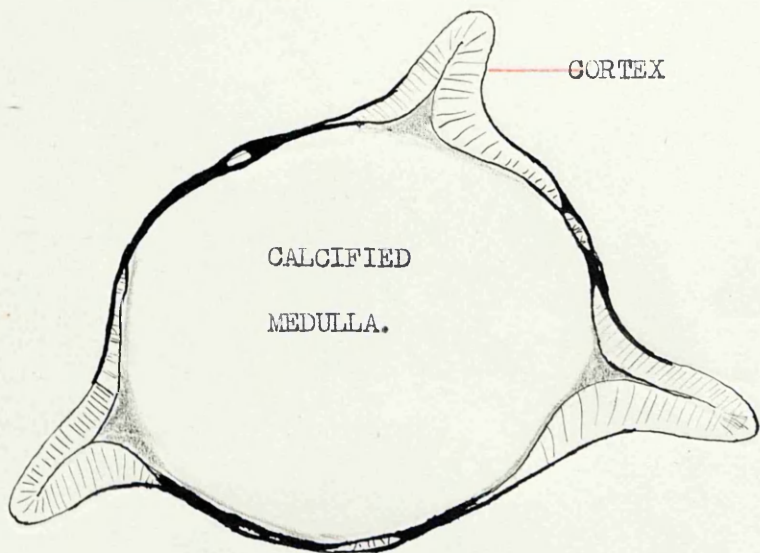

Fig. 12. DIAGRAM OF OVARIAN DERMOIDS.

EPITHELIOMA

COLON

R. OVARIAN

DERMOID

CYST

L. OVARIAN

DERMOID

CYST.

OVARIAN TISSUE

Fig. 13.   DIAGRAM OF R. ADRENAL BODY.

Fig. 13. DIAGRAM OF R. ADRENAL BODY.

CORTEX

CALCIFIED

MEDULLA.

by a body with four roughly triangular surfaces. The bulk of the
specimen was taken up by a hard mass of calcareous and ossified
material replacing the medulla. The flat surfaces of the hard mass
were clothed by a thick fibrous capsule which included small islets
of bright yellow adreno-cortical tissue. At the free edges the
fibrous capsule w s reflected off to form an integument for thickened
ridges of adrenal c rtex (Figs. 1 & 13).

The left adrenal gland ($5\frac{1}{2}$ gms.) presented natural appearances;
the cortical tissue was prominent but not definitely increased.

Para-aortic glands : In the region of the coeliac axis there was
a conglomerate mass of enlarged glands, from $\frac{1}{2}$ to 2 cms. in diameter,
irregular in shape, fibrous, and containing many chalky white calcareous
nodes.

The ovaries. Both ovaries were represented by dermoid cysts, which
filled the pelvis, the right 11 cm. in diameter, the left 8 cms. These
consisted of cavities filled with hair and inspissated sebaceous material,
with smooth thin walls. In each there was an area of thickening in the
wall incorporating ovarian tissue. In the right dermoid the ovarian
tissue was extensive, and contained numerous blood vessels, small cysts
containing clear fluid, and a large corpus luteum stretched over an
area of $3\frac{1}{2}$ cms. in diameter and $\frac{1}{2}$ cm. in maximum thickness. In the
left cyst the ovarian tissue was confined to an attenuated area, 2 cmm.
in diameter, presenting as white ovarian stroma without other
macroscopic detail. Postero-internally the interior of the right
cyst was heaped up and irregular. From here a large mass of pale

tumour tissue extended backwards and inwards to invade the wall of
the colon at the brim of the pelvis.   Numerous yellow areas of
necrosis were present in the tumour mass and, near the colon, there
was sloughing of large areas with loculi of pus in the tissues.
This was evidently the source of the peritonitis (Fig. 12).

The uterus, 5 cms. long from neck to fundus and $2\frac{1}{2}$ cms. in maximal
thickness was small, but had a thick layer of endometrium (Fig. 8).
The cervix was small.

Head: The brain (1050 gms.) showed a g neral atrophy with widening
of the the sulci and ventricles, and excess of cerebro-pinal fluid.
No other abnormality was detected.   The pineal body and the pituitary
gland were of natural size and appearance.

HISTOLOGICAL EXAMINATION

The adrenal glands and caloified para-aortic glands.

The adrenal glands and the calcified para-aortic glands require joint consideration as they present associated abnormalities.

The capsules of the para-aortic glands show fibrous thickening with lobulation by thick fibrous trabeculae. The calcified areas, of amorphous appearance, are in some cases completely invested by fibrous tissue, in others associated with uncalcified caseous material. The whole presents a picture indistinguishable from effete tuberculosis (Fig. 3). While the regressive changes are in most areas complete, a few small cellular follicles with central caseation, indistinguishable from tuberculous follicles, are present. Although tubercle bacilli have not been demonstrated, it is considered that it must be taken that all the latter appearances represent tuberculosis with almost complete healing. The same process appears to be a reasonable explanation for the large calcified and bony mass taking up the right adrenal medulla. The picture is the same, except that no cellular follicles are present and there is some imperfect bone formation in the calcified amorphous material. There is no organoid differentiation to indicate the existence either of a simple tumour of bone or of degeneration in a bone-bearing teratoma. The flat surfaces of the bony mass in the right adrenal/are clothed by a thick
                                                    -gland

fibrous capsule which includes small pseudo-adenomatous formations

of adreno-cortical epithelium.   The thickened tissue, reflected

of at the angles of the right adrenal body, presents the general

gross characters of adrenal cortex, and shows all three histological

zones in normal proportion.   Frozen sections of parts of the cortical

tissue removed before the process of decalcification show no abnormality

when examined for fat (isotropic and anisotropic).   Scattered throughout

the cortical tissue there are a few collections of altered epithelium.

In some of these pseudo-adenomatous areas the cells are large and show

gross vacuolation;   the nucleus being pressed to one side in signet-ring

fashion.   Frozen sections show the vacuoles to contain neutral fat.   In

others the cells are small and have a solid non-vacuolated eosinophilic

granular cytoplasm.   Those areas occur in all three zones of the cortex

and produce slight alteration of the normal architectural plan (Figs.

1 & 2).   The central mass is separated from the ridges of cortical

epithelium by condensations of connective tissue.   There is no trace

of adreno-medullary tissue.   The left adrenal gland shows no abnormality.

Nodules of epithelial tissue are present in the conglomerate mass of

calcified coeliac glands.   They are encapsulated by fibrous bands.

The cells are arranged in columns supported by loose stroma, with

small focal accumulations of lymphocytes in places.   The epithelial

cells are polygonal and have dense eosinophil cytoplasm which, with the

oil-immersion lens, can be seen to be finely granular.   The nuclei

are central, and hyperchromatic. No mitoses are present (Figs.3 & 4). This epithelium is sharply distinguished from the malignant epithelium in the right breast and the hepatic metastases, the cells of the latter being smaller and having vesicular oval nuclei, and scanty pale-staining cytoplasm. The fuchsinophil granules of Vines (referred to in the discussion) were not found in the coeliac epithelium or in the adrenal-cortex.

Ovarian dermoids: The cyst walls consist of thin epidermis with hair follicles and accessory skin glands. A few small islets of cartilage are the only other elements found on extensive examination. The mass of tumour arising in the heaped-up area in the right cyst wall and extending in a large mass into the colon is a squamous epithelioma. It is well differentiated and shows large cell nests with prominent keratinised centres. While the tumour is well differentiated in mass, the basal cells are large and aberrant. The tumour extends through the wall of the colon to the basement membrane of the mucosa (Fig. 7). The infiltrated bowel musculature and the region of the colon above show hypertrophy; a degree of chronic obstruction apparently having been present. The ovarian tissue incorporated in the wall of the right cyst equals a normal ovary in amount, while that of the left is slightly less. Examples of primordial follicles, early follicle formation, a few small corpora albicantes, and many small atretic follicles have all been recognised in addition to the large macroscopic corpus luteum previously referred

to.   There is abundant ovarian stroma (Figs. 5,6).

Uterus: A well circumscribed intramural fibroid takes up the whole

thickness of the uterine wall at one point.   The endometrium shows

normal premenstrual secretory characters.

Mammary glands: Both breasts show dense hyaline fibrosis.   The

basement membranes of many of the ducts and acini show hyaline

thickening and also peripheral wholed-fibrosis.   In some of the

ducts and acini this obliterative process has resulted in destruction

of the epithelium.   In others there is epithelial hyperplasia with

the production of intraduct and intra-acinar tumour of both solid and

cribriform type.   In the right breast the intraduct tumour has broken

through with the production of universal injection of the tissue spaces

with tumour cells (Fig. 9), but there is only marginal infiltration

of the surrounding fatty tissue.   Cystic change in ducts and acini

is conspicuous in the left breast.   This is associated with many

areas of intraduct tumour, but there has been no break through with

the development of frank malignancy (Fig. 10).

The hepatic tumour nodules show characters consistent with

metastases from the right breast.   The tumour epithelium consists

of small cells with loose scanty cytoplasm.   The epithelium, associated

with moderate stroma production, shows a tendency to adenomatous

formation.   The growing edge presents the characters of tumour

invasion and there is no suggestion that the masses represent

ectopic or metastatic adreno-cortical epithelium.

The pituitary gland. Sections from all levels, 50 in all, were examined. The gland shows a normal gross structure; the pars intermedia is represented by a single cleft. The basophil cells of the anterior lobe are not numerous, but are within normal limits in size and relative proportion. The basophil cells show normal granularity, vacuolation is inconspicuous and the hyaline lesion of Crooke is absent. The acidophils and chromophobes present no abnormality. The wandering cells of the posterior lobe show no abnormality.

Much of the pineal gland is calcified; no abnormality is detected.

Summary of post-mortem findings.

Spare virile habitus with facial hair of male type; female external genitalia and enlarged clitoris; mammary carcinoma with hepatic metastases; bilateral ovarian dermoid cysts; epithelioma, arising in right ovarian dermoid cyst, invading colon, with production of peritonitis; ovarian tissue, including a large corpus luteum, in wall of dermoids; right adrenal represented by ridges of thickened cortex upon a calcified and ossified medulla; epithelial tissue incorporated in mass of calcified coeliac glands; small uterus with thick endometrium.

D I S C U S S I O N.

The association of the developement of virilism in the female with adreno-cortical abnormality has long been known. The subject has been extensively studied and reviewed by Broster & Vines and their co-workers (1938). The condition of adrenal virilism (the adreno-genital) syndrome, developing in the female at, or near, puberty or in the young adult, is characterised by amenorrhoea, the development of hirsuties and a facies of male type, and a spare virile habitus, enlargement of the clitoris, sometimes distressing psychological upset, occasionally deepening of the voice. The uterus, ovaries, and breasts become atrophic. In older subjects, due to loss of plasticity of the tissues with age, changes are less marked. While my subject exhibited the classical syndrome there are also unusual features in that, though the condition was classical from 1917 to 1925, sometime between 1925 and 1941 menstruation started and continued until death in 1943. For this, as we shall see later, there is an explanation. What I wish to make very clear here is that the condition of virilism, as shown in this case, may be maintained throughout life without any tendency to develop the criteria of Cushing's syndrome, e.g. obesity, hypertension, glycosuria.

THE ADRENAL ABNORMALITY.

Boster and Vines describe numerous cases of the condition with adreno-cortical hyperplasia. In the present case the adreno-cortical abnormality is of some complexity. The right adreno-cortical tissue is certainly thickened. It is considered, however, that the anatomical peculiarity of the specimen suggests that, in the beginning, there was a uniform thickening of the adrenal cortex, and that later the medulla was replaced and expanded by a morbid process resulting in atrophy of much of the cortex and leaving only the peculiar heaped up projections of cortical tissue. But this is not an end to the adreno-cortical abnormality. It is considered that the epithelial nodes embedded in the effete tuberculous mass in the coeliac region represent ectopic adreno-cortical tissue. The arrangement of the tissue bears a close resemblance to the malignant adenoma of ectopic adrenal tissue described in part III, while the cells closely correspond to the solid non-vacuolated cells which compose some of the pseudo-adenomata in the right adrenal cortex. I find no evidence that it is metastatic tumour. The ovarian tumour is an epithelioma; the breast cancer has different cytological characters from the coeliac tissues. In extensive sectioning no evidence of malignant tissue has been found in the coeliac lymphatics. The coeliac epithelium, well circumscribed by fibrous and calcareous tissue, has evidently

been long resident there and shows, rather than invasive characters,
retrogression before an advancing fibrotic process.

Thus, from its character and arrangement, I consider that the
coeliac epithelium is ectopic, and not metastatic, and that it is
ectopic adreno-cortical tissue.   Moreover it has been previously more
extensive, and has been reduced by a chronic tuberculous process in
neighbouring glands.   The nature of the morbid process which has
occupied and expanded the right adrenal medulla is more obscure.
There is no evidence to indicate the existence of a bone-bearing
teratomatous mass.   It is considered that an effete tuberculous
process is the most reasonable explanation.   But this is in dispute
and, in view of the other anomalies of development present, degeneration
in some congenital malformation cannot be excluded as an explanation
for the adrenal medullary mass.   Either way the general argument remains,
namely, that in the right adrenal a thickened adrenal cortex has been
reduced by the evolution of a chronic process in the medulla, and that,
in the coeliac region, ectopic adreno-cortical tissue has been reduced
by a chronic process more obviously tuberculous in origin.

Ectopic adreno-cortical tissue is not unknown in adreno-virilism.
                        there
In partII‸is described an adreno-cortical tumour in the tale of the
pancreas associated with bilateral adreno-cortical hyperplasia and
Kolodny (1934) has also described a case of virilism with a carcinoma
of ectopic adreno-cortical tissue in the coeliac region.   Some ovarian
tumours associated with virilism are regarded as tumours of ectopic

adreno-cortical tissue (Novak, 1941; Thomson, 1942).   Glynn (1911)
quotes a number of cases of the allied condition of pseudo-
hermaphroditism with large adreno-cortical rests in the broad ligament.

The Ovarian Tissue.

It should be noted that a gynaecologist palpated a "gland" in
the right ovarian region, when the patient was twenty-one. Presumably
this was the right ovarian dermoid cyst.   At autopsy the patient
had abundant ovarian tissue in the wall of the right dermoid. It
cannot be postulated that the presence of the dermoid cysts interfered
with the hormonal function of the ovaries, and this contributed to the
development of virilism.   In respect of this problem it is of some
interest to recall that virilism associated with an adreno-cortical
tumour in a castrate adult woman has been recorded.   Return to normal
was obtained by removal of the tumour (Thornton, 1890; Holmes, 1925).
The dermoid cysts can only be regarded as part of a complex congenital
anomaly not specially related to the virilising process.

THE DEVELOPMENT OF MENSTRUATION.

The development of menstruation in so complete a case of virilism presents a problem in itself. Precise knowledge of the case history is not as complete as one would have wished. Nevertheless, it is clear that from puberty until the age of 22 the subject did not menstruate and that, at the end of that period, a gynaecologist reported that the cervix was rudimentary and that there was no definite uterus. From the patient's statements it would appear that menstruation had been present for many of his subsequent eighteen years of life. This seems more plausible on consideration of the autopsy findings of a small but by no means rudimentary uterus, complete with a thick layer of secretory endometrium (and a fibroid), while there is a large corpus luteum in the right ovary. I consider that the establishment of menstruation, the growth of the uterus, and the presence of a large corpus luteum in the ovary at autopsy, have been brought about by reduction of the adrenal tissue, cortical and ectopic, by the chronic obliterative processes referred to. The obliterative process.is of old standing, and this is consistent with the establishment of menstruation many years ago.

Though it is presumed that a reduction of the excessive adrenocortical tissue was sufficient to allow of the establishment of menstruation, this did not permit of a reassertion of other feminine characters. It is considered that this is mainly due to loss of plasticity of the tissues with age and indicates the hopelessness of

surgical intervention once these abnormal conditions are long developed.

THE TWO MALIGNANT TUMOURS.

The presence of two undoubtedly malignant tumours is rare. In the present case there is, in a subject of 40 years, a scirrhous carcinoma of the right breast and an epithelioma arising in the wall of an ovarian dermoid of otherwise simple structure. In both cases malignancy is undoubted. The epithelioma has produced a large mass of tumour with invasion of the colon. In the right breast there is widespread scirrhous carcinoma associated with hepatic metastases. At present sex hormones, especially the oestrogens, are playing a prominent part in the production of experimental cancer in general, and of experimental mammary cancer in particular. There can be no doubt that the patient had a very abnormal endocrine make-up and it is attractive to suggest that this hormonal upset was an important factor in the development of the two malignant growths. At the

present time the assay of hormones in the human subject is in an

embryonic stage and there has been little correlation of the

empirical data so far obtained with physiological and pathological

phenomena.   Nevertheless, the presence of two malignant tumours in

a subject with undoubted endocrine upset may at least serve as a

stimulus to the pursuit of knowledge in the field of endocrine assay

in the human subject with applications far beyond the study of rarities

such as the case described.   In regard to this question many will

recall the old practice of ovariectomy in association with mamectomy

for breast cancer.

THE NATURE OF THE ADRENO-CORTICAL

ABNORMALITY IN ADRENO-VIRILISM.

There are now many recorded cases of adrenal virilism associated with either adreno-cortical hyperplasia or tumour (Broster, 1938; Glyn, 1911). In some cases the aetiology rests with anomalies of development and there may be a familial incidence of the condition. The present case is obviously such a problem from its association with ovarian dermoids, ectopic adrenal tissue, a unilateral adreno-cortical hyperplasia; and the declaration of signs dating from puberty. On the other hand, progressive signs of virilism may suddenly arise in an apparently normal female subject with a normal puberty and menstrual history (Holmes, 1925); the condition arises apparently de novo in a previously normal endocrine system. It is abundantly evident that the primary endocrine abnormality is adreno-cortical. Thus complete return to normal can be obtained by removal of an adreno-cortical tumour (Holmes, 1925). Removal of the hyperplastic adrenal in unilateral adreno-cortical hyperplasia; or of the larger in bilateral adreno-
                hyperplasia
cortical/tumour, may result in complete return to normal (Broster, 1938), and in both cases there is at least always some amelioration of symptoms. Failure of complete return to normal does not deny the adrenal cortex a primary role as, with the passing of time, some of the changes become

irreversible as is obvious from the present case. Thus one can say
that adrenal virilism may result from some congenital anomaly
primarily affecting the adrenal cortex, or it may result from some
stimulus, unknown, acting primarily on the adrenal cortex in an
apparently normal endocrine system. That virilism may be produced
by primary endocrine abnormality other than in the adrenal cortex
is not in dispute. A syndrome indistinguishable from adrenal
virilism may be produced by the arrhenoblastoma group of ovarian
tumours (Novak, 1941). In Cushing's syndrome, which is related
to adrenal virilism, the primary abnormality may rest in either the
adrenal cortex, the pituitary gland, or, possibly, the thymus (this
thesis, part III). Clinical syndromes, involving abnormal sexuality,
have been described in pineal tumours (Zondek, 1935), and a tumour in
the interpeduncular space with a normal pituitary body (Le Marquand and
Russell, 1934-5). It is therefore understandable that in so complex a
system abnormality, productive of virilism, may arise more subtle than
present methods can demonstrate, and it is not remarkable that Broster
encountered cases of virilism in which adreno-cortical hyperplasia
was not present, and in which hemi-adrenalectomy had little effect.
But this in no way denies the view that, in virilism associated with
adreno-cortical hyperplasia or tumour, the primary endocrine abnormality
is adreno-cortical.

Writers have always had difficulty in explaining that, whereas destruction of the adrenal cortex produces Addison's disease, hyperplasia of the adrenal cortex may produce virilism in the female, and, though rarely, a condition of feminisation in the male, neither of which are in any way the opposite of Addison's disease. The theories of Grollman and of Broster & Vines implicating a special tissue, or the exclusive hyperactivity of a single function, of the adrenal cortex in virilism seek to explain this difficulty.

Grollman's Theory.

Grollman (1936) has attempted to relate the activity of the adrenal
glands in virilism to the function of a special "androgenic"/distinct
zone
from the cortex proper. In the mouse, at certain stages, there is a

prominent zone of epithelium, the juxta-medullary oR X-zone, between

the medulla and cortex proper. It has different characters from the

true cortex. A somewhat similar zone is present in the adrenal gland

of the foetus. It undergoes involution soon after birth. Goormaghtigh

(1922) claims that, in the adult human adrenal, cortical cells, which

border the medulla, resemble the cells of the androgenic zone of the

infant, and are probably a remnant of this involuted tissue. Grollman

claims that adrenal virilism is produced by a hypertrophy of, or tumour

development from the cells of this tissue. In the present case a few

zones of altered character are present in the adrenal cortex. They

occur at all levels of the cortex, otherwise the cortex presents no

abnormality. It is considered that these zones of altered character

are more probably related to the presence of the medullary mass with

attendant alterations in vascular supply to the cortex, rather than

to manifestations of a special altered function, far less the implication

of a special zone of the cortex. No illustrations of the hyperplastic

adrenals of virilism have been published showing more than a thickened

cortex proper. Furthermore some virilising adrenal tumours show a

striking resemblance to true adrenal cortex (Glynn, 1911; Holmes, 1925;

Ewing, 1928). In tumour production dedifferentiation is the rule, and

it would be a contradiction of our general knowledge to assume that such growths arose from a tissue having different characters from the cortex proper. On the other hand adreno-cortical tumours associated with virilism may be so dedifferentiated as to have little or no resemblance to cortical epithelium (Glynn, 1911; Ewing, 1928). This would be explained more reasonably by the principle of anaplasia of new growth rather than the implication of a special tissue. If such growths arose from a special juxta-medullary zone one would expect them to displace the cortex proper. No one has described an epithelial tumour of the adrenal glands with a rind of the cortical tissue proper stretched over it as one finds in tumours of the adrenal medulla. In the description of a tumour of ectopic adrenal tissue ass ciated with bilateral adreno-cortical hyperplasia (McLetchie & Scott's case) described in part III I find again no reason to implicate a special juxta-medullary zone. It is considered that present evidence does not allow of more than the assumption that the thickened adrenal cortex of virilism is an enlargement of the cortex proper and that virilising adreno-cortical tumours arise from the cortex proper.

The theory of Broster & Vines.

Broster & Vines (1938) have claimed that the essential
abnormality in adrenal virilism is the presence of fuchsinophil
granules in the adreno-cortical epithelium. They relate those
granules to a special androgenic activity of the adrenal glands
which is normally dormant in adult life, but is long persistent in
the male foetus and only transient in the female foetus. Vines used
a special staining technique on fresh adrenal tissue. I find that it
is quite impossible to obtain selective differentiation with his staining
technique and I consider that most histologists will condemn his use of
a saturated solution of aniline blue. Other histologists have failed
to get reliable results with Vines' method (I. Rannie, personal
communication; D.F. Cappell, personal communication), and it is
noteworthy that Crooke and Dible sent their adrenal glands to Vines
for an opinion (Crooke, 1935; Cohen & Dible, 1936). The difficulty
is increased when one applies the method to post-mortem tissues and,
after comparing the action of Vines' method on an adrenal gland removed
at operation, with glands from routine autopsies, I concluded that Vines's
work could only be followed up on fresh tissues. Nevertheless, in
McLetchie & Scott's case granules as fuchsinophil as fibrin were found
in the adreno-cortical epithelium (hyperplastic and malignant) using
McFarlane's picro Mallory stain (McFarlane, 1944) (This thesis part III, case I)

The claim of Broster & Vines can only be adequately assessed by the examination of fresh adrenal tissue from large numbers of normal and abnormal subjects; which has not been done. Variable results have been reported in the adrenal glands of Cushing's syndrome (Crooke, 1935). Originally Broster & Vines claimed that the presence of their androgenic granules in the adult adrenal cortex was peculiar to the pathological condition of virilism. Broster (1941) has since reported a case of feminization in a male in which the granules were claimed to be present in the adrenal cortex. From the findings in McLetchie & Scott's case I consider it possible that in the adreno-cortical epithelium (hyperplastic and tumour) of adrenal virilism and pituitary basophilism certain granules, normally present in cortical epithelium, may be prominent. I do not consider that they indicate the activity of a function normally dormant. It is unfortunate that Vines bases his side of the work to some extent on the times of differentiation of adrenal cortex and the basophil cells of the pituitary gland of the foetus. As we shall see later it is most unlikely that the foetal pituitary possesses β granules. I will designate the granules in McLetchie & Scott's case "Vines' granules" with the above reservations.

In my general argument later I will take the view that the adreno cortical hyperplasia of virilism is a simple hyperfunction.

The pituitary gland in

Adrenal Virilism.

In a case of adrenal virilism, unassociated with any additional clinical manifestations which might suggest the development of Cushing's syndrome, Crooke (1935) found no pituitary abnormality. Whereas in all cases of Cushing's syndrome he found a conspicuous degree of cytoplasmic hyalinization of the basophil cells. Crooke considered that this was the fundamental distinction between the two conditions. In the present case no pituitary abnormality was found. It does not, however, carry full weight in favour of Crooke's view, since the excessive adrenal tissue had been destroyed. This would conceivably result in a reversal of any secondary pituitary change attributable to the adrenal lesion. Anderson, Hain, and Patterson(1943). confirm Crooke's view in a single case of virilism associated with an adreno-cortical tumour, and Crooke's contention has never been denied by any worker. The relationship of adrenal virilism to pituitary basophilism will be fully discussed after the enquiry into the morbid basis of the latter condition in the next part of this thesis.

In the argument related to case I, part III, it will be shown that basophil-cell changes in the pituitary gland may be associated, in the beginning, with a clinical condition of virilism, but obesity soon supervenes (Hare's and Lescher's cases), and a full Cushing's syndrome is manifest ultimately, though, if the subject dies at an early stage there may be no more than amenorrhoea and hypertrichosis (case I, part III).

On general grounds it is considered that in the present case the maintenance of a spare virile habitus for so long a period is tantamount to there never having been demonstrable pituitary abnormality.

S U M M A R Y.

A case of virilism is described.   The condition developed at
puberty in an apparently normal female child.   While male character-
istics developed to a striking degree, the patient menstruated in the
latter part of her life.   Death occurred at the age of 40 years from
peritonitis.   At autopsy the following abnormalities were found :-
(1) The right adrenal cortex was thickened and there was ectopic
adreno-cortical tissue in the region of the coeliac axis;   both tissues
had been reduced by a chronic obliterative process.   These were
considered to be the essential abnormalities determining the condition
of virilism, and their reduction the factor which allowed the development
of menstruation, and explained the finding of a large corpus luteum in
the right ovary, and a thick endometrium at autopsy despite the striking
male facies and habitus.   (2) Mammary carcinoma with hepatic metastases;
bilateral ovarian dermoid cysts;   epithelioma arising in the wall of the
right dermoid and infiltrating the colon with production of peritonitis
The problem of the inter-relationship of all of these abnormalities is
discussed.   Attention is drawn to other conditions which may give rise
to masculinization in the female.   Theories as to the nature of the
adreno-cortical abnormality in adrenal virilism are discussed.

P A R T   II.

Figs. 1 & 2.

Fig. 1.   Section of part of right adrenal showing thickened
             ridge of adrenal cortex and the calcified and ossified
             medulla.

             X.2.   H. and E.

Fig. 2.   Area of cortex of right adrenal.   Note the disturbance
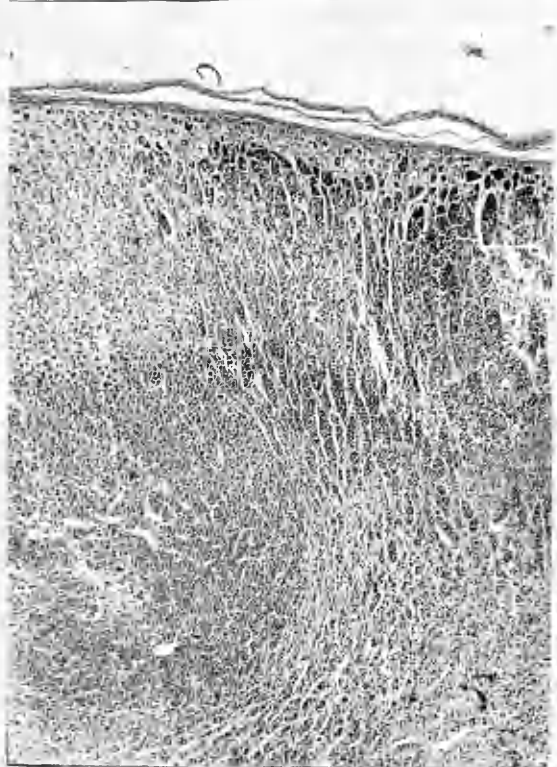   in architecture.

             X.40.   H. and E.

PART II.

Figs 1 and 2.

Fig.1.

Fig. 2.

P A R T II.

Fig. 3.   Para-aortic gland showing node of calcified effete

tuberculosis on left and foci of epithelial tissue

(dark masses) circumscribed by fibrous capsules

(white rings).

X5   H. & E.

Fig. 4.   Area from a dark circumscribed node of fig. 3 showing

ectopic adreno-cortical epithelium in loose columns
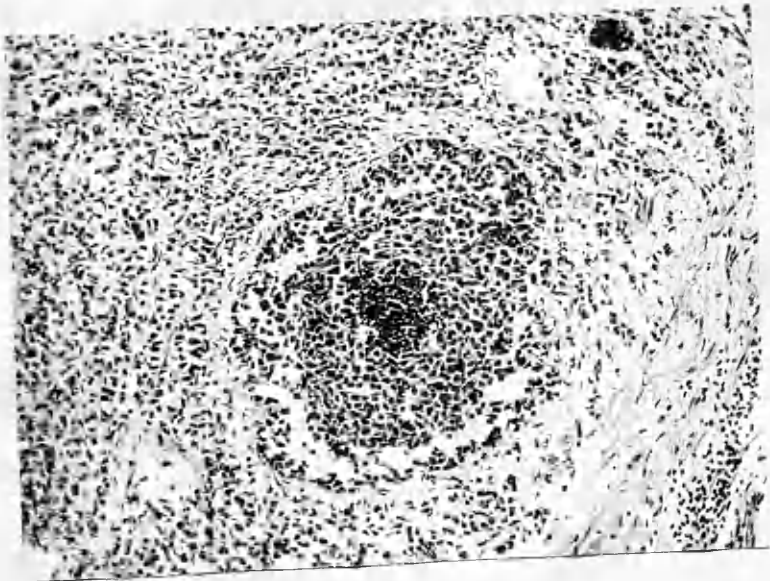
and in whorl encircling lymphocytes.

X150   H. & E.

P A R T II.

Figs. 3 & 4.

Fig. 3.

Fig. 4.

P A R T   II

Fig. 5.   Tangential cut of large corpus luteum in wall of

R. ovarian dermoid.   Note the well developed

layer of lutein cells and central clot.

Xc.5   H. & E.

Fig. 6.   Part of large corpus luteum of R. ovarian dermoid

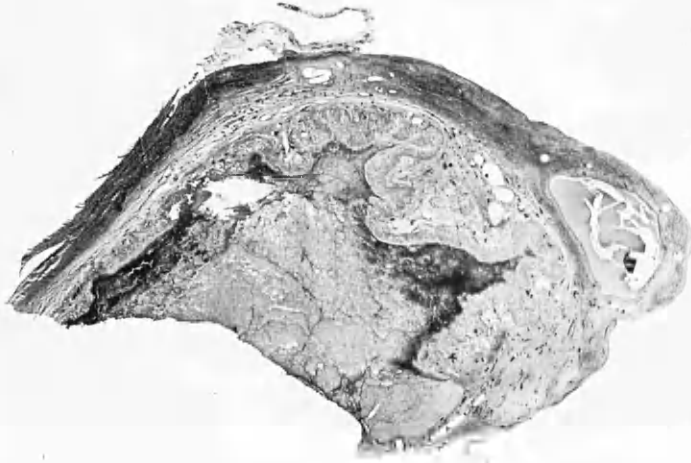X.10   H. & E.

74.

P A R T II.

Figs. 5 & 6
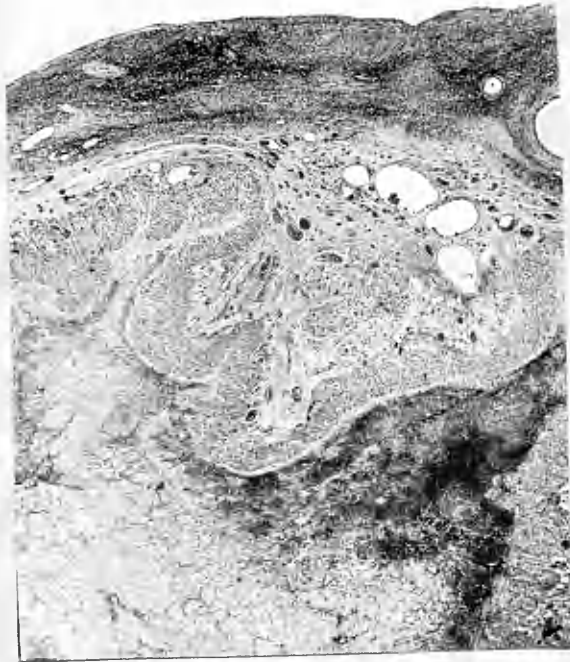
Fig. 5

Fig. 6

P A R T   II.

Fig. 7. Epithelioma invading muscular coat of
colon.

X.150.   H.& E.

Fig. 8. T.V. Section of uterus.   Note the thick
endometrium.   Part of the fibroid can be
seen on extreme right.

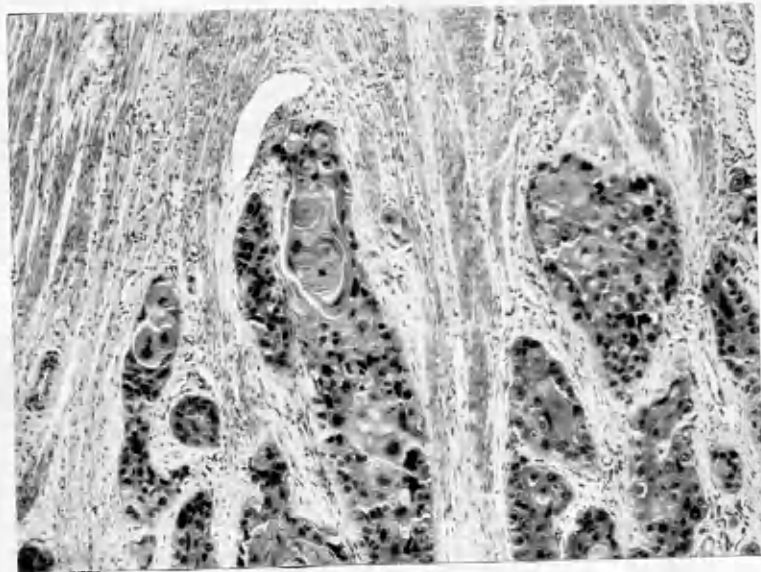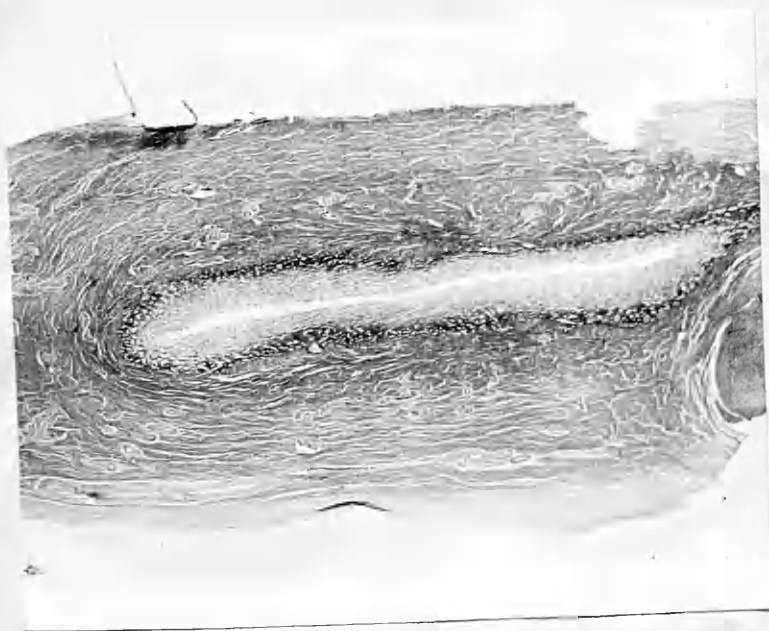X.4   H.& E.

<u>P A R T   II.</u>

Fig. 7.

Fig. 8.

P A R T   II.

Fig. 9.   Right breast showing malignant infiltration of dense

fibrous stroma and duot epithelial hyperplasia.

X.15   ·   H. & E.

Figs. 10 and 11.   Left breast showing dense stroma and

epithelial hyperplasia in cystic duots.

X.15 and X.40   H. & E.
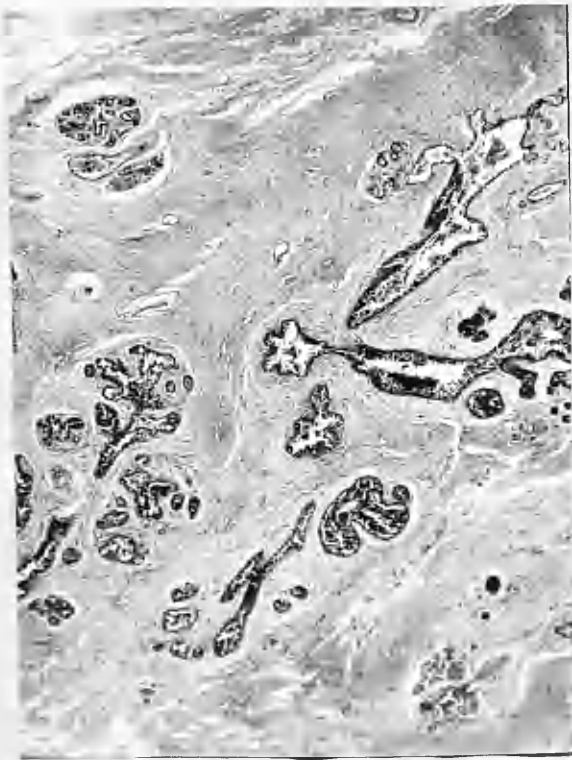
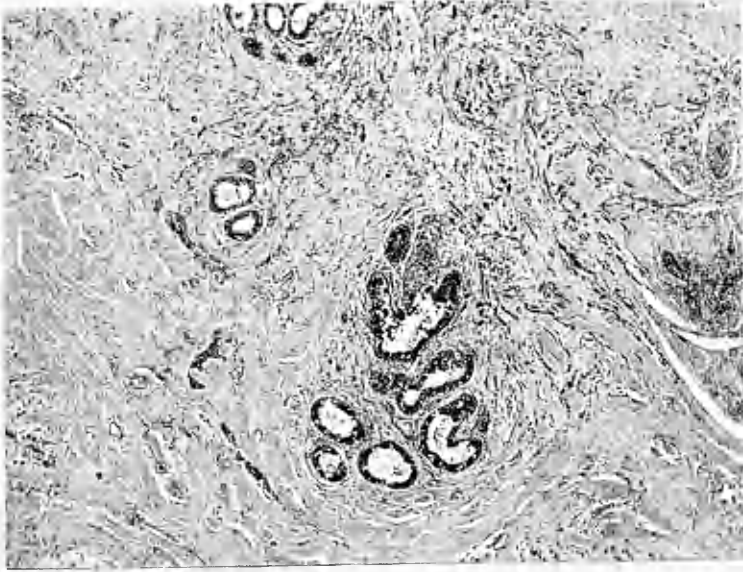# P A R T   II.

Fig. 9.

Fig. 10.

Fig. 11.

STUDIES ON THE RELATIONSHIP OF THE BASOPHIL CELLS OF THE

HYPOPHYSIS AND·THE ADRENAL CORTEX.

P A R T   T H R E E

THE PITUITARY BASOPHILISM SYNDROME

OF HARVEY CUSHING.

PART   THREE

THE PITUITARY BASOPHILISM SYNDROME

OF HARVEY CUSHING.

I N T R O D U C T I O N.

Since Cushing described the syndrome of pituitary basophilism
(Cushing's syndrome) many cases of the condition have been published.
The syndrome is characterized by an obesity sparing the limbs, by
marked hypertension, glycosuria and hyperglycaemia, osteoporosis,
characteristic cutaneous striae, polycythaemia, amenorrhoea and
hypertrichosis in the female, impotence in the male, asthenia and
diminished resistance to infection (Cohen & Dible, 1936).   Pathological
findings in the condition have been very varied in cases which have been
clinically indistinguishable (see Crooke's (1935) series.)   By 1936
the following abnormalities had been described: basophil adenoma and
basophilia (relative increase of pituitary basophil cells); adreno-
cortical hyperplasia, adenoma, carcinoma; and thymic carcinoma associated
with adreno-cortical hyperplasia.   In a few cases neither adreno-cortical,
thymic, nor anterior pituitary tumours were recorded (Oppenheimer, Globus,
Silver & Shaskin 1935; Freyberg, Barker, Newburgh & Coller, 1936; Crooke
1935; Cohen & Dible, 1936).

It seemed remarkable that such diverse pathological findings should
be associated with one clinical condition until Crooke (1935) found an
abnormality common to all.   Crooke investigated the pituitaries of a
series of twelve (now fifty - personal communication) cases of Cushing's

syndrome, many of which had been reported elsewhere. The series
contained examples of all lesions then known to be associated with
the syndrome. In every case he found a conspicuous degree of
hyalinization of the cytoplasm of the basophil cells of the anterior
pituitary; this change was found in only a few cases (9) of a large
non-basophilism series (350), and there only in a partial form in a
very few cells; in these latter cases it was not constantly associated
with any condition. Crooke concluded that basophil-cell hyalinization
was the abnormality of fundamental significance in Cushing's syndrome.
Since then a specific nuclear abnormality associated with hyalinization
of the basophil cells has been described (Severinghaus, 1938).

It is my purpose to describe three cases involving the morbid
process of pituitary basophilism. The first case is associated with
a carcinoma of an adreno-cortical rest. The second is associated with
a basophil adenoma of the anterior pituitary gland. The third case is
not associated with tumour. In this case another complex basophil-cell
abnormality in addition to basophil-cell hyalinization is present. The
abnormality appears to be the same as that described by Severinghaus,
but I place an entirely different interpretation on the appearances. It
is, therefore, my intention to describe and illustrate the hypophysis of
this case in detail. For control purposes a series of hypophyses from
conditions other than basophilism have been examined. The control series
will be included in the account of the third case.

P A R T   T H R E E

C A S E   I

CARCINOMA OF AN ADRENO-CORTICAL REST

ASSOCIATED WITH HYPOPHYSEAL ABNORMALITY.

(McLetchie & Scott's case).

The clinical history of this case was provided by Dr. L.D.W.
Scott.   The pathological work and argument are by myself.   This
case is being published in collaboration with Dr. Scott and will
be referred to in later discussions as McLetchie & Scott's case.

CASE I.

DESCRIPTION OF CASE.

Clinical History.

A female shop assistant aged 26 years was seen in July 1940, complaining of an acneform eruption of the face, hirsuties and coarsening of the features. She had first noticed the acne and the alteration in facial appearance six weeks before. Her previous health had been good and she had no complaints other than concern over the sudden change in her appearance. Her previous menstrual history was normal. She had missed two menstrual periods since the development of the acne. There was no relevant family history.

On examination, the facial features were coarse, the complexion was slightly dusky, and acne vulgaris was present over the face and brow; there was an obvious moustache and a slight growth of hair over the beard area. The hair of the head was normal. A normal degree and distribution of adiposity was present over both trunk and limbs; striae were not seen. The breasts were small. The pubic hair was of female distribution. The external genitalia were normal and there was no enlargement of the clitoris. The blood pressure was 150/90 mm.Hg, and there was no apparent cardiac enlargement. Examination of the abdomen did not reveal any palpable tumour masses.

While awaiting admission to hospital, she reported regularly and

the condition appeared to be stationary, but in the sixth week after
the original consultation she suddenly became comatose and was sent to
the Western Infirmary.   On admission she was found to be comatose and
could be roused only with difficulty; she refused to speak and resented
examination.   By clinical examination the liver was found to be palpable
one finger-breadth below the costal margin, but no other abnormality was
detected.   The blood urea was 40 mg./100 ml., and the blood sugar was
80 mg./100 ml.   The cerebrospinal fluid was not under increased pressure
and contained no increase of protein or cells.   The fundi were normal.
She remained in this condition for two days, but on the third day the
temperature and pulse rate began to rise, a temperature of 103° F. and
a pulse rate of 120 beats/min. being reached on the fourth day.   On
the fifth day she had occasional twitchings of the face and left arm,
and died after a generalized convulsion.   Post-mortem examination was
carried out 12 hr. later

Post-mortem examination.

Apart from the findings in the endocrine glands, which will be described in detail, the following changes were noted. The liver (1500 g.) was permeated with secondary carcinomatous deposits to such an extent that there was little liver tissue remaining in the left lobe. Much of the hepatic tumour was necrotic. A number of lymphatic glands in the neighbourhood of the pancreas were also invaded by secondary tumour growth. The uterus was normal, as were the kidneys apart from slight congestion. The heart (320 g.) showed slight hypertrophy of the left ventricle. The vertebrae and left femur appeared normal on section, fatty marrow being present in the latter and no bony change being evident. A localized area of congestion and dilatation of the superficial veins was present over the right occipital lobe of the brain. Subcutaneous and abdominal fat were of average amount.

The endocrine glands.

On opening the abdomen, considerable fibrous adhesion was noted in the neighbourhood of the pancreas, towards the tail of which a hard nodule could be felt. The capsule of the left adrenal was adherent to the pancreas, and owing to the difficulty of making out precise relations, the pancreas, kidneys, adrenals and aorta were removed together, fixed in Kaiserling's fixative solution and subsequently dissected. The hard nodule was then seen to be a mass of tumour tissue, 2.5 cm. in diameter, replacing the substance of the pancreas near its tail. An irregular fibrous capsule appeared to invest the tumour, separating it from the pancreas medially and from the left adrenal laterally. In order to determine the exact relationship of the adrenal gland to the tumour, large blocks of tissue comprising pancreas, tumour, and left adrenal in continuity were prepared for histological examination. When this was done it was evident that the tumour lay completely outside the capsule of the left adrenal (Plate 1, fig. 1)

The pancreas.

The pancreas, apart from the tumour, appeared to be normal.
Histological examination of the tumour showed alveoli filled with
cubical cells and supported by a delicate fibrous stroma; in some
areas the structure resembled that of the zona glomerulosa of the
adrenal cortex; in others the cubical cells were arranged in long
columns, the structure resembling the zona fasciculata. Thin-walled
vessels were present, but the growth was not very vascular (Plate 1, fig. 2).
The epithelial cells had finely granular cytoplasm and single, oval,
hyperchromatic nuclei; a few large nuclear forms were present but
aberrant forms and mitotic figures were not found. The secondary
deposits in liver and lymph glands had also this simple adenomatous
structure. A dense fibrous capsule invested the primary tumour, but
in places tumour cells had infiltrated beyond the capsule and were
arranged in masses of small polygonal cells with dense hyperchromatic
nuclei and very scanty cytoplasm. In general, the tumour corresponded
to the malignant adreno-cortical adenoma described by Ewing (1928).

The adrenals.

The adrenal glands were larger than normal, and had notably rounded edges. The right adrenal weighed 12.8 g. and the left 16 g. (allowance being made for a wedge left adhering to the pancreas). The increase in size involved the cortex of each gland (cortical hyperplasia) and the medulla on each side was normal in size and appearance. The deposition of fat and doubly refracting material in each cortex appeared normal.

Since it has been claimed that the essential evidence of androgenic overactivity in the adrenal cortex is the demonstration of fuchsinophil material in the epithelial cells (Broster & Vines, 1938), a search for this was made in the adreno-cortical tissue, the primary tumour in the pancreas, and the glandular and hepatic metastases. Small amounts of highly fuchsinophil material were found in the epithelial cells in all sites with the exception of the hepatic metastases. The staining technique employed was McFarlane's picro-Mallory stain (see this thesis, part II, for comment). In the adrenal cortex the material was present in small scattered areas in all three zones. Similar granules were not found in twelve adrenal glands from routine autopsy cases. In the primary tumour and glandular metastases it was found in scattered areas, in some of which it was plentiful, and presented a striking appearance, being disposed in discrete granules and also sharp-edged crescentic inclusions. The fuchsinophil material had no obvious relation to

autolytic or necrotic processes, being on the contrary absent in the necrotic and autolysed hepatic secondaries. According to Vines, the substance responsible for this staining reaction is labile and tends to diminish as the result of post-mortem autolysis; it is, therefore, probable that the fuchsinophil reaction would have been more plentiful had the tissue been fresher, and had the blocks of tissue examined received primary fixation. Apart from general enlargement and the presence of the granules of Vines, I could find no difference in the structure of the adrenal cortex from that of the normal adult cortex.

The pituitary gland.

Serial sections of the gland were examined. The histological technique and methods of enumeration and measurement of cells employed are described elsewhere (this thesis, part I).

The anterior pituitary was of normal size and gross structure.

The basophil cells. An enumeration of the cell types was carried out according to the method of Rasmussen. The relative number of the basophil cells (14.9%) was slightly above the maximum normal (13.6%)

and well above the mean for the age and sex of the subject, as defined

by Rasmussen (1929, 1933).   Apart from the actual enumeration, the

basophil cells appeared much more numerous than in any other hypophyses from

young subjects which I have examined.   All the basophil cells seemed of

large size, but measurement showed that they were in fact within normal

limits (see this part, Case III, page 125).   The majority of the basophil

cells showed the more extreme forms of cytoplasmic hyalinization described

by Crooke (1935), the normal granular cytoplasm being replaced by wax-like,

homogeneous, refractile cytoplasm.   Many of the cells were completely

hyalinized, others showed partial hyalinization with granules persisting

as a peripheral rim and as small juxta-nuclear collections.   The persisting

granules were mainly very small and feebly staining.   Normal granular

basophil cells were rare, only one or two being encountered in an occasional

section.   The nuclei of the basophil cells appeared normal even in the

completely hyaline cells.   Basophil-cell vacuolation was inconspicuous.

The extreme degree of the cytoplasmic alteration in the basophil cells is

evident from the illustrations (Plates 2, 3 ,figs. 3, 4).

The size and relative proportion of the acidophil and chromophobe

cells were well within normal limits.   These cells showed no cytological

abnormality.

Adenomata.   In the inferior part of the gland there were three small

adenomata, all about 1.5 mm. in diameter.   The term adenoma is used

in the conventional sense adopted in hypophyseal histology - focal

accumulations of a single cell type with loss of the normal acinous

plan.   Two of the adenomata were of papillary structure, the cell

type being similar to that of the normal chromophobe cells of the

gland.   They had indefinite edges merging imperceptibly into normal

parenchyma.   In the third adenoma there was a central zone of colloid

material, similar to the colloid of the cysts of the pars intermedia,

surrounded by a cellular zone sharply demarcated from surrounding

anterior pituitary structure, with compression of adjacent acini

(Plate 4, fig.5).   The cells of the adenoma were loosely arranged

with little stromal support, and with no suggestion of polarity.   The

cells had large oval vesicular nuclei and scanty irregular finely

granular cytoplasm;   some cells appeared as almost bare nuclei.   The

staining reaction of the cells differed slightly from the chromophobe

cells of the gland and appeared to conform to the 'indeterminate'

adenoma described by Graef, Bunim & Rottino (1936) in a similar type of

case, but the degree of post-mortem change present does not justify a

special descriptione of the tinctorial affinities of the cells.   I will

refer to the two chromophobe adenomata and the 'indeterminate' adenoma

collectively as the chromophobe adenomata.

The para intermedia was relatively large with many duct-like spaces

lined by one or two layers of columnar cells with faintly granular

cytoplasm. In places these cells showed differentiation to cells indistinguishable from anterior-lobe basophil cells and many of the dubts were packed with basophil cells, many of which showed hyalinization. The posterior lobe. The gross structure appeared normal. Wandering basophil cells were numerous in the area adjacent to the pars intermedia. The majority were hyalinized. Deep in the posterior lobe there were two irregular and incomplete gland-like spaces lined by columnar cells similar to those in the pars intermedia. These cells could be seen differentiating into cells indistinguishable from anterior lobe basophil cells. Wandering basophil cells produced from this area were scattered in the nervous matter around the spaces. Many of these basophil cells showed hyalinization.

Other endocrine glands.

The thyroid gland showed normal involutional tissue. In the right ovary primordial follicles, corpora lutea and corpora albicantes were present; the left ovary was represented by a single cyst lined by flattened cubical cells. No thymic tissue was found in the mediastinum.

Summary of post-mortem findings.

(1)   Carcinoma in substance of the pancreas with hepatic and glandular metastases.

(2)   Bilateral adreno-cortical hyperplasia.

(3)   Fuchsinophil (androgenic) granules of Vines in epithelium of both tumour and adrenal cortex.

(4)   Anterior pituitary - Basophilia (relative increase in basophil cells), extreme basophil-cell hyalinization, three chromophobe adenomata.

D I S C U S S I O N

The tumour in the pancreas.

The tumour has the structure of a malignant adreno-cortical adenoma; fuchsinophil granules are present, a common finding in the adrenal-cortical tumours associated with masculinization (Broster & Vines, 1938). The tumour is present in a site where adreno-cortical rests have been shown to occur (Ribbert, quoted by Glynn, 1911) and is associated with a group of symptoms similar to those commonly present in cases with adreno-cortical neoplasm. Both macroscopically and microscopically the tumour is shown to have no direct connexion with the adrenal glands. Accordingly it is an adenocarcinoma of an adreno-cortical rest. Hyperplasia or tumour in adrenal rests is uncommon and their association with sexual alteration is exceedingly rare (this thesis, part II).

Taking into consideration the short course of the disease in this case, it seems likely that the primary condition is the carcinoma of the cortical rest. I consider that the bilateral adreno-cortical hyperplasia is produced by the same stimulus (unknown) which induced malignant change in the adreno-cortical rest. Reasons will be given later for assuming that the hypophyseal changes are secondary to the adreno-cortical abnormality. Grollman (1936) has propounded the theory that hyperplasia or tumour of the adrenal cortex associated with masculinization is produced from a special juxta-medullary zone

of the adrenal cortex.   I have not found any indication from the
morphology of the enlarged adrenals and the adreno-cortical tumour to
indicate the implication of such a tissue in the morbid process of the
present case.(see this thesis, part II).

The hypophyseal abnormality.

Basophil-cell hyaliniation.   Crooke (1935) has shown that a conspicuous
degree of basophil-cell hyalinization is the only constant lesion in,
and is specific for, Cushing's syndrome whether there be a tumour of
the basophil cells, of the adrenal cortex, or of the thymus, and, in my
opinion, all subsequent evidence appears to substantiate this view (this
thesis, part III, final discussion).   In this case the hyalinization
is greater in degree than has ever been previously recorded;   this
finding is possibly in parallel with the large amount of abnormal
adreno-cortical tissue both as tumour and cortical hyperplasia.   Crooke
specially noted that, despite the replacement of the basophil granules
by the refractile hyaline cytoplasm, the nucleus of the hyaline basophil-
cell showed no abnormality.   The apparent normality of the nucleus is a

striking feature in this case despite the remarkable degree to which

hyalinization has proceeded so that the nuclei appear embalmed in a

dense wax-like cytoplasm.   Since the nucleus was normal Crooke concluded

that basophil-cell hyalinization was an attribute of some special alteration

in basophil-cell activity rather than a stage of cell death.   In this

case the derangement of basophil-cell activity is of extreme degree

and it cannot be denied that the accepted morbid basis of Cushing's

syndrome is present

Basophilia.   Basophilia has been previously recorded in a case of adreno-

cortical tumour associated with Cushing's syndrome (Lescher & Robb-

Smith, 1935).   Basophil-cell hyalinization was also present in this

case (Crooke, 1935).   In connexion with the importance of this findinng,

the active development of basophil cells from the pars intermedia and

associated epithelial inclusions found in the posterior lobe in the

present case is of importance.   While columns of wandering basophil

cells in the posterior lobe are often found to be continuous with the

epithelium of the pars intermedia, I have not observed in my control

series active differentiation of basophil cells from large numbers of

pars intermedia cells as obtains in the present case.   Lewis & Lee

(1927) have already drawn attention to clefts in the posterior lobe

lined with epithelial cells similar to my finding.   I have also

encountered these structures in the control series but unassociated

with production of basophil cells.   I thus consider that the active

development of basophil cells from the pars intermedia and allied
tissues is associated with the general basophilia and is evidence of
a stimulus for increased basophil-cell activity which is part of the
morbid process.

The chromophobe adenomata.   Chromophobe adenomata have been recorded
in cases with a similar pathology to our own, but associated with a
symptomatology more obviously belonging to Cushing's syndrome (McCullagh
& Cuyler, 1937; Graef et. al. 1936).   Close (1934) records the finding
of chromophobe adenomata in 10% of pituitary glands from routine autopsies.
He does not distinguish between true chromophobe and 'indeterminate'
adenomata.   Some adenomata which I have encountered in routine autopsy
material have been 'indeterminate' in character and it is probable that
Close's series contained examples of this type.   Graef et al. have
pointed out that the so-called chromophobe adenomata are rare in young
subjects, and that consider that the adenoma in their case is associated
with the morbid process and is not an incidental finding.   Since,
however, these adenomata are sometimes found unassociated with adrenal
tumour, and are not constantly associated with adrenal tumours, it would
appear that they can have only some secondary significance in relation
to the morbid process under discussion.   Cramer & Horning (1936) and
others have produced large chromophobe adenomata in the hypophyses of
rats by injections of oestrogen.   The chromophobe adenomata found in
cases of adrenal tumour may be correlated with this experimental finding,
in that large amounts of oestrogen have been shown to be excreted in such

cases (Frank, 1934; Saphir & Parker, 1936).   In Graef's case the

oestrogen excretion exceeded that of pregnancy.

The relationship of the clinical to the morbid anatomical findings.

The comatose state followed by sudden death was apparently due

to hepatic failure, coma and delirium being not uncommonly associated

with massive necrotic secondary carcinoma of the liver, though this

sudden termination preceded by convulsions is unusual and the possibility

that the coma was due to the extreme basophil-cell changes cannot be

excluded.

At no time did the patient's condition suggest that a morbid

process was at work as extensive as I have found, and on clinical grounds

the patient's condition might be regarded as one of virilism showing only

coarsening of the features, hirsuties, amenorrhoea, slight hypertension

and dusky cyanosis.   The symptomatic history of the disease was,

however, only of 12 weeks' duration and my view is that had the

patient lived longer more obvious signs of Cushing's syndrome would

have developed as a result of the specific pituitary change.   With

regard to the development of Cushing's syndrome in cases of adreno-

cortical tumour, it is of interest to consider the course of two cases

pathologically similar to ours.

Hare, Ross & Crooke (1935) reported the case of a female aged 31 years in whom the first significant clinical signs were changes in facial appearance, with obesity and the growth of a beard; one year later amenorrhoea followed a period of metorrhagia. Later, a full Cushing's syndrome developed and the patient died after a surgical operation for removal of an adreno-cortical carcinoma. Post-mortem examination showed hyalinization of the basophil cells. This clinical picture of initial virilism followed by Cushing's syndrome was also shown by the patient reported by Lescher & Robb Smith (1935). In this case, in a female aged 32 years, menstruation ceased abruptly, obesity appeared and was followed by the growth of a beard and moustache. When examined three years after the onset of the disease, she presented the typical appearance of Cushing's syndrome with obesity, hirsuties, plethoric appearance, dusky cyanosis, hyperglycaemia, polycythaemia, hypertension and marked osteoporosis. As in the previous case, death occurred after the removal of an adrenocortical carcinoma and again hyalinization of the basophil cells was found at autopsy (Crooke 1935). It would appear that the present case differs from these mainly in its short duration and only the extreme malignancy of the tumour prevented a similar course being taken. The three cases are presented as illustrating a possible sequence in the genesis of Cushing's syndrome.

On general principles, it is most unlikely that such a profound derangement of basophil-cell function as is manifested by the extreme

hyalinization in our case would only be associated with manifestations of virilism.   On the other hand, it cannot be doubted that hypercortico-adrenal conditions of long duration may be only associated with manifestations of virilism without developing evidence of multi-glandular disease, though this is more true of cases with adreno-cortical hyperplasia than with tumour (this thesis, part I).   In those cases the pituitary gland shows no demonstrable abnormality.

It will be argued later in this thesis that in Cushing's syndrome associated with adrenal tumour the sequence of events is as follows: the adreno-cortical hyperactivity results in basophil-cell overactivity which may be associated with basophil-cell increase (basophilia);   basophil-cell hyalinization is a cytoplasmic change consequent on basophil-cell overactivity.

It is evident from this case that a profound basophil-cell response may be rapidly elicited in the anterior pituitary by a hyperadreno-cortical condition without much external evidence, and it is apparent from Lecher's and Hare's cases that it takes time for unequivocal signs of multiglandular upset to develop.

Up to this point I have passed over the possibility of the hypophyseal changes in this case being antecedent to the adreno-cortical abnormality. This would be unlikely if return to normal could be obtained after removal of an adreno-cortical tumour in a case where the symptomatology left no doubt that the morbid process had passed beyond that of pure hypercortico-adrenal
stimulation.

Unfortunately, no case with the full clinical picture of
Cushing's syndrome has survived operative removal of the
tumour.   Walters, Wilder & Kepler (1934) have recorded a series
of cases under the heading of "The Adreno-Cortical Syndrome" in at
least one of which complete return to normal was obtained after
removal of an adrenal tumour.   The symptomatology of their cases
is much more suggestive of early Cushing's syndrome than of
uncomplicated hypercortico-adrenal stimulation and it is considered
that one must infer that the basophil-cell alterations of Crooke
can be secondary to an adrenal tumour and are reversible.

The importance of the general findings in this case will be
included in the final discussion on Cushing's syndrome.   The relation
of adrenal virilism to Cushing's syndrome will also be discussed later.

S U M M A R Y.

A case is described of the sudden development of a condition
of mild virilism in a previously healthy and apparently normal
adult female.   Sudden death followed a brief period of coma three
months after the onset of the first signs.   Post-mortem examination
showed a carcinoma of an adreno-cortical rest and bilateral adreno-
cortical hyperplasia.   In the anterior pituitary gland extreme
basophil-cell hyalinization, basophilia, and three chromophobe
adenomata were found. It is contended that had the patient lived
longer a fully developed Cushing's syndrome would have been manifest
in contrast to the simple condition of virilism of life-long duration
manifested by the case described in part II.   The alterations in the
pituitary gland are considered to be secondary to the adreno-cortical
abnormality.

P A R T   III

Plate 1.

Fig. 1.   Low power view of section of pancreas and adjacent
tissue of left adrenal gland. Note that the tumour
takes up the tail of the pancreas (dark mass on left)
and is separated from the adrenal gland, on right, by
fatty areolar tissue.   Note the circumscribed area
in the centre of the tumour indicating the probable
site of the original rest tissue

                                                            H & E

Fig. 2.   A higher power view of tumour of fig. 1.
Note the resemblance to adrenal cortex.

P A R T   III

Plate 1

TUMOUR OF PANCREAS (Case 1)

Fig. 1.

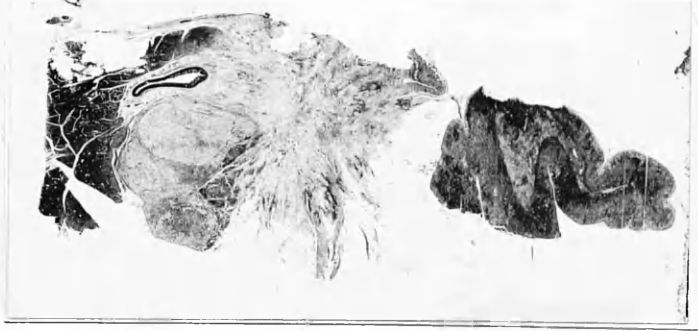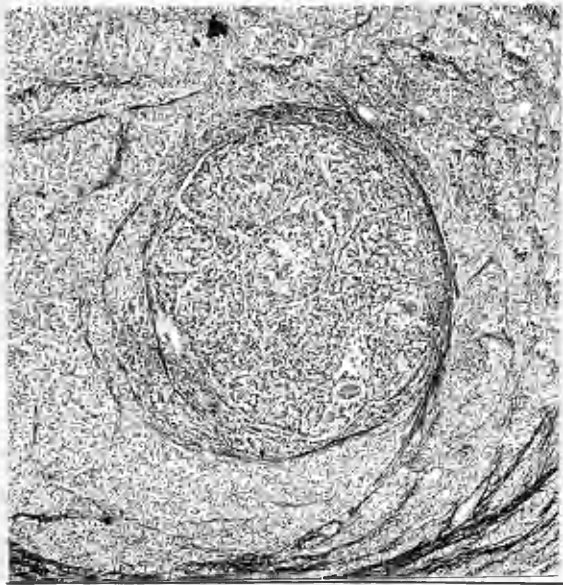Fig. 2.

P A R T   III

Plate XI 2.

Fig. 3.

A - field of anterior pituitary gland of Case 1.

B - field, rich in basophil cells, of a normal anterior

pituitary gland with same degree of post portem change

as A and stained by same method.   Compare with A.

NOTE in A the extreme degree of hyalinization of the basophil

cells seen as pale grey cells.   Peripheral granulations can

be made out as dark rims.   Areas of juxta-nuclear granulation

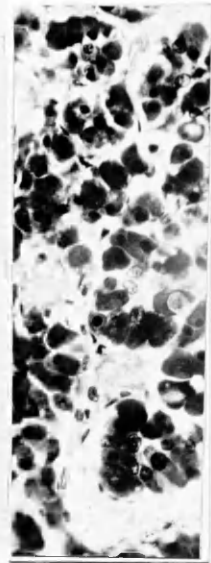and vacuolation can be made out in the hyaline cells.

NOTE the normal appearance of the nuclei of the abnormal cells.

A 210   METHOD IIA

P A R T   III

Plate II 2.

Fig 3

(A)   Field of anterior pituitary gland of case 1
with control strip of normal anterior pituitary
gland (B).
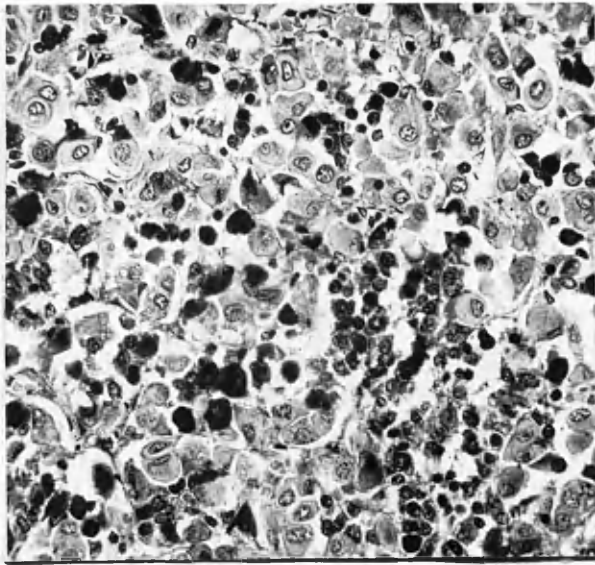
A   B
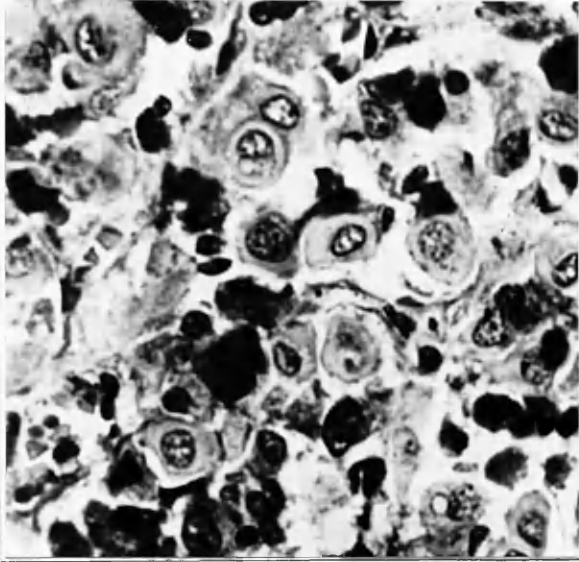
X 210   METHOD IIA

P A R T   III

Plate 3.

Fig. 4.

Enlargement of area of fig. 3(A).

NOTE the normal appearance of the nuclei of the
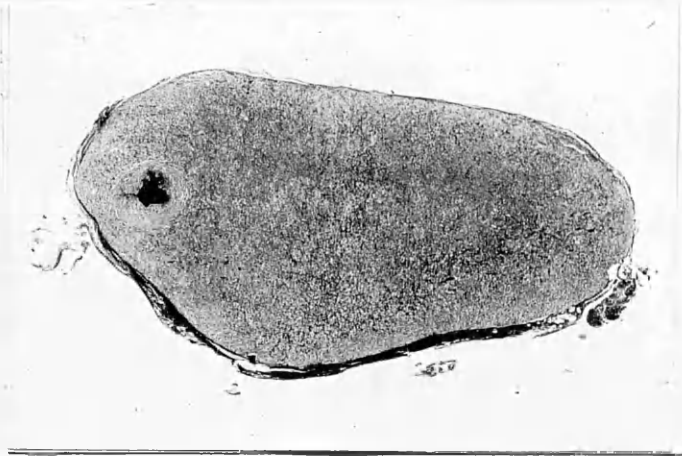
hyaline cells.

METHOD IIA

P A R T   III

Plate 4

Fig. 5.

"Indeterminate" adenoma of anterior pituitary gland,
Case 1.

Low power view of complete section of anterior pituitary
gland.   NOTE the small adenoma with central zone of
colloid (black mass).

C A S E   II.

THE REPORT OF A CASE OF CUSHING'S SYNDROME ASSOCIATED WITH A BASOPHIL ADENOMA OF THE ANTERIOR PITUITARY GLAND.

Clinical details of this case were kindly supplied by Dr. W. Gray.

Clinical history.

Before the development of her fatal illness the patient was a normal female of medium nutrition.   Menstruation started at 11 years and was regular until 1936 when it ceased.   The patient was married and had borne three normal children.   All the pregnancies were uneventful.   In 1938 the patient was under observation at Stobhill Hospital for three months, as she had become "emotional" and feared that she would do harm to her children. She was dismissed as "improved."   Two years later she was re-admitted to Stobhill Hospital with the same complaint.   It was now obvious that she had endocrine disease.   The patient, aged 37, lethargic and emotionally unstable, exhibited the following signs :-

<u>Signs of masculinization</u>. The hair of the head was dry. There

was a heavy growth of hair on the chin and moustache area. The

patient's bodily habitus was that of a rather stout male subject.

Her features were coarse and of male type. The voice was harsh.

The abdominal and genital hair and the hair on the limbs were of

female type and distribution. The breasts and clitoris appeared

normal. All the abnormal signs dated from 1938.

<u>Hypertension</u>. The systolic blood pressure varied from 190 to 240

mm. Hg., with occasional falls to 140 mm. Hg.

<u>Glycosuria</u>. The urine contained from 1 to 2% glucose. The
<u>and hyperglycaemia</u>.
                      fasting blood sugar was 190 mgm. per cent and after

the ingestion of 50 gm. of glucose rose to 300mgm. per cent within

an hour. When attempts were made to control the blood sugar with

insulin the mental and physical condition deteriorated.

<u>Pigmentation</u>   There was a dusky cyanosed facies and
<u>and abnormal coloration</u>.
                      blotchy red colouration of the abdominal

wall. The limbs showed a dark brownish-blue pigmentation.

A hypercortico-adrenal state was considered to be the basis

of the condition, though upset of pituitary function could not be

excluded. The pituitary fossa was normal radiologically. It

was decided to visualise the adrenal glands radiologically using

retroperitoneal injections of air. When this was carried out the

right side was found to be normal. The films of the left side were

not good and the examination was repeated three days later.   The

left side was found to be normal.   The patient died suddenly on

the evening following the last injection.

          Dr. F. E. Reynolds has kindly supplied the following post-

mortem report :-

POST MORTEM FINDINGS.

General Appearances.   The body was that of a well developed and

quite well nourished woman;   within the body a large amount of

fat was found.   The general appearance have been recorded in the

clinical notes but at the time of conducting the post mortem

examination pigmentation of the skin was not apparent although

there was perhaps some excess of pigment in relation to the nipples.

Body Cavities. A few old fibrous adhesions passed between the surface of the left lung and the chest wall. No excess of fluid was found in any of the body cavities and the only one in which fibrous adhesions were present was the left pleural cavity. The fatty tissues around both kidneys contained many bubbles of air.

Respiratory System. The fauces were not congested and the air passages were not obstructed. Both lungs were congested and especially in their lower lobes. In addition, two recent haemorrhages had occurred into the pulmonary substance of the left lower lobe in relation to the root of this lung. The haemorrhages were small in size and of no practical moment.

Cardio-vascular System. A large number of "flame-like" haemorrhages had occurred under the endocardium lining the cavity of the left ventricle. The mitral cusps showed patches of degenerative change in their substance and these were of a greater degree than is usual to find in women of this patient's age. No lesions of the cusps of the other orifices were found and the tricuspid and mitral openings were not dilated. The cardiac muscle was extremely pale in colour. Scattered throughout the aorta were streaks and patches of degenerative change. Although these were not excessive in degree they were much more than is usual to find at 37 years of age.

Endocrine Organs.   Both adrenal glands were large but they were
within normal limits.   The usual relationship between cortex and
medulla was unaltered and no lesions were found in either of the
bodies.   The thyroid gland was small.   The pituitary gland was
small but filled up its fossa.   The ovaries were of average size.
No fibrosis had occurred and a fairly recent corpus luteum was
present in the left ovary.

Urinary System.   The kidneys were of average size.   Their capsules
stripped easily.   Their substance was pale and contained an excess
of fluid.   The ureters were not dilated.   A considerable number
of haemorrhages had occurred in or immediately beneath the lining
of the urinary bladder;   again these were "flame-like."

Other Organs.   No lesions of primary pathological importance were
found in the liver, spleen, pancreas, alimentary tract, or brain.
In the wall of the uterus were a number of fibro-leio-myomata situated
immediately below the peritoneal covering of the organ.   The largest
of these was not as big as a lentil.

Half of one of the adrenal glands, preserved in Kaiserling's
solution, and paraffin blocks of the pituitary gland were kindly
supplied to me for examination by Dr. Reynolds.

113.

HISTOLOGICAL EXAMINATION.

The pituitary gland.

The gland had been bisected in the sagittal plane, and fixed in
formalin.   It was found that the paraffin blocks were too tough
to obtain thin sections.   After a few complete sections had been
mounted each block was divided into four parts with a sharp razor
and the resulting blocks were embedded separately.   Thin sections
were then obtained and the gland reconstructed from serial sections.
From the examination of the sections of the original blocks it was
obvious that a large basophil adenoma was present in a gland of
normal size.

The basophil adenoma of the anterior lobe.

The adenoma is roughly spherical and is 5 to 6 mms. in diameter.
It is not encapsulated but has produced a condensation of the
surrounding parenchyma equivalent to a depth of about 2 acini.
Inferiorly the tumour abuts on the capsule of the gland with the
production of slight bulging.   Posteriorly the tumour impinges on
the pars intermedia.   The pars intermedia is compressed and distorted
slightly.   Anteriorly and superiorly the tumour is covered by the
surviving parenchyma of the anterior lobe.   The surviving tissue is
judged to be equivalent to about one quarter of the normal gland.   It
shows no upset in gross structure.   The tumour is of papillary
character and shows thin-walled blood spaces clothed by thick layers
of cells.   A few areas of haemorrhage are present but there is no

necrosis. The tumour cells are of uniform type and are all small, being about 12 $\mathcal{U}$ in diameter. The cytoplasm is finely granular. The granules are smaller and closer packed than in normal basophil cells. The characters of the tumour cells are definitely basophilic. While in many cells, using the specific stains for β granules, the granules stain as intensely as mature β granules of the anterior lobe, in others the granules stain less intensely. The nuclei are small and hyperchromatic. Mitoses have not been seen. Vacuolation and hyalinization of the cytoplasm of the cells is not present. To make sure of this point some hundreds of sections were examined over a period of a year.

The surviving anterior lobe tissue.

Basophil cells are not numerous and in many sections none is present. They are all of small type, none being more than 14 $\mathcal{U}$ in diameter. The majority show normal granularity. Cytoplasmic vacuolation is inconspicuous and of inter-granular type (see case III for comparison). A small proportion (about one tength) show the cytoplasmic hyalinization of Crooke. While all degrees of the condition are present hyaline cytoplasm presents mainly as thin sub-peripheral rims. This would not have been made out without the new methods introduced. The nuclei of the basophil cells show no abnormality. The acidophil and chromophobe cells show no abnormality.

Pars intermedia.   The pars intermedia presents as a number of

colloid-filled cysts lined by a flattened non-granular cubical

epithelium.   The wandering basophil cells of the posterior lobe

are scanty but within the normal variation.   They show normal

granularity and neither cytoplasmic vacuolation nor hyalinization

is present.   The pars nervosa shows no abnormality.

The adrenal cortex.

The cortex appears enlarged and is approximately the same thickness

as the cortex of Case I, which is definitely hyperplastic (Dr. Reynolds

describes the adrenal glands as large but within normal limits).   The

gross structure of the cortex appears unaltered.   The fuchsinophil

granules of Vines are not found .   Frozen sections show no abnormality.

D I S C U S S I O N.

Considering the relatively short duration of the disease
the syndrome of pituitary basophilism is well developed. The
subject did not show as marked visible adiposity as is seen in
the fully developed condition but the pathologist noted a large
amount of fat in the body cavities.   There is no information
available concerning osteoporosis or polycythaemia.

The size and structure of the pituitary adenoma is similar
to many already described (Cushing, 1932; Russell, Evans and
Crooke, 1934; Crooke, 1935).   The fact that the cells of the
adenoma differ slightly from normal basophil cells of the anterior
lobe indicates a departure from normal consistent with the belief
that they are true tumour cells as opposed to a focal hyperplasia,
perhaps self-limiting and of the nature of a physiological process.
The degree of hyalinization of the basophil cells of the surviving
anterior lobe parenchyma and the absence of hyalinization in the
tumour cells shall be discussed in the final summation.

S U M M A R Y.

A case of Cushing's syndrome is described in a woman of
37. A basophil adenoma was present in the anterior pituitary
gland. The cells of the adenoma did not show the cytoplasmic
hyalinization of Crooke. Basophil cells in the surviving
anterior lobe were small and not numerous; about 10% showed the
hyaline lesion of Crooke, mainly in slight degree.

C A S E   III

THE REPORT OF A CASE OF CUSHING'S SYNDROME
UNASSOCIATED WITH TUMOUR ANYWHERE.

DESCRIPTION OF CASE.

History.   The patient, an unmarried male aged 32, was admitted to the surgical wards of Mr. J. S. Buchanan, Western Infirmary, Glasgow, with cellulitis and abscess formation of the left foot caused by slight trauma.   The patient had not felt well for the past three years; he had noticed his hair becoming grey and obesity developing over the past two years; he had been worried by a feeling of 'uselessness' and lack of libido for the past eighteen months; for the past year he found that he only had to shave once every three days instead of every day, as previously.   There was a clinical history of diabetes mellitus for the past 12 months.   Previous to all this he had been a normal healthy subject.

On admission the patient was grey-haired and of florid complexion. He was of average stature but was very stout, showing especially facial and abdominal obesity.   A few bluish striae were present on the abdomen.   Glycosuria, polyuria, acetonuria and polydipsia were present.   There was hypertension (B.P. 170/100).   The heart was

enlarged;   the subcutaneous and retinal vessels showed arteriosclerotic changes.   The blood picture gave R.B.C. 5.1 million, W.B.C. 7400 and Hb 90%.   Bone changes were not obvious but the patient was too ill for extensive investigation.   The Wassermann reaction was negative. Radiography of the sella turcica showed no abnormality.   The condition was diagnosed as Cushing's syndrome.

The patient's condition deteriorated both locally and generally, despite control of the glycosuria by insulin and repeated drainage of the septic area.   Ultimately attacks of blindness, giddiness and mental upset became frequent, and the patient died in coma six weeks after admission.   Post-mortem examination was carried out by Prof. J. Shaw Dunn 26 hr. later.

Summary of post-mortem findings: Marked hypertrophy of left ventricle, arteriosclerosis and bronchopneumonia.   Endocrine glands: pancreas embedded in fat but showing a considerable amount of natural-looking tissue;   adrenals (12 g.) not enlarged;   thyroid not enlarged, no abnormality;   testes small but of normal appearance;   pituitary gland of normal size;   no thymic tissue found in the mediastinum. Only the pituitary gland was taken for histological examination. The paraffin blocks of the gland were given to me by Dr. J. E. Craik.

H I S T O L O G I C A L   E X A M I N A T I O N.

The pituitary gland had been divided into four parts by cuts
parallel to the sagittal plane and fixed in Formol Zenker/corrosive
sublimate.   The posterior lobe was deficient in the blocks, only
a narrow rim of tissue being left adherent to the anterior lobe.

Anterior pituitary.   The anterior pituitary presents a normal
architecture, and the relative proportion of cells appears to be
within normal limits with differential staining.   Rasmussen's
method of differential cell enumeration is strictly applicable only
to horizontal sections of the gland.   Therefore a Rasmussen count
cannot be accurately copied in this case.   The table below shows
the results obtained on counting 17,000 cells by the method that
Rasmussen (1929, 1933) defines, but using vertical sections.   The
results are compared with a similar count carried out on a normal
adult male:

	Basophil cells.	Acidophil cells	Chromophobe cells.
CASE III	10%	38%	51%
CONTROL	8%	40%	52%

These figures are well within Rasmussen's normal limits, and this
was the impression gained on comparison of the sections with sections
from 120 other human pituitary glands.

The basophil cells.   The basophil cells show three abnormalities:

hyalinization, excessive vacuolation and an increase in size.   Only

one to two normal granular basophil-cells were seen in most of the

sections (80) examined.

Basophil-cell hyalinization.   As Crooke described, the progressive

development of hyalinization, in the cells uncomplicated by excessive

vacuolation, is seen to follow an invariable rule when it encroaches

upon the granular cytoplasm.   Beginning as a narrow complete, or

almost complete, zone situated peripherally, the hyaline change

advances towards the nucleus.   In most cells showing advanced

hyalinization, granular cytoplasm persists in two areas, one in

contact with the nucleus on that side of the nucleus which has the

most abundant cytoplasm (juxta-nuclear granulation), and another on

the periphery of the cell.   When eventually the juxta-nuclear

granulation is hyalinized the peripheral granulation is often still

partially persistent (see this thesis part I   for illustrations).

All stages from a narrow subperipheral hyaline zone to complete

hyalinization are prsent.   About 5% of the basophil cells are

completely hyaline (Plate 5, fig.6 ; Plate 6, fig.10.).   Cells

with a narrow complete sub-peripheral hyaline zone are particularly

numerous;   this type does not appear to have been observed by Crooke

(Plate 6, fig. 11).

Vacuolation in the basophil cells.   About one-fifth of the
partially hyaline basophil cells show no vacuolation of the remaining
granular cytoplasm.   The rest show varying degrees of vacuolation of
the granular cytoplasm and in most it is completely vacuolated.
Excessive vacuolation in the individual cells is not represented by a
large single vacuole but is always of multifocal origin, being repre-
sented by a conglomeration of small vacuoles.   These may be outlined
by a layer of normal ripe granules (inter-granular vacuolation) (Plate 6,
fig. 9 and **fig.** 10), but in many the outlining granules have become
scanty and are small and feebly stained.   Refractile cytoplasmic
threads are then revealed outlining the vacuoles, and to this appearance
I give the name cobweb vacuolation.   This cobweb vacuolation develops
in any area of the cell but is most common in the juxta-nuclear area.
Cobweb vacuoles often indent and compress the nucleus, which may
eventually be flattened out against the periphery of the cell.
Depending on the plane of section many configurations are produced
(Plate   6 fig.11 and Plates 7 and 8).   Where cobweb vacuolation
abuts on hyaline cytoplasm the threads outlining the vacuoles appear
 continuous with the hyaline cytoplasm.   A few very large (35) non-
hyaline cells are present.

Intrahyaline vacuoles are present in many of the cells with advanced
hyalinization (Plate 5 , fig. 6, and Plate 7).   Cobweb vacuolation is
also conspicuous in non-hyalinized cells;   stages in its development are
shown in Plate 6 fig. 11 .

The amount of basophil-cell vacuolation is much greater than in any of the glands of the control series both as regards the proportion of vacuolated cells and the degree of vacuolation in the individual cells.

<u>The nuclei of the basophil cells.</u> In hyaline basophil cells uncomplicated by excessive vacuolation, the nuclei appear normal except for a few of the completely hyalinized cells in which the nucleus is hyperchromatic and slightly distorted. The hyaline cytoplasm of the latter cells stains very deeply. The nucleus may be normal in appearance in cells with excessive cobweb vacuolation, but in the majority it is peripheral and compressed and scalloped by the vacuoles. Despite the remarkable degree to which scalloping of the nucleus may proceed in association with cobweb vacuolation, it is indeed remarkable that I have not seen a single example of nuclear pyknosis. In connection with the assessment of nuclear pyknosis in the normal anterior pituitary, basophil nuclei vary greatly in chromatin content, some being of dense structure and highly fuchsinophil, and only slight detail is made out with well-differentiated staining and high power observation. When the latter type of nucleus is compressed and scalloped little nuclear detail is discernible (Plate 7). The nucleoli of some of the nuclei of vacuolated cells appear unusually large. Many binucleate and trinucleate cells are present (4-6 in every

section examined).   No mitotic figures were found

Many peculiar dispositions of hyaline, vacuoles, normal

granularity and nucleus in the basophil cells give the impression

that constant alteration in the morbid process is occurring.

The acidophil cells show normal granularity.

Size of chromophil cells.   With regard to the size of the cells,

there is a striking abnormality present.   In every field examined the

diameter of the acidophil cells has been only a half to a third the

diameter of the basophil cells frequently the disproportion has even

been greater, while only in a few fields was an acidophil cell found

as large as the smallest basophil cell   (Plate 5, fig. 6   ).

In the glands of subjects, aged 20-40, in the control series,

acidophil cells vary from 6 to 20 ᵼ in diameter.   Acidophil cells

show great variation in size;   the basophil cells are much more constant,

the vast majority being 14-16   in diameter.   Occasionally a giant

chromophil cell is seen.   In no case has any disproportion in the

basophil/acidophil ratio been found approaching the conditions in the

present case.   Here less than 1% of the acidophil cells are over 12 ᵼ ;

0.2% have diameters over 16 ; many are less than 8   .   The majority

of the basophil cells are 18-22   in diameter, and about 4% are giant

forms of 28-36 ᵼ ;   many of these forms show the phenomenon of cupping

(Plate 5, fig. 8 ;   see Severinghaus (1938) for explanation).   The

table shows the results obtained on measuring, with the eyepiece

micrometer, approximately 1000 acidophil, and 1000 basophil, cells

from the present case, Case I, and, as a control, from sections of

the hypophysis of a normal adult male, age 34.   Both case and control

appeared to have the same degree of post-mortem change and shrinkage .

Diameter of chromophil cells (⫯)				
Over 20	20-16	16-12	12-8	> 8

Percentage of basophil cells present in various groups

	Over 20	20-16	16-12	12-8	> 8
Present Case	27	30	42	3	0
Case I	4	30	58	8	0
Control	7	29	52	12	0

Percentage of acidophil cells present in various groups

	Over 20	20-16	16-12	12-8	> 8
Present Case	0	0.2	0.7	78	21
Case I	4	10	29	56	1
Control	3	19	27	47	3

Thus, there is in the present case (1) a greater preponderance of

basophil cells of larger type than normal;   (2) a constant abnormality

of the acidophil cells, in that they were all of small type, and it is

clear (Case I) that this abnormality is not constantly associated with

basophil/hyalinization.   The chromophobe cells are all of the small
      -cell

type found in the normal adult.   No adenoma has been found on serial

section.

The posterior lobe.   Observation is limited to a rind of tissue

attached to the anterior lobe.   The wandering cells are plentiful and

of normal type.   A few show intermediate stages of hyalinization and vacuolation.   The pars intermedia is small and presents no abnormality.

Summary of anterior pituitary findings.

(1)   A conspicuous degree of basophil-cell hyalinization.

(2)   Excessive basophil-cell vacuolation, with development of the cobweb and scalloping of the nucleus.

(3)   Enlargement of basophil cells.

(4)   Great decrease in size of acidophil cells.

(5)   Relative proportion of cells within normal limits.

(6)   No adenoma present.

Anterior pituitary gland.

Comparison of cases I, II & III.

	Basophil cells.	Adenomata	Acidophil cells
Case I.	Large, excessive vacuolation with cobweb development, hyalinization.	None	Small
Case III.	Increase in number, normal size, extreme hyalinization, inconspicuous vacuolation.	3 small chromophobe adenomata	Normal.
Basophil ⌐Adenoma Case II	No hyalinization.	A large basophil adenoma.	
Surviving lobe.	Small, hyalinization much less than in I or III.		Normal.

THE CONTROL SERIES.

Six to twelve sections from different levels of each gland of a
control series of 140 hypophyses were examined by the methods previously
described (this thesis, part 1).   The glands were taken from routine
autopsies on adult subjects mainly from medical wards.   The sections
were specially examined with regard to hyalinization and vacuolation.

Basophil-cell hyalinization.   Hyalinization was found to a slight
degree in a few (1 - 3) basophil cells in sections from seven different
glands.   The finding was not constantly associated with any condition.
Thus hyaline basophil cells were encountered in one case of essential
hypertension but not in other four, in one case of alimentary carcinoma
but not in other nine, in two cases of bronchial carcinoma but not in
other seventeen, in one case of diabetes mellitus but not in other
seven, in one obese subject dying from cardiac failure, but not in any
other obese subject, in one subject over 70,killed accidentally, but not
in any other subject over fifty five.   Basophil-cell hyalinization was
not found in any of the cases of the series with endocrine abnormality
except the one case of diabetes mellitus: thyrotoxicosis (3), almost
complete ablation of the thyroid by amyloidosis (1), myxoedema (1),
islet-cell tumour of the pancreas with hypoglycaemia (2), diabetes
mellitus (8), adreno-cortical hyperplasia in a case of hypertension
(1), Addison's disease (5), thymic tumour associated with myasthenia
gravis (1); two cases of haemochromatosis with marked haemosiderin

deposits in the cells of the anterior pituitary; two cases of

parathyroid hyperplasia or adenoma associated with osteitis fibrosa.

A large number of sections from these cases were examined.

Basophil-cell vacuolation. The observation of cytoplasmic threads

outlining basophil-cell vacuoles does not appear to have been previously

described. Since it is so conspicuous a feature in the case it might

be questioned if this was a special type of vacuolation only associated

with the process of hyalinization.

Vacuolation of the basophil cells is present in all the glands of

the control series in very varying degree. It is generally more con-

spicuous in elderly subjects. The vacuolation is mainly of intergranular

type, i.e. the vacuoles are outlined by a rim of granules and no enveloping

cytoplasmic membrane (refractile thread, in section) is visible, and this

obtains even when the vacuolation in individual cells is multifocal.

Bearing no relation to the degree of vacuolation in the whole gland or

in the individual cells cobweb vacuolation is present in a considerable

number of the control series but in very slight degree and in very few

cells. It is represented by a few contiguous vacuoles with partial

disappearance of surrounding granules and appearance of the cytoplasmic

thread. Occasionally, when juxta-nuclear, the cobweb vacuoles scallop

the nucleus. The cobweb vacuolation is not associated with hyalinization

and its occurrence in the individual cases cannot be attributed to any

common factor. The greatest degrees of vacuolation are present in

elderly subjects.   Severinghaus (1938) has already recorded this

and considers that it is a reaction to hypogonadism (senile

castration).   In none of the elderly subjects in the control series

is the basophil vacuolation nearly as marked as it was in the case of

basophilism.   Inquiry into the contents of the basophil vacuoles has

not been made.   Biggart (1934), using frozen sections, found basophil

vacuoles to contain an unstainable substance and small quantities of

fat.

D I S C U S S I O N.

I intend to attempt to evaluate the various hypophyseal abnormalities
found in the case/as far as possible in the light of what is known of
            (Case III)
pathological reactions in the human hypophysis.

The pathological findings in this case of Cushing's syndrome are
unusual in that there is neither hypophyseal, adreno-cortical nor thymic
tumour, nor adrenal hyperplasia.   Such a pathological type of basophilism
is rare but not unknown (Oppenheimer et al. 1935; Freyberg et al. 1936;
Ulrich, 1936).   Nevertheless, the hypophysis is highly abnormal.   In
addition to a conspicuous degree of basophil-cell hyalinization, the
hypophysis shows: basophil-cell enlargement; excessive basophil-
cell vacuolation, associated with degranulation and the appearance of
cytoplasmic envelopes round the vacuoles,which displace, compress and
scallop the nucleus; and marked decrease in the size of the acidophil
cells.

There is no true indication in the literature of basophilism as to
the incidence of the latter abnormalities, owing to the cursory
examination and scanty description of the anterior pituitary in many cases.

The excessive basophil-cell vacuolation.

Basophil-cell vacuolation is associated with disappearance of β
granules, and I consider that the appearances in this case and the

control series indicate that cobweb vacuolation is merely an intensification of the usual process where, with increased disappearance of surrounding granules, there is revealed a refractile cytoplasmic envelope (thread in section) surrounding the vacuoles. I consider that it is probable that in 'intergranular' vacuolation the envelope is also present but is masked by the surrounding granules.

It appears unlikely that the excessive disappearance of β granules associated with the excessive vacuolation and cobweb development can be attributed to the hyaline cytoplasm in the cells complicating the normal process of vacuolation, in that the process (1) is also extreme in non-hyaline basophil cells in the same gland, (2) is present in the control series unassociated with hyalinization or with any other obvious abnormal factor, and (3) is absent in the hyaline basophil cells in cases 1 and 2.

Though I am convinced that cobweb vacuolation is merely a further development of ordinary intergranular vacuolation and is therefore cytoplasmic and not nuclear, Severinghaus (1938), Rasmussen (1936) and Graef, Bunim & Rottino (1936) have interpreted what appear to us to be similar appearances in hyaline basophil cells in Cushing's syndrome as nuclear 'blisters.'

Severinghaus (1938), commenting on Crooke's findings states (p.84) that on examining sections from a case (Graef's) of Cushing's syndrome in 1934, 'I reported similar changes, which I characterized as a general degranulation of the basophils of the hypophysis .... Nuclear changes in these basophils were to me, however, more striking than the cytoplasmic.

Very few cells had nuclei. In most cases they were extremely
lobulated, frequently with vesicular bulges, not unlike thin-walled
blisters which occupied most of the volume of the cell. Deeply
shrunken, elongated, pyknotic nuclei were numerous.' Therefore
Severinghaus clearly interpreted the vacuoles as nuclear structures.
In the diagrammatic illustrations provided the artist has been tardy
to follow the written descriptions and at the most has drawn the vacuoles
as herniations of the nucleus, the nuclear membrane being drawn mainly
intact.

Rasmussen reported that in the anterior pituitaries of three cases
of Cushing's syndrome, in addition to basophil-cell hyalinization, 'the
nuclei of these abnormal cells may be enlarged andlobulated as if ballooned
out in several places. These nuclear changes have been stressed by
Severinghaus ... The nuclei of some of these cells may appear essentially
normal.'

Graef et al. state that the nuclear blisters, 'exhibited no
chromatism with any of the stains employed.'

Cobweb vacuolation can be easily made to simulate nuclear 'blistering.'
With the staining methods used (this thesis, pt.1) nuclear and cyto-
plasmic structures have always been brilliantly differentiated. The
process of cobweb vacuoles compressing the nucleus is obvious when the
plane of section passes through the long axis of the crescentic scalloped
nucleus, but when the plane is tangential only a small area of indented
nucleus is present. In many staining methods advocated for the anterior

pituitary, the nucleus, granules and hyaline cytoplasm of the basophil cells appear as slightly different shades of blue. The lightly stained cytoplasmic threads of the vacuoles appear, with these stains, as continuations of the nuclear chromatin threads. Cobweb vacuolation is then easily mistaken for a continuation of nuclear structure.

I have always found that persisting granules studding the threads which outline the vesicles give the reactions of cytoplasmic and not nuclear material, and I am of the opinion that the appearances are completely explained as a result of compression and scalloping of the nucleus by the cobweb vacuoles.

Diminution in size of the acidophil cells in basophilism has only been recorded once before, as far as I can ascertain - in the Rabbe-Krause case of Cushing's original series (Cushing, 1932). The decrease in size was not so marked as in the present case.

The significance of the abnormalities of size and vacuolation of the chromophil cells.

It cannot be held that these abnormalities of size and vacuolation present in the chromophil cells of the hypophysis represent a congenital anomaly of the hypophysis, in that the subject was a normal healthy virile male until three years before death and was of normal stature. Furthermore, these abnormalities must have only some secondary significance in relation to the morbid process of basophilism, in that they are inconstant findings in basophilism (I did not find them in cases 1 and 2; and they are not associated with any particular

pathological type, since similar vacuolation was found in Graef's case

of adreno-cortical carcinoma, and decrease in size of acidophil cells

occurred in the Rabbe-Krause case of basophil adenoma.

Castration during active sexual life is the only condition which

is known to produce in the human hypophysis a degree of basophil-cell

vacuolation similar to that found in the present case (Biggart, 1934).

In the castrated rat, in addition to excessive basophil-cell vacuolation,

the basophil cells increase in number and size, while the acidophil cells

decrease in number and size (Severinghaus, 1938). In the hypophysis of

the castrated human, of which few critical examinations have been made,

only excessive basophil-cell vacuolation and perhaps basophil-cell

increase have been reported (Biggart, 1934). There is no detailed

account of the type of vacuolation, though Rasmussen (1938) depicts

it as a single large vacuole taking up the bulk of the cell. I have

reason to believe that this is by no means always the case, in that in

the virtually castrate state of senility, as seen in the aged subjects

of the control series, any excessive basophil-cell vacuolation is not

represented in this form in the large number which I have examined, but

is represented by a conglomeration of small vacuoles in the basophil cells

specially affected.

The castrate/and Cushing's syndrome have much in common clinically,

state

while gonadal atrophy and fibrosis are frequently present in Cushing's

syndrome (Cushing, 1932). Unfortunately, no microscopic examination

of the tests of my case was made.

I consider that the increase in size and excessive vacuolation of the basophil cells and the decrease in size of the acidophil cells are likely to be correlated with the findings in the castrated rat and are to be interpreted as representing a phase of hypophyseal reaction secondary to hypogonadism. Why these changes should be inconstant in basophilism is unknown, but not incompatible with our knowledge of the anterior pituitary, in which change, even in morbid processes, is probably constantly occurring. An example of this is the finding by Severinghaus of a high basophil-cell count in the hypophysis from a case of Addison's disease as opposed to the constant finding by others of great basophil-cell decrease in this condition. The unusual finding is interpreted as a consequence of death occurring during a phase of basophil-cell regeneration, which must be rare in this condition (Severinghaus, 1938).

There is possibly another factor concerned with the increase in size of the basophil cells; while many of the very large ones show gross vacuolation, others, unassociated with vacuolation, show an advanced degree of hyalinization. The latter cells have frequently two or more nuclei, and I consider that their enlargement is probably more correctly to be interpreted as a manifestation of functional hypertrophy as distinct from the enlargement associated with gross vacuolation.

The hyalinization of the basophil cells.

While the abnormalities of chromophil-cell size and vacuolation must be considered as secondary changes bearing a very general interpretation to the morbid process of Cushing's syndrome, this cannot be said of the conspicuous degree of basophil-cell hyalinization which is present.

Crooke considers that hyalinization of the basophil cell is an indication of altered physiological activity, as opposed to a stage of cell death, since the basophil-cell nuclei are entirely normal. Severinghaus (1938) contends that it is a stage to cell death since he found nuclear breakdown (blistering) associated with hyalinization. My interpretation of the nuclear appearances favours Crooke's view as to the essential nature of the lesion. Moreover, it is apparent from the examination of the other two cases of basophilism that basophil-cell abnormality additional to hyalinization is inconstant.

The presence of basophil-cell hyalinization has never been denied in any case of Cushing's syndrome reported since Crooke's original communication, except in one instance - a case described by Pons & Pappenheimer (1937). I do not consider that it is clinically a case of basophilism and, moreover, only one-half of the gland was examined and basophil cells were scanty in that half. Basophil-cell hyalinization has been found in two cases of basophilism associated with unique pituitary lesions, not included in Crooke's original series -

a metastasizing basophil carcinoma (Cohen & Dible, 1937) a large

chromophobe adenoma (Fuller & Russell, 1936).   I now add this third

type - a conspicuous degree of hyalinization of the basophil cells in

a case of basophilism without tumour. Rasmussen (1938) has already

made very brief reference to basophil-cell hyalinization in such a type.

These findings contrast with the inconstant and exceptional finding

of a few hyaline basophil cells in conditions other than Cushing's

syndrome (Crooke, 1935; Rasmussen,1936; my control series), and

especially with the absence of basophil-cell hyalinization in so many

cases of endocrine abnormality in my control cases.   All these

substantiate Crooke's hypothesis that the abnormal activity of the

basophil cells, of which hyalinization is the morbid manifestation,

is the essential abnormality of Cushing's syndrome.   Special weight

is added to this view by the present case in which no tumour or hyper-

plasia of the basophil cells of the hypophysis, the adrenal cortex or

of the thymus is present, and in which the peculiar nuclear appearances

in many basophil cells are accounted for by the effects of vacuolation

in the cells.

The interrelationship of the different pathological types of Cushing's
        syndrome.

Many writers have taken definite views either that basophilism

is essentially a hyper-adreno-cortical or a hyper-basophil condition

(see Pardee, 1938).   While neither view is justified on morbid

anatomical grounds when all the pathological types are considered, attention must be drawn to the correlation existing between many of the pathological types of basophilism in addition to the common bond of basophil-cell hyalinization. Thus, it is the rule rather than the exception to find adreno-cortical hyperplasia associated with basophil adenoma (Freyberg et al. 1936); and again in some cases of adreno-cortical tumour, basophilia, that is a relative increase in the basophil cells, is present (Lescher & Robb-Smith, 1935; Case II). In McLetchie & Scott's case basophil cells are actively developing from the 'ducts' of the pars intermedia in contrast to the other cases of basophilism which I have examined and the glands of the control series, in none of which is such active basophil-cell increase evident.

The above findings suggest that hypersecretory processes of the basophil cells and the adrenal cortex are complementary, the one producing the other.[x] Since an organ need not necessarily enlarge with hyperactivity, I consider that in those cases of basophil adenoma and adreno-cortical tumour in which there is not enlargement of the complementary organ (adreno-cortical hyperplasia, basophilia) either hyperactivity of the complementary organ is present but is not expressed in enlargement or the organ was not in a phase of hyperactivity at the time of death, but was so earlier.

In Addison's disease, destruction of the adrenal cortex is accompanied by great decrease in the number of basophil cells of the hypophysis (Crooke & Russell, 1935; this thesis PartIV). These

[x] The relation of adreno-cortical tumour to the adrenal virilisim and to Cushing's syndrome is dealt with later.

facts suggest that the basophil cells and the adrenal cortex constitute a functional complex. Cushing's syndrome is in almost every respect an opposite condition to Addison's disease (McQuarrie, Johnson & Ziegler, 1937). One might therefore claim that while Addison's disease is the symptom of hypofunction of the basophil-adreno-cortical complex, Cushing's syndrome is a symptom of hyperactivity of the complex.

The thymic tumours of Cushing's syndrome are associated with adreno-cortical hyperplasia. Thus whether in Cushing's syndrome there is a basophil adenoma, adrenal tumour or thymic tumour, there exists sufficient correlation on morbid anatomical grounds to indicate the eventual production of a common inter-endocrine abnormality quite apart from the finding of basophil-cell hyalinization. But in the case which I have described there is no absolute morbid anatomical evidence of basophil, adreno-cortical or thymic hyperactivity. However, it must be kept in mind that the pathological findings in the hypophysis of basophilism are only those obtaining at a point of time in a complex morbid process possibly undergoing constant alteration. I have already noted that, in the present case, although the basophil cells were not increased in number, large binucleate and trinucleate forms were numerous. A few binucleate (and trinucleate) basophil cells are encountered in most human hypophyses. The proportion is very variable throughout any gland and no definite statement could be given about the proportion in the present case, with reference to

normal, without a very extensive statistical survey.   Nevertheless,
inspection of the sections gives the impression that large binucleate
forms are abnormally numerous.   It is possible that this is an
indication of basophil-cell hyperactivity.

Thus apart from the common bond of basophil-cell hyalinization
the rather unique case of basophilism described offers possible
correlation with the common morbid anatomical types of basophilism in
that there is evidence suggestive of basophil-cell hyperactivity.   The
final elucidation of the problem depends on the significance of basophil-
cell hyalinization.   While this problem must ultimately rest with the
experimental worker, I consider that certain observations at least
suggest a solution, though the conclusions reached must be taken with
reserve as the evidence is based on histological change from a
quantitative aspect which is never a certain guide.

The significance of basophil-cell hyalinization.

Crooke has noted two special points with reference to the
basophil adenoma of Cushing's syndrome: (1) the cells of the adenoma
did not show the hyaline change; and (2) while the basophil cells of
the surviving parenchyma showed hyalinization, it was not so conspicuous
as in the hypophyses from other morbid types of basophilism (adrenal
tumour, thymic tumour) in five of the six cases of basophil adenoma
examined by him.   This was also the finding in the case II, previously
described, where no hyalinization was present in the large adenoma

while hyalinization was mainly limited to minute subperipheral rims

in about one-tenth of the basophil cells of the surviving parenchyma.

In the case reported by Cohen & Dible (1936) a metastisizing basophil

carcinoma was associated with a clinical syndrome containing practically

every sign ever recorded in Cushing's syndrome. Hyalinization was not

present in the cells of the carcinoma. In the very scanty surviving

gland parenchyma hyalinization was present in slight degree in a few

basophil cells and was demonstrated with difficulty (Cohen & Dible, 1937).

In the first place it would appear that the cells of the basophil

adenoma of Cushing's syndrome do not obey the same laws as the basophil

cells of the parenchyma in that they do not undergo hyalinization - a

factor perhaps not inconsistent with our knowledge of the autonomy of

tumour cells. Secondly, especially from the evidence of Cohen &

Dible's case, it would appear that the basophil tumour, when present,

is the significant abnormality in the hypophysis and that basophil-cell

hyalinization is a subsidiary phenomenon since the latter may be

relatively inconspicuous. Since this evidence indicates that hyper-

activity of the basophil cells is the essential lesion in basophilism,

and since basophil-cell hyalinization is a constant finding in all

pathological types of basophilism, it would be reasonable to assume that

cytoplasmic hyalinization is a change consequent on basophil-cell hyper-

activity. As we shall see later this assumption goes far to unify all

the varied pathological pictures which pituitary basophilism is heir to.

My explanations of the various findings in Cushing's syndrome
are summarized below.

Mechanism of pituitary basophilism.

Basophilism may be initiated by a hypersecretory tumour of the
basophil cells of the hypophysis, of the adrenal cortex, and possibly
of the thymus.   The basophil adenoma produces adreno-cortical hyperfunc-
tion, which is usually expressed in enlargement of the adrenal cortex.
The adreno-cortical tumour produces basophil hyperactivity which may
be associated with active increase in basophil cells (basophilia).   The
inter-glandular relationships of the thymus are obscure, but the mechanism
in Cushing's syndrome is understandable in that adreno-cortical hyper-
plasia and basophil-cell hyalinization are constantly associated with
the thymic tumour of Cushing's syndrome.   Basophil hyperactivity,
which is common to all these mechanisms, acting on all other endocrine
glands results in the multiglandular disturbance, the outward mani-
festation of which is Cushing's syndrome.   Basophil-cell hyalinization
is a cytoplasmic change consequent/hyperactivity of the basophil cells
and hence is common to all these mechanisms.   In the case of the
basophil adenoma the tumour cells do not obey the ordinary laws
applicable to the basophil cells of the hypophysis and do not develop
hyalinization.   The reaction of the basophil cells of the surviving
gland in the hypophysis containing a basophil adenoma is only of
secondary significance and only slight hyalinization is usually produced.

In some cases, such as the one I have described, no tumour is present. It would appear reasonable to assume that in these cases there is a generalized basophil-cell abnormality in which phases of hyperactivity alternate with hyalinization, though this may be secondary to adreno-cortical hyperactivity expressed usually by hyperplasia. Consequent on the multiglandular abnormality produced by basophil-cell hyperactivity certain secondary changes may occur in the hypophysis: excessive basophil-cell vacuolation, decrease in size of acidophil cells, the development of chromophobe adenomata. The excessive vacuolation of the basophil cells with cobweb development and consequent nuclear distortion may be, at some phases of the disease, one of the most conspicuous morbid appearances in/hypophysis.

<u>P A R T   III</u>

<u>Plate 5.</u>

<u>Fig. 6</u> shows two basophil cells, one (completely
hyalinized) shows intra-hyaline vacuoles.

<u>Fig. 7.</u> A hyaline cell showing a single vacuole among
the persisting granules.

<u>Fig. 8.</u> A large hyaline basophil cell "cupping" three
immature cells; one appears, in this plane of
section, to be internal.   NOTE the small size
of the surrounding acidophil cells (orange-red).

P A R T   III.

Plate 5.

Hyaline basophil cells unassociated with excessive
vacuolation;   from the pituitary gland of Case III

Fig. 6.
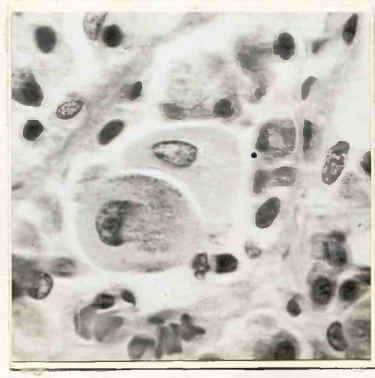
Fig. 7.
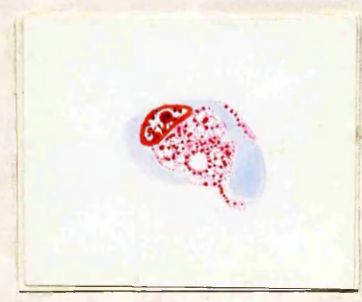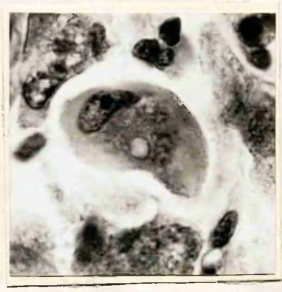
Fig. 8.
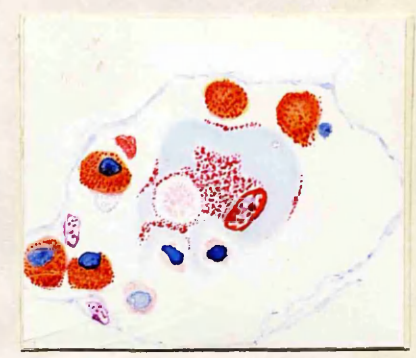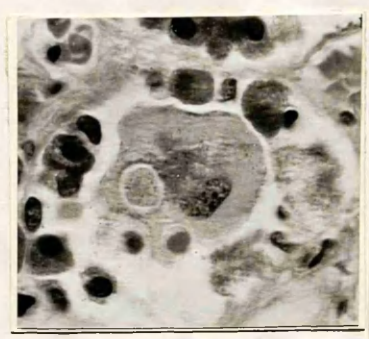
X 800   METHOD IIA

P A R T III

Plate 6

P A R T   III.

Plate 6.

Gross cytoplasmic vacuolation in basophil cells of pituitary gland (Case III).

Fig. 9.

Fig. 10.

Fig. 11.

X 800

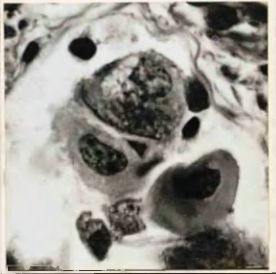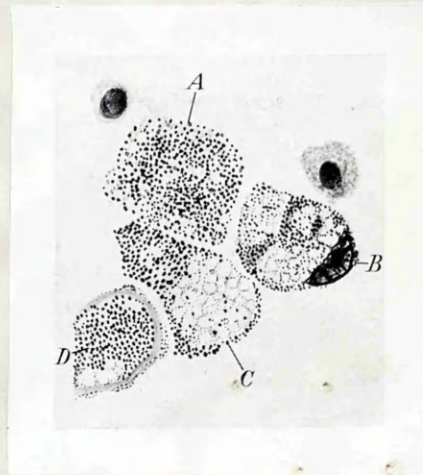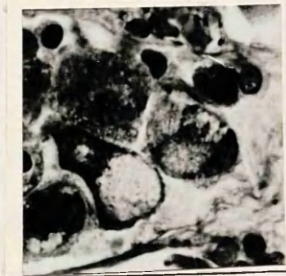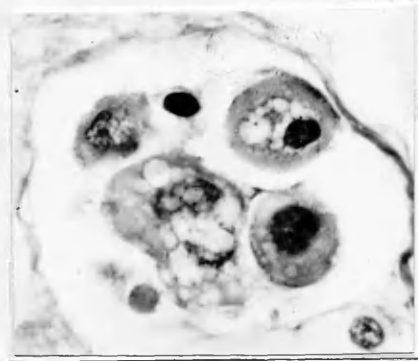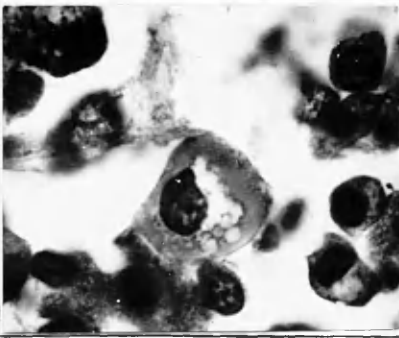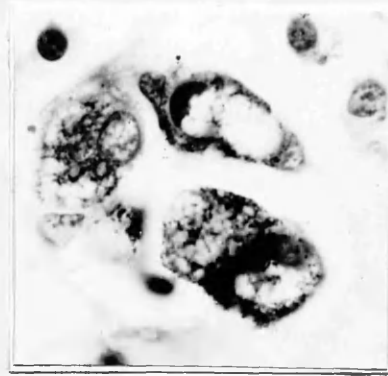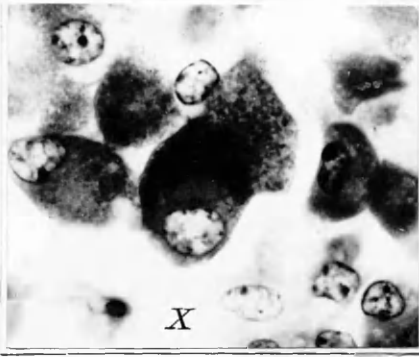METHOD IIB
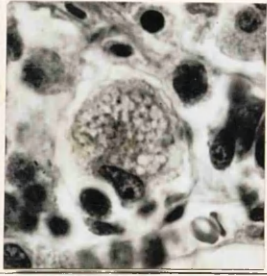
P A R T   I I I

Plate 7.

Three fields showing cobweb vacuolation, hyaline
cytoplasm, and nuclear displacement and scalloping
in basophil cells (Case III).
Contrast with field, X, of basophil cells from a normal
pituitary gland.

P A R T   III

Plate 8.

Diagrammatic illustration of the complexity of hyaline cytoplasm,

intergranular vacuolation, cobweb vacuolation, displacement and

scalloping of nucleus in basophil cells of pituitary gland of

Cushing's syndrome.

N - nucleus; H - hyaline cytoplasm; C.V. - cobweb vacuolation;

G.V. - intergranular vacuolation; G - normal basophil granulations;

X - a binucleate hyaline cell; Y - a large cell of type X cut

tangentially; note what is seen of the scalloped nuclei.

Compare with drawings shewing Severinghaus's interpretation of the
abnormal basophil-cells of Cushing's syndrome (small diagram on
right), taken from Severinghaus (1938).   The following is Severinghaus's
description :-

"Figs.16, 17 and 18.   Basophiles from the anterior lobe of an
18-year-old girl with Cushing syndrome.   Note basophilic, but non-
granular (hyaline) cytoplasm and progressive states in the vacuolation or
blistering of the nucleus.   Only a small percentage of normal nuclei were
present in the basophiles of this advanced case.   Formal fixation."

P A R T   III

Plate 8.
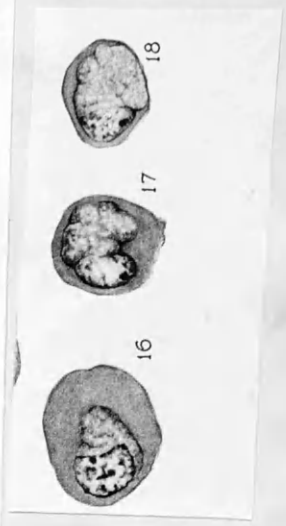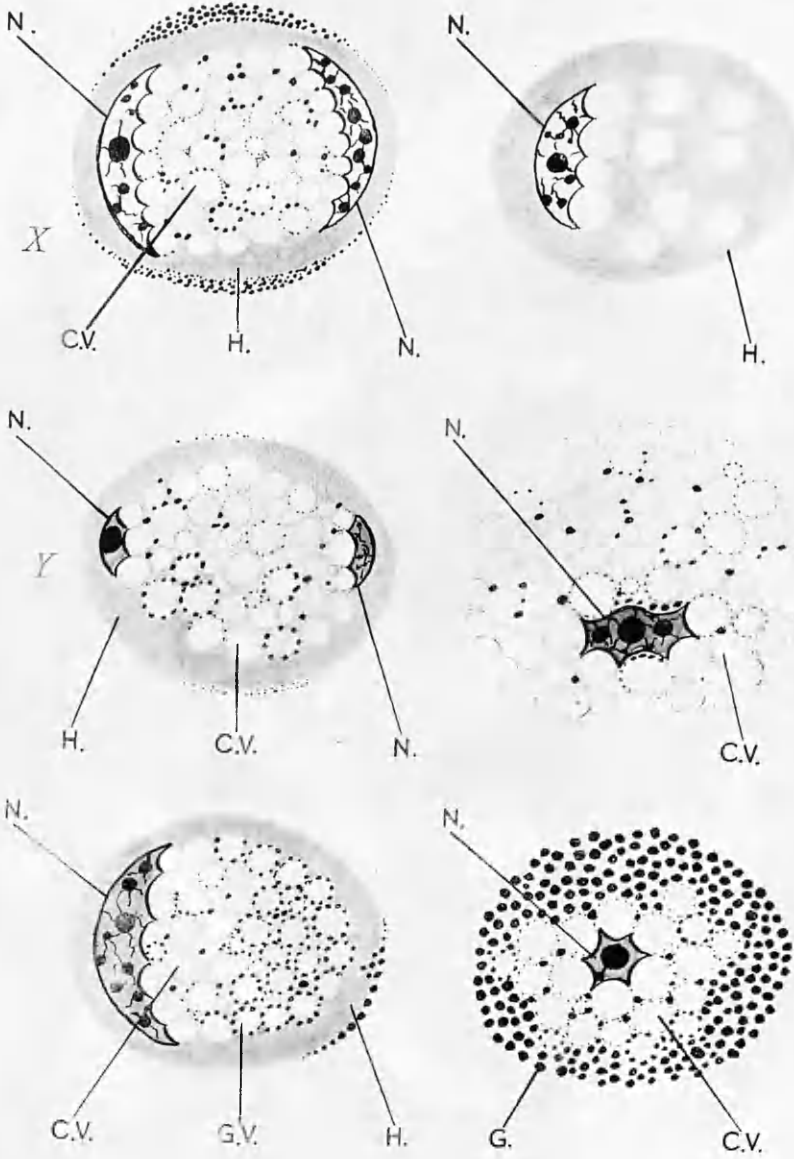
THE RELATIONSHIP OF THE MORBID PROCESSES OF ADRENAL VIRILISM AND CUSHING'S SYNDROME.

It has already been stated that in adrenal virilism the primary endocrine abnormality is adreno-cortical. It appears that from a like adrenal lesion Cushing's syndrome may arise. I believe that the difference between the adrenal abnormalities of virilism and of Cushing's syndrome of primary adrenal origin is quantitative and not qualitative. It is considered that up to a certain degree a primary adreno-cortical hyperfunction acts independently and produces the condition of adrenal virilism, and that, beyond a certain threshold, adrenal hyperfunction induces a complementary basophil reaction, resulting in Cushing's syndrome. The evidence for this contention is as follows :-

(1) No qualitative distinction has been substantiated between the adreno-cortical abnormality of both conditions in the female. Broster (1940) claims that in the male the adrenal glands of Cushing's syndrome do not show Vines' reaction. This has not been substantiated and in cases of Cushing's syndrome in the female the reaction has sometimes been reported positive, sometimes negative (Crooke, 1935).

(2)   Either adreno-cortical hyperplasia or tumour is the rule in Cushing's syndrome.   The adrenal tumour is almost certainly a primary endocrine abnormality.   Only in a minority of cases can adrenal hyperplasia be considered a possible primary endocrine abnormality.   Thus adrenal hyperplasia in Cushing's syndrome is commonly associated with a basophil adenoma, more rarely with a thymic tumour or no tumour anywhere.   Thus Jonas (1935) in a review of 35 fatal cases quotes only five cases where there was claimed to be no tumour anywhere and in two of these cases the pituitary gland received a scanty examination.   The basophil adenoma is almost certainly a primary endocrine abnormality.   The thymic tumour may also be primary. Where there is no tumour anywhere, which is rare, it is not certain whether adrenal hyperfunction or basophil-cell hyperfunction is primary. Accordingly one can say with certainty that Cushing's syndrome is rarely produced by a primary adreno-cortical hyperplasia.

(3)   It is certain that pure adrenal virilism is commonly produced by adreno-cortical hyperplasia.   Thus Broster performed unilateral adrenalectomy on thirty three cases of masculinization, many of which showed frank adreno-cortical hyperplasia, and were more or less completely restored to normal by the operation.   Broster does not describe a single case of pure adrenal virilism associated with adreno-cortical tumour in his collection (Broster & Vines 1938).

(4) When we come to discuss the position of adreno-cortical tumour there is an immediate difficulty. Some have shown to a striking degree the syndrome of pure adrenal virilism without obesity and when examined in the light of Crooke's work at autopsy have not shown basophil-cell hyalinization (Crooke, 1935; Anderson, Hain and Patterson, 1943). But subjects with adrenal tumour may die early in the course of the disease and though the morbid basis of Cushing's syndrome may have developed, they may not show the symptoms of pituitary involvement. This is demonstrated by my case (Case I) where the gross alteration of the basophil cells leaves no doubt that the morbid process implied much more than simple virilism. Without attempting to disentangle a confusing literature I think that this holds for the majority of cases of adreno-cortical tumour in the female. Thus, in addition to signs of virilism Kolodny's case showed marked hypertension (Kolodny,1934); five cases reported by Walters, Wilder and Kepler (1934) showed some or all of the following signs - hypertension, obesity, glycosuria, purplish abdominal striae; Graeff's case showed obesity and glycosuria (Graef, Bunim and Rottino, 1936): accordingly there is much to indicate that adreno-cortical tumour more commonly leads to the development of Cushing's syndrome than to a pure condition of virilism.

(5)   Scowen (1942) has recently reviewed the evidence which shows

that the adrenal cortex is the principle source of androgenic substances.

It has been shown that adreno-cortical tumour is associated with a higher

output of derivatives of androgens in the urine than adreno-cortical

hyperplasia, though the lower figures for the one overlap the

higher for the other (Crooke & Callow, 1939).   Thus, in so far

as androgenic output is concerned there is evidence that, as a rule,

cortical tumours have greater activity than cortical hyperplasia.

(6)   The adrenal cortex has always been associated with a hormone

essential for life.   This function is now identified with two

activities - namely, the production (1) of substances having a potent

effect on membrane permeability, and hence on the distribution of inorganic

ions in the body, and (2) of substances having an important effect on

carbohydrate metabolism (Scowen, 1942).   How those substances act

physiologically is unknown, but from the evidence in the next part

of the thesis it will be argued that they act pathologically only when

there is corresponding reactions in both the adrenal cortex and the

basophil cells.   In simple adrenal virilism there is no evidence of any

hyperfunction of the basophil cells and the effects of a pathological

activity of those substances are not manifest.

From this evidence I base my theory of the inter-relationship of

the morbid processes of adrenal virilism and Cushing's syndrome.   It

is as follows :-

Adrenal virilism is the symptom of a simple hyperfunction of the adrenal cortex. The excessive production of androgenic substances, a normal product of the adrenal cortex, produces the alteration. It is possible that other normal products of the adrenal cortex are produced in excess simultaneously, but in the presence of a normal anterior pituitary gland they are prevented from having any pathological action. Primary adrenal hyperfunction above a certain high degree, usually the result of adreno-cortical tumour, produces a complementary hyperfunction of the basophil cells of the hypophysis equivalent to the activity of a basophil adenoma. From this abnormality the multiglandular disturbance of Cushing's syndrome is produced and, to some extent, the effect of overproduction of androgens is obscured by other abnormalities, e.g. the development of obesity. Equally well a primary basophil adenoma induces adreno-cortical hyperfunction and the excessive production of androgens plays its part in the morbid process. Just as certain lower degrees of adrenal hyperfunction exert only a ⚹ pathological overactivity of one function (androgenic) it is possible that low degrees of basophil hyperfunction may act independently of the adrenal cortex and produce certain signs, e.g. possibly obesity, hypertension.

It is considered that mild degrees of the abnormality implicated in "basophilism without tumour" account for what Broster (1940) describes as that "large clinical group of hefty hairy women who cannot be labelled Cushing's syndrome on the one hand or virilism on the other."

Problems of Aetiology.

In some cases, as I have already stated, the morbid process of
adrenal virilism rests in anomalies of development.   Apart from this
the ultimate causation of both adrenal virilism and Cushing's syndrome
is obscure and is largely the, as yet insoluble, problem of tumour
development.   In Cushing's syndrome the primary aetiological factor
may be variously -

(1) a generalised hyperfunction of the basophil-cells, a basophil
adenoma or carcinoma;

(2) a generalised hyperfunction of the adrenal cortex, cortical adenoma
or carcinoma;

(3) a thymic tumour.   In the endocrine glands the distinction between
a focal hyperplasia, perhaps self limiting and of the nature of a
physiological process, and an adenoma, in the sense of an autonomous
new growth having no regard to physiological needs, is not clear.   It
is therefore tempting to consider that there is a progressive connection
between diffuse hyperplasia, focal hyperplasia, adenoma and carcinoma, and
that a tumour, though we do not pretend to know what determines malig-
nancy, is a further progression of the same factors which determine
hyperplasia.   In the case of Cushing's syndrome there is an immediate
check to such a train of thought, for the cells of the basophil adenoma
are entirely different from the basophil cells of the gland proper.
Whereas the latter show hyalinization, the cells of the adenoma do not.

Thus, they have different metabolic activities and we have to accord to the adenoma cells that inexplicable autonomy common to all tumour cells. This also holds for the adreno-cortical tumours. The presence of a malignant adrenal tumour and hyperplastic cortical tissue in Case I is unique. Where there is an adreno-cortical tumour the unaffected adrenal is of normal or small size. I have already stated that the hyperplastic adrenals may have been produced by the same stimulus (unknown) which resulted in the development of tumour in the new growth. There may be another explanation. In this case the degree of cyto-plasmic hyalinization of the basophil cells is greater than has ever been recorded. It is possible that the cortical hyperplasia is secondary to this reaction. The fact that cortical hyperplasia is not usually associated with tumour suggests that the tumour arises de novo in a normal endocrine system.

Where there is a generalised hyperfunction of a tissue the problem is clearly different from that of tumour. One of the most important reactions of puberty is the maturation of chromophobe cells to basophil cells. This is true for the pigeon (Schooley and Riddle, 1938). It also appears to be true for the human subject. A proper investigation has yet to be made. I could find no mature basophil cells in the hypophyses from two stillborn infants, and only two or three small mature basophil cells in any section of the hypophyses from two children aged 9 and 10 years respectively. While in the hypophysis of a child of thirteen they were numerous. From what has been said of the reation

of the basophil cells to the adrenal cortex it is, therefore, probable that at puberty a new balance is struck between those tissues. It is probable that this may be at times in error. There is evidence to suggest that it is frequently in error, though transiently. One of the most common signs of rapidly developing pituitary basophilism is the development of severe facial acne vulgaris. It is possible that the acne of puberty has a similar basis. Equally well one sometimes notices the development at puberty of obesity, particularly around the buttocks in males, which passes off in later years. To me this suggests a temporary maladjustment of basophil-cell - adreno-cortical relations. It is possibly from greater degrees of such maladjustment that basophilism without tumour springs.

The part of the thymic tumour in basophilism is still sub judice. One has yet to be removed in this condition.

S U M M A R Y.

1.   A case of basophilism, unassociated with hypophyseal, adrenal or thymic tumour and without adrenal hyperplasia, is described.

2.   In the anterior pituitary the following abnormalities are present:

(a)   a conspicuous degree of basophil-cell hyalinization;

(b)   an excessive degree of basophil-cell vacuolation;

(c)   multifocal basophil-cell vacuolation in the individual cells is associated with excessive disappearance of granules and with the revelation of a refractile cytoplasmic envelope surrounding the vacuoles (cobweb vacuolation);

(d)   the nuclei of the basophil cells are normal but in many cells cobweb vacuolation is associated with displacement of the nuclei to the periphery of the cells and with compression and scalloping of the nucleus by the vacuoles;   binucleate basophil cells are numerous;

(e)   the basophil cells are increased in size.

(f)   the acidophil cells are greatly reduced in size.

3.   These findings are contrasted with the findings in, (a) two other cases of basophilism, (b) a large series of hypophyses from non-basophilism cases and (c) other recorded descriptions of the abnormal basophil cells of basophilism.

4.   On the basis of the evidence gathered, it is pointed out that:

(a)   the abnormalities of size and vacuolation found in the chromophil cells are:

(i) inconstant in basophilism, (ii) not associated with any particular pathological type of basophilism;

(b) the cobweb vacuolation and nuclear scalloping in the basophil cells is similar to what Severinghaus and others have interpreted as nuclear 'blistering.'

5. It is suggested that the abnormalities of size and vacuolation in the chromophil cells represent a phase of reaction to hypogonadism, and are only of secondary importance in the morbid process of basophilism.

6. The correlation between the different pathological types of basophilism is briefly discussed.

7. It is considered that basophil-cell hyperactivity is the essential abnormality of Cushing's syndrome, and that basophil-cell hyalinization is a cytoplasmic change resulting from hyperactivity.

8. The relationship of the morbid processes of adrenal virilism and pituitary basophilism is discussed.

STUDIES ON THE RELATIONSHIP OF THE BASOPHIL CELLS OF THE

HYPOPHYSIS AND THE ADRENAL CORTEX.

P A R T   F O U R.

SOME OBSERVATIONS ON ADDISON'S DISEASE.

P A R T   F O U R.

SOME OBSERVATIONS ON ADDISON'S DISEASE.

Introduction.

Crooke & Russell (1935) have shown that the classical syndrome of Addison's disease, whether due to tuberculosis or a destructive atrophy of the adrenal cortex, is accompanied by diminution of the basophil cells of the anterior pituitary gland.   It is my purpose to describe the pituitary glands from five cases of Addison's disease.   The findings of Crooke & Russell are verified.   In addition I shall describe a case of exceptional interest in which, although there was gross destruction of the adrenal glands of long standing, the basophil cells of the hypophysis were not diminished nor was the condition heir to the syndrome of Addison's disease.

THE PITUITARY GLAND IN ADDISON'S DISEASE.

Material.

Serial sections of the pituitary glands from five cases of Addison's disease were examined by the methods described in part I, particularly using the staining techniques 1A and 2A.   The five cases all showed the classical syndrome of asthenia, pigmentation, hypotonia, (the systolic blood pressure being less than 80 mm.Hg. in all cases), loss of appetite and gastro-intestinal upset.   Two were males aged 34 years and 42 years, with bilateral adrenal tuberculosis.   The others, a female of 37 years and males of 33 years and 36 years, were all cases of adrenal atrophy, the adrenal glands being reduced to minute structures in all cases.   Some had been treated with cortical preparations and high salt diet, the longest period of treatment being one year.   A summary of the clinical and post-mortem findings is provided in an appendix.

The findings in the pituitary glands were essentially similar in all cases.   The glands were all within normal limits of size and none appeared small.

HISTOLOGICAL FINDINGS.

Gross structure.   No abnormality of gross structure is present.

There is no interstitial fibrosis.

Anterior lobe.

Relative proportion of different cell types:

(a) **Basophil cells.**   When examined by methods 1A and III a striking

paucity of basophil cells is noted.   No areas rich in basophil cells

are visible to the naked eye in sections from any level.   Micro-

scopically only a few scattered cells or small clusters of ten to

twenty cells are seen.   The relative proportion of basophil cells

in all cases is judged to be in the region of a few thousandths of

the total cell count (see fig. 1, page 180).

(b) **Acidophil cells.**

As compared with the glands of the control series (this thesis,

part III), examined by method IB, a reduction of acidophil cells is

present in four cases.   In three the reduction is slight and is judged

to be little beyond the lower limits of normal.   In one case (case 2)

the reduction is of greater degree and the relative proportion of

acidophil cells is judged to be in the region of 5 to 10%.   In one

case (case 5) no reduction is evident.   No analysis by Rasmussen's

method has been carried out, but it is certain that the reduction, when

present, is slight compared with the gross reduction of basophil cells.

(c)   Chromophobe cells.

The depletion of mature chromophil cells is accounted for by an increase of chromophobe cells.   These cells constitute between 80% and 90% of the total in all cases.   The majority are of the small type found normally.   A small proportion, judged to be between 2% to 5%, are abnormally large forms (16 to 24 μ).

Cell characters.

(a)   Basophil cells.

Their size is within normal limits.   An occasional large form (24 to 28 μ), is present, but smaller forms (14 μ) predominate. Some of the cells have a shrunken appearance and the granules, though staining deeply, are small and have an angular appearance.   They appear to correspond to the basophil cells found in the pituitary glands of children which I have examined.   Nuclear pyknosis is present in a few cells and all stages to the completely degenerate agglutination cell (see this thesis, part I) are seen.   Evidence of cell death is not a prominent feature and the majority of basophil cells have normal nuclear and granular characters.

(b)   Acidophil cells.

The acidophil cells show no abnormality.

(c)   Chromophobe cells.

The chromophobe cells were studied principally with method 1A at a stage of differentiation where mature β granules are bright red

(acid fuchsin), acidophil cells and chromophobe cells are yellow
(picro-orange), and the stroma is blue (aniline blue). The large
chromophobe cells show a pale blue spongioplasm. Some are highly
vacuolated. In many, fine granules are developing from the spongioplasm
in irregular areas. Some of those granules are stained blue, others are
faint to deep red. Those cells are considered to be immature basophil
cells. The small chromophobe cells show no abnormality. The nuclei
of the large chromophobe cells are normal.

Pars intermedia.

The pars intermedia shows no abnormality.

Posterior Lobe.

In three of the glands the reduction of wandering basophil cells
is so extreme that they are rarely encountered. In case I only one
cluster of five cells is present in forty sections taken from all
levels. In two of the glands (cases 4 and 5) the wandering cells
are not numerous but are within normal limits. The majority of the
wandering cells show no abnormality. In case 3 a few large chromophobes
are present. In case 4 the granules are small and the cells have a
shrunken appearance.

D I S C U S S I O N.

The findings in the five cases verify Crooke & Russell's description of the pituitary gland in Addison's disease.   The gland is of normal size, "The chromophobe cells are increased in number, and a variable proportion of exceptionally large examples is present.   The acidophil cells are reduced but seldom conspicuously. Constant features are the extreme reduction of the basophil cells" - "somewhere between one-fiftieth and one five-hundredth of the average normal" - - - " and the presence of a series of abnormal basophil transitional cells."   "The reduction in number of the basophil cells in the anterior lobe of the pituitary is considered to be a constant change, and the most significant change in the other ductless glands following destruction of the supra-renal cortex in Addison's Disease." Crooke & Russell examined a series of 12 cases, and in 5 applied the differential counting methods of Rasmussen (Crooke & Russell, 1935). The gross reduction of mature basophil cells in my cases is a striking feature.   No such reduction is present in other cases of the control series, which includes cases where there is ablation of an endocrine gland - one case of almost total obliteration of the thyroid by amyloidosis, one case of a polycystic pancreas with total absence of islets.

While the majority of observers reach similar conclusions to Crooke & Russell there are some exceptions.   The distribution of basophil

cells in the anterior pituitary is so variable that one requires serial sections, preferably on the horizontal plane, and specific staining before any statement about relative proportions can be made. All discrepant results are doubted on the grounds of inadequate technical examination save one (Crooke & Russell, 1935; Rasmussen, 1938). This case, described by Severinghaus (1938), was of a woman dying at the age of 61, with classical symptoms of Addison's disease over a period of 2 years and a total illness of 4 years duration. The disease was well controlled by salt, the blood pressure being maintained at 136/80 mm.Hg. The final acute manifestations lasted only 11 days. At autopsy an abnormally high basophil-cell count (25%) was found in the anterior pituitary gland. The result has been verified by Rasmussen (1938). Severinghaus concludes that in Addison's disease destruction of the adrenal cortex leads to disappearance of mature basophil cells from the hypophysis, but there may be transient periods of active regeneration of basophil cells and he explains his curious result by death taking place at one of these fortuitous periods. While experimental evidence was not at first in agreement with such a hypothesis this was probably due to technical difficulty in examining the fresh animal hypophysis. Grollman and Firor (1935) now report a striking decrease, up to total absence of mature basophil cells in the hypophysis of adrenalectomised dogs.

Crooke & Russell divide the large chromophobes into different types of abnormal basophil transitional cells according to different

staining reactions. I do not consider that their rather elaborate detail is justified from the examination of glands from autopsy material and I do not consider that their staining technique is sufficiently controllable to allow of the various distinctions made. I agree with them, however, that they represent transitional basophil cells. Crooke & Russell describe them as being abnormal in that they did not encounter them in normal glands and rarely in glands from routine autopsies. In my control series a few of those cells have been seen in almost all glands from subjects over forty. In a few glands, no doubt abnormally, they appear to be almost as numerous as in Addison's disease. Severinghaus (1938) has produced much evidence to show that in the anterior pituitary gland there are chromophobe/chromophil cycles. Though there is some evidence of true degeneration of basophil cells, in the sense of a process to cell death, in Addison's disease; this was inconspicuous in my series. Crooke & Russell state that "a considerable number of the basophil cells have pyknotic nuclei." I consider that the main process at work in Addison's disease is an arrest of the normal process of maturation of β granules.

In their cases of Addison's disease Crooke & Russell found no change in the wandering cells, save in two cases where there were very minor alterations; a few abnormal basophil transitional cells in one, abnormally pale basophil cells in another. Kraus (1927) and others have recorded a reduction corresponding to that in the anterior lobe in some cases. In three of my five cases there is no doubt that the

wandering cells are reduced.   It is considered probable that in

all cases of Addison's disease there is a decrease of basophil cells

in the posterior lobe, though this is much slower in development than

in the anterior lobe, and, due to the extreme normal variation in

wandering cells, it is only in advanced cases that decrease can be

made out.

Crooke & Russell believe that the depletion of mature basophil

cells in the pituitary gland of Addison's disease is always secondary

to the adrenal lesion since the adrenal atrophy is of a destructive

character as opposed to the simple atrophy of Simmonds's disease,

while the tuberculosis lesion of the adrenals is doubtless the primary.

The adrenal atrophy of Addison's disease appears to be the result

of the organization of an acute nectrotizing process.   In the earliest

stages areas of cortical cells are seen to have disappeared and the

resulting spaces are occupied by round cells and macrophages, while

there is generalised vascular engorgement.   All stages can be

traced from this stage to the shrunken fibrotic mass enclosing

disjointed remnangts of the surviving tissue (Crooke & Russell, 1935).

The atrophic adrenal glands in my control series of Addison's disease

show in 2 cases the late stage, in the third, (case 1), it is at a

subacute stage.   In all these cases the medulla is reduced as well

as the cortex and is involved in the organizing process.   In case 1

the left adrenal is reduced to a minute structure,1.4 cms. x 1 mm., and

consists of minute irregular islets of adrenal tissue (cortical and

(medullary) separated by vascular nodes of round cells held by a delicate stroma. The right adrenal is represented by an area of thin-walled blood spaces cuffed by round cells. Adrenal tissue is confined to a few microscopic collections of highly vacuolated epithelial cells. In the other cases destruction of the adrenal medulla is of less degree than of the cortex.

The aetiology of the primary necrosis which leads to the so-called adrenal "atrophy" of Addison's disease is unknown. But, in view of the destructive nature of the condition and the involvement to some extent of the adrenal medulla, I consider that a vascular lesion is probably the basis of the condition, and that the condition is the result of survival after the usually fatal catastrophe of "haemorrhage into the adrenals." A vascular lesion in a main adrenal vessel, such as embolism or thrombosis, would seem unlikely since the lesion is bilateral. While bilateral thrombosis of the central medullary vein would produce this type of lesion a special factor, peculiar to the adrenals, would require to operate for the condition occurs in an age group not associated with primary vascular degeneration. Relatively large size and great muscular content are prominent features of the central vein of the adrenal medulla and, no doubt, it plays an important part in the regulation of the blood supply of the organ. It is considered possible that "haemorrhage into the adrenals" is produced by bilateral spasm of the adrenal veins due to an aberration of some normal physiological mechanism, and that the adrenal atrophy of Addison's disease is the end result of a corresponding lesion of less degree.

Such a mechanism would result conceivably in greater destruction of the more vascular cortex than of the medulla, which would agree with the morbid anatomical findings. Accordingly from the examination of the cases and on general grounds I agree with Crooke & Russell that the adrenal atrophy of Addison's disease is a primary abnormality.

Barker (1929) reported a type of atrophy where the medulla was normal and the cortex was reduced to a narrow strip. This is a contention in itself difficult to prove since islets of cortical tissue are frequently present in the medulla. Were such a condition to be substantiated a vascular accident would not be clearly admissible as the aetiological factor and it suggests the selective action of some agent on the cortex. Dunn and his co-workers have recently shown that alloxan, a simple condensation product of urea, when exhibited in animals leads to a selective necrosis of the islets of Langerhans, which leaves a minimum of sclerosis when the dead islet tissue is absorbed (Dunn, McLetchie & Sheehan, 1943; Dunn and McLetchie, 1943). It remains to be seen whether further experimentation will show that such a mechanism could operate naturally, but the observation opens new fields in the problem of the degeneration of highly spec-ialised tissues to which the adjective "toxic" was so frequently applied. A comparable mechanism would be conceived to operate in cases where there is a selective atrophy of the adrenal cortex with minimal sclerosis though, equally well, a primary assault on the basophil cells of the hypophysis would lead to a similar result.

The present evidence favours a primary destruction of the adrenals, the result of a vascular accident, as the most likely and common mode of production of the atrophy of Addison's disease.

The basophil cells of the pituitary gland are manifestly of great importance and there can be little doubt that their gross depletion constitutes an important factor in the morbid process of Addison's disease.   It is considered that this view will be even more justified from the speculations entailed by the next case to be described.

S U M M A R Y.

A description is given of the pituitary gland in five cases
of Addison's disease.   The Addison's disease was due/either to
tuberculosis or a destructive atrophy of the adrenal glands.
Constant features are the gross reduction of the basophil cells
and the presence of numerous basophil transitional cells.   The
acidophils may be reduced but not conspicuously.   The chromophobe
cells are increased in number.   It is considered that the basophil-
cell depletion is due to an arrest of the normal process of maturation
of β granules.   It is considered that the basophil-cell reaction is
secondary to the adrenal destruction in all cases.   The main findings
are in agreement with the classical description of Crooke & Russell.

C A S E   6.

THE REPORT OF A CASE OF ADRENAL INSUFFICIENCY ASSOCIATED WITH AN ABNORMALLY HIGH PROPORTION OF BASOPHIL CELLS IN THE ANTERIOR PITUITARY GLAND.

The case to be described is of a woman, aged 64 years, showing no signs referrable to Addison's disease, save mild pigmentation of the face, who died during an attack of diarrhoea and vomiting which followed the injection of typhoid vaccine. Post-mortem examination revealed adrenal destruction of a degree and chronicity as great or greater than that commonly described in classical Addison's disease. The endocrine factor preventing the development of Addison's disease appeared to rest with the anterior pituitary gland. In contrast to the findings in Addison's disease of basophil-cell depletion, the proportion of mature basophil cells in the anterior pituitary gland was beyond the upper limits of normal.

C A S E 6.

Case History.

The patient, a multipara aged 64, had, up to her fatal illness, led a very active life and enjoyed good health.   She had had no serious illnesses and her weight had remained constant for many years.

She was admitted to the Western Infirmary, Glasgow, for treatment for rheumatoid arthritis, under Dr. Douglas Adams.   She complained of joint pains with swelling and stiffness of joints over a period of four months.   The pain, of a burning character, affected one joint at a time, most frequently the wrist and interphalangeal joint, but at some time most of the small joints of the body had been affected.   The pain, accompanied by stiffness and, sometimes, slight swelling of the joint, was brought on by movement, especially knitting.   The patient had no other complaints.   Her appetite was good and there was no history of asthenia or gastro-intestinal upset

General Examination.   The patient was well nourished.   Her face was rather pale.   There was no cyanosis or oedema.   The wrist joints showed swelling and slight limitations of movement.   A few hard subcutaneous nodules were present on the flexor surfaces of the wrists.   The blood pressure was 160/95 mm.Hg.   The erythrocyte sedimentation rate was 28 mm in one hour (Westergreen).   The Wasserman reaction was negative.   No other abnormality was noted.   The patient was given intramuscular protein shocks on 4 occasions (T.A.B. vaccine;

25, 50, 100 and 150 million organisms respectively) over a period
of 14 days.   On the first 3 occasions the temperature rose sharply
to 103°-104° but fell within a few hours.   The respiration and pulse
were not markedly affected, and the patient's general condition good.
The patient's condition deteriorated rapidly after the last "shock."
She became very weak and developed severe diarrhoea and vomiting.
The pulse became very feeble.   Intra-venous glucose-saline drip was
initiated and, since the attack resembled the crisis of acute adrenal
insufficiency, an injection of eucortone was given, but the patient
died within a few hours.

The post mortem examination was carried out by Dr. A. C. Lendrum.
The following is his report:

P O S T   M O R T E M

External Appearances.   The body is that of a well-nourished, well developed woman, with an adequate, if not slightly excessive, amount of subcutaneous fat.   There is no oedema.   There is a fine brown pigmentation over the brow and malar region, only made out in good daylight.   The pigmentation resembles slight sunburn - this was a sunless period of the year.

Thorax: The pericardium is normal.   The heart (280 gms) shows no abnormality of the valves or endocardium.   The foramen ovale is closed. The coronary arteries are mildly atheromatous, but the lumen throughout is adequate and nowhere roughened.   The myocardium is slightly soft, but of normal thickness and uniform appearance.

The aorta shows a slight degree of atheroma in the distal part;   its elasticity and pliability are definitely good for the age, as indeed are all thea arteries of the body.

The lungs (left 350 gms., right 500 gms.) lie free apart from slight apical adhesions on the left side.   The trachea, bronchi and pulmonary vessels show nothing of note.   In both lungs there is slight apical starring;   the remainder of the pulmonary parenchyma shows merely slight congestion.   The tracheo-bronchial glands are slightly enlarged.

There is no obvious evidence of **thymic** tissue in the mediastinum.

The **thyroid** gland of average size is pale and somewhat adenomatous.

Abdomen.   The **oesophagus, stomach and intestines** show nothing of note.   The faecal material in the large intestine is somewhat paste-like in consistence, but is not of a dysenteric nature, and there is no evidence of an acute

congestive change in the alimentary tract.

The liver (1500 gms.) shows slight fatty change but is otherwise apparently normal. The gall bladder contains one stone about 6 mms. in diameter, mainly cholesterol; this has not produced any obstruction of the biliary circulation into the bladder. The spleen (170 gms) is slightly enlarged and definitely soft, it is not quite the spleen of an acute inflammatory process. The pancreas is fairly well preserved and apparently normal.

On the right side in the situation of the adrenal gland, three small dark spherical bodies, each about 3 mms. in diameter, are founding lying in the fat. On the left side the adrenal gland appears as a calcified mass invested by a thick fibrous capsule $2\frac{1}{2}$ cms. in length, with a thickness of 12-15 mms. No real glandular tissue is made out. A search was made for ectopic adrenal tissue, particularly in the region of coeliac axis, in the pancreas, broad ligament and ovaries. None is present.

The kidneys (each 110 gms.) show on section a normal architecture with slight pallor of the cortical parenchyma and distinct congestion. The capsules strip easily from a smooth surface.

None of the tissues of the body appear to be abnormal in their fluid content.

Head. The brain (1300 gms.) shows no abnormality; the pituitary gland is of full natural size.

I was present at the autopsy and carried out all of the histological examination.

H I S T O L O G I C A L   E X A M I N A T I O N.

The <u>left adrenal body</u> consists of a mass of calcified amorphous material,
lobulated by thick fibrous bands.   There is no trace of adrenal tissue.
The general arrangement is that of effete tuberculosis.   The small spherical
<u>right adrenal bodies</u> consist of dense fibrous tissue engulfing minute islets
of adreno-cortical epithelium.   Many of the epithelial cells are highly
vacuolated, and the cell columns are broken up by strands of fibrous tissue.
Lymphocytes infiltrate the epithelial tissue and are also present as focal
aggregations in the fibrous stroma.   A few minute islets of adrenal
medullary tissue are also present.

The <u>thyroid</u> gland shows pseudo-adenomatous areas with acini larger
than normal.   The gland has a full colloid content, and the epithelium is
of normal flattened type.

The pituitary gland.

The gland is of normal gross structure and is of average size.

<u>Anterior pituitary gland.</u>

The chromophil and chromophobe cells show no cytological abnormality.
As compared with the glands of the control series (this thesis, part III)
the relative proportion of acidophil cells is within normal limits, while
the relative proportion of basophil cells is well beyond the upper limits
of normal.   Using method 1A (this thesis, part I) deep red areas take up
approximately a half of the total area in sections from many levels.   At

P A R T   III

Fig. 1   Fig. 2
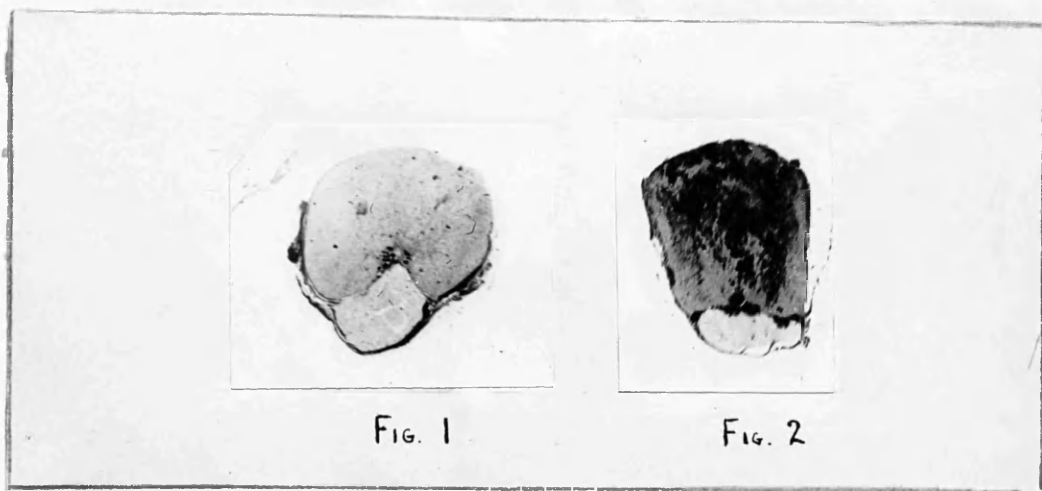
The figures show complete sections of whole pituitary glands stained
by method 1A [β granules, bright red (acid fuchsin); all other
cellular elements, bright yellow (picro-orange); stroma, blue
(aniline blue).]

Both figs. x 3.

Fig. 1; section of pituitary gland of Case 2 of classical
         Addison's disease.

Fig. 2; section of pituitary gland of the present case,
         Case 6.

NOTE the great area of fig. 2 taken up by confluent dark areas
(basophil-cell rich), while in fig. 1 no such areas are present
(dark dots are photographic artefacts).   The conglomeration of
small black discs in fig. 1 represents the colloid in the cysts of
the pars intermedia.   NOTE the wandering cells in fig. 2 as dark
masses infiltrating the pars nervosa.   The pars intermedia is not
a conspicuous element in this gland.

one level the proportion is only a sixth but a high proportion of scattered basophil cells is made out microscopically. The relative proportion of mature basophil cells is judged to be more than 30% of the total cell count. The proportion found is only approached by three cases in the control series; one male subject of 60 years dying from the effects of prostatic hypertrophy, one male of 50 dying from cardiac failure, and one very obese female diabetic of 48 years. Basophil-cell increase is not common to the age group as the relative proportion is well within the normal variation in twenty-five other subjects of the control series, all over 50 years of age. The increase in mature basophil cells was verified by all the other staining methods described. Thus there appears to be no reason to doubt that there is true basophilia (relative increase of basophil cells). This offers a striking contrast to the pituitary glands of the five cases of Addison's disease where, as described, no basophil-cell rich areas were visible to the naked eye and the relative proportion of mature basophil cells was judged to be in the region of a fraction of one per cent (compare figs. 1 and 2).

The pars intermedia shows no abnormality.

Posterior lobe. Wandering basophil cells are numerous, but within normal limits.

C A S E   6

D I S C U S S I O N.

Addison's disease is produced by gross destruction of the adrenal cortex, commonly by tuberculosis or atrophy.   Barker (1929) states that clinical symptoms become prominent only when at least 90% of the cortical tissue has been destroyed.   While the medulla may be involved in the destructive process, this may be only to a slight extent, or not at all, and medullary involvement does not appear to be necessary for the development of the syndrome (Crooke & Russell, 1935; Wells, 1930).   In the present case the amount of cortical tissue persisting is only a fraction of a hundredth part and is less than the amount persisting in some of the control cases of classical Addison's disease.   The medullary tissue is correspondingly reduced.   Nevertheless, Addison's disease was not manifest.   Indeed, apart from the joint condition, the patient enjoyed good health, and the only sign referable to Addison's disease was the pigmentation of the face, so slight that it was only appreciated in strong daylight.   It is reasonable to ascribe the terminal gastro-intestinal crisis and collapse which followed the protein shock therapy, to acute adrenal insufficiency; the latent adreno-cortical deficiency being unmasked under the stress of the reaction.

The failure of development of Addison's disease cannot be ascribed to a time factor for the adrenal lesions are of a chronic nature.   The left adrenal gland has been totally destroyed by tuberculosis.   The lesion is manifestly of long standing.   The lesion of the right adrenal has not the

183.

characters of tuberculosis. The microscopic appearances are similar

to the late stage of the so called "atrophy" of classical Addison's disease.

The distribution of the adrenal tissue in three small nodes is, however,

unusual and it may be that the morbid process is not the same as in the

classical atrophy. It is considered possible that chronic tuberculous

involvement of adrenal blood vessels near the aorta may have produced

a slow and unequal starvation atrophy. No such lesion was evident at

the post-mortem. While it is not possible to state the age of the lesion

it is probably of some years duration, though it is considered that it

occurred some time after the chronic tuberculous process on the left side.

The basophil cells of the anterior pituitary gland are important

elements in the endocrine system and it can hardly be doubted from the

evidence discussed previously that their gross depletion is an important

factor in the morbid process of Addison's disease. Therefore in the

present case it is considered that the failure of development of symptoms

of Addison's disease in the presence of the gross and chronic adrenal

destruction is associated with the absence of depletion of mature basophil

cells in the hypophysis. Nor is it considered that Severinghaus's finding

of basophilia in the presence of symptoms of Addison's disease contradicts

such a supposition, quite apart from the possible explanation of a temporary

regeneration of basophil cells in his case. It should be noted that in

Severinghaus's case the disease was well controlled by salt, that the

patient first experienced symptoms at the age of 57, and that it took two

years for the disease to develope sufficiently to allow of a diagnosis of

Addison's disease. (All of my control cases of Addison's disease and Crooke & Russell's cases were of a more inexorable nature). Severinghaus does not give great detail of his case and does not describe the adrenal lesion, but it is considered that it has much in common with the present case. Accordingly it is considered that the high basophil-cell-content of the pituitary gland is the important endocrine factor in the failure of development of symptoms of Addison's disease in the present case.

As to why basophil-cell depletion did not take place in the anterior pituitary gland in the presence of such gross adrenal destruction, I can only indulge in speculation. In the first place, the adrenal lesion is of a dual character. The left adrenal gland was presumably obliterated many years ago by tuberculosis, and, with this deficiency, adreno-cortical - basophil-cell relationships may have become so adjusted that the subsequent loss of the right adrenal was compensated for by active basophil-cell increase. In the second place the age of the subject may be an important factor. The patient was 64 at death. My control cases of Addison's disease are all much younger, while Crooke & Russell's series contains only two subjects over 50; one of 53 and one of 63. It may be that in the latter part of life the cortex becomes less important in the endocrine system. Whatever the true explanation may be, I consider that the implications of this case and the findings in classical Addison's disease indicate that the depletion of mature basophil cells in the anterior pituitary gland is an essential part of the morbid process of Addison's disease.

ADDISON'S DISEASE AND PITUITARY BASOPHILISM.

Addison's disease is, in most respects, the opposite of Cushing's syndrome.   McQuarrie, Johnson and Ziegler (1937) give the following table of comparison between the findings in Addison's disease and a case of Cushing's syndrome (patient M.V.) :

T A B L E

DATA	ADDISON'S DISEASE	PATIENT M.V.
Clinical		
Nutrition	Emaciated	Adiposity of trunk, neck and face
Skin -	Pigmented	Dusky, florid face; acrocyanosis; purplish striations
Hair	Normal or sparse	Hirsutism; hypertrichosis of extremities.
Eye balls	Endophthalmos	Exophthalmos.
Sex function	Diminished	Diminished
Blood pressure	Reduced	Elevated
Body temperature	Subnormal	Normal
Basal metabolism	Reduced	$\pm 0\%$ + 16%
Plasma CO_2 combining power.	Decreased	Greatly increased
Gastrointestinal	Hypochlorhydria;	Achlorhydria; anorexia to normal appetite.
Blood volume	Reduced	Normal
Skeleton	Normal	Normal to slight osteoporosis
Voice	Normal	Masculine; rough.

TABLE   (continued)

DATA	ADDISON'S DISEASE	PATIENT M. V.
Blood Constituents		
Sugar	Hypoglycaemia	Hyperglycaemia (frank diabetes).
Sodium	Decreased	Increased
Potassium	Increased	Decreased
Calcium	Slightly increased	Slightly decreased.
Magnesium	Increased	Decreased
Chloride	Decreased	Decreased
Bicarbonate	Decreased	Greatly increased
Inorganic phosphorus	Slightly increased	Slightly decreased
Non-protein nitrogen	Increased	Normal
Serum proteins	Increased	Decreased.
Response to Therapy.		
Cortin	Beneficial	Not helpful
NaCl solution	Beneficial	Not helpful
K Cl solution	Harmful	Beneficial.
Insulin	Harmful	Beneficial.

McQuarrie, Johnson and Ziegler contend that, since Addison's disease
is the symptom of adreno-cortical destruction, the opposite condition,
Cushing's syndrome, is the symptom of adreno-cortical hyperfunction.
Their error lies in that they did not appreciate the precise nature of the
morbid processes involved.   In my final summation of the morbid processes
of Cushing's syndrome I considered that hyperfunction of the basophil cells

was a "sine qua non." The evidence indicated that the basophil cells and the adrenal cortex were so closely inter-related that (1) they could be conveniently regarded as a functional complex in that a high degree of hyperfunction of the one was followed by a like alteration in the other, and (2) the primary morbid process in Cushing's syndrome could be regarded as a hyperfunction of the complex. The nature of the morbid process in Addison's disease carries similar implications in that a high degree of adreno-cortical hypofunction is followed by a like change in the basophil cells. It has already been presumed that in respect of androgenic activity the adrenal cortex has a relative autonomy, but in respect of its other functions it would appear that they only have a pathological effect when the cortical abnormality is of high degree and is associated with a like alteration in the basophil cells.

The inter-endocrine relationships which I have discussed are the most obvious which I have encountered, and are considered of primary importance. The reduction of the adrenal glands to minute proportions is a striking feature of Simmonds's disease (Sheehan and McLetchie, 1943), and also is the most immediate effect of experimental hypophysectomy in animals (Grollman, 1936). The pituitary destruction involves both acidophil and basophil cells. In acromegaly and gigantism, which is associated with adenoma or diffuse hyperplasia of the acidophil cells, adrenal hyperplasia is produced. Nevertheless it is not considered that the same close relationship exists between the adrenal cortex and the acidophil cells, as with the basophil cells. Thus primary adrenal hyperplasia does not

produce acidophil-cell hyperplasia. The adrenal destruction of Addison's

disease results in depletion of the acidophil cells of the pituitary gland,

but only to a slight degree, in contrast to the gross depletion of basophil

cells. While adrenal hyperplasia is produced in acromegaly this is in

common with a generalised splanchnomegaly. While the destruction of

cells
acidophil/ in Simmonds's disease may contribute to the production of

adrenal atrophy, it is almost certain, from all the previous evidence

discussed, that this is produced in part by the destruction of the basophil

cells. Thus there is little evidence for placing the relationship of the

acidophil cells to the adrenal cortex closer than the relationship of the

acidophil cells to most other endocrine glands, as opposed to the more

obvious reciprocal relationship which exists between the adrenal cortex and

the basophil cells.

THE PART OF THE WANDERING BASOPHIL CELLS OF THE

POSTERIOR LOBE OF THE HYPOPHYSIS IN MORBID PROCESSES.

The intimate relationship between the adrenal cortex and the

basophil cells of the hypophysis refers to the basophil cells of the

anterior lobe.   The wandering basophil cells of the posterior lobe

require separate consideration.   The proportion of wandering cells is

extremely variable in glands from routine autopsies and so far no correllation

between the numbers of cells and any morbid condition has been substan-

tiated (Scriba, 1936; Rasmussen, 1936), though Kraus (1935) claims that

there is an increase in persons abnormally overweight.   Their depletion

in Addison's disease cannot be directly connected with loss of weight, a

variable feature of the syndrome and connected more obviously, in some

cases, with gastro-intestinal upset, in others with widespread tuberculosis.

In the allied condition of Simmonds's disease, produced by post-partum

necrosis of the anterior pituitary gland, Sheehan has already stressed

the curious fact that, despite gross destruction of the anterior lobe,

loss of weight is not a feature of the condition (Sheehan, 1939; Sheehan

& McLetchie, 1943).   It is important to recognise that in post-partum

necrosis of the pituitary gland the pars intermedia and posterior lobe

are usually spared completely.   In the case described by Sheehan &

McLetchie destruction of the anterior lobe is as great as has ever been

described.   The bulk of the pars intermedia is intact and the posterior

lobe is not involved in the primary destructive process, though it is

somewhat shrunken.   In this case wandering basophil cells were

identified in the posterior lobe.   Though this subject had classical

symptoms of severe Simmonds's disease for a period of seven years, the

general body nutrition remained good.   The findings suggest a linkage

between the wandering cells and nutrition but this will be denied by some

general considerations to follow.

In Cushing's syndrome the wandering cells undergo the same alterations

as the basophil cells in the anterior lobe, but to a less degree.   Hyalin-

ization of the basophil cells of the anterior lobe is present in descending

degree in Cases I, III and II (This thesis, part III).   The degree of

hyalinization of the wandering cells is greater in case I than in case

III, while no hyalinization is present in case II.   In each case the

degree of hyalinization of the wandering cells is less than that in the

basophil cells of the anterior lobe of the same gland.   Cytoplasmic

vacuolation of the basophil cells of the anterior lobe is of great degree

in case III.   It is only in this case that vacuolation is present in the

wandering cells and it is only of slight degree.*   It was already concluded

that in Addison's disease depletion of wandering cells is a slower process

than that of the basophil cells of the anterior lobe.   Thus from these

two conditions it would appear that the wandering cells undergo the same

reactions as their brothers in the anterior lobe but to a less degree.

If this law is generally applicable we are drawn into deep waters.   As

has been stated, Kraus maintains that the wandering cells are increased

* (this thesis, page   )

in people abnormally overweight.   Now if we conceive of a subject having

a transient period of basophilia in the anterior lobe this would be

reflected in an increase in the wandering cells in the posterior lobe;

admittedly slower in development, but, equally well, slower to pass off.

Thus a posterior lobe basophilia may merely be the shadow of a long past

anterior lobe basophilia.   I mention this to indicate the difficult

nature of the evidence.

In Addison's disease the depletion of wandering cells, when present,

is absolute, i.e. there is no corresponding replacement by chromophobe

cells as obtains in the anterior lobe.   For this there are two possible

explanations :-

(1)   There may be continual cell death and replacement of wandering cells

in the posterior lobe and in Addison's disease the replacement is stopped.

(If cell death does take place normally it must be a slow process, as I

have rarely encountered wandering cells in a process of dissolution).

(2)   In Addison's disease the essential process may be one of death of

mature basophil cells.   I favour the first view.

The wandering cells arise from the pars intermedia, which is probably

a vestigial structure.   In children the pars intermedia and wandering

cells are represented by tubulo-racemore glands composed of a non-granular

epithelium having a similar origin to the anterior lobe (Rasmussen,1936A).

It would appear that the development of wandering basophil cells takes

origin from the same stimulus which causes maturation of chromophobe

cells to basophil cells in the anterior lobe at puberty.   I consider

that the wandering cells of the posterior lobe follow the same

reactions as the basophil cells of the anterior lobe, but to a less

degree, simply because they are essentially the same as their brothers

in the anterior lobe but have a much smaller blood supply.   It is not

considered that they make any special contribution to the pathological

processes in which they are involved.

In conclusion.

This paper has been written from the viewpoint of the morbid anatomist. The writer cannot pretend to have read, far less digested, all the mass of literature now available on other aspects of the problem. While morbid anatomy has many limitations it has the merit that the changes observed occur in the compass of a human life, and from the observed facts it is sometimes possible to enunciate fundamental principles. The conditions I have dealt with are all uncommon, but a proper understanding of them has wider applications. In the future important contributions to diagnosis by endocrine assay are to be looked for. But, in the beginning, knowledge can only be accurately acquired, in so far as the human subject is concerned, by the investigation of the normal and of the abnormal with a known lesion. Equally well the value of hormone substitution therapy can only be assessed in the beginning by trial in cases where the morbid process is accurately defined. From this we can proceed to the diagnosis and treatment of those milder and more occult manifestations of the classical syndromes which encompass the great bulk of endocrine disease.

AND THEN MY HEART WITH PLEASURE FILLS,

AND DANCES WITH THE BASOPHILS.

A C K N O W L E D G M E N T S.

O R M S K I R K.

12 FEB. 1944.

S Y N O P S I S.

SUMMARY OF CASES OF ADDISON'S DISEASE.

Case I.

Addison's disease: atrophy of adrenal glands
Male aet 36.

Clinical history : Discharged from Army as a case of Addison's

disease a few months before his death.   He gave himself injections

of cortical preparations (Organon) and took an excess of sodium

chloride in diet.   Classical signs and symptoms of Addison's disease

were present, and some days before admission to Western Infirmary he

showed vomiting, a fall in B.P. to 80/45 and pallor.

On admission - semi-comatose; B.P. 35/?; pulse imperceptible;

brown pigmentation of hands, face, umbilical region, and buccal

mucosa.   Treated with eucortone, intravenous glucose-saline, and

DOCA.   Died one day after admission.

Post-mortem.

Fair nutrition; heart, 250 grms.; oedema of lungs; thyroid,

fairly large and pale; thymus, not enlarged; liver, 1150 grms.,

wasted; pancreas, normal in appearance; kidneys, 145 grms. and

150grms., spleen, 135 grms.

Adrenal glands: On dissection of the area of adrenal glands, small

patches of collapsed cortical tissue, brownish in colour, found in

fat at site of both adrenal glands.   The appearances point to almost

complete atrophy of adrenals with fatty replacement.

Histological description in text.

Case 2.

Addison's disease; atrophy of adrenals glands;

female aet 37

Clinical history : 4 months history of asthenia, attacks of vomiting, marked loss of weight, pigmentation of skin. Generalised pigmentation most marked on face, neck, elbows, hands, and in buccal cavity. B.P. 70/44, later 90/65 when treated with eucortone and high salt diet.

Post-mortem.

Heart, 160 grms.; lungs, liver, spleen, all small; thymus, prominent; thyroid, normal size, ample colloid, but many alveoli are small and epithelium is cubical. Adrenal tissue - minute bodies about 1cmm. x 2 mms.,showing minute islets of cortical and medullary tissue engulfed by young adult fibrous tissue; zones of round cell infiltration present.

Case 3.

Addison's disease; bilateral adrenal tuberculosis;

male, aet 42.

Clinical history. Nineteen months history of asthenia, loss of weight, anorexia, pigmentation of face and limbs, attacks of vomiting. Treated with eucortone and high salt diet for 12 months. B.P.110/65 later 80/60. 2/8/41 adrenal grafts planted in rectus muscle; sepsis supervened; died 6/8/41.

Post-mortem.

Well developed male; deep brown pigmentation of skin; heart, 255 grms; healed tuberculosis of r. lung; bilateral pleural effusions, each 1½ pints; thyroid, no abnormality; thymus, not enlarged; liver and spleen, small; r. adrenal gland, entirely replaced by caseous tuberculous tissue; l. adrenal gland, almost entirely replaced by caseous tuberculous tissue, kidneys, normal.

Case 4.

Addison's disease; atrophy of adrenal glands.

male aet 33.

Nine months history of asthenia, attacks of dizziness, brownish pigmentation of face.   B.P. 84/55; temperature, low; improvement with eucortone and high salt diet, but relapsed and died four months after start of treatment.

Post-mortem.

Emaciated; diffuse brown pigmentation of skin most marked on face; heart, 290 grms., thymus, enlarged; thyroid, small (20 **grms.**); liver, 1300 grms.; adrenal glands, reduced to tiny atrophic structures, each about $1\frac{1}{2}$ cms. x a few mms.   Histologically, small rind of cortical and medullary tissue supported by young adult vascular fibrous stroma showing areas of round cell infiltration

Case 5.

Addison's disease; bilateral adrenal tuberculosis

male, aet. 34.

Soldier; three months history of asthenia. Admitted semi-comatose to military hospital; B.P. 50/?; pigmentation of face and hands; rallied with intra-venous glucose saline and eucortone; improved on eucortone and high salt diet. Attacks of vomiting supervened and caused death one month later.

Post-mortem.

Heart, 300 grms.; active tuberculous focus in r. lung. Liver, spleen, kidneys - no abnormality. Thyroid, large; thymus, large. Both adrenal glands almost entirely replaced by caseous tuberculous tissue. Haemorrhage into r. gland.

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