STUDIES ON THE STRUCTURE AND FUNCTION OF THE HUMAN ADRENAL CORTEX

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I declare that the work embodied in this thesis is my own and that the thesis was written by myself.

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

The adrenal gland in the human subject was first described by Bartholomaeus Eustachius in 1563. By 1883 a reasonably accurate account of the gross anatomy, and gross histology of the human adrenal had been given by various authors (Gottschau, 1883). Detailed knowledge of the finer histological, cytological and cytochemical structure of the mammalian adrenal cortex has been obtained by various investigators over the past 60 years (Bourne, 1949; Jones, 1957). Most of this work was however done on species other than the human. Just as mediaeval anatomy was dependent on the studies of Galen on the pig, so, until recently, knowledge of the cytology of the human was dependent on studies of convenient laboratory animals. Anatomy arose from its mediaeval nadir when Vesalius dared to dissect the human body; parallel cytological opportunities have arisen in the study of the human adrenal cortex since the advent of adrenalectomy as a form of treatment for advanced mammary cancer (Huggins & Bergenstal, 1951). Fresh tissue for study by modern techniques is now available for the first time.

Such tissue has been studied histologically and histochemically by Symington et al. (1955). This group suggest that there are three types of cell in the human adrenal cortex:

I. The glomerulosa cell. The glomerulosa has been shown by Giroud et al. (1958) in the rat and by Ayres et al. (1958) in the ox to be

the source of aldosterone.

II. The clear cell in the zona fasciculata, characterized by a large content of sudanophil lipid, and by a low content of histochemically demonstrable RNA, alkaline phosphatase and succinic dehydrogenase.

III. The compact cell of the zona reticularis, characterized by a low content of sudanophil lipid, and a high content of histochemically demonstrable RNA, succinic dehydrogenase and alkaline phosphatase.

The same group have shown as did Rogers and Williams (1947) that stimulation of the adrenal cortex by ACTH or by external stress causes a loss of lipid in the zona fasciculata, the so-called phenomenon of lipid depletion. The clear cells in the zona fasciculata lose their lipid and become rich in RNA and stainable enzymes, thus assuming the form of compact cells. The degree of lipid loss varies with the degree of stress. When lipid depletion occurs there is a concomitant increase in output of corticosteroids. At the same time there is of course an increase in the number of compact cells in the gland; the inference was then made that as Yoffey (1953) had suggested the zona reticularis was an actively functional zone.

This was directly opposed to the view of Bennett (1940) that the zona reticularis in the cat was a senescent zone, in

that cell division occurs peripherally, and cell death centrally. It did not agree either with the idea that the lipid depleted adrenal cortex seen in patients dying of severe stress was an indication of adrenal cortical exhaustion (Stoner et al., 1953). If indeed the compact cells of the zona reticularis are actively functional, then it is strange that the stressed adrenal at death which contains many of these cells should be considered as being exhausted.

The term adrenal exhaustion occurs throughout the literature on adrenocortical physiology and stress, at times without a clear definition of its meaning.

Selye (1946) defined the stage of exhaustion in his general adaptation syndrome as "The sum of the non-specific reactions which ultimately develop as the result of very prolonged exposure to stimuli to which adaptation had been developed, but which could no longer be maintained." He believed (1946) that corticoid administration might aid resistance in the intact animal, thus implicitly subscribing to the idea of adrenal exhaustion, but later (1950) stated that "lack of corticoids was rarely an important limiting factor of resistance in conditions of grave systemic stress." Yet (1950) he uses the phrase "exhausted adrenal", admitting however (1946) that plasma corticoid levels may rise at death. The third stage of the

general adaption syndrome seems to be exhaustion of the whole body accompanied by certain morphological degenerative changes in the adrenal cortex.

Morphological changes do of course occur in the adrenal cortex in response to stress, notably loss of cortical lipid (Sarason, 1943; Selye, 1946; Symington et al., 1955). Lipid loss, loss of birefringent material and negative ketosteroid staining reactions are said (Stoner et al., 1953) to indicate loss of preformed hormone and therefore loss of secretory function. The ketosteroid stains have been shown to indicate nothing more than carbonyl groups in formalin fixed tissue (Karnowsky & Deane, 1954) and so no direct inference as to function can be drawn from such studies.

It is doubtful whether excessive stimulation of the adrenal can produce failure of adrenal secretion (Sayers, 1950). While there are similarities between the reaction to severe stress and the phenomena of adrenal cortical failure as seen in the Waterhouse-Friederichsen syndrome, there is no unequivocal proof, particularly in the human subject, that administration of corticosteroids increases the resistance of the intact organism to stress (Swingle & Remington, 1944; Sayers, 1950). This makes it unlikely that the rate or quantity of corticoid secretion is a limiting factor in survival. Nevertheless there have been

suggestions that clinically in the human subject administration of corticoids increases resistance to severe infections (Thorn, et al, 1951) but in most of these cases the picture was obscured by simultaneous administration of antibiotics.

In attempting to assess adrenocortical function at death and to correlate it with function, we are hampered by the fact that the methods which are applicable to patients at operation, i.e., estimation of adrenal vein corticosteroids and enzymic synthetic power *in vitro*, are not practicable on post-mortem material (Grant *et al.*, 1957). It is therefore, necessary to use indirect methods. An attempt was made to study the relationship of post-mortem lipid depletion to adrenal function using such methods. The term adrenal exhaustion is defined as a state wherein the secretion of adrenal cortical hormones is insufficient for the needs of the organism.

The present investigation was intended to investigate:

1. Human adrenal function at death.
2. The site of cell replacement in the human adrenal cortex.
3. The detailed cytology of the human adrenal cortex.

During the latter cytological investigation it became evident that no lamellar endoplasmic reticulum was present, and that its place was probably taken by

endoplasmic reticulum of the vesicular type. To prove that the many vesicles seen were actually endoplasmic reticulum it was necessary to examine:

4. Ultra centrifuged cell fractions to attempt to correlate components resembling these vesicles with the presence of RNA.

M A T E R I A L S A N D M E T H O D S

(A) STANDARD TECHNIQUES

Human adrenal glands were obtained at necropsy (Groups 1A & 1B) and by two-stage bilateral adrenalectomy (Groups 2, 2A & 2B).

Group 1A. The adrenals were removed from 74 subjects at necropsy. These subjects had died in a large general hospital; they did not represent a random sample of deaths in that hospital but were removed mainly from patients likely to be grossly stressed, e.g., severe burns or severe infections. Necropsy was in all cases performed within 6 hours of death.

Group 1B. The adrenals in this group were removed from 344 subjects at necropsy. This series was a random sample of deaths in the same hospital, and was examined merely to ensure that the series 1A was not too grossly biased.

Group 2. Adrenals were obtained by a two-stage operation from 40 female subjects suffering from advanced mammary carcinoma, as described by Symington et al., 1956.

Group 2A. The first gland was removed without prior hormone therapy and served as a control "normal" gland.

Group 2B. The second gland was removed after an interval varying from 7 to 21 days; 200-400 units of ACTH gel were administered over the three days prior to operation in all but 6 of the cases. The glands were all subjected to the stress of the first operation.

The adrenal glands from all groups were fixed in 10% neutral formalin and embedded in paraffin and gelatin. Sections of the paraffin blocks cut at 6 μ were stained with Harris's haematoxylin and eosin, Heidenhain's iron haematoxylin and methyl green and pyronin (Brachet, 1953). Gelatin sections cut at 12 μ were stained with Sudan IV, and the degree of lipid depletion was estimated in all cases (Table I) (Currie & Symington, 1955).

Mitotic counts were carried out on paraffin sections stained with haematoxylin and eosin and iron haematoxylin. A 1/6" objective was used and 100 high power fields were counted, contripetally from the capsule. Only cells with recognizable chromatin skeins, devoid of a nuclear membrane on further inspection with a 1/12" oil immersion objective, were accepted as being in mitosis. The count was expressed as mitotic figures per 100 high power fields. Such an area contains approximately 10,000 cells.

The adrenal glands examined were all free of metastatic cancer or other lesions.

Blood was obtained by cardiac puncture from 55 of the cases in Group 1 above. The delay between death and removal of the blood varied from a few minutes up to 6 hours, in most cases being less than 3 hours. The plasma 17-OH corticosteroid level was estimated by Silber's modification of the method of Silber

and Porter (1954). In 5 cases duplicate estimations were performed on specimens removed immediately and several hours after death.

Group 3. Adrenals from 20 similar patients to those in group 2 were subjected to detailed cytological study. Six of these were control normal glands and 4 had been subjected to ACTH stimulation. The material from the other 10 was unsuitable because of technical deficiencies.

Group 3A. Small blocks were fixed in formol dichromate (Regaud's fluid modified) a mixture of 80 parts of 4% potassium dichromate and 20 parts of 40% formaldehyde solution, and in Altmann's fluid a mixture of equal parts of 2% osmium tetroxide and 5% potassium dichromate. The former mixture does not preserve neutral fats well but provides tissue which sections fairly readily; the latter provides tissue which is difficult to cut but in which fat is preserved. After 4 days fixation, the tissue was postchromed in 3% potassium dichromate for seven days. During these procedures it is important not to allow the pH of the mixture to fall below 4, or mitochondria tend to be washed out in free chromic acid, which is present due to dissociation of potassium dichromate below pH4. The material was then vacuum embedded in paraffin wax and sectioned at 3-5 μ.

Group 3B. Similar blocks afterchrome-osmium fixation were

post osmicated in 2% OsO_4 in an oven at 37°C. This (Gatenby & Beams, 1950) is of course essentially the Weigl-Mann-Kopsch Golgi method. Sections were cut at 5-10 μ.

Sections from (A) and (B) were examined unstained and after staining by the Bensley-Cowdry aniline acid fuchsin method. In the case of the non osmicated tissue, sections were stained also by Regaud's iron haematoxylin. Thus both mitochondria and Golgi apparatus could be studied.

Group 3C. Blocks from 6 unstimulated and 4 stimulated glands were fixed in Zetterqvist's buffered osmium mixture, washed, dehydrated and embedded in methacrylate. Thin sections were cut either on a Porter-Blum or on a Cooke and Perkins thermal advance microtome, using the standard glass knives. Tissue obtained from another 10 adrenals was not satisfactorily fixed, though the delay between removal from the body and immersion in fixative was never more than 1 minute. Often, however, the tissue was subjected to prolonged ischaemia due to surgical manipulation before actual removal from the body. The sections were viewed on either a Philips EM 75B or EM 100 microscope.

(B) THE ORIENTATION PROBLEM

The localisation problem in the adrenal cortex is acute

inasmuchas cells in various zones are normally distinguished on staining reaction rather than shape or arrangement. Phase contrast is thus not useful for achieving tissue orientation. Excellent as osmium tetroxide is as a cytological fixative, it has the disadvantage of rendering tissues refractory to most stains (Gatenby & Beams, 1950; Baker, 1958). It was observed by Overton in 1890 that hydrogen peroxide removes osmium precipitate from tissue sections; this fact was applied to the staining of thick sections of osmium fixed methacrylate embedded tissue. The test material used was rat colon which contains many tissue components; it was fixed in 1% osmium tetroxide, embedded in methacrylate and sectioned at 1-2 μ. on a Porter-Blum ultramicrotome.

Results (Table II)

It was found that only toluidine blue and the periodic acid Schiff technique gave adequate results without prior treatment of the section with hydrogen peroxide. As a contrast methylene blue and the polychrome methylene group gave unsatisfactory pictures, including even the Wright Giemsa method of Condie et al., 1958).

Both iron and alum haematoxylins do stain to some extent but are slow and difficult to differentiate. The common cytoplasmic stains stain very slowly and produce a muddy hue. After treatment with hydrogen peroxide (50 vols. for 30 mins. or 100 vols.

for 10 mins.) results are obtained with iron and alum haematoxylin and with the common cytoplasmic stains entirely comparable to those obtained on formalin fixed tissues. The exception is eosin. Treatment with hydrogen peroxide accelerates staining by the PAS technique, but has no effect on toluidine or methylene blue.

Alternative methods of removing osmium were tried: Mallory's bleach, potassium iodate, sulphurous acid chlorine, dioxan and turpentine. Of these only Mallory's bleach and turpentine are effective. Mallory's bleach is as effective as hydrogen peroxide. Turpentine is almost as effective but rather slower; it allows staining with 2% alcoholic eosin whereas hydrogen peroxide does not. Potassium iodate, sulphurous acid and chlorine are partly effective but tend to remove the sections from the slide. Dioxan is ineffective.

It was found that pretreatment with Mallory's bleach, i.e., potassium permanganate followed by oxalic acid mimicked the action of peroxide exactly. A brown staining of the material may however persist impairing the final picture though not the staining process (Binet, 1894). Sulphurous acid (Monckeberg & Bette, 1899) and chlorine (Mayer, 1881) are partially effective but tend to remove the section from the slide; they are cumbersome to prepare. Potassium iodate suffers from both of these disadvantages and in

addition takes at least 24 hours to act. Dioxan (Asana, 1940) is quite ineffective. Any action attributed to it may be due to contamination with organic peroxides. These reagents are all oxidising agents; the remaining substance turpentine is in a different class. It owes its action partly to its solvent effect on the fats to which osmium attaches. Since however it has much more effect in removing osmium than xylol some additional mechanism must be involved. Its effect is slower than that of hydrogen peroxide and differs therefrom in two particulars. Tissue will stain with 2% alcoholic eosin after turpentine, but not after peroxide. Turpentine unlike the others has a specific differentiating effect on Golgi staining, removing ordinary fat but leaving the Golgi network.

These staining methods would seem to be more useful in obtaining tissue orientation in electron microscopy than the current methods. Phase contrast microscopy in the examination of osmium fixed material is adequate only in the examination of organs where there is a clear arrangement of tissues. In an organ where it is necessary to identify different cell types lying side by side and where the cells are defined from one another largely by staining reaction something further is necessary. Houck and Dempsey (1954) found that while satisfactory nuclear staining could be obtained in osmium fixed

methacrylate embedded material cytoplasmic staining was always poor. Exceptions to this rule were silver precipitation methods and the PAS reaction (Weiss, 1957). The recent gallocyanin chrome phloxin method (Runge et al., 1958) gave good results in the authors' hands but takes 24-48 hours to perform. Peroxide treatment followed by haematoxylin and phloxin or one of the trichrome methods is quicker, simpler and more in line with normal histological practice.

An essential step in rendering osmic fixed material stainable by the ordinary techniques is the removal of the black osmium precipitate. The exact nature of this precipitate is not known nor is the chemistry of osmium fixation. It seems likely (Gatenby & Beams, 1950) that osmium tetroxide (OsO_4) in aqueous solution forms perosmic acid (H_2OsO_5) and that in the process of fixation this is reduced to lower oxides of osmium which are insoluble and form the black precipitate. Baker (1958) suggests that the deposit is osmium dioxide (OsO_2). The essentially particulate nature of osmic staining even at the electron microscopic level suggests that deposition occurs at specific sites almost certainly at double bonds, and probably at those of unsaturated lipids and of the side groups of such amino-acids as tryptophan and histidine, and similarly at the sulphydryl groups of amino-acids (Bahr, 1954; Baker, 1958). It would seem likely

that stains attach themselves at the same sites probably by ionic linkages and that in osmium fixed tissue the stains are partially or completely blocked from attaching to these sites by their prior occupation by the osmium tetroxide. Osmium has little affinity for nucleoproteins; this may account for the fact that basic dyes are more effective in staining osmium fixed tissues than acidic dyes. Thus the normal nuclear dyes do stain osmium fixed tissue; the cytoplasmic dyes do not. Toluidine blue may be effective because of its affinity for nuclear DNA and cytoplasmic RNA. Removal of osmium precipitate may involve re-oxidation of OsO_2 to the soluble OsO_4, leaving the reaction sites free for staining. It is not clear on this hypothesis why though phloxin stains adequately after peroxide treatment, the closely related eosin does not.

Several methods of removing osmium tetroxide have been practised. Hydrogen peroxide is the simplest and best. It has been used in recent years only by Ruthmann (1958) who found that ribonuclease was ineffective on osmicated tissue and that the inhibition of ribonuclease was removed by washing in peroxide; by Chou (CIT. Baker, 1958) and by Swift and Rasch (1958) who used it to remove osmium from thin sections to be viewed on the electron microscope. None of these authors used it to facilitate staining.

(C) ULTRACENTRIFUGATION

Preparations of mitochondria and microsomes from ox adrenal cortex were obtained by Dr. J.K. Grant, Department of Biochemistry, University of Edinburgh, as follows:

Adrenal cortical tissue, one hour after removal from the ox at the slaughterhouse was sliced and homogenised in 0.88 μ. sucrose. The homogenate was then centrifuged for 10 minutes at 1,200 g. to remove nuclei. Centrifugation was repeated and mitochondria were obtained from the supernatant by centrifuging for 10 minutes at 7,000 g. in a Spinco ultracentrifuge. After removal of the mitochondria, further centrifugation at 105,000 g. in the Spinco for 30 minutes produced a microsomal fraction.

These two fractionswere then:

1. Fixed in formalin, paraffin embedded, sectioned at 5 μ. and stained with pyronin.
2. Fixed in formol dichromate, paraffin embedded, sectioned at 5 μ. and stained with aniline acid fuchsin.
3. Rapidly frozen in an ethyl alcohol-dry ice mixture. Frozen sections were cut in a cryostat and stained by the tetrazolium reaction for succinic dehydrogenase.
4. Fixed in Zetterqvists buffered osmium mixture and embedded in methracrylate. Thin sections were cut and examined with the electron microscope.

RESULTS

ADRENAL LIPID PATTERNS

MITOTIC COUNTS

PLASMA CORTICOSTEROID LEVELS

MITOCHONDRIA

GOLGI APPARATUS

ELECTRON MICROSCOPY

ULTRACENTRIFUGATION

ADRENAL LIPID PATTERNS

In group 1 all grades of lipid depletion were found (Tables III and IV). Loss of lipids from the zona fasciculata is accompanied by an alteration in the appearance of the cells from the vacuolated clear form to a deeply eosinophil compact form and by the appearance in these cells of histochemically demonstrable ribonucleic acid and alkaline phosphatase (Symington et al., 1955). Total lipid depletion is uncommon and occurs notably in burns (Table III). In the small series (1A) the degrees of lipid depletion was found to be reasonably representative as compared to the larger group (1B). In group 2, as shown previously (Symington et al., 1956) there was little or no lipid depletion of the glands which were removed without prior stimulation (group 2A). The group which were removed after the stimulation of prior operation and ACTH administration (group 2B) showed moderate lipid depletion (2-3+).

MITOTIC COUNTS

In group 1A the mean mitotic count in the adrenal cortex post-mortem is 1.2 per 100 high power fields. In only one case, that of a man dying 15 hours after the onset of a coronary thrombosis, was a higher count found - 8 figures per 100 high power fields (Table V). In group 2A a similar count was found

while in group 2B in 9 out of 40 specimens the count was grossly elevated to a level in one case of 35 figures per 100 high power fields. The remaining 31 cases showed little or no elevation above the normal. Of these 31 cases 6 had not received ACTH, while the dosage of ACTH and the lapse of time between administration of ACTH and operation varied widely from case to case. These factors were difficult to control because this was mainly a retrospective investigation and because at any time the experimental plan might become secondary to clinical considerations. Mitotic figures were found only in compact RNA containing cells, notably in the zona reticularis in the non-lipid depleted control gland, but were seen wherever compact cells were present, i.e., in a fairly peripheral position in a lipid depleted adrenal (Figs. 1 & 2).

PLASMA CORTICOSTEROID LEVELS

The mean normal 17-OH corticosteroid level in the plasma of normal young subjects during life was found to be 19.5 µg. per 100 ml. range 11-27 µg. At death the level was found invariably to be normal or elevated (Table IV) to a mean of 45.5 µg. No correlation was noted between the degree of adrenal cortical lipid depletion and the plasma 17-OH corticosteroid level. A fall in the level was found in specimens

removed several hours after death when compared with specimens removed immediately after death (Table VI).

MITOCHONDRIA

Most compact cells contain many mitochondria, which may appear either as rods and filaments, but more frequently as granules (Figs. 7, 8, 9, 10). The compact cell of the ACTH treated fasciculata is as rich in mitochondria as that of the untreated reticularis. The clear cell of the unstimulated fasciculata contains fewer mitochondria, all having a granular form, and compressed between the large lipid globules. Even in the unstimulated fasciculata a few cells are present which are rich in mitochondria. The glomerulosa cell is almost as rich in mitochondria as the compact cell, but the mitochondria are rather smaller. A few fuchsinophil globules 2-4 μ. in diameter, are seen occasionally in both fasciculata and reticularis; these may be microbodies.

GOLGI APPARATUS

As shown by osmium impregnation a distinct Golgi apparatus is present only in the clear cell (Figs. 11, 12, 13). Here it takes the form of a network lying in between the lipid globules; a similar appearance is seen in lipid containing glomerulosa

cells. In compact cells only a few osmic granules are seen. No success was obtained with Aoyama impregnations.

ELECTRON MICROSCOPY

In the control specimens, three cell types were found; the compact cells of the zona reticularis, clear cells in the zona fasciculata, and glomerulosa cells. In the ACTH-treated specimens, compact cells were found in the zona reticularis and fasciculata. The glomerulosa cells were unchanged.

Compact cell (Figs. 14, 15, 16, 17)

These cells were examined in the control zona reticularis and the ACTH-treated zona fasciculata and reticularis. The cell membrane at the low resolution employed is single. Where two cells are in apposition it is plane; where a little more space intervenes between the cells it is convoluted; at points where three or more cells meet, the membrane is thrown into micro-villi. These measure 1.5 μ x 150-500 Å (Carr, 1958).

The cytoplasm contains small vacuoles which tend to swell up in poorly fixed material, but which in the best material available range in diameter from 500 Å to 1000 Å. Between the vacuoles lie osmophil granules 100 Å in diameter, presumably the granules of Palade.

Mitochondria are numerous, usually elongated (2.5 μ x 0.4 μ)

though, of course, they may appear as circular profiles depending on the plane of section. The internal reflections of the mitochondrial membrane are more often tubular than cristate but both forms are seen even in the same mitochondrion.

A number of black osmophil masses, often called lipid bodies, are seen. These are characterised by punctate osmophilia, vacuolation and relatively small size (.5 μ). Occasionally, in material which has been processed for too long a time, lipid bodies show marked dissolution of the osmophil material resembling the 'Ringkorner' phenomenon of the light microscopist (Baker, 1958). A system of flattened vesicles and associated membranes present in a few compact cells probably represents the Golgi apparatus. The nucleus shows a clear perinuclear space and occasional well marked indentations; no other special features are present. The appearance of the compact cell in the zona reticularis and in the ACTH stimulated zona fasciculata is the same except that elongated mitochondria are not seen in the latter site. Cells are sometimes seen whose appearance suggests a transition between the compact cell and the clear cell; lipid accumulation within the cell is focal and the lipid globules do not show vacuolation. Micro-villi are present (Figs. 18 & 19).

Clear cell (Figs. 20, 21)

Here the cell membrane is plane, and lacks micro-villi.

Very occasionally groups of two or three nuclei are seen without any cell membrane between them. They appear to form a syncytium. The cell contains many large lipid bodies, which may indent the nucleus. These are larger, 1.5 μ, than those of the compact cell and do not present the characteristic vacuolation or punctate osmophilia already described. The mitochondria are fewer and smaller than in the compact cell and are usually spherical or oval in shape. Mitochondria are often seen in close relation to fat globules; very occasionally osmophil material has been seen within a mitochondrion, or mitochondrial membrane has been seen surrounding a partially sequestrated portion of a lipid globule. There is no conclusive evidence of a relation between lipid globules and mitochondria other than that imposed by the spatial limits of the inside of the cell. Granular osmophilic bodies about 2.5 μ in size and characterised by a granular osmophilic internum are seen in some cells often in relation to lipid bodies. These are presumably microbodies.

Glomerulosa cell (Figs. 22, 23)

This is like the compact cell in most features. The nucleus however, is a little larger in relation to the volume of the cytoplasm. The cell membrane is plane or almost so; long micro-villi such as are seen in the compact cell are not present.

Basement membranes (Fig. 24)

In all zones a basement membrane surrounds groups of cells, or occasionally single cells. At the antivascular pole of the cell no basement membrane is evident and in the zona reticularis and zona glomerulosa micro-villi lie in the extracellular space. At the vascular pole of the cell the micro-villi are smaller or absent and lie deep to the basement membrane. The capillary walls show no special features; small vesicles are prominent in the endothelial cell cytoplasm. Occasional villous structures project into the lumen. The endothelial cells have a basement membrane and are therefore separated from the cortical cells by the endothelial cell basement membrane, a space containing granular osmophil material, and the cortical cell basement membrane.

ULTRACENTRIFUGATION

The mitochondrial fraction gave a negative pyronin reaction and positive tetrazolium and acid fuchsin reactions. The electron micrographs (Fig. 26) showed a fairly pure mitochondrial fraction, with a fair degree of rupture of membranes.

The microsomal fraction gave a positive pyronin reaction and a negative tetrazolium and acid fuchsin reaction. Electron microscopically it consisted of membranes arranged in vesicles,

often broken, with a few attached osmophil particles in the 100-150 Å range, probably the granules of Palade (Fig. 27).

DISCUSSION

ADRENAL CORTICAL FUNCTION AT DEATH

MITOSIS

LIGHT MICROSCOPIC HISTOLOGY AND CYTOLOGY

MITOCHONDRIA

GOLGI APPARATUS

ELECTRON MICROSCOPY:

- Cell types
- Microvilli
- Endoplasmic reticulum
- Golgi apparatus
- mitochondria
- microbodies
- lipid bodies
- ACTH-stimulation

ZONATION

ADRENAL CORTICAL FUNCTION AT DEATH

Since the function of the adrenal cortex is to secrete adrenocortical hormones, it follows that the only satisfactory measure of function of this gland, as opposed to the ability of the target organs to respond to adrenal cortical hormones, is the amount of corticosteroid that the cortex can synthesize and secrete. The measurement of hormone levels in blood or urine will not give an index of secretory function, since these levels are dependent on other factors than actual secretion. A direct estimate of adrenal function can be obtained by assessing the power of the gland to perform one of the steps of steroid synthesis, e.g., the conversion of deoxycorticosterone (DOC) to corticosterone. This can be done at adrenalectomy; at the same time the adrenal 17-OH corticosteroid output is estimated by cannulation of the adrenal vein, and the peripheral plasma steroid level is measured (Grant et al., 1957). This procedure gives a direct index of adrenal cortical function, but is not suitable for application to the study of post-mortem material, since the power of enzymic synthesis falls to zero shortly after death. At post-mortem only indirect methods are available. It is dangerous to infer the state of function from hormone levels alone and as dangerous to draw such inferences from histological appearances. An attempt has been made to derive some

idea of function at death from consideration of three factors together - plasma corticoid levels, the degree of adrenal cortical lipid depletion and the adrenal cortical mitotic count.

The phenomenon of adrenal lipid depletion in response to stress has been discussed previously (Symington et al., 1955) and the histological findings in the present series agree with the previous findings. The common finding at death is moderate lipid depletion (2-3+). Total lipid depletion is rare occurring only twice in group 1A and in 6% of group 1B. Total lipid depletion occurs only in patients who have died of an illness involving overwhelming stress, notably in severe infections and in extensive burns. It is not reasonable to argue as has been done that total lipid depletion means that the gland is no longer capable of adequate function since even morphologically the totally lipid depleted gland is not a homogeneous entity. Some such glands show in haematoxylin and eosin preparations a peripheral extension of undegenerated compact cells and of alkaline phosphatase (Figs. 3 & 5). Others show in addition cytolysis of the peripheral cells with pseudo-acinous degeneration and loss of alkaline phosphatase activity (Figs. 4 & 6). In operation cases as shown previously (Symington et al., 1957) the first adrenal to be removed commonly shows little depletion,

while the second removed after ACTH stimulation shows moderate lipid depletion (2-3+).

In considering the value of post-mortem blood corticoid levels as an index of adrenal cortical function it is necessary to consider firstly whether these values give a true indication of the actual values at death, and secondly if they do how the values at death relate to secretory function at that time. In several cases where duplicate estimations were performed on samples removed immediately after and several hours after death the level fell somewhat in the interim (Table VI). The true plasma level at death is therefore somewhat higher than these figures indicate; these values can therefore be regarded as a true indication that the plasma level at death is much higher than in the normal subject. This is compatible with the opinion of Sayers (1950) that the administration of corticoids does not prolong life in stressed organisms.

This is not necessarily to be interpreted as meaning that the actual secretion of corticosteroids is high in the hours immediately before death, since the high plasma levels may be due to other factors. The high terminal level follows a relatively rapid rise starting 24 to 48 hours before death. The corticosteroid level at any time is dependent on a number of factors - adrenal stimulation by ACTH and the resultant adrenal

cortical output, the rate of hepatic conjugation of steroid, the renal excretion of conjugated steroid, and the rate of utilization of steroid by the peripheral tissues. Sevitt (1950) suggested on the basis of splenic and peripheral blood eosinophil counts that there might be adrenal cortical hyperfunction at death. In the agonal state, however, impaired hepatic conjugation (Sandberg et al., 1955) and impaired renal excretion of steroid have been demonstrated (Symington et al., 1955). These factors can account for the high plasma corticoid level without invoking adrenal hyperfunction, and a high corticoid level from whatever cause could account for the terminal eosinopenia. Yet, however inactive the adrenal may be it is still capable at death of secreting further, since Sandberg et al. showed that administration of ACTH in the last hours of life could still evoke further secretion of steroid. In the absence of blood ACTH studies it is difficult to arrive at an estimate of anterior pituitary function in the last hours of life. The lack of correlation between adrenal lipid depletion and levels of plasma corticosteroids indicates that at death these levels are dependent on factors other than adrenal function, and proves also that no matter how depleted the gland may be it is still capable of putting forth as much corticosteroid as the body can utilize.

The use of post-mortem mitotic counts as an index of adrenal

function involves similar difficulties; the mitotic count on post-mortem material may or may not reflect the actual mitotic activity at the time of death and, even if it does, the mitotic activity may not reflect the secretory activity of the gland. Mitotic counts on post-mortem material tend to be difficult to interpret. In some tissues a mitotic figure initiated before death may pass to completion after the death of the organism (Bullough, 1950), that is in the time lag between somatic and cellular death. This factor would not seem to be important in the present study since counts on post-mortem specimens agree closely with those on freshly fixed specimens from operation patients not stimulated with ACTH. Presumably a highly specialized cell like that of the adrenal cortex dies relatively soon after somatic death. Sufficient time will not elapse to allow figures already started to pass to completion. It seems certain that these figures reflect accurately the mitotic rate in the cortex at death.

It is less certain whether the mitotic rate is related to secretory function at death. It is clear from group 2B that the adrenal cortex is capable of a mitotic response; the eliciting factors are doubtful. In the rat (Cater & Stack-Dunne, 1953) growth hormone and to a lesser extent ACTH have been shown to stimulate mitosis. In these operation cases the main stimulus

was the use of a crude ACTH preparation, probably containing traces of growth hormone, in addition to the minor stress effect of the first operation and possibly removal of the first adrenal gland. A precise analysis of the time relations between the mitotic response and the administration of ACTH was not possible as this was a retrospective investigation. Previous hormone studies on these and similar patients (Grant et al., 1957) indicate that at the time of the removal of the second gland after ACTH stimulation, the plasma corticosteroid is high and this is, of course, due to increased secretion. Thus, at the time of operation a high mitotic count is associated with increased secretion. At post-mortem the converse may be true - the low adrenal mitotic count may be associated with a lowered adrenal secretion. Since in one case dying 15 hours after the onset of a coronary thrombosis the mitotic count was high and since the cells found in the stressed gland at death are the same in appearance as those found in the stressed gland at operation, i.e., compact cells, it is likely that the lack of cell division at death is associated not with adrenal exhaustion but with lack of stimulation. It is unlikely that the same cells which, in the gland removed at operation, are capable of both active secretion and of cell division should at death be incapable of either; and more likely that they retain the capacity both to divide and to secrete, but are being stimulated to neither.

The conclusions from this study of adrenal function are not indisputable but are consonant with the known facts. The stress of a severe disease process produces an increase in ACTH secretion followed by an increase in adrenal cortical lipid, usually partial, rarely complete. At this point, when the state of the gland is like that of second side adrenalectomy specimens, there may be a rise in mitotic count. The one case at post-mortem showing a rise in mitotic count may have been at this stage. When the disease process becomes irreversible, hepatic conjugation of steroid falls, renal excretion falls and possibly tissue utilization falls. The plasma steroid level rises due to peripheral blockage and ACTH production is depressed. Adrenal output of corticoid now falls due not to intrinsic failure of the adrenal cells, but to mere lack of stimulation. Similarly, the rate of cell division in the adrenal cortex falls. The adrenal cortex is now inactive from the point of view both of secretion and of cell division, but is still lipid-depleted because the process of restocking with lipid may take several days.

At death the adrenal cortex is inactive but not exhausted. Therefore, while complete lipid depletion at death indicates that the gland has been highly active it does not give any indication of the state of function at the time of death.

"Adrenal exhaustion" probably does not result from stress alone, but is always due to an intrinsic adrenal lesion, the best example of which remains the completely haemorrhagic gland of the Waterhouse-Friderichsen syndrome.

MITOSIS

The study of mitosis in any organ is usually carried out on material which has been removed from the experimental animal not long after death. In this study material was used which was not fixed for as much as 24 hours after death. This raises two questions; whether cell division can start after somatic death; and whether cell divisions started before somatic death may finish before cellular death but after somatic death. In the former case results might be erroneously high, in the latter erroneously low. Cells of different types vary in their capacity to divide after somatic death. This division probably takes place in an anaerobic environment, using anaerobic glycolysis as a source of energy. Tumour cells are capable (Bullough, 1950) of division for up to two hours after the death of the organism. Similarly spermatogonia and primary spermatocytes in the rat testis can both survive, and enter mitosis for many hours after death (Roosen-Runge, 1953). The epidermal and intestinal epithelial cells of mice on the other hand do not

enter mitosis after death but can complete divisions started before somatic death. No inference can be drawn from these findings as to what happens to adrenal cortical cells in the time interval between somatic and cellular death, but since the findings in post-mortem specimen agree with those in freshly fixed operation specimens from patients not stimulated with ACTH, the delay in fixation does not seem to vitiate the results.

There is good evidence that in most species cell division takes place in the outer part of the cortex. This view was originated by Gottschau (1883). He suggested that cells divided in the outer layers and migrated actively centripetally, dying in the zona reticularis which was thus a senile or at any rate a senescent zone. Later authors differ in the exact site they appoint as the so-called germinal zone. In the guinea-pig Hoerr (1931) in a careful and authoritative study found that mitotic figures occur almost entirely in the boundary zone between the zona glomerulosa and the zona fasciculata. Degenerate cells characterised by the classic criteria of pycnosis, karyorhexis and karyolysis occur in the zona reticularis. This concept was strengthened by the work of Zwemer and his colleagues (Zwemer Wotton and Norkus, 1938; Wotton and Zwemer, 1943) in the rat, the mouse and the cat. These workers modified the theory of cell migration by suggesting that the source of the cells was not the

transitional zone but a group of undifferentiated fibroblast-like cells in the capsule of the organ. Mitchell (1948) found that in the rat mitotic figures occurred in the zona glomerulosa and outer zona fasciculata. Blumenthal (1940) found mitotic figures in the guinea-pig only in the glomerulosa and fasciculata; mitosis became less frequent with advancing age.

Not only the naturally occurring cell division, but also cell division started by artificial means e.g., the administration of testosterone occurs in the capsule, glomerulosa and outer fasciculata (Nathanson & Bruce, 1941). The presence of a lipid free intermediate zone in the adrenal cortex of the rat, lying between the glomerulosa and the fasciculata was pointed out by Cater and Stack Dunne (1953). Whereas other workers believed that such a zone was the site of maximum cell division (Hoerr, 1931), Cater and Stack Dunne showed that stimulation with ACTH produces:

A. An increase in lipid in this zona intermedia.

B. An increase in mitosis in the glomerulosa and in the outer fasciculata but not in this distinct intermediate zone. This mitotic response is much exaggerated if STH be used.

Another means of investigating the problem of the origin of cells in the adrenal cortex is the use of vital dyes which act as

markers for a particular group of cells. The cell migration theory was initially strengthened by the findings of Salmon and Zwemer (1941) that after subcutaneous administration of trypan blue there was progressive inward migration of cells containing dye, till after 20 days the reticularis contained dye. Unfortunately after injection of a vital dye into the blood a high plasma concentration is present for a considerable time; moreover Salmon continued the injections for a period of some twenty days. The work was repeated by Calma and Foster (1943) who in more carefully controlled conditions failed to find evidence of actual cell migration and felt that the previous results were due to generalised absorption of the dye by the cortical cells. After a single injection of trypan blue in rats Baxter (1946) distinguished two different types of trypan blue absorption by cells. The capsular cells and glomerulosa cells store the dye in the form of many small particles; these persist throughout the duration of the experiment suggesting that no cell migration is taking place. Little dye was segregated in the zona fasciculata. Reticularis cells take up the dye in two distinct ways. Most took it up in the form of irregular masses, generally in relation to the amount of natural lipofuscin pigment already present in the cell. These cells were considered to be the same as Hoerr's dark cells. Other cells

took up the dye diffusely into both nucleus and cytoplasm. This was believed by Ludford (1933) to be the sign of a dead or dying cell. Baxter (1946) found cell division to be most common in the glomerulosa, and in the capsule. Mitosis was rare in the fasciculata. His findings confirm the peripheral origin of cells in the rodent adrenal cortex but cast grave doubts on the possibility of migration of these newly formed cells.

This is in keeping with the findings of Whitehead (1933, 1943) who found that in the mouse adrenal most mitotic figures were to be found in the glomerulosa and that no degenerative changes were to be found in any part of the cortex. This led to the suggestion that after cell division peripherally the germinal layer migrated out due merely to the volume of new tissue formed, acting as a sort of cambium layer. This view was supported by Nicander (1952) in a comparative study of a considerable range of species. In general, however, careful studies of the site of cell division have been done only on the rodent.

The concept of peripheral origin of cells is further supported by transplantation and regeneration experiments. Ingle and Higgins (1938) noted a marked relationship between the amount of capsule left after adrenal enucleation and the degree of regeneration that occurred. They suggested that

regeneration occurs from the capsule, possibly with the aid of surviving fragments of glomerulosa. These findings were independently substantiated by Baker and Bailiff (1939). Greep and Deane (1947) on the other hand enucleated adrenals in the rat, and found that a centripetal proliferation occurred rather from the remaining cortical cells below the capsule than from the capsule itself. They preferred to call this centripetal proliferation rather than migration. No evidence of division of capsular cells was seen, but mitotic figures occurred all the way down the length of the proliferating column of cells. Experiments with adrenal cortical grafts show, for the most part, survival of the graft only in the presence of capsule and glomerulosa (Jones & Spalding, 1954) though Coupland (1956) showed that growth could take place from the reticularis.

From this mass of evidence the position in the rodent is fairly clear. Cells divide in the glomerulosa but probably not in the capsule. The pressure of cell division presses the older cells in. The death of cells in the zona reticularis allows this to occur. There is probably some cell replacement at all levels in the cortex. No true migration however occurs.

Few studies have been done on the larger mammals or on man. Dempster (1955) showed that in the dog regeneration took place by proliferation of islets of cells in the capsule; this does not of

course necessarily imply that this is the normal mode of cell replacement. Graham (1916) showed very clearly that in the guinea-pig after experimentally induced chloroform necrosis of the adrenal cortex, regeneration occurred by cell division in the glomerulosa and outer fasciculata, and attempted to draw similar conclusions from the examination of a series of adrenals from patients who had succumbed to fulminating infections. He suggests that mitosis occurs peripherally, and is found in the reticularis only in the child. The series was small and the evidence not at all convincing.

In the present study mitotic figures have been found only in one particular type of cell - the compact cell of the reticularis and stimulated fasciculata, and are found only rarely, 1 in 10,000 cells. It is difficult to reconcile these findings with those seen in rodents. No mitotic figures have been seen in the human glomerulosa, yet in the rodent glomerulosa they are common; it may be that mitotic figures do occur in the zona glomerulosa of human adrenal cortex, but are not seen partly because of the small size of the glomerulosa in the human, and partly because the glomerulosa in the human is less active than in the rodent. The human environment as far as salt and water balance is concerned is more stable than that of the rodent.

The adult human adrenal cortex is clearly different from that

of the infant; Crowder (1957) has shown that development of the human foetal adrenal occurs by division of the coelomic epithelium in a subcapsular position, with inward movement of cells. This is akin to the more primitive pattern of the relatively undifferentiated rodent adrenal. In the human however zonation is fully differentiated, and the reticularis is marked off from the glomerulosa more distinctly than in the rodent by a zone of cells containing masses of fat; it is difficult to imagine the latter dividing.

Division is slow or absent peripherally and present though rarely marked centrally; thus the reticularis is subjected to little if any pressure from the periphery and degenerative changes, such as are seen in the cat (Bennett, 1940) are rare or absent.

It is interesting that most of the mitotic figures seen were in metaphase. Whitehead (1933) found that the commonest stage to be seen was the monospireme followed by the dispireme, monaster and diaster in that order. Schrader (1952) suggests that the duration of mitosis varies with tissue, animal and temperature; the duration varies from 10 minutes to several hours; metaphase and anaphase are rapid while prophase and telophase are slow. In other words the stages which are most readily recognisable in histological sections are of short duration. The only

detailed account of the time relations of mitosis is that of Lewis and Lewis (1917) who found that, out of a total time range of 67-205 minutes metaphase occupied 2-10 minutes and anaphase 2-3 minutes. It may be that at least some of the dark cells described by Hoerr (1931) and some of the very dense compact cells in the present material are in either very early prophase or very late telophase, and therefore have not been identified as mitotic figures.

It is not surprising that only the RNA rich compact cell in the human adrenal cortex shows mitosis. The presence of considerable amounts of RNA in rapidly dividing tissue is said to be usual (Caspersson, 1939; Brachet, 1953) and indeed the RNA fraction of embryos has been shown to be effective in promoting the growth of cells in culture (Fischer, 1939). Growth of adrenal cortical cells under ACTH stimulation is accompanied by an increase in cytoplasmic RNA, then an increase in chromidial and mitochondrial content later by an increase in nuclear DNA and finally by cell division (Fiala, Sproul & Fiala, 1956). Similar studies in liver have shown that cell growth resulting from STH stimulation involves increased mitosis, increased RNA synthesis and increased DNA synthesis.

The mitotic studies presented here show that the human adrenal cortex is capable of a mitotic response; the stimulus

involved is not clear. Hormones of varying types have been shown to affect mitosis. Bullough (1950) showed that in the epidermis mitosis was probably related to hexokinase activity and was stimulated by insulin and STH and probably by ACTH; though this latter may merely have been due to contamination. Adrenalin oestrogen and cortisone on the other hand were inhibitory. There is no question here of organ specificity of hormones.

As long ago as 1941 testosterone was shown by Nathanson and Brues to stimulate adrenal cortical mitosis in the rat. STH and to a lesser extent ACTH have the same effect (Cater & Stack Dunne, 1953). The former is not an organ specific effect, since STH produces an increase in mitosis in both liver and epidermis. In scurvy where there is a well marked stress effect and therefore ACTH secretion there is a mitotic response in the guinea-pig adrenal (Howard & Cater, 1959).

In the present series the stimulus to cell division may have been exogenous ACTH, the use of a preparation containing traces of STH, endogenous ACTH due to the stress of the first operation, or merely the effect of the ablation of one of a pair of organs. Precise analysis of the phenomenon is not possible as the investigation was largely retrospective, and the dosage of ACTH

was not uniform either in quality or time of administration. No definite statement can be made as to whether ACTH has an organ specific effect in promoting cell division in the human adrenal cortex, but it appears likely that this is the case.

Only one patient showed an elevation of mitotic count at death; this patient died 15 hours after the onset of a coronary artery thrombosis. When the anterior pituitary of a patient who has died soon after the onset of an acutely stressing disease is examined it is adjudged to be highly active on the grounds of degranulation of the mucous basophils. If on the other hand death occurs several days after the onset of the catastrophe, the mucous basophils return to their previous state (Currie et al., 1955). Probably this one case died in the stage of anterior pituitary hyperactivity, while the others survived till anterior pituitary function had once more fallen to normal. What is clear is that the human adrenal cortex is capable of a mitotic response and that its mitotic inactivity at death indicates not lack of ability but lack of stimulus.

LIGHT MICROSCOPIC HISTOLOGY AND CYTOLOGY

The histology of the human adrenal cortex at a fairly superficial level is familiar. A capsule of connective tissue surrounds a mass of oriented epithelial cells. The capsule is

continuous with a stroma of connective tissue, partly collagenous but mainly argyrophilic which surrounds and supports the cells. The gland was first divided into three zones by Arnold (1866) on the basis of gross arrangement of the connective tissue and vascular framework. In the outer zone the connective tissue is arranged around roughly spherical clusters of cells, the zona glomerulosa; in the middle zone the connective tissue forms a tubular sheath around long columns of cells, the zona fasciculata; and in the innermost zone the connective tissue is more irregularly arranged around anastomosing cords of cells (Elias & Pauly, 1956).

The blood vessels fall into the same pattern as the connective tissue (Arnold, 1866; Bennett, 1940; Harrison, 1957). From the subcapsular plexus arterioles pass into the glomerulosa to form arciform capillary loops; thence into the straight sinusoids of the fasciculata. These sinusoids have only feebly phagocytic walls. From here blood passes into the tortuous branching capillaries of the zona reticularis and thence into the medullary sinusoids and veins. A few arteries, the arteria recta, pass straight through the cortex into the medulla without branching; but in the main the cortical capillary supply is a portal supply to the medulla. Blood flow can be controlled it would seem either by variation in the calibre of these arteria recta

(Harrison, 1957) or by variation in the degree of contraction of the muscle in the walls of the medullary veins. The nerve supply to the adrenal cortex is scanty; a few fine fibres pass to the glomerulosa cells; the rest of the nerve fibres seen in the cortex are either vasomotor, or passing through to the medulla. This is the conventional picture of the histology of the human adrenal cortex; and accords well with the appearances seen in the 450 specimens examined. However the zona intermedia described by Maximow and Bloom (1959), a zone of compressed lipid free cells lying between the glomerulosa and fasciculata, while present in the rodent is not present in the human gland.

The glomerulosa cell in man is a cell about 12 μ. in diameter, with a fairly deeply staining nucleus in which the detail of a peripheral nuclear membrane surrounding the nuclear sap, and enclosing several masses of basophil material can usually be made out. The cytoplasm contains a varying amount of fat which appears as small vacuoles in paraffin preparations; this is never so plentiful as in the fasciculata. Mitochondria of granular or filamentous form are numerous and scattered throughout the cytoplasm. There is some evidence that in the rat the lipid content of these cells may vary with the plasma sodium and potassium levels; in man the lipid content of these cells does vary at death in an unanalysed fashion. Adrenals removed at operation generally

have a lipid laden glomerulosa, accompanied by a high mitochondrial count. The Golgi apparatus in animals is described as a network of black channels concave towards the nucleus (Bennett, 1940; Bourne, 1949; Reese & Moon, 1938). In man on osmic impregnation a black osmium precipitate extends as a network throughout the cell, surrounding the lipid globules. There is good reason to believe that this is in fact impregnation of the endoplasmic reticulum. The glomerulosa cell contains a moderate amount of RNA, succinic dehydrogenase and alkaline phosphatase (Symington et al., 1955).

The clear cell of the unstimulated fasciculata is larger, about 20 μ. in diameter, with a pale vesicular nucleus in which several nucleoli may be present; these are never deeply eosinophil as in the compact cell. The cytoplasm in haematoxylin and eosin preparations is foamy and reticular in appearance due to the dissolution of fat. Much sudanophil material is present in the form of large globules which give a strongly positive reaction for cholesterol. The cells contain little RNA, and that is strung out like beads on a string in the spaces between the lipid globules. A few cells contain many mitochondria mostly orientated to the vascular pole of the cell but most contain only a few mitochondria which are all granular in form. The mitochondria occupy a similar position in the cell to the stainable

RNA, i.e. in the spaces between the lipid globules. Prolonged osmication gives the impression of an intricate meshwork of Golgi reticulum extending like a basket round the lipid globules. It is notable that the cells are so distended with lipid that the cytoplasmic membranes seem to be pressed together. The clear cells contain little alkaline phosphatase and succinic dehydrogenase (Symington et al., 1955).

The compact cell found in the reticularis and ACTH stimulated fasciculata is small, about 10 μ. in diameter. The nucleus is deeply staining with basic dyes, the nuclear membrane not always apparent. In about one in five of the cells this appearance is marked to the point almost of pyenosis, without however any other signs of degeneration. Prominent deeply eosinophil nucleoli are frequent. The cytoplasm is granular and deeply eosinophil; occasionally in cells with very deeply staining nuclei, this eosinophilia is also marked. This variation may correspond to the dark and light cells of Hoerr (1931) recently described ultrastructurally by Lever (1955) and may represent some variation in functional status. These cells contain little fat, but give a markedly positive reaction for RNA, succinic dehydrogenase and alkaline phosphatase and in most though not all cases contain many mitochondria. Filamentous forms make up at least 50% of the mitochondria. No Golgi

apparatus is demonstrable, apart from a few small black grains. A striking feature of these cells is that their cell membranes do not lie in close juxtaposition, but are separated at their anti-vascular poles by spaces 1-2 μ. across, across which, in well stained iron haematoxylin preparations, a few fine wisps of cytoplasm may be seen. These probably represent microvilli and may be compared to the so-called striated border in the small intestine.

MITOCHONDRIA

In man there is a distinct variation in mitochondrial content in the three types of cell. The glomerulosa cell has many mitochondria of which some but not all are rod shaped. The clear cell of the unstimulated fasciculata has many fewer, almost all granular and all lying in the interstices of cytoplasm between the fat globules. The compact cell of the zona reticularis and ACTH-stimulated fasciculata usually contains many mitochondria, all granular but varying grossly in size from the barely visible to fuchsinophil masses 2-3 μ. in diameter. The change from clear to compact cell which occurs under the influence of ACTH is accompanied by an apparent increase in the number of mitochondria in the cells concerned.

Despite the considerable species variation in other morpholo-

gical characteristics there is fairly close agreement between those findings and mitochondrial distribution in other species. In the mouse Miller (1950) found that the mitochondrial content of cells varied inversely with lipid content. Here in the glomerulosa mitochondria are rod shaped, in the fasciculata they are rather obscured by fat while in the reticularis they are numerous and rod shaped. A zona intermedia with a low content of mitochondria is present. Such a zone is not present in the human adrenal cortex.

Stimulation with insulin which of course produces ACTH secretion causes an apparent increase in mitochondria, while hypophysectomy produces a corresponding decrease. In the pigeon (Miller & Riddle, 1942) a corresponding mitochondrial increase occurs on stimulation, while a similar decrease follows hypophysectomy. In birds however the phenomenon is not as apparent as in the mammal, due to the intermingling of lipid and non-lipid containing cells. Any lipid depletion that occurs is thus obscured.

In the hamster (Kniggo, 1954) the mitochondria are granular in the glomerulosa, and rod like elsewhere. Fat is of course almost absent in this species. Mitochondria are rather sparse in the inner fasciculata and tend to decrease on hypophysectomy throughout the gland. In the rat (Deane & Greep, 1946) the

mitochondria are granular and tend to be rather larger in the zona fasciculata. They decrease in the fasciculata but not in the reticularis on hypophysectomy. The phenomenon of inverse relation to fat content is borne out by Gain and Harrison (1950) who found that in the rat and in the guinea-pig mitochondria were plentiful in lipid free and fewer in lipid containing cells. They linked this finding however to some functional inferences which are not entirely acceptable; namely that a secretory cycle can be discerned in which the cells containing mitochondria are functionally inactive while the cells containing much fat and therefore fewer mitochondria are functionally active.

A secretory cycle was also postulated by Bennett (1940) in the cat. Bennett found that in the glomerulosa mitochondria were frequent and rod shaped, in the outer fasciculata or secretory zone they were shorter and lay between the fat vacuoles while in the inner zona fasciculata or post secretory zone they were yet shorter. The zona reticularis was designated as a senescent zone and was characterised by marked pleomorphism of mitochondria. These findings and interpretations are similar to those of Hoerr (1931) who worked with the guinea-pig. Here the mitochondria are rod like in the glomerulosa, granular in the fasciculata and again rod like in the reticularis with a few lumpy fuchsinophil masses. More mitochondria were found in the dark than in the light cells.

The idea that there are two basic types of adrenal cortical cell, that containing many mitochondria and that containing few mitochondria is attributable largely to Miller and Riddle (1942). It seems likely that the undoubted variations in lipid content of cells are a secondary phenomenon. A clear differentiation between the two cell types has been shown only in the fasciculata and reticularis, depending presumably on the production at the time of examination of glucocorticoid. Variations in lipid and mitochondrial content of the glomerulosa do occur with the plasma levels of sodium and potassium and presumably therefore with the mineralocorticoid production. Variations in the appearance of the zona glomerulosa do occur in the human at death but these have not been analysed due to the absence of data on plasma electrolyte levels in most cases at death.

Cain and Harrison (1950) believe that the fat in the cells may be active steroid, and that it is the fat containing cells which are active. This view is untenable because the content of active steroid in the adrenal cortex has been shown to be very low. Moreover the histochemical methods which were alleged to demonstrate steroid in fact do nothing more than show the presence of carbonyl groups in formalin fixed tissue.

It would be equally difficult to prove the converse - that the cells which contain many mitochondria are functionally more

active. The current view of the function of the mitochondrion is that it is a primary site within the cell of oxidation reduction reactions; it would seem reasonable to infer that a large number of mitochondria indicates rapid oxidative turnover with in the cells and that this in its turn implies cellular secretory activity.

GOLGI APPARATUS

A Golgi apparatus has been demonstrated in the adrenal cortex of various species. The general view is that in the glomerulosa and fasciculata a diffuse black network is present, while in the reticularis the Golgi apparatus is more compact. In the cat Bennett (1940) using Da Fano's silver technique showed in the glomerulosa a conical mass of black channels with its base turned towards the nucleus. In the secretory zone a tangled skein goes out between the lipid globules, this skein becomes dense in the post secretory zone and still more dense in the senescent zone. Hoerr (1931) failed to demonstrate a Golgi apparatus elsewhere than in the glomerulosa, and in that site only with a silver method. Miller and Riddle (1942) described a branching black network extending between the lipid globules in the lipid containing cells. Cain and Harrison using the Aoyama technique on rat material found a black juxtanuclear cap which extended

strandwise across the cell; this was never found in the glomerulosa where no Golgi apparatus was ever demonstrable.

ELECTRON MICROSCOPY

Cell types

As would be expected from their light microscopic appearances, the cells of the human adrenal cortex, as seen by the electron microscope, fall into three types. The compact cell of the reticularis and ACTH-stimulated fasciculata is characterised by the presence of a plentiful vesicular endoplasmic reticulum, usually abundant rod-like mitochondria and microvilli. The characteristics of clear cell of the unstimulated zona fasciculata are the absence of a prominent endoplasmic reticulum, fewer spherical mitochondria, a plane cell membrane and many large osmophil lipid bodies. The zona glomerulosa cell is slightly smaller with a proportionally bigger nucleus, fairly prominent endoplasmic reticulum and frequent, often elongated mitochondria.

This cell classification is very different from that of Lever (1955) who in the rodent adrenal classified cells in a manner similar to that of Hoerr (1932). Light cells are characterised by the presence of clusters of polyhedral sacs; dark cells and glomerulosa cells by the absence of these sacs.

Similar sacs were found in this material only in tissue which had suffered a gross fixation delay. Such delay is, of course, more frequent, and fixation artefact more difficult to avoid in a study of human material. So far as the human adrenal is concerned, these sacs would seem to be fixation artefacts and thus not a reliable criterion for cellular classification. Nevertheless in the zona reticularis a definite variation in the density of endoplasmic reticulum and in mitochondrial count among cells does exist.

Microvilli

The striking feature of the human compact cell is the presence of microvilli; these filamentous prolongations of the plasma membrane are more prominent in the human than in the rodent, where they were described by Lever (1955). In the mouse (Zelander, 1959) microvilli are found in all zones but are more prominent in the inner part of the zona fasciculata. Similar structures are present in many other sites, e.g., parathyroid, liver and intestinal epithelium. Their function is unknown, but they may provide additional surface area through which transport can occur. Alternatively they may act merely as a reserve of spare membrane to allow the cell to expand and contract in varying physiological states. The demonstration by Zelander (1959) that microvilli are present in all zones of the mouse

adrenal cortex makes it unlikely that they have any topographical functional significance. Microvilli project into the intercellular extravascular space, and are most prominent and numerous on the non-vascular pole of the cell. On light examination of a group of compact cells a small space containing tiny wisps of material with an affinity for iron haematoxylin can sometimes be seen between them; this is not visible in the clear cells, whose membranes lie in close juxtaposition. The wispy material is presumably clumped microvilli.

Endoplasmic reticulum

The endoplasmic reticulum in the adrenal cortex is of the smooth or vesicular type, as described by Palade (1956).

Differential centrifugation of homogenates of ox adrenal cortex under the conditions used to prepare liver microsomal fractions, gave a fraction which was strongly pyroninophilic but was not fuchsinophilic, and did not contain histochemically obvious succinic dehydrogenase. The electron microscopic appearances of this fraction - vesicles with related osmophil particles - resemble the vesicles seen in the human adrenal cortex. It is reasonable to describe the latter as endoplasmic reticulum. Osmophil granules of ribonucleoprotein are not attached to the membranes of this type of reticulum but lie between the vacuoles. The adrenal cortex resembles the testis in paucity of rough reticulum. It

It is difficult to know whether these vacuoles form a true reticulum, or are merely an aggregate of independent spaces. The latter is the more likely explanation as only occasionally are more than two intercommunicating vacuoles seen.

Golgi apparatus

A few groups of large vesicles with associated membranes which correspond to the classical ultrastructural criteria of Dalton and Felix (1956) have been seen in the zona reticularis and the zona glomerulosa but not in the zona fasciculata. This does not correspond with the findings on light microscopic examination of human adrenal stained by the osmium Golgi techniques where a diffuse Golgi reticulum is found in zona fasciculata and in some of the more fatty cells of the zona glomerulosa; and it is likely that the endoplasmic reticulum is being impregnated, as has been shown to be the case in the gastric parietal cell (Hally, 1959). The sacs which Lever (1955) suggests are Golgiform, do not conform to the criteria of Dalton and Felix.

Mitochondria

Belt and Pease (1956) have shown that in the rat the mitochondria of the zona glomerulosa process cristae, while those in the inner zones have tubules. The mitochondria in the outer

part of the mouse adrenal cortex, on the other hand, are tubulosaccular, while centrally they are recti-membranous or cyclomembranous (Zelander, 1959). In the present study the only clear zonal variation in mitochondrial structure is the presence of elongated forms in the glomerulosa cell and the compact cell, and the total absence of these forms in the clear cell. Both cristae and tubules are present in mitochondria in all zones. Mitochondria containing lipid masses led Lever (1956) to suggest that a transformation took place between the mitochondrion and the lipid globule. Only once during this study was a mitochondrion seen, which contained aggregates of osmophil material.

Microbodies

Another organelle present chiefly in clear cells that are not totally filled with lipid is the microbody: this was originally described in regenerating liver by Rouiller and Bernhard (1956); Belt (1958) suggested that in the adrenal cortex the microbody is a common precursor of both lipid globule and the mitochondrion. Microbodies are certainly present in the human adrenal cortex, but their significance is unknown. They probably correspond to the liposomes described by Deane and Greep (1946) in the rat, and by Knigge (1954) in the hamster. These are

deeply fuchsinophil structures, 2-4 μ in diameter, and, like the lipids in the mitochondrial capsule, resist extraction with fat solvents after chromation and show, thereafter, notable fuchsinophilia.

Lipid bodies

The suggestion that the osmophil globules in zona fasciculata are fat is of course only an assumption. None of the other osmophil substances, e.g., amino acids, such as tryptophan or histidine (Bahr, 1954) are likely to occur in globular aggregates. Examination of parallel thick sections shows that the globules occur in the same place as the fat space of the traditional spongiocyte; and these of course, are well known to correspond to the sudanophil globules seen in a gelatin embedded section. The punctate osmophilia and vacuolation, and the smaller size of the lipid bodies in the compact cell, is probably due to varying unsaturation of the fats, and suggests either breakdown or build up of lipid.

ACTH-stimulation

The only significant change seen on ACTH-stimulation in this investigation is the appearance in the zona fasciculata of compact cells similar in electron microscopic appearance to the compact cells of the zona reticularis. A possible fallacy is

that by chance compact cells were examined which had this form before ACTH-stimulation. In the ACTH treated glands, however, blocks taken from a peripheral part of the gland showed the typical compact cell appearance, and it is extremely unlikely that compact cells were present in this situation, before ACTH-stimulation. Moreover, the changes correspond accurately to the light microscopic changes, and thus suggest genuine ultrastructural change. The relationship between variations in ACTH production, whether after hypophysectomy or stress has been described in the pigeon by Miller and Riddle (1942), in the rat by Deane and Greep (1946), in the mouse by Miller (1950) and in the human by Rogers and Williams (1949) and Symington et al. (1955). Lever (1956) has observed the ultrastructural changes in rodents and Ashworth et al. (1959) in the rat. In general, it seems that ACTH-stimulation leads to an increase in mitochondrial content, an increase in RNA and in endoplasmic reticulum, and hypophysectomy to the reverse. In hypophysectomised animals, Lever (1956) found a reduction in the numbers and osmophilia of mitochondria which was restored by ACTH. A feature of the ACTH treated rat adrenal was the appearance of deficiencies in the mitochondrial membranes, showing continuity between the internum of the mitochondrion and the cytoplasm. This was not, however, observed in the present study. The significant finding

in the ACTH treated material was the appearance in the zona fasciculata of cells apparently identical in the detail of their fine structure to the cells of the unstimulated zona reticularis. On stimulation, the lipid globules disappear, mitochondria round off and become more frequent, endoplasmic reticulum becomes more plentiful and microvilli appear.

ZONATION

The significance of the existence of varying zones within the human adrenal cortex is manifold. Embryologically the various zones come into being due to the migration in from the subcapsular area of undifferentiated cells derived from the intermediate cell mass (Crowder, 1958). In the embryo centripetal migration of cells does undoubtedly take place, but in the infant when the foetal cortex disappears the connective tissue between the cell cords becomes more marked and more obvious than in the foetus, so that the cord-like arrangement of cells which was initially a reflection of centripetal migration becomes no more than a reflection of the fibro-vascular architecture of the gland. Increasing differentiation of the cells follows with the varying environments which cells in the various zones are subjected to. The peripheral cells receive blood first; those within receive more anoxic blood. Perhaps herein lies the origin of the

functional significance of the zonation of the cortex. There is little doubt that in the rat (Ayres et al., 1958) and the ox (Giroud, et al., 1958) aldosterone is synthesized in the zona glomerulosa. It is less certain where the other hormones, the glucocorticoid group and both male and female sex hormones, are secreted. Some workers believe that sex hormone is produced in the zona reticularis, and glucocorticoid in the zona fasciculata. This is based largely on the use of stains which are said to demonstrate steroids, the so-called ketosteroid effect. It has been shown (Karnowsky & Deane, 1954) however that these stains demonstrate merely carbonyl groups in formalin fixed tissue. Later work (Mitchell, 1960) suggests that they demonstrate merely carbonyl groups in tissue fixed in any fashion. Moreover micro-chemical estimations show that only very minute amounts of steroid are present in the cortical cells. It seems therefore that any attempt to prove functional zonation based on histochemical estimation of steroids is based on a fallacy. Bennet (1940) on the basis of such phenylhydrazine staining in the cat adrenal cortex suggested that the secretory zone was the zona fasciculata and that the zona reticularis in which he saw degenerate cells was a zone of dying cells. In the human the zona reticularis is very much alive, to the degree that cell division occurs in it. Moreover the cytological and ultrastructural characteristics of

the zona reticularis are not those of degeneration. Yoffey (1953) suggested that the zona reticularis was the site of glucocorticoid production. This is supported by the findings of Symington and his colleagues who have found that there is an increase in the zona reticularis type compact cells after ACTH-stimulation, in parallel with the increase in corticoid output. This view implies that the active zone spreads out into the zona fasciculata under the influence of ACTH. A somewhat similar view is held by Tonutti et al. (1954) who believe that the adrenal cortex consists of two zones the outer composed of outer zona fasciculata and zona glomerulosa and the inner composed of zona reticulosa and inner zona fasciculata. After ACTH-stimulation there is an increase in the so-called transformation fields between the inner and outer zones. It is clear that the site of formation of aldosterone has been proved and that there is strong presumptive evidence that glucocorticoids are elaborated in the compact cells of the zona reticularis before ACTH-stimulation and in the zona fasciculata after ACTH-stimulation. The site of formation of adrenal androgens and oestrogens is uncertain.

S U M M A R Y

1. The human adrenal cortex has been studied at death by correlation of plasma hydrocortisone levels, with histology. It has been proved that the totally lipid depleted adrenal cortex seen at death is not functionally exhausted.

2. The pattern of cellular replacement in the human adrenal cortex has been studied. Cell replacement in the human adrenal cortex takes place only in the compact cells of the zona reticularis and ACTH stimulated zona fasciculata. The centripetal theory of cell growth does not hold for the adult human adrenal cortex.

3. The distribution of mitochondria and Golgi apparatus in the human adrenal cortex has been studied. Mitochondria are plentiful in the compact cell and the glomerulosa cell, but fewer in the clear cell. True Golgi impregnation has not been demonstrated except as a network in the clear cells which probably represents impregnation of the endoplasmic reticulum.

4. The ultrastructure of the human adrenal cortex has been studied. Three cell types are found:

(i) the compact cell in the zona reticularis and ACTH-stimulated fasciculata, characterised by the presence of frequent mitochondria, prominent vesicular endoplasmic reticulum, microvilli and scanty lipid. The vesicular component has been

shown by cell fractionation to be endoplasmic reticulum.

(ii) the clear cell characterised by fewer mitochondria, less endoplasmic reticulum, absence of microvilli and prominent lipid globules.

(iii) the glomerulosa cell characterised by relatively frequent mitochondria, dense endoplasmic reticulum, a variable lipid content and small or absent microvilli.

(iv) ACTH-stimulation has the effect of altering the fine structure of the clear cell to approximately that of the compact cell.

TABLE I

Grading of adrenal cortical lipid depletion

Percentage loss of lipid	Grade
0-25	0
25-50	+
50-75	+ +
75-95	+ + +
over 95	+ + + +

TABLE II

Staining	Without peroxide		With peroxide	
	time	result	time	result
Mayer's haemalum	30-40 mins.	satis-factory	5 mins.	good
Iron haemato-xylin, Weigert	30-40 mins.	satis-factory	5 mins.	good
Toluidin blue	5 mins.	good	5 mins.	good
Pyronin	30 mins.	nil	15 mins.	satis-factory
Eosin water sol.	45 mins.	nil	45 mins.	poor
Eosin alcohol sol.	45 mins.	nil	45 mins.	poor
Phloxin	30 mins.	poor	15 mins.	good
Masson's Trichrome	1 hour	poor	10 mins.	good
Mallory's Triple Stain	1 hour	poor	10 mins.	good
van Gieson	30 mins.	poor	5 mins.	good
P.A.S.	30 mins.	satis-factory	5 mins.	good

TABLE III

Relationship between complete adrenal lipid depletion at death (++++) and the cause of death (group 1b)

Condition	No. of cases	Completed lipid depletion (++++)	
		No.	Per cent.
Control	100	0	0
Acute infections	127	8	6
Peritonitis	67	4	6
Burns	50	9	18
Total	344	21	6

TABLE IV

Relationship between plasma level of 17-OH corticosteroid at death and lipid depletion of the adrenal cortex. The cross-hatched area represents the normal range of plasma 17-OH corticosteroid level during life

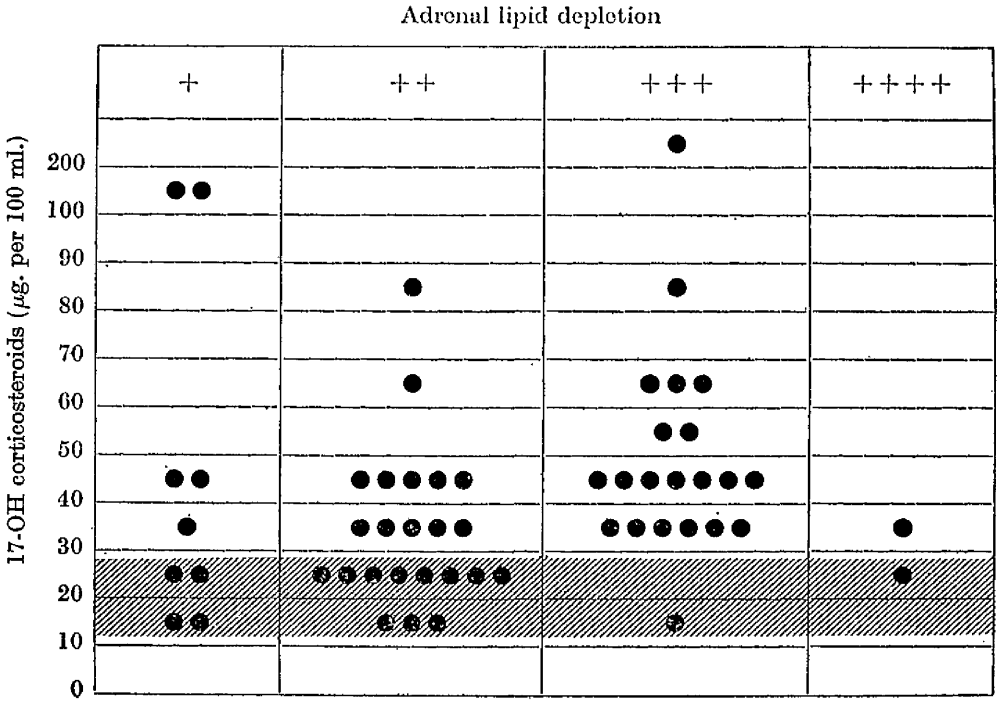


TABLE V

Mitotic counts in adrenal cortex per 100 high-power fields from post-mortem (group 1a) and adrenalectomised (group 11) patients

Group	No. of cases	Mitotic count		S.D.
		Mean	Range	
1a. Post-mortem	74	1.2	0.8	1.2
11a. Bilateral adrenal-ectomy - first side	40	0.9	0.2	0.7
11b. Bilateral adrenal-ectomy - second side	40	3.6	0.35	6.3

TABLE VI

The fall in post-mortem plasma 17-OH corticosteroid with increase in interval between death and collection of the specimen

Case	Time after death of removal of specimen	Plasma level of 17-OH corticosteroid (μg. per 100 ml.)
1	30 min.	37
	3 hr 45 min.	28
2	30 min.	65
	1 hr 30 min.	58
3	30 min.	69
	2 hr 0 min.	33
4	15 min.	33
	2 hr 0 min.	24
5	30 min.	20
	3 hr 0 min.	15

THE ADRENAL CORTEX AT DEATH

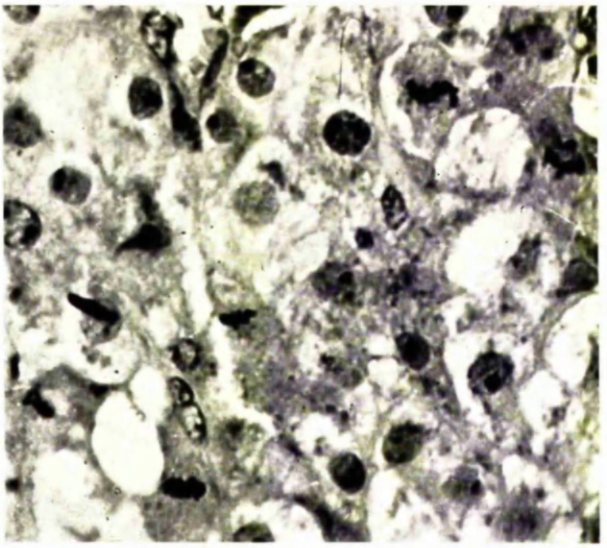


FIG. 1.—Human adrenal cortex. Five mitotic figures are seen, all in metaphase and all in compact cells. Hæmatoxylin and eosin. ×500.

FIG. 2.—Adrenal cortex. Mitotic figure in compact cell. Metaphase. H. and E. ×800.

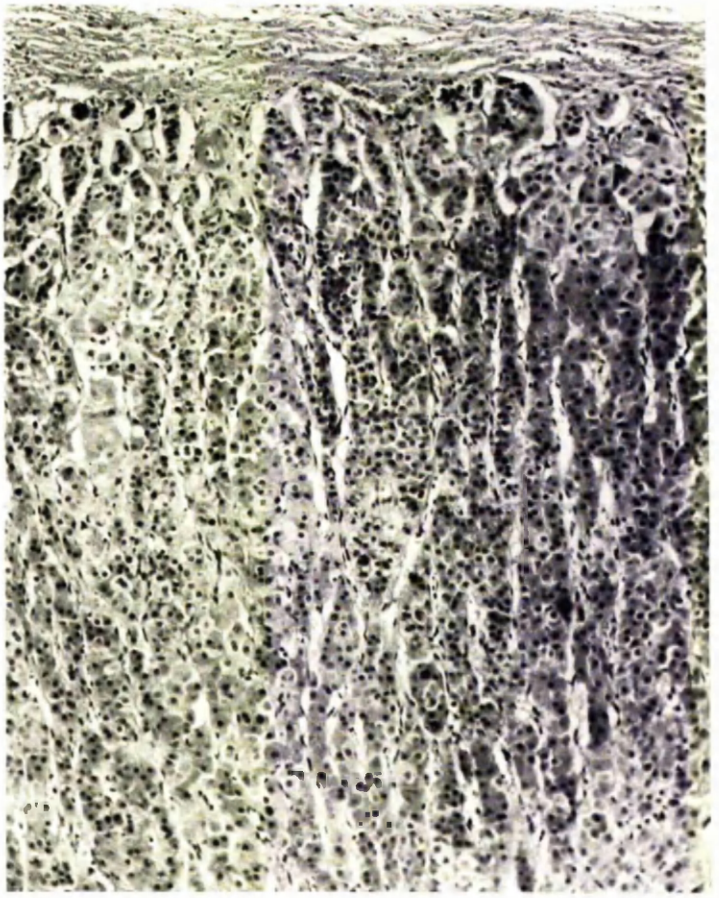


FIG. 3.—Adrenal cortex. Compact cells extend to the periphery of the gland, but no degenerative changes are present. H. and E. ×160.

THE ADRENAL CORTEX AT DEATH

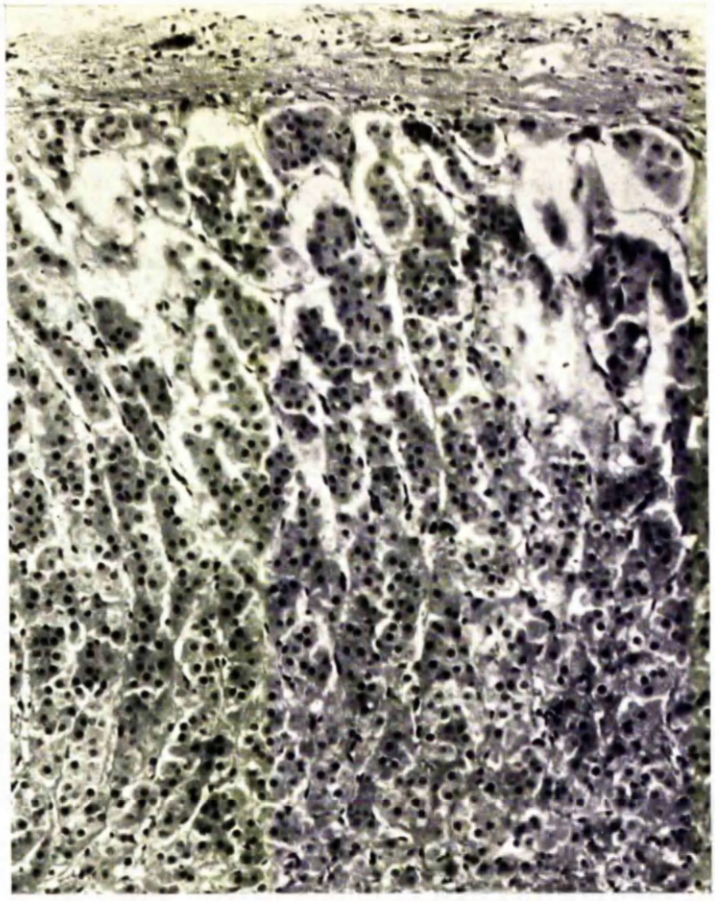


FIG. 4.—Adrenal cortex. Compact cells extend to the periphery of the gland. Cytolysis and lumen formation are prominent. H. and E. × 160.

FIG. 5.—Adrenal cortex. Alkaline phosphatase extends to the periphery of the gland. Mannheimer and Seligman azo coupling method. × 100.

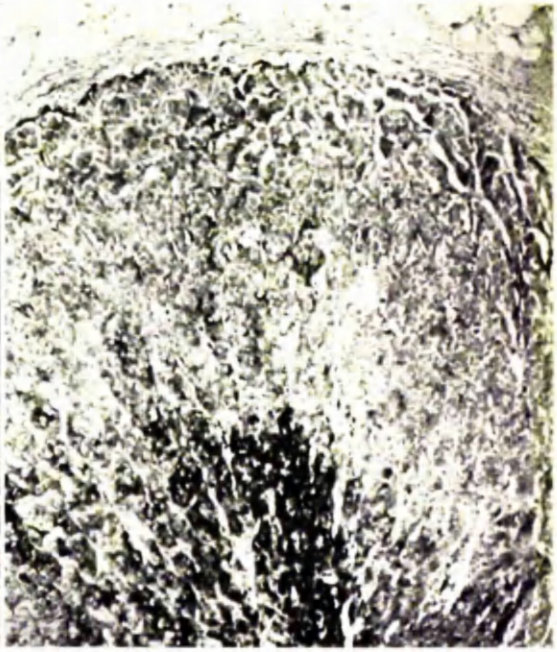


FIG. 6.—Adrenal cortex. Peripheral alkaline phosphatase activity absent. Mannheimer and Seligman. × 160.

Fig. 7.

Control: Compact cells of zona reticularis stained with aniline acid fuchsin and methyl green. Many mitochondria are present. X 1200.

Fig 7

Fig. 8.

Control. Clear cells of zona fasciculata, stained with aniline acid fuchsin and methyl green. Mitochondria are fewer in most cells. The occasional cell contains many mitochondria. The lipid has dissolved out and appears as vacuoles. X 1200.

Fig. 9.

ACTH treated compact cells of zona fasciculata stained with aniline acid fuchsin and methyl green. Mitochondria are frequent. X 1200.

Fig. 10.

Control. Cells of zona glomerulosa stained with aniline acid fuchsin and methyl green. The capsule appears in the bottom left corner of the picture. Mitochondria are frequent. The lipid is heavily osmicated and appears as brown granules. X 1,200.

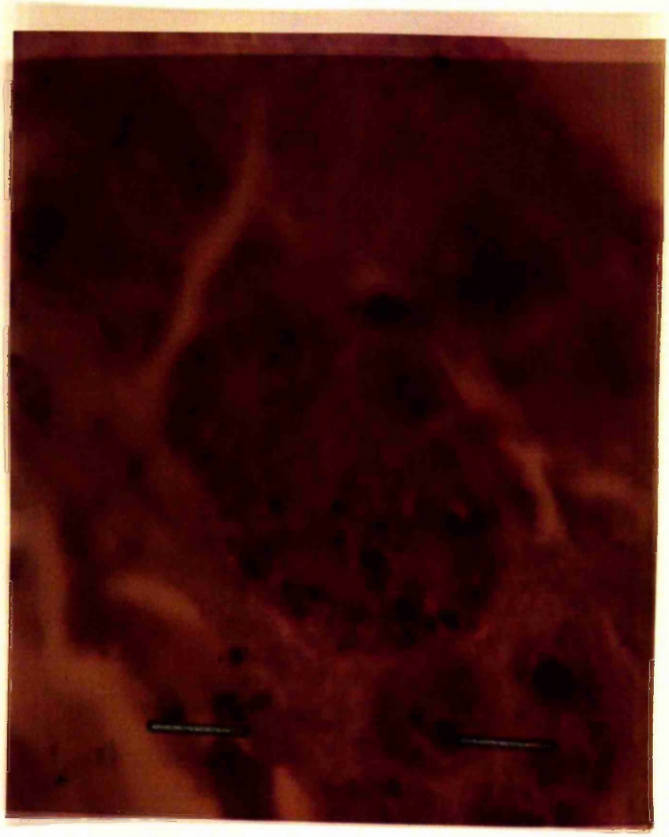


Fig. 11

Control. Compact cells of zona reticularis stained by the osmium Golgi technique (Weigl-Mann-Kopsch) and treated with turpentine. The cytoplasm is filled with dense osmophil granules. X 1800.

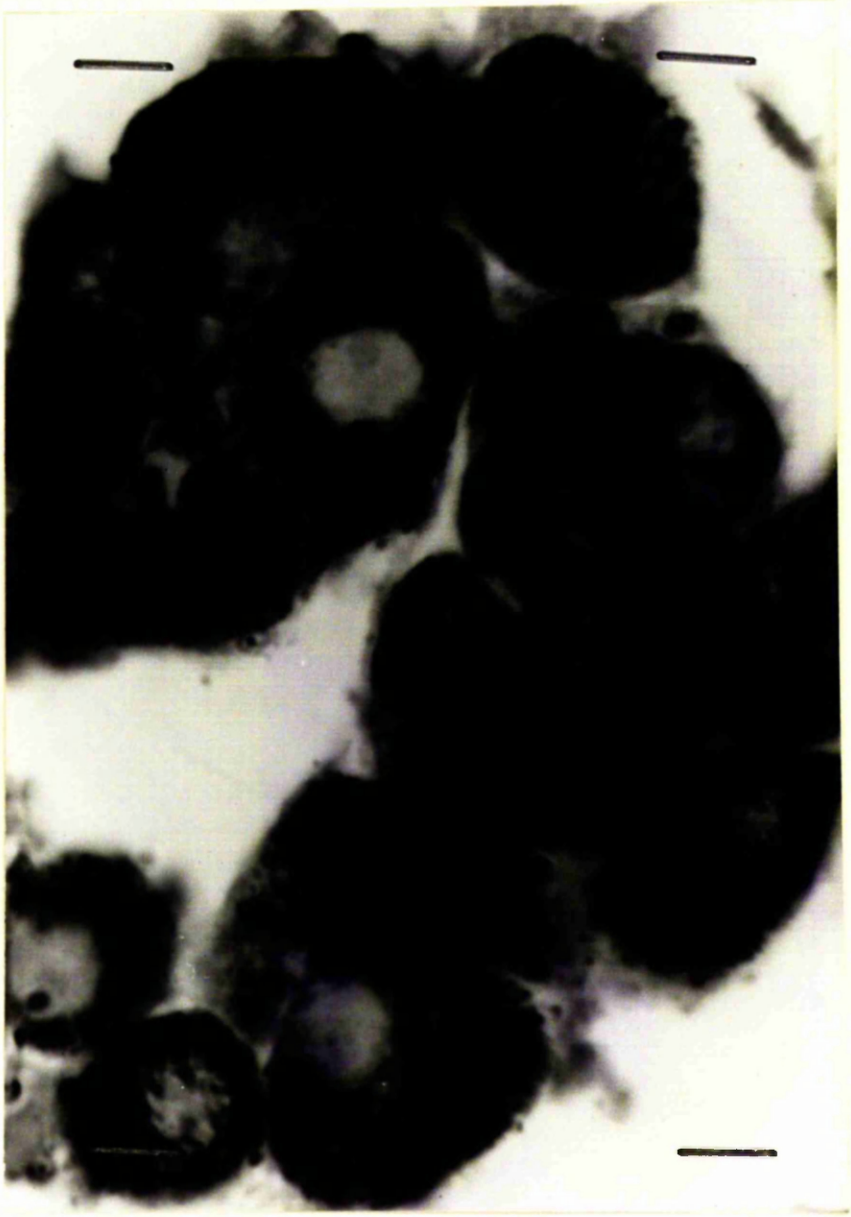


Fig. 12.

Control. Clear cells of zona fasciculata stained by the osmium Golgi technique and treated with turpentine. The cytoplasmic vacuoles from which lipid has been washed by the turpentine are surrounded by osmophil granules. ×1800

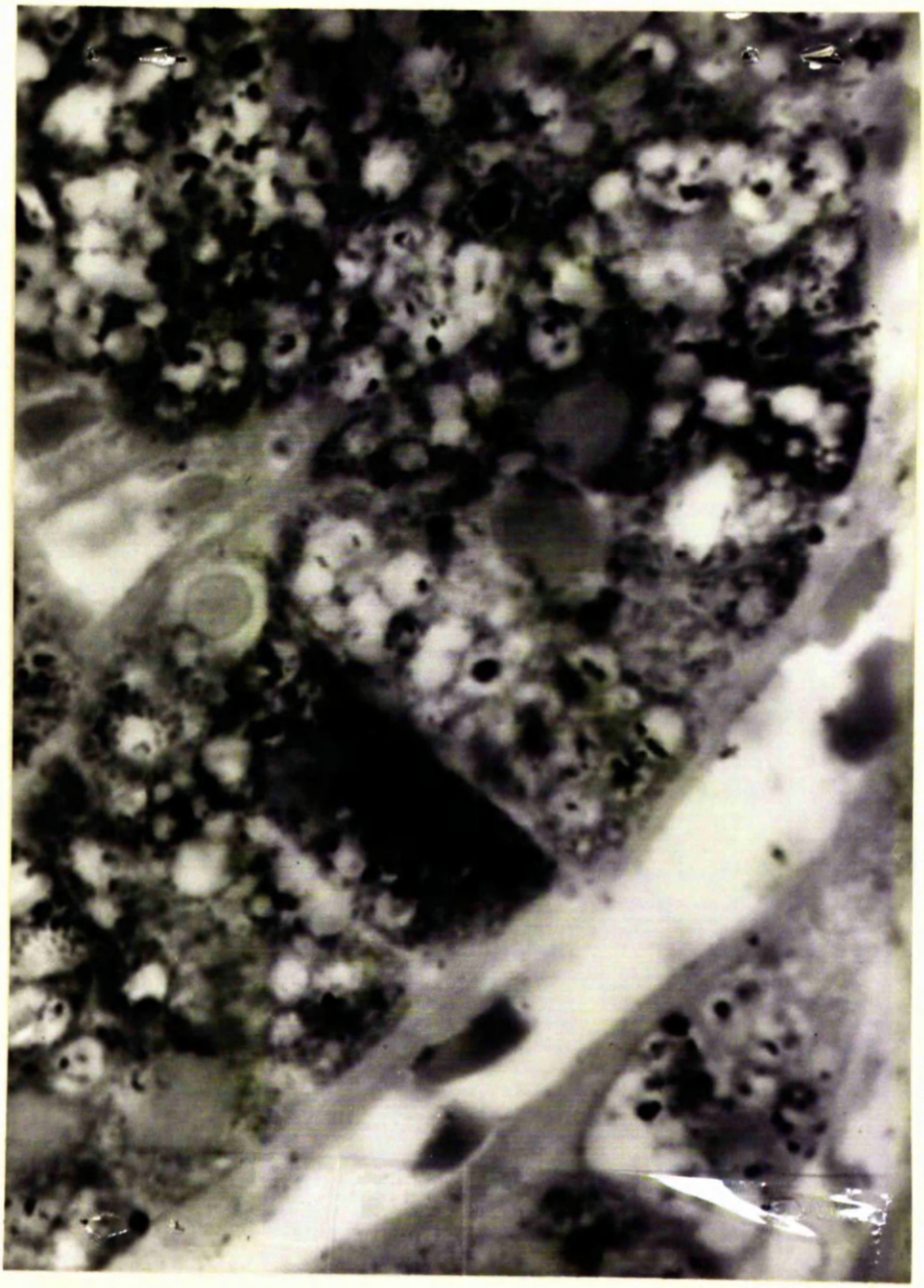
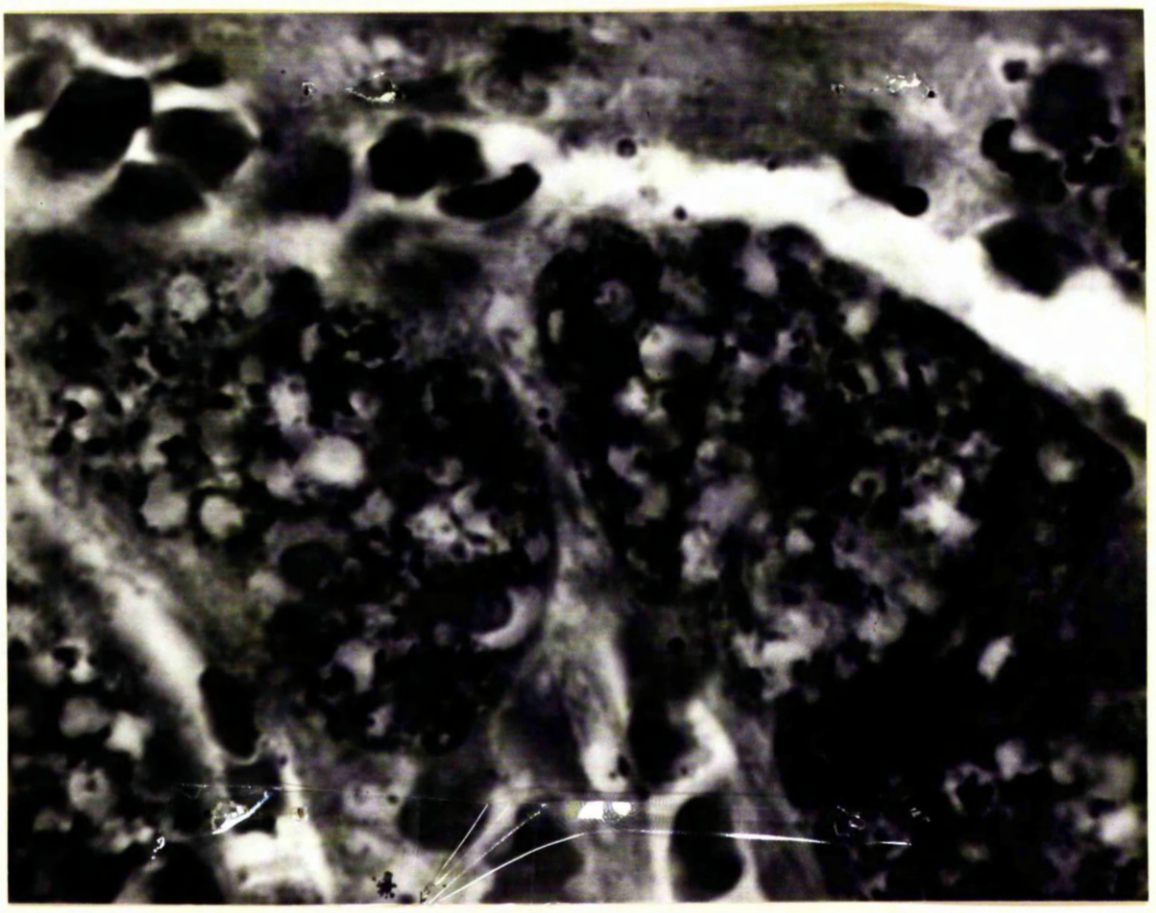


Fig. 13.

Control. Cells of the zona glomerulosa stained by the osmium Golgi technique. ×1800



ELECTRON MICROGRAPHS

Throughout the following illustrations:

Mv = microvilli

Mt = mitochondria

M = cell membrane

N = nucleus

B = microbody

G = Golgi apparatus

G^{Va} = Golgi vacuole

G^{Ve} = Golgi vesicle

G^{M} = Golgi membrane

BM = basement membrane

L = lipid globule

P = palade granules

V = vesicles of endoplasmic reticulum

PN = perinuclear space

The figures 1, 2, 3 etc. denote individual cells.

Fig. 14.

Control. Compact cells of zona reticularis. One cell occupies most of the field. Note the frequent mitochondria and prominent endoplasmic reticulum. Lipid bodies are small and vacuolated. X 15,000.

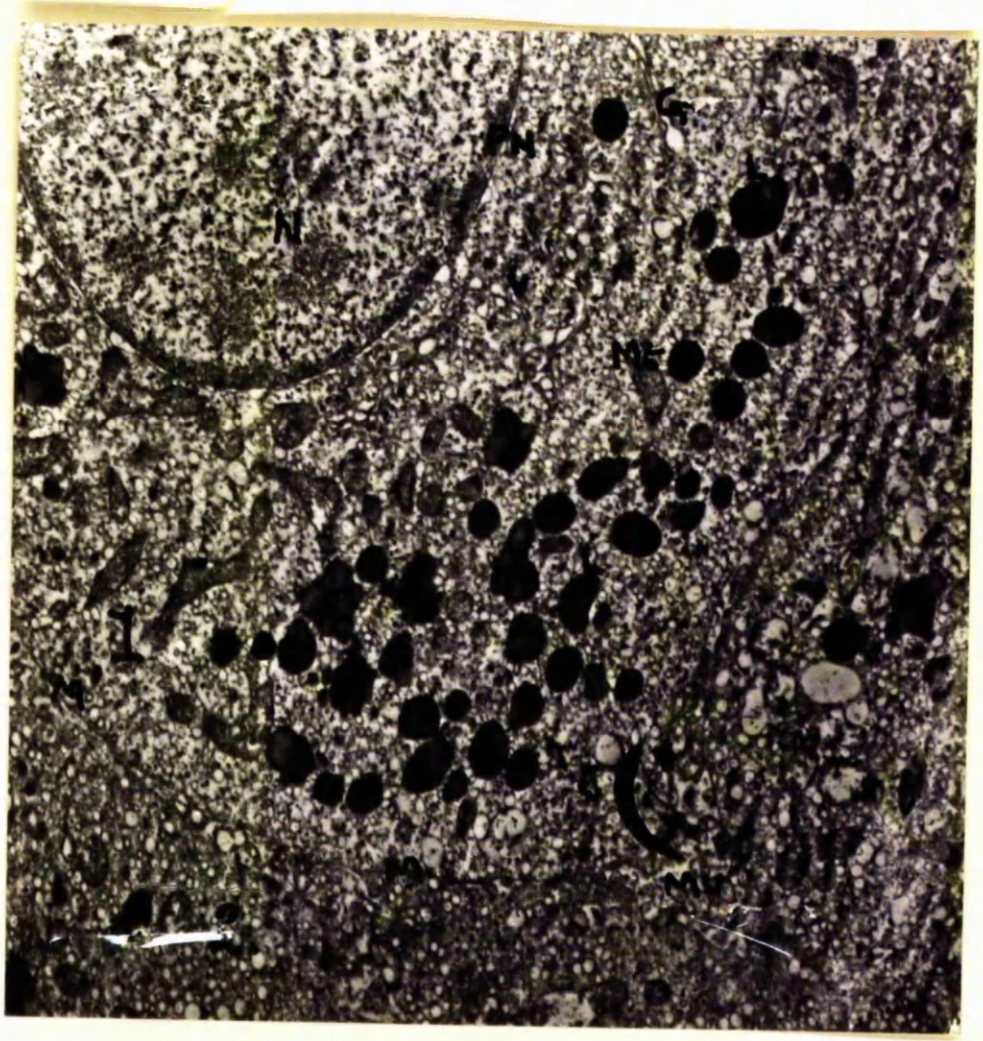

N
I
M

Fig. 15.

Control. Adjoining parts of two compact cells of zona reticularis. The mitochondria have a tubular internum. Palade granules lie between the vesicles of the endoplasmic reticulum. The lipid bodies show vacuolation and punctate osmophilia. X 25,000.

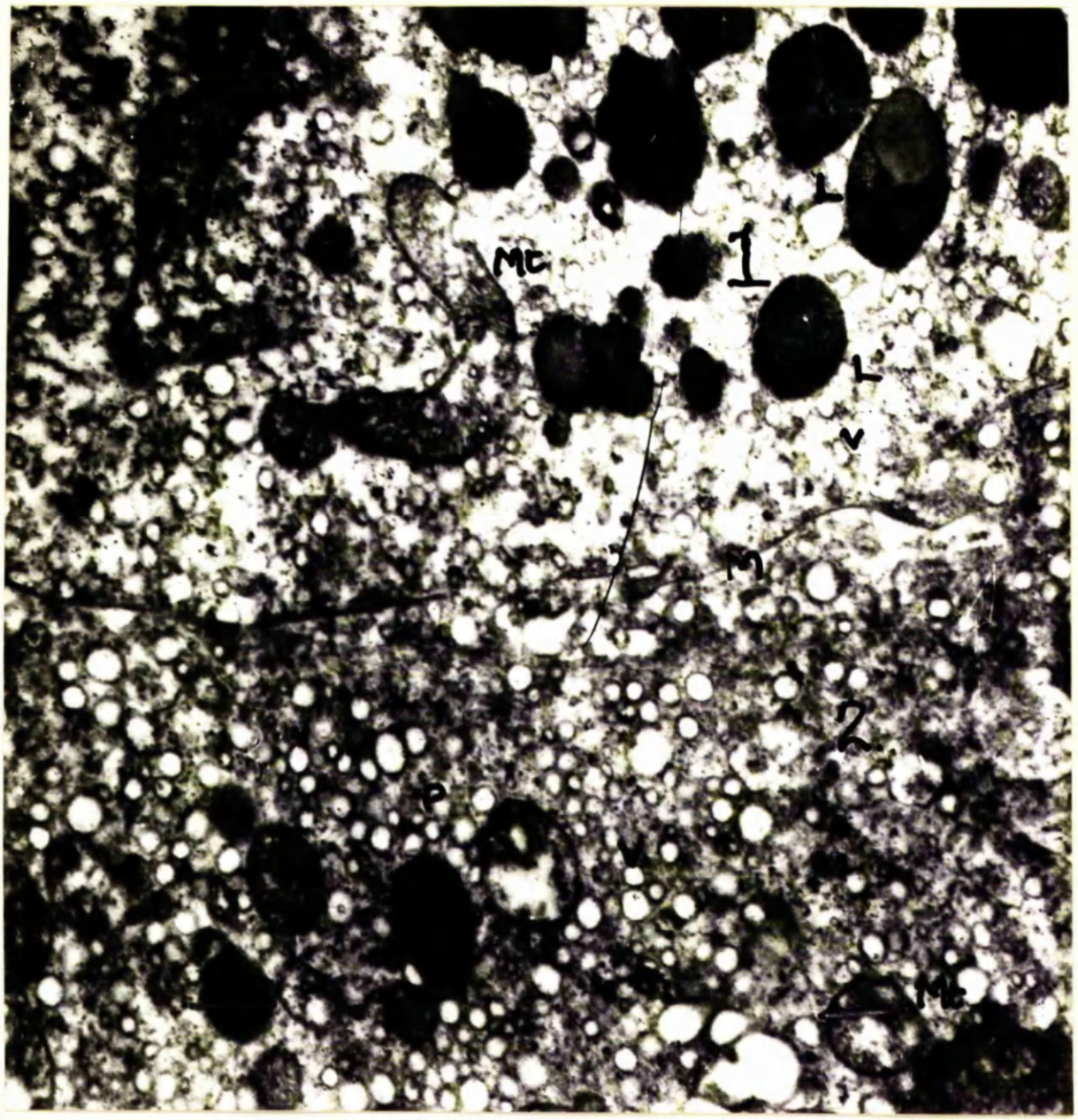

MC
1
L
L
V
M
2
P
V
MC

Fig. 16.

Control. Compact cells of zona reticularis. The cell membrane is arranged in complex interdigitating microvilli. X 42,000.

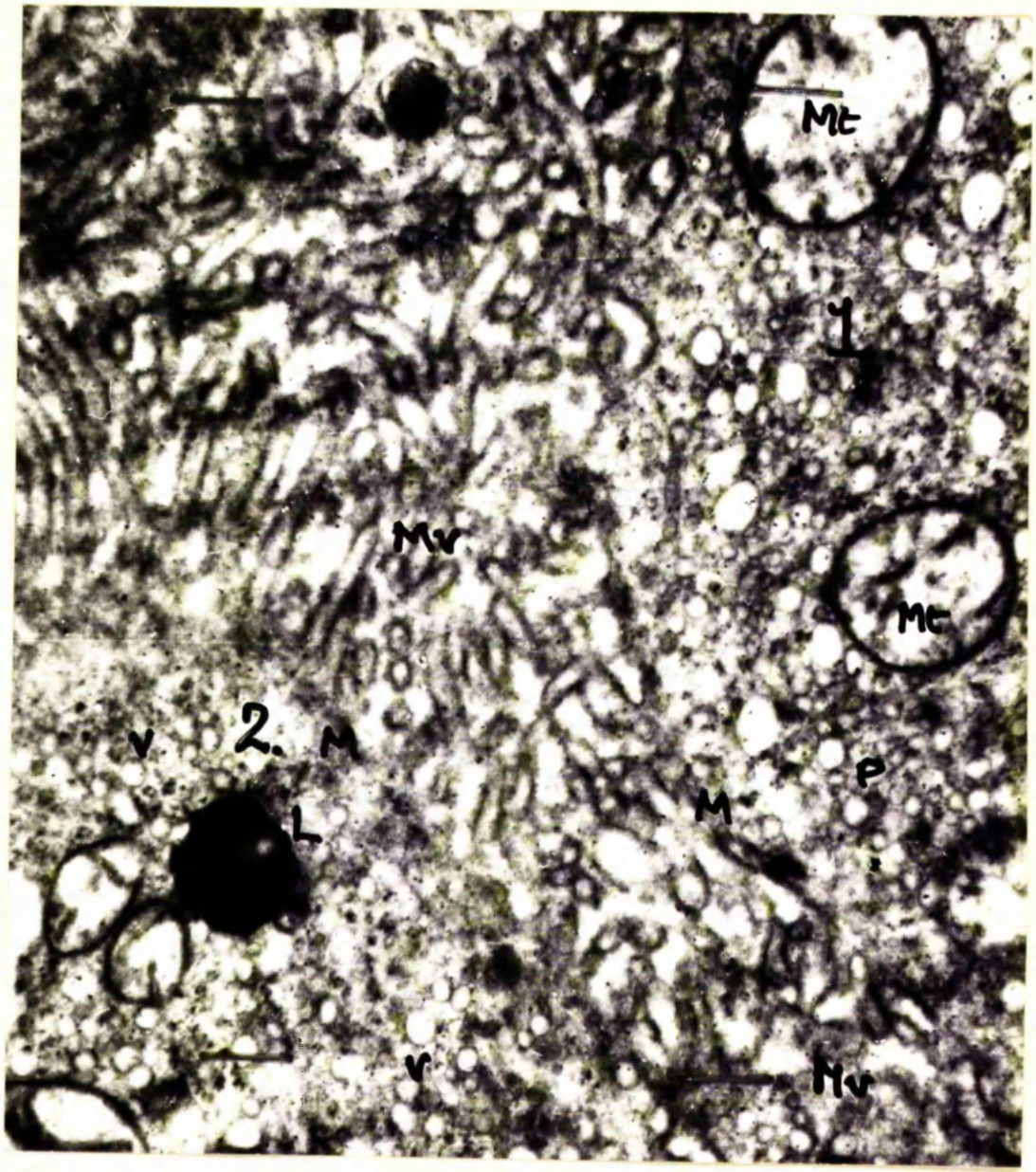

Mt
1
Mv
Mt
V
2.
M
L
M
P
V
Mv

Fig. 17.

Control. Compact cell of zona reticularis. The margin of a cell is shown with microvilli and basement membrane. The group of vacuoles, vesicles and membranes constitutes a Golgi apparatus. × 35,000

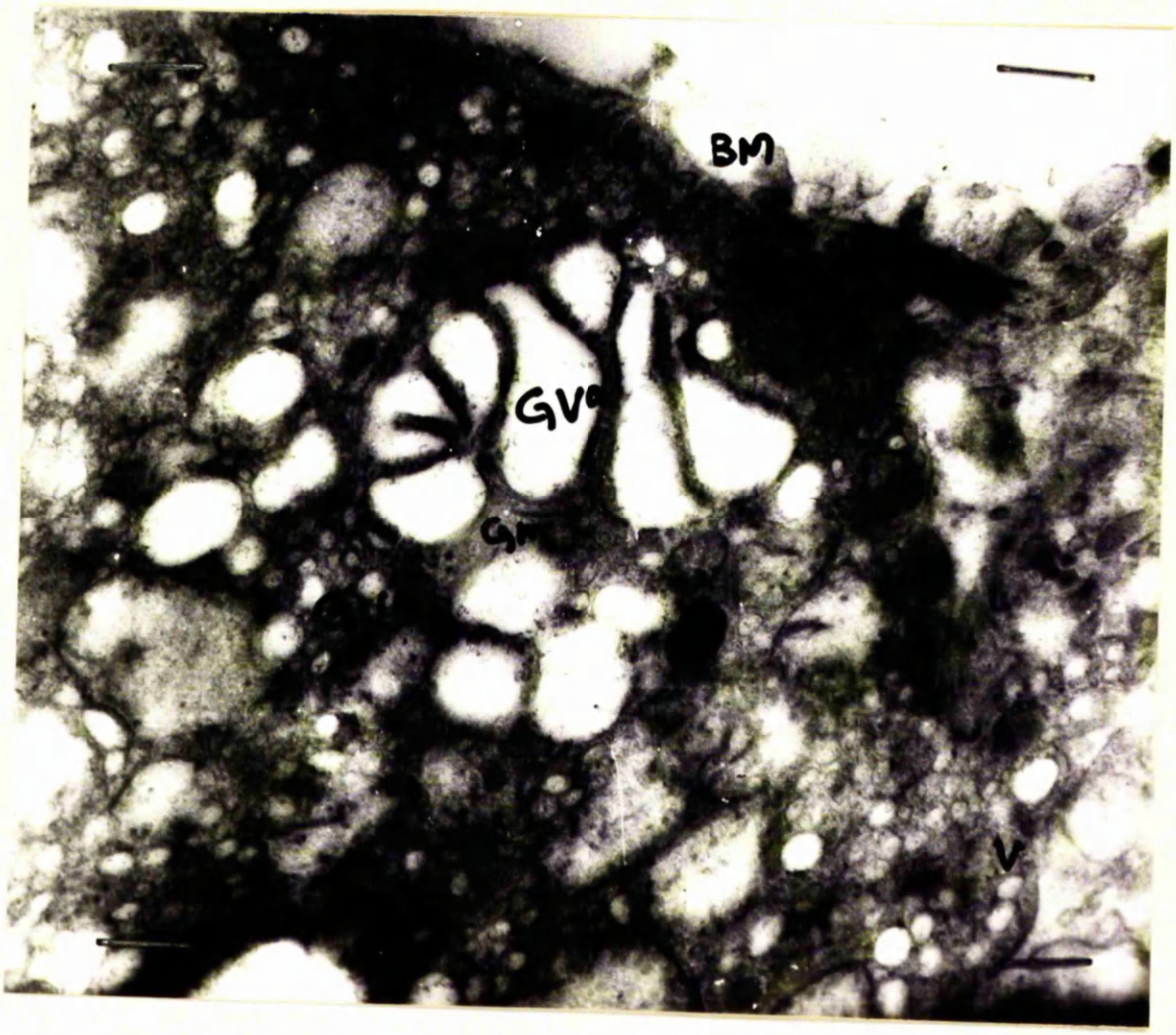

BM
GVC
GF
V

Fig. 18.

ACTH stimulated. These cells from the zona fasciculata contain many mitochondria and a prominent vesicular endoplasmic reticulum. Microvilli are present. Large lipid bodies are present at one point. X 12,000.

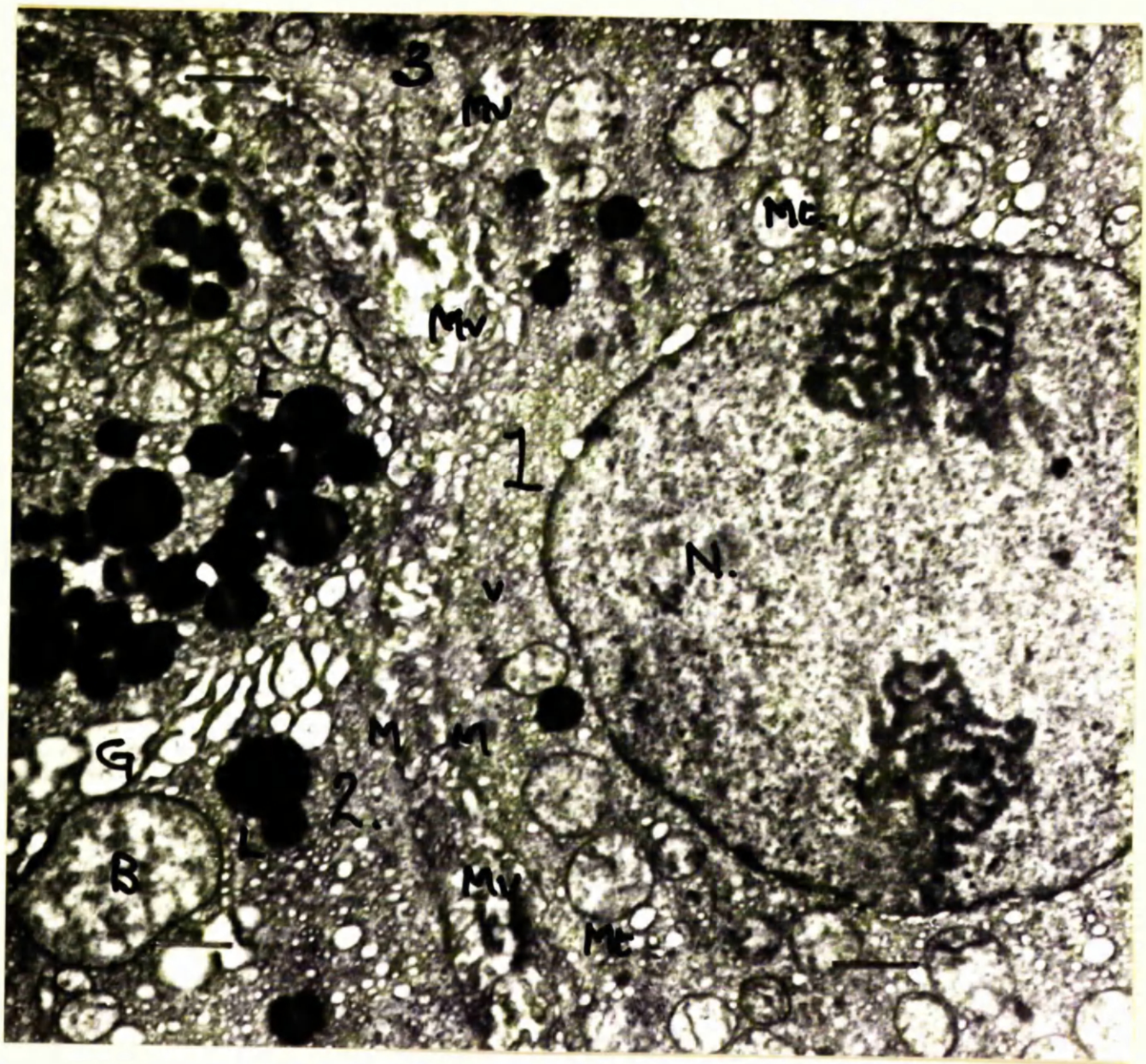

3
Mv
Mc
Mv
1
N.
v
M
M
G
2.
L
B
Mv
Mc

Fig. 19.

ACTH stimulated. The same area at higher magnification. Both large lipid bodies and microvilli are present. X 25000

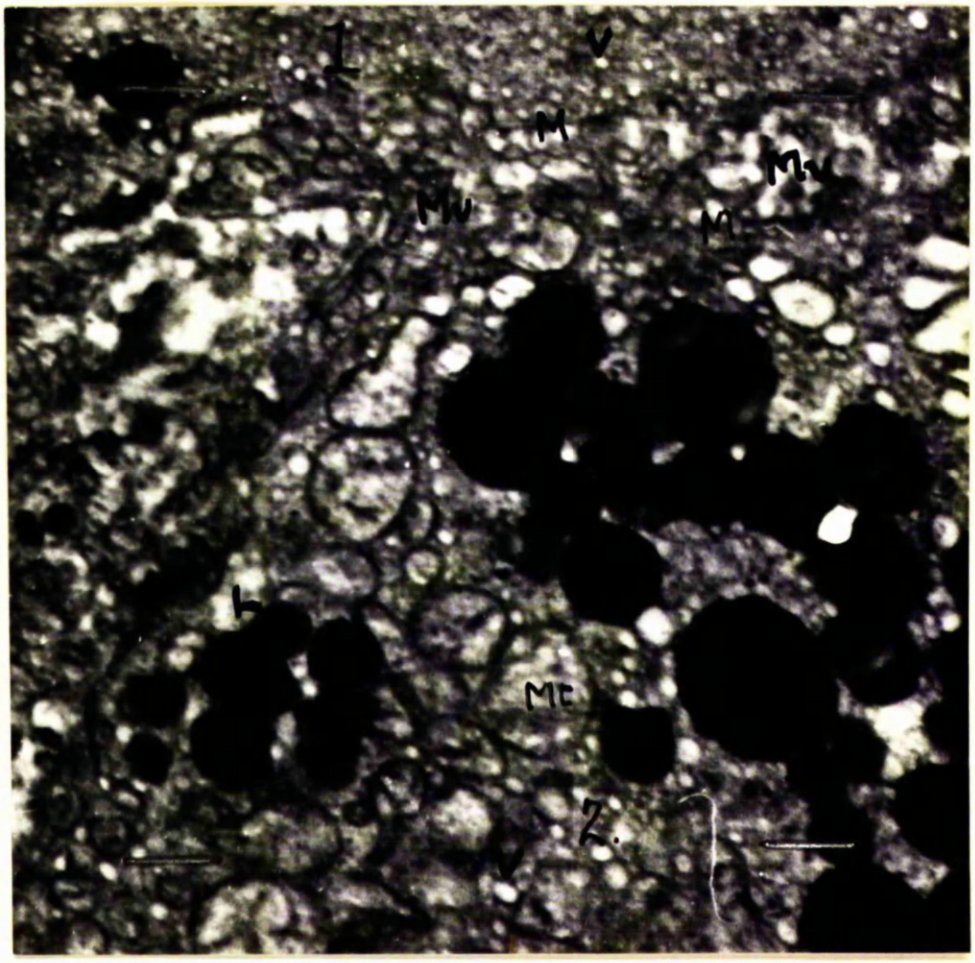

I
V
M
MV
MV
M
MI
Z.

Fig. 20.

Control. Clear cells of zona fasciculata. The prominent feature is the presence of many large lipid bodies. ×10,000

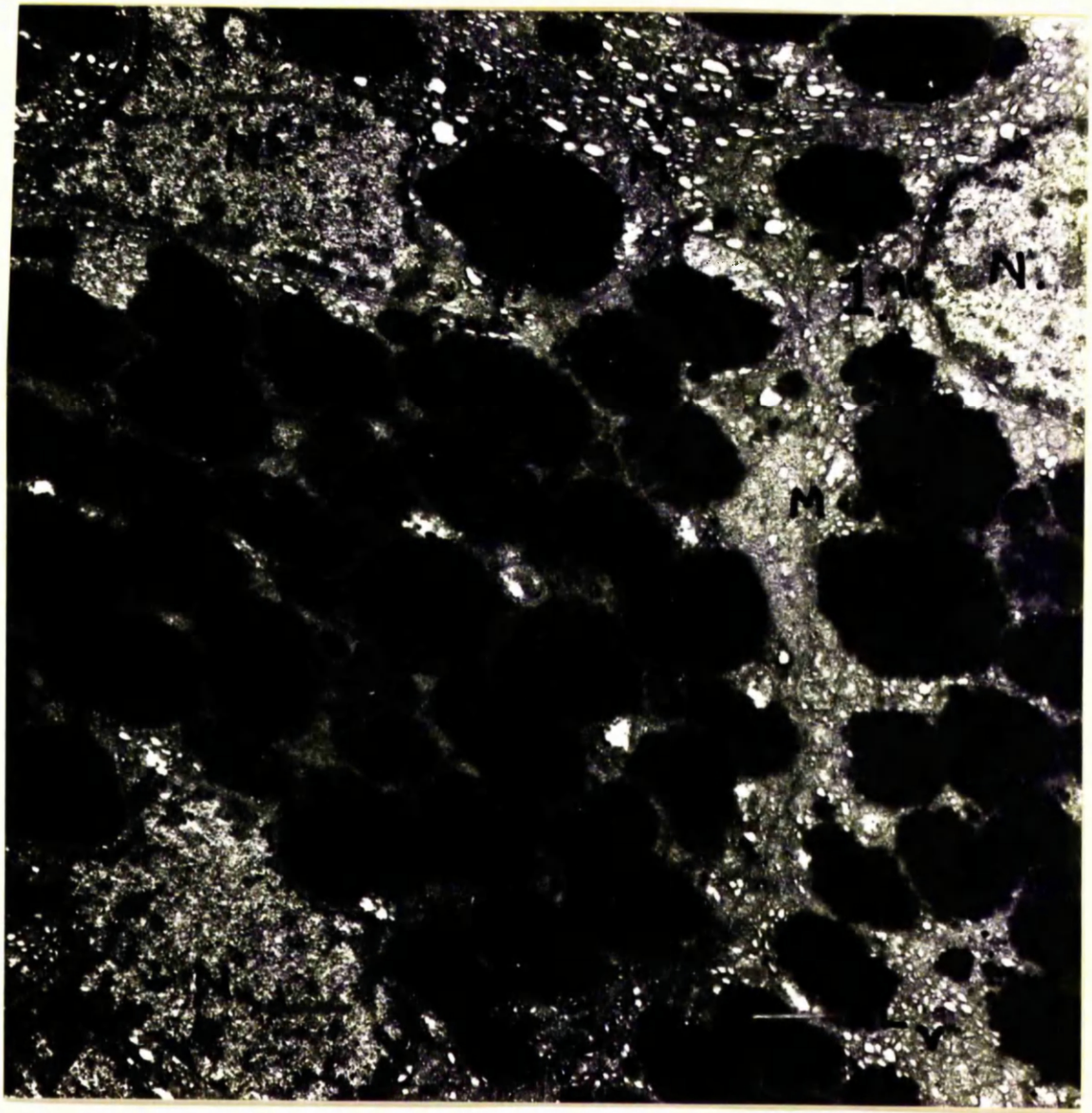

N
N
M
V

Fig. 21.

Control. Clear cells of zona fasciculata. Most of the cytoplasm of these cells is occupied by lipid. The intervening area seen here is composed of the vesicles of the endoplasmic reticulum. Mitochondria are not frequent. ×48000

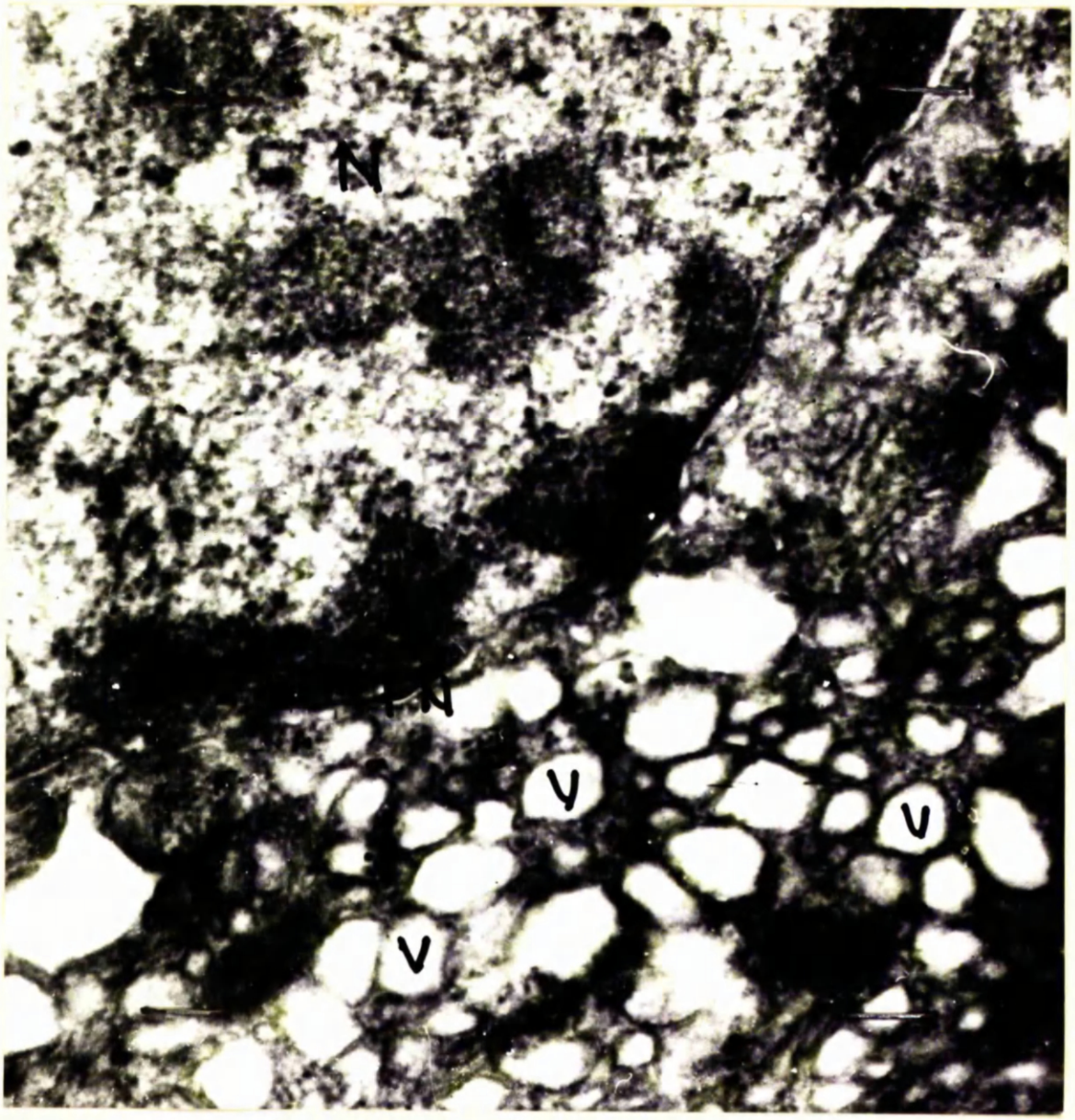

N
N
V
V
V

Fig. 22.

Control. Zona glomerulosa. The endoplasmic reticulum is dense and mitochondria fairly frequent. The cell membrane is plane. X 24,000.

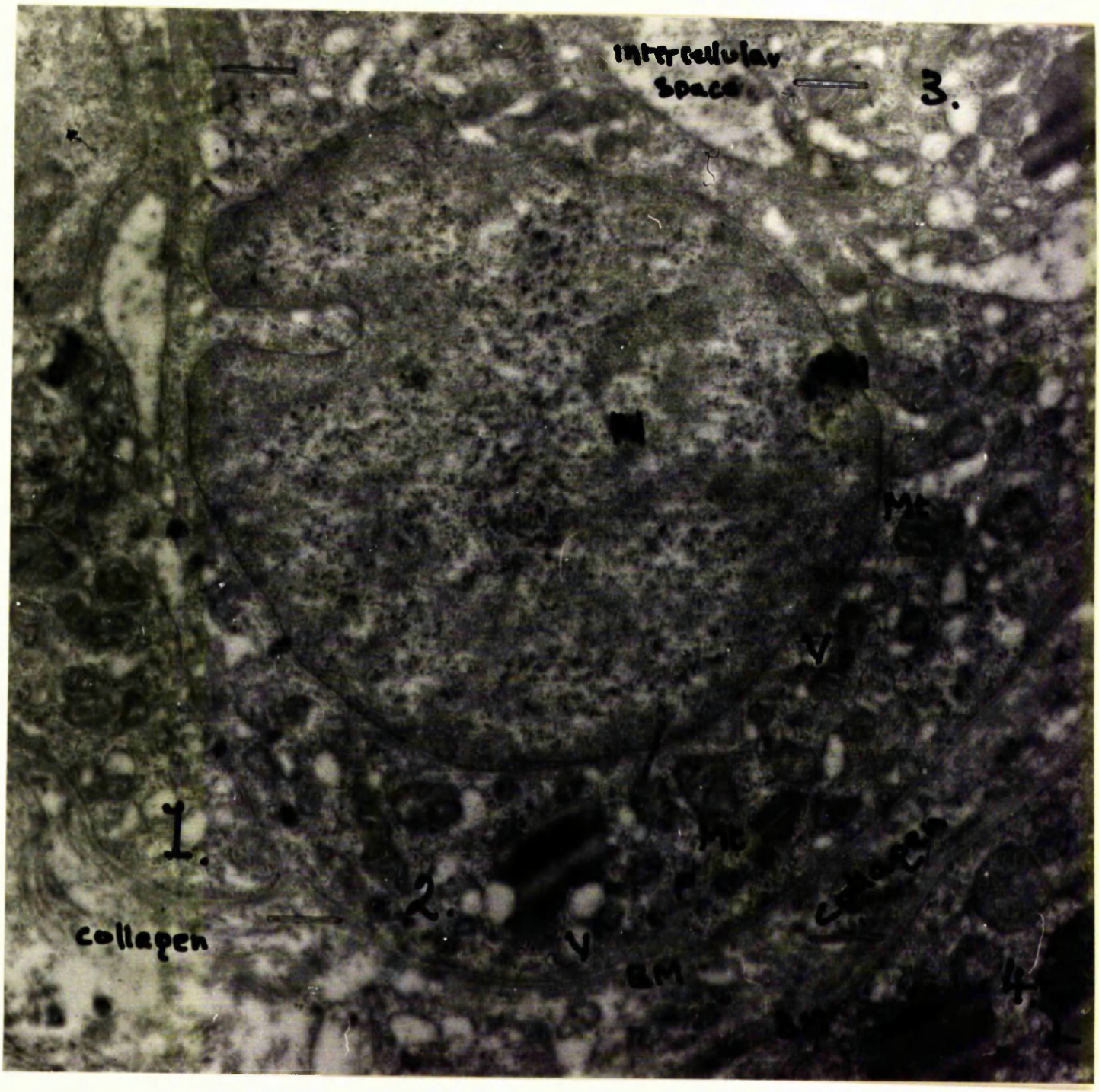

intercellular space
3.
Mt
V
1.
2.
collagen
V
BM
4

Fig. 23.

Control. Zona glomerulosa. A microbody bounded by a membrane and with granular internum is present. X 32,000.

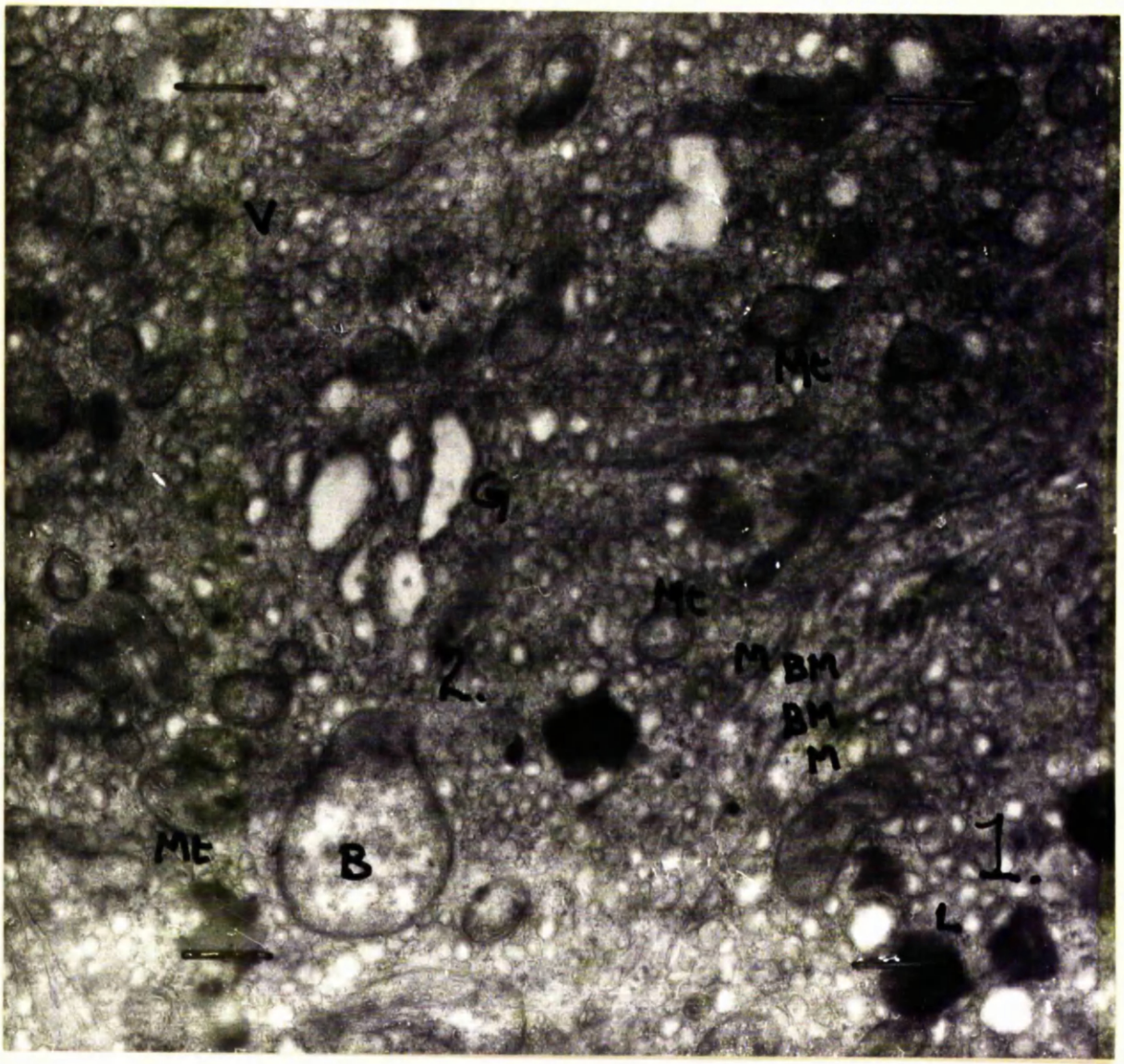

V
Mc
G
Mc
L
M
BM
BM
M
Mc
B
L
L

Fig. 24.

ACTH stimulated. Zona fasciculata. Two adjoining cells are separated from a capillary by a space containing granular osmophilic material. X 12,000.

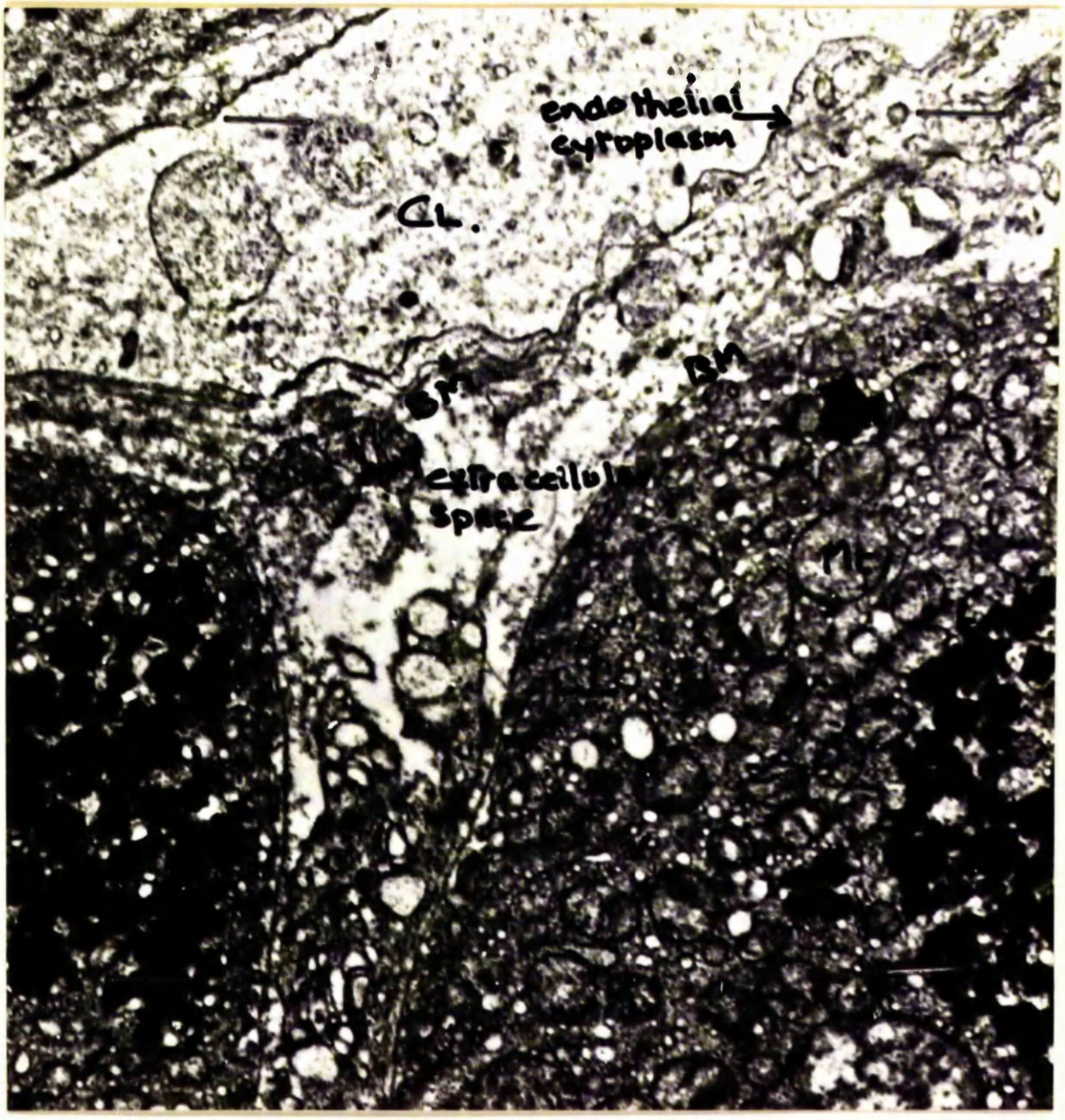

endothelial cytoplasm
GL.
BM
Extracellular space

Fig. 25.

Control. Zona glomerulosa. Parts of two cortical cells are shown, separated from the capillary lumen by the endothelial cell cytoplasm, the endothelial basement membrane, a space containing granular osmophilic material, and the cortical cell basement membrane. A microvillus is present on the endothelial cell. ×32000

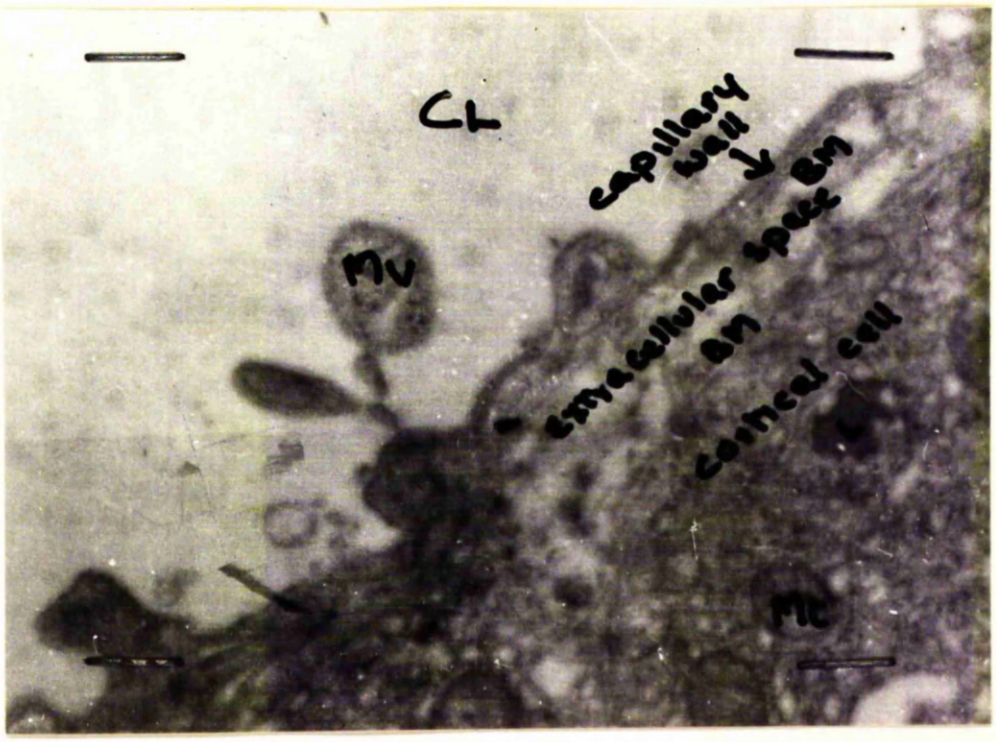

CL
capillary wall
Mv
extracellular space
BM
cortical cell
MC

Fig. 26.

Mitochondrial fraction showing rupture of mitochondrial membranes. ×25000

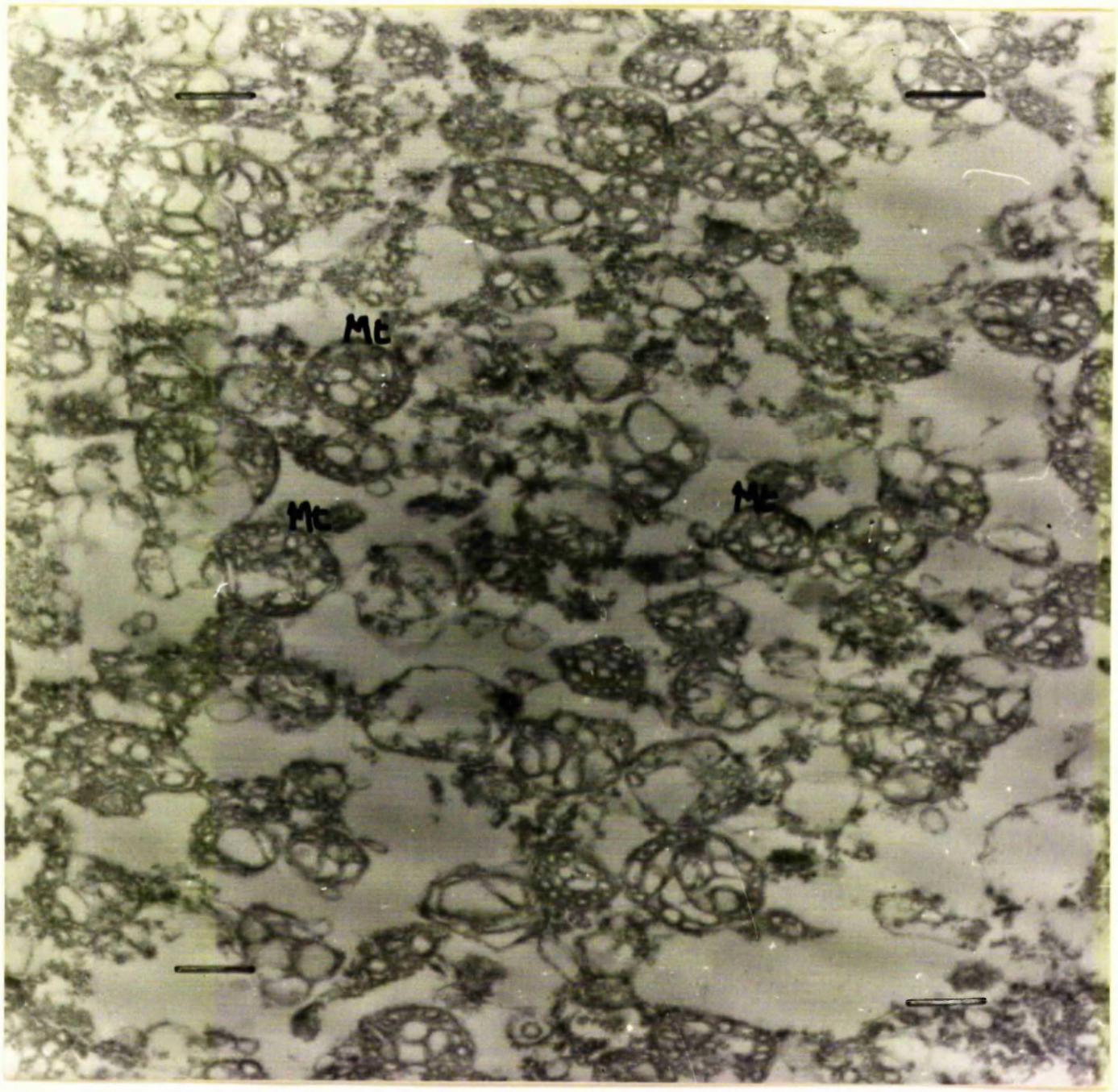
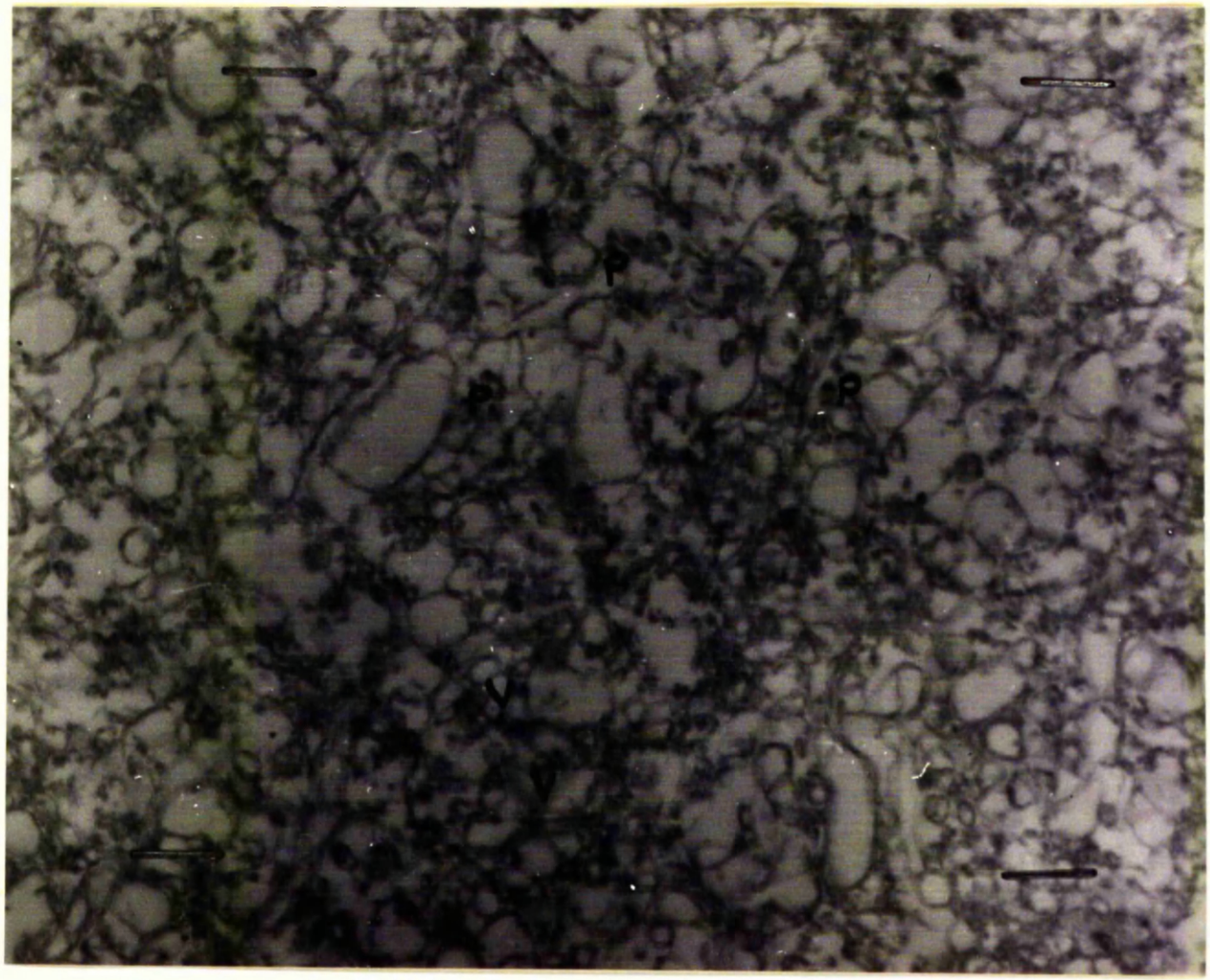

Mt
Mt
Mt

Fig. 27.

Microsomal fraction showing fragments of membrane often arranged in vesicles with related Palade granules. ×25000


P
P
V

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Summary of Thesis for the Degree of Doctor of Philosophy Presented to the University of Glasgow

by

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1. The human adrenal cortex has been studied at death by correlation of plasma hydrocortisone levels, with histology. It has been proved that the totally lipid depleted adrenal cortex seen at death is not functionally exhausted.

2. The pattern of cellular replacement in the human adrenal cortex has been studied. Cell replacement in the human adrenal cortex takes place only in the compact cells of the zona reticularis and ACTH stimulated zona fasciculata. The centripetal theory of cell growth does not hold for the adult human adrenal cortex.

3. The distribution of mitochondria and Golgi apparatus in the human adrenal cortex has been studied. Mitochondria are plentiful in the compact cell and the glomerulosa cell, but fewer in the clear cell. True Golgi impregnation has not been demonstrated except as a network in the clear cells which probably represents impregnation of the endoplasmic reticulum.

4. The ultrastructure of the human adrenal cortex has been studied. Three cell types are found:

(i) the compact cell in the zona reticularis and ACTH-stimulated fasciculata, characterised by the presence of frequent mitochondria, prominent vesicular endoplasmic reticulum, microvilli and scanty lipid. The vesicular component has been shown by cell fractionation to be endoplasmic reticulum.

(ii) the clear cell characterised by fewer mitochondria, less endoplasmic reticulum, absence of microvilli

and prominent lipid globules.

(iii) the glomerulosa cell characterised by relatively frequent mitochondria, dense endoplasmic reticulum, a variable lipid content and small or absent microvilli.

(iv) ACTH-stimulation has the effect of altering the fine structure of the clear cell to approximately that of the compact cell.