SOME OBSERVATIONS ON THE PARS TUBERALIS OF THE HYPOPHYSIS OF THE GUINEA PIG (A LIGHT AND ELECTRON MICROSCOPICAL STUDY)

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ABSTRACT

The pars tuberalis of the normal, young adult guinea pig, studied by means of light and electron microscopy, was seen to be composed of a number of cell types. Three granulated cell profiles exhibiting ultrastructural characteristics of protein synthesis and storage were seen, the granules of the first averaging 121nm, those of the second, 114nm and those of the third, 229nm. The first of these was the dominant granulated cell profile observed. The second was seen in only two animals and was very similar to the first, distinguishable only by its greater amount of rough endoplasmic reticulum, some of which was dilated. Therefore, these two profiles are thought to represent the same cell type. The third cell profile was relatively rare and light microscopical staining procedures demonstrated it to be the only chromophilic cell type in the pars tuberalis, reacting positively to both alcian blue and periodic acid-Schiff.

Two nongranulated cell profiles were also observed, both being very numerous. One was primarily identified by its cytoplasmic filaments, approximately 100Å in diameter, and the second was recognized by the presence of abundant, dilated rough endoplasmic reticulum. The latter cell profile was almost always seen to border on a follicular lumen which contained 'colloid'. The 'colloid' itself reacted positively with alcian blue, periodic acid-Schiff, chrome

alum haematoxylin, aldehyde fuchsin and Azur II.

Larger cystic structures were also observed and these are described.

Many fenestrated capillaries were seen between the pars tuberalis and the median eminence along their lengths. Blood vessels surrounded entirely by tuberal tissue, on the other hand, were usually seen to be small arterioles. Neither the light nor the electron microscopical examination, however, provided evidence of a general innervation of the pars tuberalis.

Possible functions of the tissue and their control are discussed in light of these findings.

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CONTENTS

INTRODUCTION

Light microscopical examination has yielded valuable information as to the existence and position of the pars tuberalis which lies on the surface of the tuber cinereum (of the hypothalmus) from which it derives its name (Tilney, 1913). It has been described in nearly all vertebrates, notable exceptions including the fish, whose pituitary embryology is substantially different, thus making determination of homologous areas difficult to establish, and Ophidians and Lacertilians, whose embryonic pars tuberalis anlagen develop into atypical tuberalis tissue or seem to disappear altogether (see reviews of Hanström, 1966 and Wingstrand, 1966).

The pars tuberalis originates from two lateral lobes of the oral epithelium of Rathke's pouch which grow and extend anteriorly as a forked tongue adjacent to the developing neural tissue. Eventually, the fork-shaped anlage fuses along its midline to form a continuous unit, growing closer to the neural element. Capillaries condense between the oral and neural elements, after which the neural tissue, the capillaries and the epithelium fuse together (Tilney, 1913, Herlant, 1964). In the adult animal, the pars tuberalis is usually continuous with the pars distalis although in a few species, the tuberal lobe extends further and becomes isolated from the rest of the adenohypophysis, e.g., Anura (Green, 1947). It is the establishment of this common pattern of origin and development in most mammals, amphibians, reptiles and birds to which the pars tuberalis owes its discrete identification.

Light microscopy, however, has not produced much information at the cytological level which is relevant to the function of the pars tuberalis. The presence of 'colloid' deposits in the adenohypophysis and in the pars tuberalis in particular has long been recognized in many animals (e.g., cat, Tilney, 1913, Atwell, 1929; porpoise, Wislocki and Geiling, 1936; guinea pig, Kirkman, 1937; rabbit and cat, Dawson, 1937) but the significance of these deposits has not been established. Concerning the cells themselves, a few investigators have reported a substantial number of basiphils (Tilney, 1913, Kutas, 1958) and PAS^{+} cells in the human and Rhesus monkey (Pearse and van Noorden, 1963, Fand, 1965, Knowles and Kumar, 1969, Conklin,1968), but most studies on mammals, avians and reptiles agree that the majority of cells are either chromophobic or very faintly basiphilic (Guizzetti, 1925, Wislocki and Geiling, 1936, Severinghaus, 1936, Dawson, 1937, Wingstrand, 1951, Allanson et al., 1959, Beck, et.al., 1969, Klein et al., 1970, Allanson, 1971, Doerr-Schott, 1971, Dierickx et al., 1971, Cameron and Foster, 1972a, Stoeckel et al., 1973a & b). As a result, light microscopical methods comparable to those utilized in the study of the pars distalis to obtain evidence of cellular function (i.e., ablation of different endocrine organs or the administration of various drugs followed by an examination of the altered cytology as indicated by changes in the staining patterns of the cells) have not been applicable. In more recent years, the results of fluorescent antibody techniques have provided evidence of some LH-containing cells in the pars tuberalis of the human (Midgley,

1966) and of the rat (Baker and Yu, 1975) while others report ACTH-containing cells in the human (Pearse and van Noorden, 1963).

The small size of the pars tuberalis and its position surrounding the neural stalk has made it difficult to isolate for the purposes of obtaining pure extracts for injection experiments or for bioassay. Injections of tuberal extracts have resulted in the stimulation of the interstitial cells of the testis and luteal tissue in the rat (Legait, 1969), but this could be due to contamination by tissue from the anterior pars distalis as easily as from influences of the pars tuberalis. A similar doubt could be expressed with regard to bioassays which have indicated a TSH, ACTH or gonadotrophic content (Reichlin, 1963).

The relatively small size of the pars tuberalis, of course, is no impediment to an electron microscopical investigation and in recent years, an increasing number of ultrastructural studies have been appearing. Together with the increasing use of tissue culture techniques, enzyme techniques and the more established methods of organ removal and drug injection, electron microscopical investigations provides a broader scope for further study. First, however, the normal ultrastructural characteristics of tuberal tissue must be more firmly established and it is for this reason that such an examination of the guinea pig pars tuberalis has been undertaken. As far as can be determined, no other work on this subject has been carried out.

Electron microscopical examination of the pars tuberalis has largely involved study of the rat (Kobayashi et al., 1963, Stutinsky et al., 1964, Oota and Kurosumi, 1966, Rinne,

1966, Klein et al., 1970, Kotsu, 1971, Unsicker, 1971, Kotsu and Daikoku, 1972, Stoeckel et al., 1973a & b, Costoff, 1973, Stutinsky et al.,1973, Brandi and Peillon, 1973, Dellmann et al., 1974), although the cat (Green and van Breemen, 1955, Morato and Ferriera, 1960, Dellmann et al., 1974), rabbit (Allanson et al., 1959, Young et al., 1965, Cameron and Foster, 1972a), Rhesus monkey (Knowles and Kumar, 1969), frog (Dierickx et al., 1971, Doerr-Schott, 1971, Vandenberghe et al.,1973), mouse (Unsicker, 1971, Stoeckel et al., 1973a, Dellmann et al., 1974), doormouse (Stoeckel et al., 1973a, Dellmann et al., 1974), chicken (Grignon and Guedenet, 1968, Dellmann et al., 1974), newt, hamster and cattle (Dellmann et al., 1974) have also been examined. Except for the earliest studies, all reports either agree or are consistent with the view that the tissue contains many granulated cells showing ultrastructural evidence of protein synthesis and secretion; that is to say, small cytoplasmic granules appear to originate in a well developed Golgi zone, they are sometimes observed very near the plasmalemma and the cells contain rough endoplasmic reticulum (Young et al., 1965, Oota and Kurosumi, 1966, Rinne, 1966, Grignon and Guedenet, 1968, Knowles and Kumar, 1969, Klein et al., 1970, Dierickx et al., 1971, Doerr-Schott, 1971, Kotsu and Daikoku, 1972, Cameron and Foster, 1972a, Stoeckel et al., 1973a & b, Vandenberghe et al., 1973, Dellmann et al., 1974). Light microscopical correlation studies show that in many of these animals the granulated cells are either chromophobic or very faintly PAS^{+} (Allanson et al., 1959, Klein et al., 1970, Dierickx et al., 1971, Doerr-Schott, 1971, Cameron and Foster, 1972a, Stoeckel et al., 1973a & b). These

'chromophobic' cells, however, have not been recognized as being ultrastructurally similar to any cell type of the pars distalis, and hence, it has been suggested that perhaps the granulated cells of the pars tuberalis may produce a secretory product(s) which is, as yet, unidentified (Morato, 1965, Dierickx et al., 1971, Cameron and Foster, 1972a, Kotsu and Daikoku, 1972, Stoeckel et al., 1973b, Dellmann et al., 1974).

It should be noted that the reported granulated cells of the pars tuberalis are not restricted exclusively to one type. In animals reported to have largely chromophobic cells, a few typical chromophiles are seen (e.g., Dawson, 1937, Beck, et al., 1969, Legait and Contet, 1969). Similarly, electron microscopical reports frequently indicate at least one other morphologically distinct cell type which is usually recognized as an invasive pars distalis cell (Rinne, 1966, Cameron and Foster, 1972a, Stoeckel et al., 1973b, Dellmann et al., 1974) although these cells are relatively uncommon.

It follows that information on the ultrastructure of the guinea pig pars distalis is extremely relevant to the interpretation of any evidence concerning granulated cells in the pars tuberalis. Smith (1963) reports three cell types in the guinea pig pars distalis as follows: acidophils containing ~400nm granules, basiphils containing ~ 200nm granules and nongranulated stellate cells containing "fibrilles". These limited findings are backed partially by the light microscopical studies of several investigators who found evidence leading them to conclude that there is only one type of basiphil in the guinea pig pars distalis (Kirkman, 1937, Hagquist, 1938, D'Angelo, 1963).

In contrast, however, Peterson (1957) reported two types of mucoid cells in the pars distalis of the guinea pig. Similarly, Amat (1970) reported histochemical staining differences between a thyrotroph and a second type of basiphil in this tissue. Ultrastructural evidence more in line with these findings was given in a paper by Amat and Boya (1970) in which they describe presumed mammatrophs, somatotrophs, thyrotrophs, gonadotrophs and prolactin cells together with a nongranulated stellate cell. They make no mention of an ACTH cell. Their classification was based on morphological comparisons (including granule sizes) with recognized cell types in other animals.

The basis for comparing pars tuberalis cells with pars distalis cells in the guinea pig, therefore, appears to be weak. A supplementary examination, however brief, has been thought necessary, not so much to confirm or refute the preceding reports, but rather to aid in the interpretation of any granulated cells found in this study.

Many of the ultrastructural reports on the pars tuberalis mention the presence of nongranulated cells. The literature, however, is often unclear as to their distribution and frequency and their morphology is not well defined. The absence of a more active interest in these cells may in part be due to the fact that the function of nongranulated cells in the pars distalis (see reviews of Dingeman and Feltkamp, 1972, and Vila-Porcile, 1972) is still not understood. There does seem to be a general concensus, however, that there are nongranulated cells in the pars tuberalis which exhibit a poorly developed Golgi apparatus, contain little endoplasmic reticulum and are

frequently associated with at least some type of follicle (Oota and Kurosume, 1966, Kotsu and Daikoku, 1972, Cameron and Foster, 1972a). Cameron and Foster reported more specifically on a sizeable number of stellate interstitial cells in the rabbit pars tuberalis, the cytoplasm of which was characterized by abundant microfilaments. They observed that these filaments were not disassociated when the tissue was incubated with cytocholasin B. In contrast, Morato and Ferriera (1960) described nongranulated cells in the cat pars tuberalis with characteristics substantially different from those generally reported by other investigators. They found two kinds of nongranulated cells, one dark and one light, the latter associated with 'colloidal' deposits and possessing many cytoplasmic vacuoles to which small osmiophilic grains were attached. They suggested that the dark and light cells represented different stages of the same cell type.

In the light of these reports, it would seem appropriate to observe carefully the nongranulated cell population of the guinea pig pars tuberalis and to note its similarities and dissimilarities to the above observations and also, to compare it with the nongranulated cells of the pars distalis as reported by others.

On the question of glycogen, numerous authors have reported its presence in the cells of the pars tuberalis (in the rat, Oota and Kurosumi, 1966, Klein et al., 1970, Stoeckel et al., 1973a & b, Dellmann et al., 1974; in mice, Siperstein, 1955, Stoeckel et al., 1973a, Dellmann et al., 1974; in the frog, Dierickx et al., 1971; in the doormouse, Stoeckel et al., 1973a, Dellmann et al., 1974;

in the hamster, cat, cattle and newt, Dellmann et al., 1974). Stoeckel et al. (1971) stated that the glycogen content of the granulated cells of the pars tuberalis of the mouse distinguishes them from the rostral ACTH cells in an area where these two types of cells come into contact. The above findings are based, in most instances, on the ultrastructural morphology of tissue fixed with glutaraldehyde followed by osmium tetroxide. Only Stoeckel et al. 1973b) supported their findings with a histochemical technique. On the other hand, Cameron and Foster (1972a) reported that glycogen was not seen either on the ultrastructural level or on the light microscopical level using a periodic acid-Schiff reaction with diastase control. In addition, Knowles and Kumar (1969) made no mention of glycogen in the tuberal tissue of the Rhesus monkey. In this study, therefore, an attempt has been made to investigate the glycogen content of the guinea pig pars tuberalis.

The question of how the pars tuberalis is regulated is not yet clearly answered. While reports concerning certain experimental animals describe neurosecretory material entering the tuberal tissue (Beck and Daniel, 1960, Beck, et al. 1969, Daniel and Prichard, 1970, Naik, 1972, Stutinsky et al., 1973), those concerning normal animals show much disagreement. Papers on the electron microscopical examination of the rabbit (Cameron and Foster, 1972a), frog (Dierickx et al., 1971), doormouse (Stoeckel et al., 1973a, Dellmann et al., 1974), rat (Unsicker, 1971) and monkey (Knowles and Kumar, 1969) report nerve fibres in the tuberal tissue, although there is considerable discrepancy as to the type of nerve fibres observed; peptidergic and adrenergic both have been

mentioned. In contrast, other ultrastructural studies on the pars tuberalis of the frog (Doerr-Schott, 1971), rat (Stoeckel et al., 1973a & b, Dellmann et al., 1974), mouse (Stoeckel et al., 1973a, Dellmann et al., 1974) and in the hamster, cat, cattle, chicken and newt (Dellmann et al., 1974) have not found evidence for innervation of the tissue.

The reports resulting from light microscopical investigation also lack agreement. Neurosecretory fibres have been observed entering the tuberal tissue of the rat (Arko and Kivalo, 1958, Okomoto and Ihara, 1960) and some amphibians (Legait and Burlet, 1966). Nerve fibres have also been reported in the pars tuberalis of the cat (Metuzals, 1959). Szentagothai et al. (1968), reporting on the cat and dog, and Beck et al. (1969) reporting on the goat, observed rare nerve fibres in the tuberal tissue but only in a small and restricted area at a low level of the stalk. Furthermore, Wingstrand (1951) observed only autonomic (perivascular) nerve fibres in the avian tuberal tissue. In contrast, Beck and Daniel (1960) report the absence of both nerve fibres and neurosecretory material in the sheep and goat and Fuxe (1964) reports no direct adrenergic innervation (using an immunofluorescent technique) in the mouse, rat, guinea pig, hamster, rabbit and cat.

Alternatively, it is possible that the pars tuberalis may be regulated via a blood supply. While it is not within the scope of this study to investigate the guinea pig hypophysial vascular system, this aspect has been examined by others (Boerner-Patzelt, 1954, Weatherhead, 1968). They have found that the portal vascular system of this animal resembles that of other mammals (e.g., vole, Enemar, 1957;

mouse, Enemar, 1961; rat, Glydon, 1957, Cambell, 1966; rabbit, Ternby, 1972); that is to say, the anterior hypophysial artery feeds the primary capillary plexus which is located between, and runs parallel to, the pars tuberalis and the infundibular stem. The primary capillary plexus in turn gives rise to the long and short capillary loops which ultimately feed the wider portal vessels, many of which pass through the pars tuberalis en route to the pars distalis. The intimate location of the primary plexus and the portal vessels in relation to the cells of the pars tuberalis in many animals may be responsible for the observation that the pars tuberalis is an extremely vascular region of the pituitary gland (Wislocki and Geiling, 1936, Stevens, 1937, Harris, 1955, Allanson et al., 1959, Bloom and Fawcett, 1968).

Apart from the portal vascular system, there might be other explanations for the blood vessels reported in the pars tuberalis. In an embryologic study of the hypophysial blood supply of the guinea pig, Weatherhead (1968) reports that as the pars tuberalis anlage grows anteriorly over the prospective median eminence, it contains some capillaries of its own which eventually join those of the primary plexus. A similar description of the development of the pars tuberalis vasculature has been reported in the mouse (Enemar, 1961). In the frog, Dierickx et al. (1971) also reports a separate vascularization of the pars tuberalis, the efferent capillaries of which anastomose with the capillary plexus of the median eminence. Likewise, Wingstrand (1951) reports on the embryonic development of the avian hypophysis in which the general pattern is that as the pars tuberalis anlage developes, it carries with it

a capillary supply. Not until some time after birth does the epithelium of the pars tuberalis come to enclose the portal vascular system. However, cytoplasmic granules of the avian pars tuberalis cells are seen in about the 6-day old embryo.

While the nature of the vascularization of the pars tuberalis is not clearly understood, the studies cited above do not preclude the possibility that the regulation of this tissue may be a function of its vascular system.

The major anatomical divisions of the mammalian hypophysis were established by Rioch, Wislocke and O'Leary (1940). Several of these divisions are important enough to mention now so as to facilitate the understanding of anatomical regions mentioned in this thesis. Diagram I (page 30) may be referred to. The adenohypophysis (originating from the oral epithelium of Rathke's pouch) includes the pars distalis (PD), the pars intermedia (PI) and the pars tuberalis (PT). The neurohypophysis (originating from the floor of the brain) includes the pars nervosa (PN), the infundibular stem (IS) and the median eminence (ME). Two further important terms must be kept in mind, the neural stalk, which refers to the tissue of both the IS and the ME, and the hypophysial stalk, which refers to the neural stalk plus the surrounding adenohypophysial tissue (including the PT).

In conclusion, the aim of this study is to describe the PT in the adult guinea pig with respect to its gross anatomy, its light microscopical characteristics and its ultrastructural cytology. Electron microscopical experiments in this study mainly involve male animals which, through the absence of morphological changes related to

the estrus cycle, are thought to be more advantageous for the determination of clear ultrastructural cell types. Lastly, a brief examination of the guinea pig PD is carried out in order to supplement the information available on this tissue for the purposes of evaluating the possible morphological uniqueness of granulated cells of the PT.

MATERIALS AND METHODS

Animals

Twenty-seven Hartley albino guinea pigs were used in this study. After their arrival at the St. Mary's Hospital Medical School animal house, they were placed in quarantine for three weeks before being used for any experiments. They were fed SG1 pellets and water ad libitum. Animals were killed between 9 weeks and one year of age. At the time of sacrifice, they weighed at least 300 grams in the case of females and at least 400 grams in the case of males.

Sacrificing

Animals were killed by one of two methods. One procedure involved killing the animal outright by breaking its neck. The pituitary gland in these cases was quickly dissected out and immersed in the appropriate fixative. The second method involved killing via the process of perfusing the anaesthetized animal with fixative.

Perfusion technique

An ether soaked cotton wool pad and an animal were placed together in a closed wooden box. When the guinea pig gave no reflex responses, it was removed and pinned out on a cork board with the etherized pad held in position around its nose. The chest cavity was then opened. Upon exposing the heart, 0.05 cc. of heparin was injected into the ventricle. The pericardial membrane was then removed, the lower tip of the heart cut off and a small tear made in the atrium to provide an outlet for the perfusate. Dextraven 110 was next perfused through the animal by means of a

cannula fitted to a hospital infusion set which was hung from about four feet to give a reasonable flow pressure. Forceps were used simultaneously to hold the cannula in place and to close the open end of the heart. Dextraven 110 was administered for several minutes until the liver and the skin about the nose began to look pale. The cannula was then disconnected from the dextraven source and reconnected to a second infusion set which supplied the required fixative.

A small amount of fast green dye was added to the fixative in the cases of formal cadmium calcium chloride, formal calcium chloride and gluteraldehyde in order to create a built-in marker by which the effectiveness of the perfusion could be judged. The yellow colour of Bouin and Bouin-Hollande fixatives was an adequate marker on its own. Colouration of the skin around the eyes was a good indication that the fixative had probably reached the pituitary gland.

The perfusion of fixative was usually continued for a minimum period of five minutes after which the cranium and a large portion of the brain was removed, leaving the pituitary gland (attached to a small piece of hypothalmus) in place. This was then carefully freed from the surrounding meninges and immediately immersed in a small amount of fixative for some additional time. Before immersion, the colouration of the gland was checked to determine the actual success of the perfusion.

Light microscopy

Fixatives used for examining the pars tuberalis under the light microscope included formal cadmium calcium chloride (FCC, Baker, 1944), formal calcium chloride (FC, Baker, 1944),

Bouin and Bouin-Hollande (Langeron, 1942). Tissue which was fixed in Bouin or Bouin-Hollande (for three to five days) was rinsed repeatedly in 70% ethanol to remove excess fixative before continuing with the process of dehydration and embedding. Tissue fixed (overnight) in FCC was rinsed in running tap water for two hours to remove excess cadmium before proceeding with dehydration and tissue fixed (overnight) in FC was taken immediately through the dehydration and embedding schedule. For all pituitary glands used in light microscopical experiments, the dehydration process involved taking the material through a series of graded ethanols - 15 minutes each in 70% and 90% alchohol and one hour in absolute ethanol (four changes of 15 minutes each). The tissue was then cleared in chloroform overnight and vacuum embedded the following day in 57°C wax (three changes of 15 minutes each) before being cast in Leuckhart's L pieces.

Blocks containing the whole pituitary gland attached to the brain were cut serially on a Spencer microtome in either the vertical, transverse or horizontal plane at a thickness of 5μ for most staining techniques and at 8μ and 10μ for some of the neurosecretory stains and for all silver impregnations. Some sections were mounted directly on to degreased glass slides using a drop of albumin solution as an adhesive. Others were floated on to a 50°C water bath containing approximately 0.002% each of gelatine and potassium dichromate before mounting on glass slides.

Erlich's haematoxylin and 1% alchohol soluble eosin was used for general purposes and Jordon and Baker's pryonin methyl green (PMG, 1955) with an RNAase control

was used for cellular RNA detection. To check for evidence of innervation of the PT, Bargmann's chrome alum haematoxylin (CAH, 1949), Gabe's aldehyde fuchsin (AF, 1953) and Kerr's alcian blue (AB, 1965) were used to detect neurosecretory substances and Bodian's silver (protargol) impregnation (1937), to detect nerve fibres. The specificity of the neurosecretory stains was checked by omitting the oxidation step for some slides and noting that substances continuing to stain were not neurosecretory (e.g., 'colloid'). As a control for the silver impregnations, one slide of cerebellum tissue which had been fixed in Bouin-Hollande was processed in each tray of pituitary slides to be impregnated. A double impregnation was routinely performed as suggested by Bodian. After dewaxing the slides and bringing them down to H_2O, they were placed in the prescribed protargol solution for 24 hours, washed, reduced and washed again before being returned to a fresh protargol solution for a further 24 hours. After repeating the reduction procedure, the normal schedule was resumed to completion. Every other Bodian impregnated section was counterstained with either Gabe's AF or with Foley's counterstain (Humason, 1972), thus aiding the distinction between nervous and non-nervous tissue and, in the case of Gabe's AF, also demonstrating neurosecretory material. Periodic acid-Schiff (PAS, Pearse, 1968), aldehyde fuchsin (AF, Gabe, 1953), alcian blue (Kerr, 1965), lead haematoxylin (LH, MacConaill, 1947 - when staining Bouin fixed sections, pretreatment with 0.2N HCL as per Solcia et al., 1968, was carried out) and a combination of AB/PAS/Orange G (APG) were used to investigate the tinctorial properties of the cells of the pars tuberalis. The

quality of these techniques was always checked by examining their effectiveness on the cells of the pars distalis. Lastly, the presence of glycogen was investigated by use of Best's carmine method and the PAS reaction, both with diastase controls.

In addition, thick sections cut from specimen blocks prepared for electron microscopy were examined under the light microscope. One micron sections mounted on glass slides were stained with Azur II (0.5% Azur II in 3% sodium tetraborate). Sections two microns thick were also mounted on glass slides and subsequently treated with a solution of absolute ethanol which had been saturated with potassium hydroxide to remove the TAAB resin (Imai et al., 1968). After three rinses in absolute ethanol, these sections were stained with PAS.

Table 1 shows which fixatives were used for each of the histological techniques.

Routine electron microscopy

Guinea pigs were perfused with half strength glutaraldehyde (Karnovsky, 1965) at room temperature, after which the pituitary gland was removed and immersed in full strength fixative for a further two hours, again at room temperature. The tissue was then stored in sodium cacodylate buffer (pH 7.2) at 4° C overnight. The specimen was further dissected to obtain five pieces of PT attached to the neural stalk, each piece representing a precise area of the tissue as follows; three ventral areas (anterior, central and posterior) and two dorso-latteral areas (anterior and posterior). These bits were then placed in separate vials, post-fixed in

TABLE 1

	FCC	FC	Bouin	Bouin-Hollande	Glutar-aldehyde
H & E	X	X	X	X	
PMG	X	X			
PAS	X	X	X	X	X
APG	X	X	X	X	
AF	X	X	X	X	
AB	X	X	X	X	
LH	X	X	X		
BODIAN'S METHOD			X	X	
CAH			X	X	
BEST'S CARMINE	X		X		
AZUR II					X

Palade's osmium tetroxide (1952) for one hour at 4° C, rinsed in distilled water and bulk-stained in 3% aqueous uranyl acetate for one hour. Again, the tissue was washed in distilled H_2O followed by dehydration through graded alchohols, similar to the procedure for light microscopy. Embedding necessitated placing the pieces into a mixture of equal parts absolute ethanol and TAAB resin for one hour and afterwards into 100% TAAB resin overnight. The pieces were then embedded in fresh TAAB resin and placed in a 56° C oven to polymerize for twenty-four hours.

Sections were cut on a Huxley ultramicrotome (Cambridge Institute Company). One micron thick sections stained with Azur II were used to locate the tissue of the pars tuberalis in the block and to select particular areas for electron

microscopical examination. Thin sections showing either a silver or pale gold interference colour were mounted on degreased 200-hole copper grids. In some cases when serial sections were desired, 100-hole copper grids coated with 2% amyl acetate were used instead.

Sections were stained with Reynold's lead citrate (Venable and Coggeshall, 1965) for two minutes, the solution having been previously centrifuged. Sodium hydroxide pellets were placed in the staining chamber to absorb CO_2 in order to reduce contamination. Grids were examined on an AEI-EM6B electron microscope at 60 kV.

Selection of female animals for routine electron microscopical studies

While it has not been possible in this study to examine thoroughly the effects of the estrus cycle on the cells of the PT, it was thought appropriate to determine, for the record, at what stage in the cycle each of the female guinea pigs was at the time it was sacrificed. In order to do this, three female animals were observed for three consecutive cycles and vaginal smears were taken twice daily during the time the vagina was not closed by a covering membrane. Vaginal cells were obtained by inserting a flat wooden toothpick into the vagina after which a smear was prepared on a degreased glass slide. The slide was then immediately immersed in 100% methanol for at least 15 minutes to fix the cells. The smears were stained by the Papanicolaou method (1942) and examined to identify cornified cells, nucleated epithelial cells and leucocytes and to note changes in the proportions of these types of cells.

Two female animals were ultimately sacrificed, one in

estrus and the other in diestrus at approximately mid-cycle.

Granule and microfilament measurement technique

Micrographs of granulated cells from each of the animals studied by routine methods were randomly selected. The absolute magnification (at least X15,000) for each photograph was determined by accurately calibrating the microscope using a platinum line graticule (2,160 lines/mm). Twenty granules, selected randomly from each of 40 cells, were measured along their longest axis to the outside membrane by means of a Lumex optical micrometer with a mm scale marked off to 2mm. The mean value ($\pm$ standard error) and standard deviation of the granule measurements for several categories of cells were calculated. In addition, the student's t-test was used to check for significant differences between the granule sizes of the various categories of cells.

Photographs of 10 cells, enlarged to at least X75,000 and corrected to absolute magnification, were used for the measurements of cytoplasmic filaments. Ten longitudinally cut filaments were selected randomly from each photograph and their diameters measured.

Method of fixing tissue with potassium permanganate for electron microscopy

The hypophysial stalks of two animals which had been killed outright were immersed in freshly made 2% unbuffered potassium permanganate (pH 7.2-7.4) for two hours at room temperature, after which the tissue was rinsed in 25% ethanol for two 10-minute changes. The tissue was then cut into several smaller pieces, dehydrated and embedded following the same schedule as in routine processing. No attempt

was made to orientate these fragments. Two pieces from each pars tuberalis were examined under the electron microscope for the presence of glycogen.

Incubation of the PT with cytocholasin B in preparation for electron microscopy

The PT of two male guinea pigs which had been killed outright were used in this experiment. Each PT was divided into four pieces which were then placed each in culture media as follows: a control medium containing 97% 199 medium with 2% calf's serum and 1% DMSO, and three other culture media consisting of the control medium combined with cytocholasin B to give concentrations of 50µg, 100µg and 150µg of cytocholasin B/ml of medium. The pieces were incubated for 5½ hours at room temperature, after which they were rinsed in two changes of Hank's solution and two changes of sodium cacodylate buffer before being fixed in glutaraldehyde followed by osmium tetroxide in the normal manner. Dehydration and embedding followed standard procedures.

Ultrastructural examination of the guinea pig pars distalis

Tissue fragments of the pars distalis from two male and two female animals were fixed and embedded following the routine method for electron microscopy. Six fragments (4 from males and 2 from females) were sectioned, stained and examined in the normal manner.

TABLE 2

Summary of experiments carried out with respect to fixatives used and sex of the animals

Light microscopical experiments - 13 animals sacrificed (5♂, 8♀)

Fixatives	FCC	FC	Bouin	Bouin-Hollande
Perfused		1♂	1♂	2♂
	2♀	2♀	2♀	
Immersed			1♂	
		1♀*	1♀	

* Fixation by perfusion was attempted but was unsuccessful and therefore the pituitary gland was dissected out and immediately immersed in fixative.

Electron microscopical experiments - 14 animals sacrificed 12♂, 2♀)

Fixative	Glutaraldehyde	Potassium permanganate
Perfused	7♂	
	2♀	
Immersed	1♂, 2♂**	2♂

** Pituitary gland was incubated for 5½ hours in culture medium for the cytocholasin B experiment before immersing in fixative.

RESULTS

Gross anatomy of the guinea pig hypophysis with particular respect to the pars tuberalis

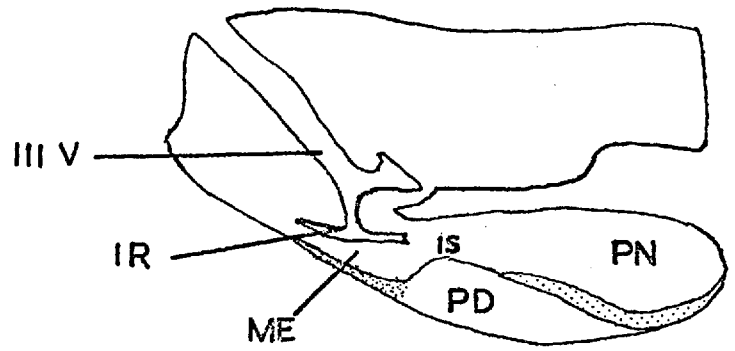
The guinea pig pituitary gland is horizontally orientated at the base of the diencephalon, its long axis running parallel to that of the brain. As in other rodents, and indeed most vertebrates, the guinea pig adenohypophysis is composed of the pars distalis, the pars intermedia and the pars tuberalis, and the neurohypophysis is composed of the pars nervosa, the infundibular stem and the median eminence. These anatomical regions are illustrated in diagram 1. The pars tuberalis is seen to wrap around much of the hypophysial stalk as drawn in diagrams 2b and 3. The infundibular recess of the third ventricle runs parallel to nearly the entire length of the pars tuberalis (diagram 3).

Light microscopical results

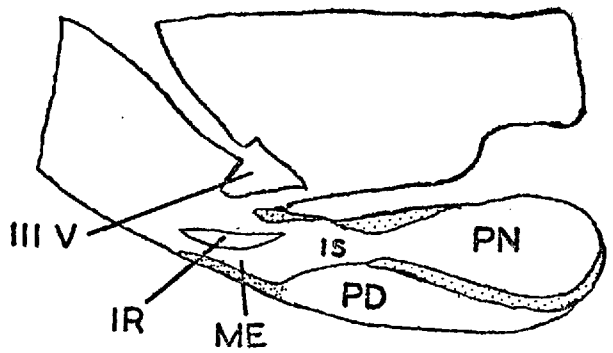
(Figures 1 - 63 at the back of the volume are micrographs which will be referred throughout the results)

The cells of the guinea pig PT are arranged in long cords spread over the neural stalk. The thickness of the tissue varies, but it is usually between two and five cells deep except in the posterio-ventral and anterio-dorsal regions where it may be up to twelve cells deep. There is no precise point of division between the PD and the PT although the transition is relatively abrupt as seen by a sharp increase in the number of chromophiles in the PD as compared to the largely chromophobic cells of the PT. The primary capillary plexus lies between the tuberal tissue and the adjacent nervous tissue, most capillaries being seen to run parallel to the long axis

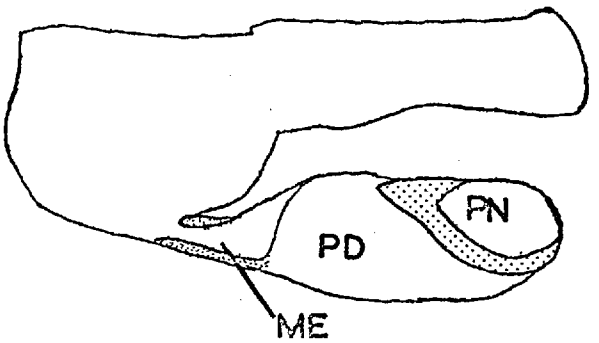
dorsal
anterior posterior
ventral



sagittal section

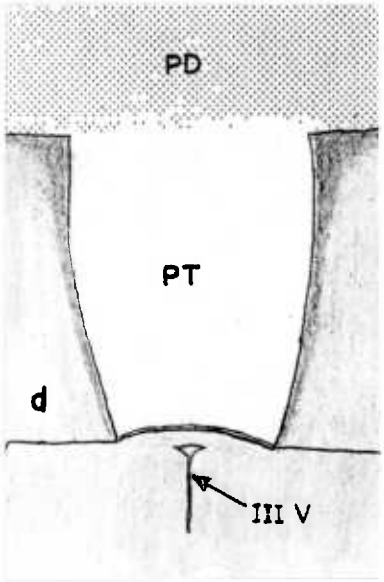


medial sagittal section



parasagittal section

Diagram 1. Tracings of three vertical sections of the guinea pig hypophysis attached to the brain to illustrate the location and shape of the tissues comprising the gland. PN - pars nervosa; PD - pars distalis; ME - median eminence; IR - infundibular recess; IS - infundibular stem; III V - third ventricle; ▨ - pars intermedia; ▩ - pars tuberalis. X13



anterior

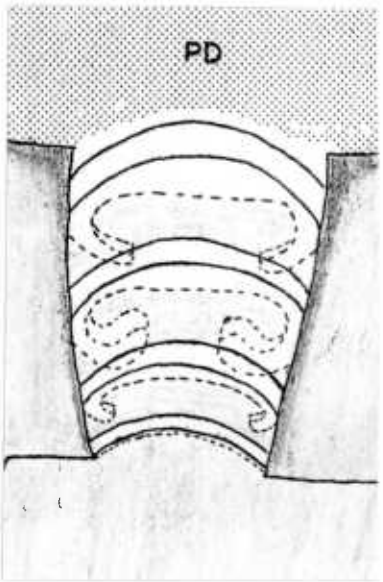


Diagram 2a. Sketch of the PT (ventral view) lying at the base of the diencephalon (d). The PT is attached to the PD posteriorly. The brain is shown to be cut transversely at the anterior extreme of the PT to illustrate the third ventricle (III V).

Diagram 2b. Same view adding four hypothetical transverse sections of the PT to show the relative changes in the dorsal extent and the depth of the tissue along its length. The third ventricle has not been drawn in for simiplicity.

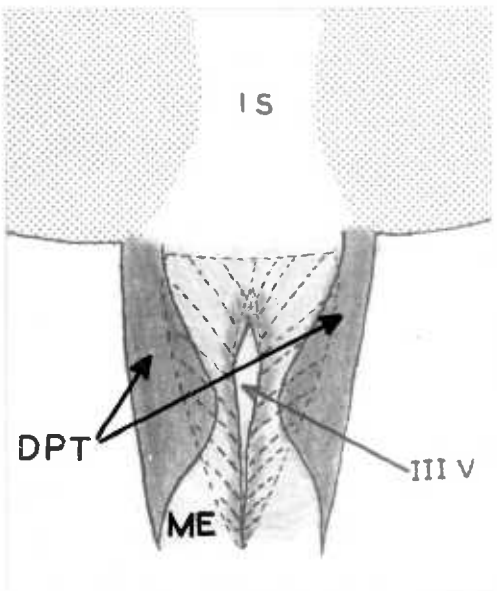


Diagram 3. Sketch of the PT as viewed from its dorsal surface (DPT - dorsal pars tuberalis) to further show how it wraps part way around the median eminence (ME). The narrow opening represents the third ventricle (III V) cut in the horizontal plane, just dorsal to where it fans out (dotted lines) internally within the median eminence. The latter is also cut in the horizontal plane at the point where it is continuous with the diencephalon. IS - infundibular stem; ⁘ - epithelial tissues of the pars intermedia and the pars distalis.

of the ME. The shape of the cords conforms with the presence of the small blood vessels lying against and within the PT. These features are seen in Figures 1 - 8.

As stated above, the PT is composed largely of chromophobic cells; more specifically, the majority of cells do not stain with orange G, eosin, PAS, AB, AF or LH. An infrequently seen cell type (Figures 5 - 7), usually located adjacent to a blood vessel, stains strongly with PAS and AB when the tissue has been fixed with FC or FCC, and faintly when fixed with Bouin or Bouin-Hollande. Moderate PAS staining of this cell is also seen in 2μ sections fixed in glutaraldehyde/osmium tetroxide from which the TAAB embedding resin has been removed by potassium hydroxide. TAAB embedded 1μ sections stained with Azur II also reveals the darkly stained granules of this cell type. This mucoid cell type is seen to be fusiform to rounded in shape and to have an eccentrically placed nucleus. Although rare, it is seen in all areas of the tuberal tissue.

Both the periodic acid-Schiff reaction and the Best's carmine method with diastase control give no evidence for glycogen in the cells of the PT. A moderate amount of cytoplasmic RNA, however, is indicated by staining the tissue with PMG, with and without an RNAase control.

'Colloid' substance (Figures 5 and 7), staining positively with PAS, CAH, AB and AF occurs regularly throughout the PT. It also stains deeply with Azur II, sometimes exhibiting metachromasia. It is found in the centre of a group of cells, the nuclei of which are located away from the lumen. When the oxidation step is omitted in the above mentioned staining methods, the luminal substance(s) continues to dye strongly. This contrasts with the reaction of neurosecretory material in the ME

which will be described shortly.

'Colloidal' deposits are also seen in very large cystic structures (Figures 5 and 9) which often occur in the posterio-ventral PT, but occasionally are seen in other regions. The luminal content stains identically with that previously described except that Azur II staining always reveals extensive metachromasis. The 'colloid'-filled lumina of the cysts are bordered by a tightly packed wall of chromophobic cells which are frequently either cuboidal or columnar in shape. At random, border cells appear which possess many cilia extending into the lumen (Figure 9). In many cysts, other cells occur immediately behind the border cells and have a plasma membrane characterized by many villous processes which tend to extend across stretches of extracellular space. Similar processes may also be seen on the basal surfaces of the border cells themselves.

No evidence for the presence of neurosecretory material is seen in the PT after staining sections serially with AB, AF or CAH. Using these same stains, the tractus hypophyseus (containing axons from the supraoptic and paraventricular nuclei) is seen to contain positively staining Herring bodies and some small beaded fibres which are confined largely to the internal ME and which continue to occur through the IS (Figure 8). As a control to check whether these results are evidence of neurosecretory material, the oxidation step has been occasionally omitted before staining. The ME, the IS and the PN in these instances are found to be void of positively staining substances. Bodian's silver impregnation is seen to reveal many argyrophilic fibres of the tractus hypophyseus, and also many finer fibres in the external ME, presumably nerve fibres

of the tuberohypophysial tract. Fibres are never seen entering the parenchyma of the PT, in contrast to numerous fibres which are seen to enter and transverse the tissue of the PI in the same sections.

Routine electron microscopical results

The systematic examination of five areas of each of ten animals, eight males and two females, was carried out. The first results to be reported deal with the eight male animals, for although a comparable morphology is seen in the females, it will be described later with respect to its variations.

Five distinct cell profiles and a transition cell profile are observed in the PT. A characterization of each follows.

Cell profile I (Figures 10 - 15). This cell profile is the predominant cell found in six of the eight male animals examined, representing a quarter to a third of the cells observed. It is evenly distributed throughout the entire tissue, is about the same size as other cells and often has an angular shape with one or several processes. Its plasma membrane makes a variable degree of contact with the basement membrane. Its numerous cytoplasmic granules are the primary identifying feature of this cell type. The granules average 121nm in length (see Table 3, page 39). They have a very electron dense core and a variably sized halo (i.e., an electron lucent space between the core and the enveloping membranes). The cytoplasm contains a moderate amount of flat, rough endoplasmic reticulum (RER) which may assume either a snake-like path through the cell (Figure 11) or may form small stacks (Figure 13). A single, relatively large Golgi region is present in which may be seen smooth and coated vesicles,

small electron dense granules with rather large haloes (Figure 13) and occasional electron dense content in lamellae which appear to be budding. A moderate number of elongated mitochondria are seen, the cristae of which may be variable in their orientation.

The cytoplasm is seen to contain numerous polyribosomes. Microtubules are also present and are occasionally very striking in cellular processes where their long axes are orientated parallel to the processes. A variety of lysosome-like bodies are seen, including multivesicular bodies and dark bodies, the latter of which may be spherical, dumb-bell-shaped or ring-shaped. Lipid-like inclusions are also observed, though infrequently.

Paired centrioles occupy a perinuclear position (Figure 13). Cilia are also seen to originate from profile I cells. Transverse views of cilia are rarely encountered, but of the few seen, either a 9+1 or a 9+2 tubular formation is observed, the axial tubule(s) being difficult to discern.

Desmosomes, usually without attached filaments, are occasionally seen joining profile I cells to cells of profiles IV and V described below. The plasmalemma is also seen to possess occasional pinocytotic-like invaginations. Groups of granules are sometimes seen aggregated against the plasmalemma adjacent to the basement membrane (Figure 14), while less often, the membrane of a single granule appears to fuse with the plasma membrane. Exocytotic-like profiles (Figure 15), however, are rarely observed.

Cell profile II (Figures 17 - 20). This cell profile appears distinct from that just described due to its more extensive RER which is observed to form deeper stacks and the cis-

ternae of which are marginally dilated, revealing an amorphous, homogeneous matrix (Figures 17 and 20). Coexisting with the stacked RER is some spherically dilated RER which appears to contain a similar amorphous matrix substance. Occasional cytoplasmic myelin-like structures are seen and very rarely, a small number of filaments measuring 90Å in diameter (Figure 40).

This cell profile appears in only two of the eight male animals studied. Correspondingly, the number of profile I cells in these animals is seen to be greatly reduced, and in some areas, totally absent. The frequency, distribution, size and shape of profile II cells are comparable to that of cell profile I and their granules similarly average 114nm in length (see Table 3, p. 39), although haloes around the dense cores are consistently reduced to a minimum. The active looking Golgi zone is also comparable to cell profile I, as are all other organelles, including the presence of granules aggregated against the plasma membrane (Figure 18).

Cell profile III (Figures 21, 22, 24, 25). Although this is the least frequently observed profile (perhaps one or two seen per electron microscopical section), it is observed in all areas of the PT. Cells of this type are usually found adjacent to a blood vessel. They are round to fusiform in shape and contain a round to oval nucleus eccentrically placed. Due to their frequency, shape and location, they are considered to correspond with the mucoid cells found in light microscopical preparations.

Under the electron microscope, this cell type is easily identified by its larger cytoplasmic granules which average 229nm in length (see Table 3, p. 39). As in the previous two cell types, the very electron dense granules of profile III

cells are membrane-bounded; occasionally, however, there is an additional medium density zone within the enveloping membrane (Figure 22). The granules of cell profile III are never seen to fuse with the plasma membrane.

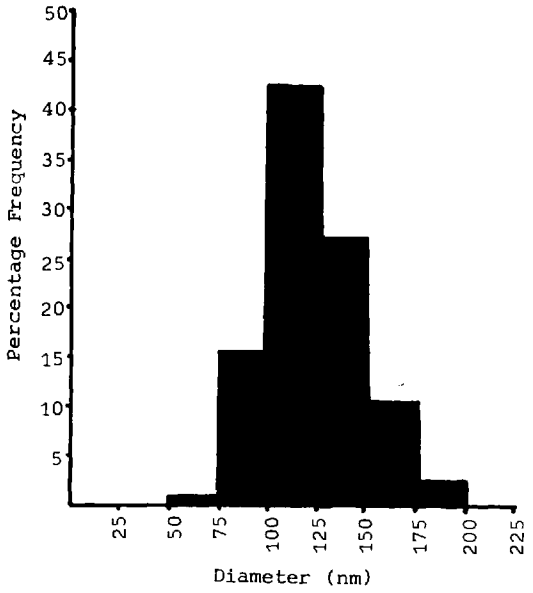
The RER tends to form several layers near the edge of the cell, but it is also found generally throughtout the cytoplasm (Figures 21 and 22). The cisternae are just slightly dilated and a moderately dense amorphous matrix is seen within. Cell profile III contains a large Golgi zone which frequently contains smooth and coated vesicles, immature looking granules and lamellae which are dilated at the ends (Figure 22). Also, prominent mitochondria with closely packed transverse cristae are typical. Some free ribosomes, an occasional microtubule and very rarely, a few filaments are seen in the cytoplasm. The plasma membrane contains noticeably more pinocytotic-like invaginations than are found in the other cell types.

Desmosomes, cilia, centrioles, lipid-like inclusions and lysosome-like bodies are as described for the previous two granulated cell types. In one instance, a profile III cell appeared to be extruding myelin-like substances (Figures 24 and 25).

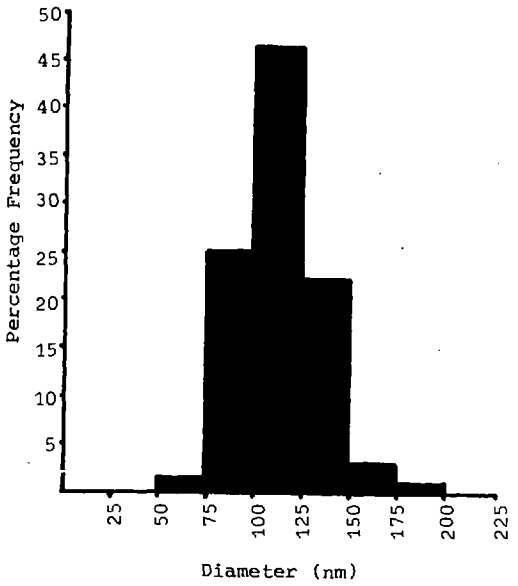
Histograms showing the distribution of granule sizes for profiles I, II and III are seen on page 38. Table 3 on page 39 shows, for each profile, the mean lengths of the granules in the individual cells sampled and the mean length, standard deviation and standard error for the profile sample as a whole.

Cell profile IV (Figures 26 - 31, 51) This cell profile is the only one not seen to be evenly distributed throughout the tuberal tissue; it has not been

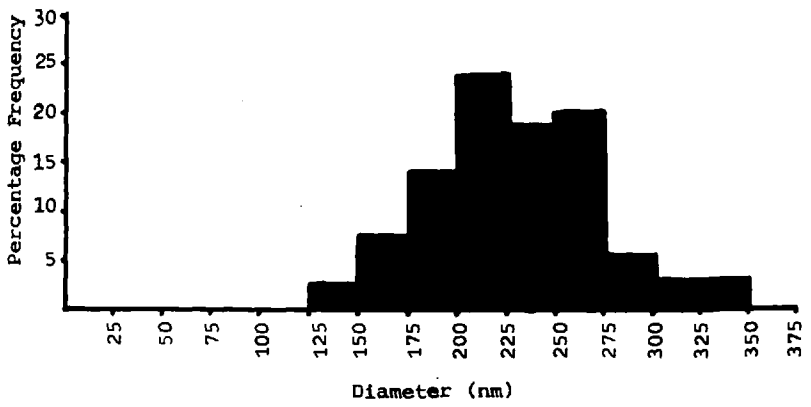
Histograms showing the distribution of granule sizes for each cell profile



cell profile I



cell profile II



cell profile III

TABLE 3

Mean granule diameter in nanometers per cell and overall mean, standard error of the mean (S.E.M.), standard deviation (SD), and range of granule sizes per cell profile.

Cell Profile I (20 cells)		Cell Profile II (10 cells)	Cell Profile III (10 cells)
101	106	113	206
125	114	99	224
117	122	112	238
135	109	135	227
133	130	111	225
121	111	115	230
125	128	98	224
120	133	120	249
133	120	113	242
113	128	122	227
overall mean	121	114	229
SD	±25.125	±20.720	±44.199
S.E.M.	±1.256	±1.465	±3.125
range	65 - 195	50 - 180	140 - 345

The student's t-test reveals significant differences in the mean diameter of all three cell profiles.

observed in the dorsal region. It is identified by the large quantity of dilated, round RER in its cytoplasm. The cisternae are observed to contain a small amount of flocculent substance and, on occasion, inclusions in the form of either small vesicles or dense particles are seen (Figure 29).

Cells of profile IV are usually cuboidal or elongated in shape and are invariably seen to border a 'colloid'-filled lumen. The polarity of the cells is characterized by an eccentrically placed nucleus located near the basal pole, a centrally located Golgi body and frequently a periluminal filamentous network (Figure 26). The fine filaments of such networks often run parallel to the lumen extending from tight

junction to tight junction, the latter being components of the junctional complexes of the periluminal plasma membrane.

Several layers of long lamellae and a moderate number of smooth vesicles are found in the Golgi zone of many profile IV cells (Figure 29). Occasionally the lamellae are seen to be dilated at their ends (Figure 30). No granules or electron dense particles, however, are observed in the membrane structures of the Golgi apparatus. The mitochondria appear more electron dense than those in other cell profiles and they are not numerous.

The cytoplasm is also seen to contain polyribosomes, some microtubules, a few random microfilaments and an occasional multivesicular body. A few membrane-bound granules, ranging in size from 100nm to 600nm, are usually present, the matrix of which is granular (Figures 27 and 28); they are quite probably lysosomes. Centrioles are frequently observed near the luminal margin of the cell (Figure 36). They are sometimes seen in pairs perpendicularly orientated to each other. Cilia have never been observed to originate in this cell type

Several characteristics make the plasma membrane distinctive. In many places, the basal and latteral plasma membrane is increased by means of microvillous-like extensions. Two neighbouring profile IV cells having interdigitating plasma membranes are illustrated in Figures 31 and 51. Desmosomes are numerous. They are usually associated with filaments when joining a cell of this type to a nongranulated cell (i.e., cell profiles IV and V) and are usually unassociated with filaments when joining a cell of this type to a granulated cell (i.e., cell profile I, II or III).

Several features of the luminal surface include micro-

villi (Figure 32) and what appear to be vesicles fused to the plasma membrane (Figures 26 and 52). In other places, however, the apical surface may be seen to be very smooth (Figures 29 and 31).

A similar fusion between vesicles and the plasmalemma is infrequently observed at the basal pole of the cell (Figure 28). The number of profile IV cells surrounding a 'colloid'-filled lumen varies greatly from only a few to many. The lumen plus the associated cells is referred to as a follicle.

Cell profile V (Figures 39 - 44). The last cell profile to be described is also nongranulated. It is seen evenly distributed in all parts of the PT and is readily recognized by an abundance of filaments in its cytoplasm. Examination of the latter at high magnification reveals a beaded structure 90 - 110Å in diameter. A transverse view of a filament revealing an electron lucent core and a dense wall from which four or five spikes may be seen to radiate is shown in Figure 42. Filaments fill the entire cytoplasm of some cells, but in other cells, they are frequently thinned out somewhat, particularly towards the cell periphery. The cells themselves, like cells of profiles I and II, often have an angular shape with one or several processes. The eccentrically placed nuclei are usually rounded but they may, though infrequently, be indented.

A small amount of short, flat RER is seen among these filaments and near the cell periphery. The mitochondria are moderate to few in number. One or several small Golgi apparatuses are frequently seen at the cellular margin (Figure 39), although in some instances a more centrally located Golgi body is present which tends to be larger and not unlike that of cell

profile IV (cf page 40). Electron dense substances are not observed within the Golgi membranes.

Polyribosomes, multivesicular bodies and lysosome-like dark bodies are observed in the cytoplasm, the latter being rod, round or dumb-bell-shaped. Also, very small cytoplasmic disruptions are not uncommon in these cells (Figure 41). Microtubules are infrequently seen. Neither cilia nor centrioles are ordinarily observed.

A few microvillous-like extensions of the plasma membrane are sometimes observed. Desmosomes are numerous and like those of profile IV, they are usually associated with filaments when joining a cell of this type to a nongranulated cell and are unassociated with filaments when joining a cell of this type to a granulated cell. Pinocytotic-like invaginations are often observed along the plasma membrane.

Sometimes, particularly in the dorsal region of the PT, cells of profile V are seen to be associated with 'colloidal' deposits (Figure 44).

Transition cell profile (Figures 45 and 46). Certain cells appear to provide evidence of a transitional morphology between cells of profile IV and profile V. They are identified primarily by the cytoplasmic coexistence of numerous filaments ($\sim$ 100Å in diameter) and spherical RER of the type found in cell profile IV. Characteristics of the transition cell profile which are similar to those of cell profile IV and V include the high frequency and the morphology of desmosomes, microvillous-like extensions of the plasma membrane and cellular associations with 'colloid' deposits.

Other cytological observations concerning the eight male animals now follows.

Cysts. Due to the random method of selecting the elec-

tron microscopical sections in this study, only a few examples of cysts were observed. Furthermore, as their bulk is quite large by electron microscopical standards, the observations are not necessarily comprehensive. Nevertheless, they are considered to be of considerable interest.

Like the follicles previously described, cysts are 'colloid'-containing structures bordered by cells; the number of associated cells, however, and the lumen itself, is much larger. A cyst is always seen to be separated from the rest of the tuberal parenchyma by basement membranes and a wide extracellular space. Furthermore, most of the cells are of a different type to those already described in this study.

Thus, the cells forming the wall are frequently columnar or cuboidal, a fact also noted by light microscopy. Neighbouring cells are joined to each other by junctional complexes situated near their apices. Under the electron beam, most cells are characterized by a dark cytoplasm containing what appears to be much smooth endoplasmic reticulum (SER), some large dark bodies which may be lysosomes, some scattered bundles of microfilaments, a few strands of flat RER and rather fatter mitochondria than in previously described cells (Figures 53 and 54). The nucleus is frequently indented. Between the nucleus and the lumen, a small Golgi zone is frequently observed. Microvilli or microvillous-like extensions may be observed on all cell surfaces. This cell profile will be referred to as Dark Cell I.

Among the dark cells bordering on the lumen, cells with a lighter cytoplasm are found at random intervals. While the cytoplasmic characteristics vary, making classification difficult and uncertain, these cells appear to fall into two

categories. One is very similar to the dark cells except that the organelles and cytoplasm are less dense. Polyribosomes are easily discerned and the SER contains a moderately dark matrix (Figure 50). Many long cilia are seen to arise from the luminal edge and project into the lumen. In cross section, the tubular arrangement in these cilia is usually seen to be 9+2 (Figure 49 - note, however, that one cross section is suggestive of 9+3). A second type of light cell (Figure 48) contains abundant filaments, a number of dark mitochondria, some flat RER and a few dark bodies. Nearer the luminal edge, many small vesicles are seen. This kind of cell is usually seen to be protruding somewhat into the lumen and this appearance is accentuated by microvilli on the luminal surfaces.

On one occasion only, all the columnar cells of the cyst were seen to be identical to those of cell profile IV (Figure 51). Of particular note, the luminal plasma membrane exhibits possible evidence of actual penetration of electron lucent vesicles through the plasma membrane (Figure 52). Also, some pinocytotic-like invaginations are seen.

Surrounding the cells which are in immediate contact with the lumen, there are usually several layers of a second kind of dark cell (Figure 47 - Dark Cell II). Its cytoplasm normally contains many free ribosomes, broad mitochondria, some flat strands of RER and a few dark bodies, some of which could be lipofuscin inclusions. Its plasma membrane possesses many microvilli which extend into the broad intercellular spaces existing between cells of this kind. In many places, desmosomes with filaments join cells of this type together.

Luminal substances. 'Colloid' is the name which has been

given to the content observed in follicles and cysts in most light microscopical studies describing these structures in the pituitary gland. In the case of cysts, on the ultrastructural level, this content is largely seen to be a relatively dense, amorphous substance. It tends to be flocculent near the cell boundaries, coexisting with a few myelin-like particles (Figures 50 and 51). It then becomes more dense and homogeneous, filling the entire cavity.

In the smaller follicles delimited by cells of profile IV, the substance(s) appears to be more variable. Its density varies from follicle to follicle (contrast Figure 31 with Figure 44). In some instances, the luminal content appears to be homogeneous, while in others, electron dense, particulate material is present (compare Figures 31, 32, and 37). Sometimes, evidence of an accumulation of small, round membranous structures is seen, a finding also verified in tissue fixed in potassium permanganate (Figures 34 and 35). Figure 33 shows a portion of the content of a follicle cut very near the tangential surface of the bordering cells, illustrating another occasional finding, that is, an aggregation of more electron dense particles near the edge of the cell (including transverse cuts of microvilli) than in the lumen generally.

Vascular and perivascular morphology. Between the PT and the neural stalk lies the primary capillary plexus, some capillaries of which encroach perpendicularly onto the tuberal tissue (Figure 58). The endothelium of these capillaries, both on the surface facing the median eminence and on the surface facing the pars tuberalis, contains numerous fenestrations, each with a closing membrane (Figure 46). Fenestrated capillaries are also seen on the external ventral surface of the

tissue. The endothelial cells are surrounded by a basement membrane and a wide perivascular space in which perivascular cells, collagen fibres and an interstitial matrix are found. Rarely is a plasma cell observed. A second basement membrane is seen adjacent to the parenchyma of the PT.

Although fenestrated capillaries are found more deeply within the PT, more commonly, the blood vessels so situated are arterioles (Figure 60). The endothelium of the latter possess numerous pinocytotic vesicles. Smooth muscle cells are seen next to the endothelium and these also contain many pinocytotic vesicles (Figure 61). Nerve fibres containing small synaptic vesicles (30 - 50nm in diameter), microtubules and round mitochondria can be observed next to the smooth muscle cells in some sections. Arterioles are found in all areas of the tissue.

Nerve fibres. Only upon two occasions were nerve fibres seen to have penetrated the basement membrane and entered the PT. In one instance, the fibre penetrated some tuberal tissue that had protruded into the normal area of the median eminence. The fibre contained some electron lucent vesicles ($\sim$ 50nm in diameter) and several dense cored vesicles ($\sim$ 120nm in diameter). In the other instance, the probable fibre, containing many microtubules, was obliquely cut and, therefore, had a honeycomb appearance. Both examples were located in the posterio-ventral region of the tissue. Normally, however, nerve fibres are not found in the PT except, as mentioned above, in association with smooth muscle cells in the perivascular spaces.

Electron microscopical results
concerning female guinea pigs

The vaginal smears examined in this study, when stained by the Papanicolaou method, do not reveal decisive changes in the ratios of cornified cells, nucleated epithelial cells and leucocytes to correspond to each stage in the estrus cycle as reported in earlier studies (Stockard and Papanicolau, 1917, Sella, 1922, Young, 1937). As in these earlier reports, however, the vaginal membrane is found to be broken for about four days of the cycle. During this period, the vaginal smears observed in this study are usually seen to contain the three kinds of cells mentioned above, each well represented. The only change in their proportions occurs at about twenty-four hours after the vaginal membrane opens when almost no leucocytes are present. This, therefore, is considered to coincide with estrus, a criterion also used by Donovan and Lockhart (1972). The estrus cycle is observed to be approximately sixteen days long, day one beginning with estrus.

Two female guinea pigs, one in estrus and one in diestrus (killed on the eigth day of the cycle) have been examined. Cells of profiles I, III, IV and V are easily identified in the young female and no change in the distribution or location of these cells is noticed. There are, however, far fewer granules in the cytoplasm of many of the profile I cells in both animals (Figure 16). No other differences, however, have been observed between the cells of the female and the male animals.

Electron microscopical results concerning potassium permanganate fixed tissue

Immersing normal guinea pig liver in 2% unbuffered potassium permanganate is found, in this study, to fix cellular alpha glycogen particles (Figure 55). Following an identical procedure to fix tuberal tissue, scattered, electron dense, fine particles are found in the cytoplasm of some profile II cells (Figure 56). These probably represent a small amount of beta glycogen. No similar findings are observed in other cell types.

As previously mentioned, an accumulation of membranous structures is seen in the lumen of some follicles in tissue fixed in this manner.

Electron microscopical results concerning tissue incubated with cytocholasin B

Cytocholasin B appears to have no effect on the cytoplasmic filaments in the cells of profile V when the tuberal tissue is incubated in any one of three concentrations of this drug (i.e., 50μg/ml, 100μg/ml and 150μg/ml). While cells are easily identified in all cultured specimens, increased vacuolation and increased numbers of disrupted cells are seen in the incubated tissue as compared with the normal. Also, the delimiting membranes of a great many granules were observed to be fused with the plasma membranes.

Electron microscopical results concerning the pars distalis of the guinea pig hypophysis

Six specimen fragments (four from male animals and two from females) of the pars distalis have been examined specifically to see if the PD contains cells corresponding to any of those described in the PT. A cell type similar to cell profile III is identified in the PD by its cytoplasmic

granules, which usually measure between 200nm and 250nm in diameter, and by its comparable RER. A second cell type is similar to cell profile V in that the cytoplasm contains many filaments measuring about 100Å in diameter. Also, the RER is sparce and the Golgi apparatus is poorly developed. Cells corresponding to the other types observed in the PT have not been seen.

DISCUSSION

The guinea pig pars tuberalis has been observed to compare with the general light microscopical picture of many other animals (human, Guizzetti, 1925; porpoise and whale, Wislocki and Geiling, 1936; rat, Severinghaus, 1936, Klein et al., 1970, Stoeckel et al., 1973a & b; rabbit, Dawson, 1937, Allanson et al., 1959, Cameron and Foster, 1972a; cat, Dawson, 1937, avian, Wingstrand, 1951; goat, Beck et al., 1969; frog, Doerr-Schott, 1971, Dierickx et al., 1971; mouse and doormouse, Stoeckel et al., 1973a & b) in that it is mainly composed of chromophobes. Only a very small number of cells were observed to be PAS^+/AB^+.

Purves and Griesbach (1951) and Halmi (1952) demonstrated that the mucoid cells of the rat PD could be separated into two groups, those that were PAS^+/AF^- and those that were PAS^+/AF^+. The former group was considered to be gonadotrophs and the latter, thyrotrophs. Heath (1965) demonstrated that using a performic acid-alcian blue-periodic acid-Schiff-orange G stain, pituitary cells staining purple (as a result of staining positively with both alcian blue and periodic acid-Schiff) were a type of gonadotroph. Accordingly, the $PAS^+/AB^+/AF^-$ cells observed in this study could be gonadotrophs.

Ultrastructural examination has produced a more extensive characterization of the PAS^+/AB^+ cell which appears to be identical to cell profile III. The low frequency and perivascular location of this cell type is similar in both light and electron microscopical sections. Furthermore, the PAS staining of 1μ araldite sections adjacent to electron

microscopical thin sections confirms this correlation. The size of the granules in cell profile III (average, 229nm in diameter) and, to a lesser extent, the cells' position near a blood vessel and the ultrastructure of its cytoplasmic RER(cf page 37) makes this cell profile very similar to gonadotrophs described by Farquhar and Rinehart (1954), Barnes (1962), Salazar (1963), Dekker (1967), Kurosumi (1968), Kurosumi and Oota (1968), Heath (1970), Costoff (1973) and Zambrano et al. (1974). The ultrastructural identification of cell types in the guinea pig PD is, unfortunately, not well established. Smith (1963) did not identify a gonadotroph as such, and Amat and Boya (1970) describe a gonadotroph with granules ranging from 170 to 200nm in diameter, but did not describe any cells with granules measuring nearer to 230nm on the average. In the brief examination of the PD carried out in this study, however, a cell which appeared identical to that of cell profile III was found not infrequently.

The PT in other animals has been observed to contain a few mucoid cells which also have been regarded as gonadotrophs on the basis of the cells' PAS^+ staining affinity and their ultrastructural features (Knowles and Kumar, 1969, Klein et al., 1970, Stoeckel et al., 1973b). Midgley (1966) reports LH-containing cells in human tuberal tissue using fluorescent antibody techniques. Baker and Yu (1975) recently reported that in an immunocytochemical analysis of the cells of the rat PT with antisera to all the adenohypophysial hormones, only LH-containing cells were demonstrated. These were, however, rare throughout most of the PT, only becoming somewhat more frequent near the PD. Further support for the view that there may be some gonadotrophic

function in the PT comes from the work of Legait (1969) who found that PT extracts injected into prepubertal rats resulted in the stimulation of the interstitial cells of the testes and luteal tissue. These reports, together with the evidence found in this study, makes the interpretation that the mucoid cell found in the PT of the guinea pig is a gonadotroph very probable.

Ultrastructural examination of the PT showed that the cells corresponding to the chromophobes seen with the light microscope are comprised of four cell profiles, two granulated and two nongranulated. The two granulated cell profiles (I and II) contain granules of similar dimensions (average diameter, 121nm and 114nm respectively). Application of the student's t-test to granule measurements, however, has revealed significant differences between the two cell profiles. Furthermore, cell profile II is readily distinguishable by its prominent RER, being both more abundant and having cisternae which are slightly more dilated than in cell profile I. The other cellular organelles, however, are comparable in the two cell profiles, particularly the moderate number of mitochondria with their irregularly orientated cristae and the tendency of some granules to aggregate at the plasma membrane. Also, the number of cells of profile II is seen to be in approximately the same proportion and of the same distribution in two animals studied as cell profile I is in other animals. Therefore, these cells are considered to be of the same type, cell profile II being somewhat more active in terms of protein synthesis than cell profile I. The reason for this difference (observed only with regard to male animals) is obscure as the animals' ages and weights

were similar in all cases; the male guinea pigs were over 400 grams and between nine weeks and one year of age. Possibly, however, this age range does allow some physiological differences between the animals which might be relevant to the function of these cells, and therefore, might account for the ultrastructural differences observed. To this point, the age of puberty and first fertile ejaculation of the male guinea pig is reported to occur between the ages of eight and twelve weeks (Webster and Young, 1951, Lane-Petter and Porter, 1963, Asdell, 1964, Paterson, 1967). In the absence of knowledge concerning the specific function of this cell type, further speculation is difficult.

The size of the granules of cell profiles I and II suggest that they could be thyrotrophs comparable to those reported in the rat (Farquhar and Rinehart, 1954, Lever and Peterson, 1960), sheep (Hopkins and Thornburn, 1972), rabbit (Foster et al., 1969), mouse (Barnes, 1962, Sano, 1962) and in cattle (Mikami (1970). Kutas (1958) reported the presence of thyrotrophic hormone in the PT of cattle and of the human on the basis of bioassays of the tissue and the demonstration of AF^+ cells. The difficulties, however, of obtaining pure PT extracts for bioassay, free from contamination by factors of the PD, necessitates weighing such evidence cautiously. Certainly, the cells of profiles I and II in this study were AF^-. While the specificity of aldehyde fuchsin for the thyrotroph cell is widely accepted, perhaps it is well to note that Amat (1968) and Marescaux et al. (1960) reported that an AF^+ cell in the PD of the guinea pig is indeed a thyrotroph. Similarly in this study, AF^+ cells were observed in the PD but not in the PT. Furthermore, cells of profiles I and II are PAS^-, indicating that thyrotrophin, a muco-

polysaccharide, is not present. Lastly, Baker and Yu (1975) found no indication of TSH-containing cells in the rat PT using an immunological technique. Hence, the evidence suggests that the cells of profiles I and II are not thyrotrophs.

Some studies have concluded that the ACTH cell is a chromophobe (Siperstein, 1963, Racadot, 1963, Knutson, 1966). It might be considered, therefore, that cells of profiles I and II are ACTH cells.

On the ultrastructural level, there has been much controversy over the identification of the ACTH cell. On the one hand, several investigators have argued that it is a non-granulated, follicular cell (Farquhar, 1957, Finerty and Keller, 1961, Rennels, 1964, Bergland and Torack, 1969, Schecter, 1969). Others have presented evidence that the ACTH cell of the PD is a granulated cell (Siperstein, 1963, Kurosumi and Oota, 1966, Kurosumi and Kobayashi, 1966, Kurosumi, 1968, Conklin, 1968, Mikami and Diamon, 1968, Siperstein and Miller, 1970, Paiz and Henninger, 1970, Foster 1971, Cameron and Foster, 1972b). The ACTH cells described in these studies have been characterized as containing granules averaging between 150nm and 250nm; the granules tend to be 'haloed' and the RER has been seen to be composed of single, short and rather flat cisternae appearing throughout the cytoplasm. As cells of profiles I and II have similar characteristics, it might be argued that they are ACTH cells. Although the size of the granules in these two cell profiles is considerably smaller, for the present it might be considered that a species variation could account for this difference. Stutinsky et al. (1964) and Klein et al. (1970) concluded that many of the granulated cells of the PT in the rat may be ACTH cells.

A comparison, however, between the granulated PT cells and the recognized ACTH cells of the same species has been the basis for the view maintained by a number of investigators that the PT does not contain ACTH cells (rat, Stoeckel et al., 1973a & b; rabbit, Cameron and Foster, 1972a; frog, Doerr-Schott, 1971, Dierickx et al., 1971). Unfortunately, neither Smith (1963) nor Amat and Boya (1970) identified an ACTH cell in the pars distalis of the guinea pig and hence, it is more difficult to draw any conclusions as to the possible ACTH content of cell profiles I and II. In this study, however, a brief examination of the PD does not reveal any cell type similar to profiles I and II.

MacConaill's lead haematoxylin (1947) has been used to stain cells of the PD in fish (Stahl, 1958, Olivereau, 1964, Mattheij, 1968), quail (Tixier-Vidal et al.,1968) and dog (Solcia et al., 1969). These investigators considered the positively stained cells to be MSH and ACTH cells. In this study, large cells in the PD were observed to stain intensely with this dye and cells of the PI were also seen to stain moderately. The cells of the PT, however, did not appear to exhibit any positive staining with lead haematoxylin. If this technique were accepted as a reliable indication of ACTH and MSH cells generally (noting that such a premise is open to question as only one of the above reports relates to mammals), then the presence of ACTH cells in the PT might have to be ruled out.

Fluorescent antibody techniques have produced evidence of ACTH cells in the human (Pearse and van Noorden, 1963). Baker and Yu (1975), however, using antisera to porcine β^{1-24} ACTH and β^{17-39} ACTH found no pos-

itively reacting cells in the rat PT, although positively reacting cells were observed in the PD.

The evidence as stated above, does not provide a firm basis for stating whether or not cells of profiles I and II are ACTH cells.

If the cells of profiles I and II are not considered to be ACTH cells, there remains the possibility that they represent a type of pituitary cell, the function of which is unknown. That the PT contains granulated cells of unknown function has been, in fact, the opinion of most investigators working on tuberal tissue in recent years (Legait and Burlet, 1966, Oota and Kurosumi, 1966, Dierickx et al., 1971, Kotsu and Daikoku, 1972, Cameron and Foster, 1972a, Stoeckel et al., 1973a & b, Dellmann et al., 1974, Baker and Yu, 1975). These reports observed granules in a tuberal cell to range anywhere from an average of 100nm (rabbit, Cameron and Foster, 1972a) to a range of 180nm - 250nm (chicken, Dellmann et al., 1974). The mean diameter of granules of profiles I and II is seen to be about the same as that in the rat (Rinne, 1966, Klein et al., 1971, Stoeckel et al., 1973a & b, Dellmann et al., 1974). Clearly, the evidence suggests that cells of profiles I and II, like granulated cells of the PT in other animals, are involved in protein synthesis, storage and release, possibly of a hormone (as ultrastructurally indicated by substantial quantities of RER, the appearance of granule formation in the Golgi apparatus, the abundant membrane bounded granules in the cytoplasm and the aggregation of some of these granules at the plasma membrane). It would follow that a 'hormone(s)' from these cells is secreted, presumably into the portal vascular system, and eventually arrives at a target organ. It has been suggested that the PD itself could

be the target of a PT 'hormone(s)' (Morato, 1965, Kotsu and Daikoku, 1972). Alternatively, the target organ could be far removed from the pituitary gland as in the case with target organs of other adenohypophysial hormones. Dellmann et al.(1974) report that under a variety of experimental conditions including thyroid inhibition with PTU, adrenalectomy, castration, hypophysectomy, water deprivation, NaCl addition to the diet, hypercalcemia induced by vitamin D_2 supplements to the diet and metyrapone treatment there were no changes in the granulated cells of the PT with the exception of gonadotrophs. Similarly, Oota and Kurosumi (1966) observed no change in the PT tissue after adrenalectomy, castration or thyroidectomy and Kotsu (1971) and Kotus and Daikoku (1972) found no change after hypophysectomy and adrenalectomy.

Discounting their cytoplasmic filaments, cells of profile V resemble the nongranulated PT cells reported by others (Kobayashi et al., 1963, Young et al., 1965, Oota and Kurosumi, 1966, Knowles and Kumar, 1969, Kotsu and Daikoku, 1972, Brandi and Peillon, 1973, Stoeckel et al., 1973a, Dellmann et al., 1974) insofar as they typically have a poorly developed Golgi apparatus and scant RER. The abundant filaments throughout the cytoplasm, however, make this cell type distinct, closely resembling only the interstitial cells reported in the PT of the rabbit (Cameron and Foster, 1972a) and possibly the 'border' cells in the newt (Dellmann et al., 1974), although in the latter case the description given is inadequate to make an accurate comparison. The filaments in both the rabbit interstitial cells and the cells of profile V measure approximately 100Å in diameter and transverse sections suggest a tubular morphology.

With respect to diameter size, the filaments found in cell profile V are similar to the intermediate filaments described in other tissues (smooth muscle cells, Somlyo et al., 1973; precardiac cells, Rash et al., 1970a; oviduct cells, Wrenn and Wessells, 1970; glial cells, Spooner et al., 1971; salivary gland, Spooner and Wessells, 1970; fibroblasts, Goldman and Follett, 1969, Goldman, 1971, Goldman et al., 1975; macrophages, Allison et al., 1971, Daems and Brederoo, 1973; fibroblasts, chondrogenic cells and nerve cells, Ishikawa et al., 1969; developing skeletal muscle, Ishikawa et al., 1968). Unlike cytoplasmic thin filaments (~ 40-70nm in diameter) which have been observed to disassemble or not appear under the influence of cytocholasin B, a fungal metabolite (axonal growth cones, Yamada et al., 1970; glial cells, Spooner et al., 1971; salivary glands, Spooner and Wessells, 1970; oviduct tissue, Wrenn and Wessells, 1970), intermediate filaments seen in the oviduct tissue, the glial cells and the salivary gland were not affected by the drug. In this study and in that of Cameron and Foster (1972a), the filaments observed in nongranulated cells of the PT were not affected by cytocholasin B. Other investigators have found that thin filaments may bind with heavy meromysin which ultrastructurally forms arrowhead complexes along the filaments (Ishikawa et al., 1969, Allison et al., 1971). Again, this formation has not been seen to occur in the 100Å filaments of the same cells. Ishikawa et al. (1968) found that intermediate filaments did not bind fluorescene-labelled antibody directed against myosin or actin, proteins of thick muscle filaments and at least some thin filaments (Adelman et al., 1968). Goldman et al. (1975) also found that the thin filaments

of 3T3 cells bind with actin-antibodies while the intermediate filaments do not. Also, Rash et al. (1970b) found that intermediate filaments were not extracted by several chemical agents which did extract actin and myosin filaments from the cytoplasm of cells. Lastly, intermediate filaments, unlike thin filaments, have been observed by others to have a tubular appearance in cross section (Schmitt, 1968, Goldman and Follett, 1969, Somlyo et al., 1973).

The above evidence indicates that the morphological differences of these two groups of filaments are not artificial ones resulting from the tissue preparation techniques employed and that the chemistry of the filaments is indeed different. The basic protein component of intermediate filaments is not presently known although Uehara et al. (1971) suggest that it may be tropomyosin.

Several investigators have suggested that intermediate filaments might be involved with the maintenance of cell shape (Goldman, 1971, Daems and Brederoo, 1973, Kennedy et al. 1974). If such were the case, cells of profile V might provide sustentacular support to the tuberal tissue as a whole, a view also put forward by Cameron and Foster (1972a). The many desmosomal contacts made between this type of cell and all other types might be functional to the sustentacular role.

Filaments have also been reported in the cytoplasm of nongranular PD cells of the guinea pig (Smith, 1963), rabbit (Salazar, 1963), delphinid (Harrison and Young, 1969) and foetal rabbit (Schecter, 1969, 1971). They have been observed in the nongranular PI cells of the rabbit (Cameron and Foster, 1971) and in thyrotrophs of thyroidectomized lizards (Forbes, 1972).

Cell profile IV appears to be almost unique in compar-

ison with cells described in the PT of other animals. Examination of the literature has revealed only one ultrastructural description of a similar kind of cell. This report (Morato, 1960) describes and displays photographs of a follicular cell with many large cytoplasmic vesicles to which densely osmiophilic grains are attached. These grains appear as though they might be ribosomes. Morato's description, unfortunately, does not provide further information and his micrographs indicate considerable lack of adequate fixation. A better comparison, therefore, cannot be made.

Cell profile IV, although primarily recognized by its abundant, spherical RER, is seen to exhibit other specific morphological features which perhaps give better indications as to its function. Very few cytoplasmic granules are observed and the Golgi zone, located between the nucleus and the apical pole, is of a moderate size and does not appear to be involved with granule condensation and packaging (several membrane-bounded granules are seen in many of these cells, but their size varies greatly - from 100nm to 600nm - and their cores are seen to be finely granular - they are perhaps lysosomes). From these observations, it would seem that cells of profile IV are not involved with protein synthesis and storage, or at least not with the same mechanism of protein synthesis and storage as occurs in other types of pituitary cells (e.g., gonadotrophs, thyrotrophs). Of more particular note is the evidence suggesting a transfer of substance(s) between the follicular lumen and the cells. Near the periluminal plasmalemma, the RER decreases sharply and many vesicles are observed. Not infrequently, some of these smaller vesicles may be seen to be fused with the plasmalemma or

appear as though 'budding' off the plasma membrane. Occasionally, invaginations are observed which are rather like irregularly shaped pinocytotic or exocytotic profiles. If such morphology is indicative of a transfer of substances, the direction of the transfer cannot be stated. In the same context, the additional presence of microvilli might suggest an absorption in the direction of the cells, but this is more speculative.

The presence of junctional complexes at the apical pole of the lateral plasma membrane of profile IV cells indicates that there is a probable selective storage of the luminal content. The filamentous network often observed in the apical zone of these cells, the extremes of which are seen to be associated with the tight junctions and gap junctions, is considered to be similar to a terminal web. These networks might well aid in the stability of a cellular seal around the follicular lumen. Any hypothetical transfer of material into or out of the lumen, therefore, probably would involve the intercellular activities of the cells of profile IV. Hence, it is suggested that the activity of the Golgi apparatus and the endoplasmic reticulum of these cells is involved with the chemical reactions which either produce 'colloid' or use 'colloid' in another capacity.

Microvilli also occur on the lateral and basal plasmalemma. As they clearly increase the surface area of the plasma membrane, they might be considered to function in the absorption of large amounts of substances from the intercellular fluid.

There are many reports which describe nongranulated cells in the adenohypophysis (Rinehart and Farquhar, 1955, Farquhar,

1957, Salazar, 1963, Smith 1963, Kagayama, 1965, Yoshida, 1966, Dent and Gupta, 1967, Yamada and Yamashita, 1967, Kurosumi, 1968, Cardell, 1969, Harrison and Young, 1969, Heath, 1970, Phifer et al., 1970, Schecter, 1971, Pearson and Licht, 1973). These cells usually have been referred to as either stellate and/or follicular cells. They have been considered to have a possible phagocytic function by some investigators (Yamashita, 1969, Farquhar, 1971, Dingemans and Feltkamp,1972, Vila-Porcile, 1972, Vila-Porcile et al., 1973). Others have thought that follicular cells are ACTH cells (Farquhar, 1957, Finerty and Keller, 1961, Rennels, 1964, Bergland and Torack, 1969, Schecter, 1969). This study has not produced evidence to support either of these views unless the various particulate substances found in follicular lumena might be argued to result from a phagocytic process.

Mainly because of their abundant, round RER, the profile IV cells in the guinea pig PT are ultrastructurally distinct from any follicular cells reported to date as far as can be determined. While they may be involved with a phagocytic process, alternatively, these cells may be involved with the depositing and storage of 'colloid' in a follicle for a positive function, perhaps not unlike thyroid cells.

In this study, a cell bearing the same major morphological features of both cell profiles IV and V was observed. Furthermore, while cell profile V was not ordinarily seen to border on a 'colloid'-filled lumen, it was found to do so occasionally, particularly in the dorsal area of the PT where profile IV cells are never found. This tends to suggest the possibility, at least, of different physiological states of the same cell resulting in a corresponding change in the

morphology.

Increases in the number and size of 'colloid' deposits in the adenohypophysis has been reported by some investigators to occur with age (horse, Höser, 1941; rat, Wolfe, 1943; human, Shanklin, 1949; hamster, Spagnoli and Charipper, 1955; mouse, Blumenthal, 1955; rat, doormouse and mouse, Stoeckel et al., 1973a). Blumenthal reports that 'colloid'-filled follicles become cystic with old age. This might explain the occurance, in this study, of a rather small cyst completely surrounded by cells of profile IV instead of the usual Dark Cell I. Also, there are a number of cellular characteristics common to both follicles and cysts in the guinea pig PT: junctional complexes, a comparable cellular polarity, small cytoplasmic vesicles and microvilli on all sides of the plasma membrane. Lastly, it has been observed in the guinea pig that in the posterior PT, very near the PD, there is a marked increase in the number of profile IV cells, and the follicles of this small region are always very much larger than normally found in the rest of the PT. Cysts are also seen to be much more numerous in the PD-PT transitional zone than elsewhere (a finding also noted by Kirkman, 1937). The high incidence of both follicles and cysts in this region, therefore, is thought to support Blumenthal's statement.

That cysts and follicles might represent minute parts of the hypophysial cleft which become isolated in development has also been considered (Kirkman, 1937, Yoshida, 1966, Kurosumi, 1968, Vanha-Perttula and Arstila, 1970, Dingemans and Feltkamp, 1972). Again, cellular similarities between nongranular follicular cells, cysts and hypophysial cleft cells have been observed by other investigators (Dingemans

and Feltkamp, 1972, Vila-Porcile, 1972). This study, however, has not provided any further evidence to expand or reinforce this hypothesis.

Unfortunately, during the experimental stage of this study, it was not known that bulk staining of tissue in aqueous uranyl acetate removes glycogen from cells (cardiac cells, Rash et al., 1970a; platlets, personal communication, A. Hoyes) and hence, all routinely prepared specimens in this study are subject to this extraction. However, on the basis of small disruptions in the cytoplasm of profile V cells and the evidence of fine particles in the cytoplasm of profile V cells fixed in potassium permanganate, it is thought possible that a variable but small amount of glycogen is present in cells of this type. In support of this interpretation, the cytoplasm of many smooth muscle cells was seen to be comparable. It is not possible to state that glycogen is not present in other types of cells, but similar evidence has not been observed in either the granulated cell profiles or in cell profile IV. The negative results of the PAS reaction and Best's carmine technique, both with diastase control, can only be explained if the amount of glycogen is very low and hence, not adequate for detection by these methods.

Glycogen has been reported in both the granulated and nongranulated cells of the PT in a number of animals (rat, Oota and Kurosumi, 1966, Klein et al., 1970, Stoeckel et al., 1973a & b, Dellmann et al., 1974; mouse, Siperstein, 1955, (foetal and newborn), Dellmann et al., 1974; frog, Dierickx et al., 1971; doormouse, hamster, cat, cattle and newt, Dellmann et al., 1974). Stoeckel et al. (1971) stated that the glycogen content of the granulated cells of the PT

in the mouse distinguishes them from the rostral ACTH cells in an area where these cells come into contact. Dellmann et al. (1974) reported the presence of glycogen in all the animals observed in their study with the exception of the chicken. However, their micrographs seem to indicate that glycogen is not always present in granulated cells of the rat and in addition, glycogen could not be seen in any of their micrographs of feline or bovine tuberal tissue. Klein et al. (1970) reported that the amount of glycogen in the PT of the rat varied inversely with the granule content. In the PT of the rabbit, Cameron and Foster (1972a) reported that glycogen was not seen either on the ultrastructural or the light microscopical level using the PAS reaction with distase control. Knowles and Kumar (1969) make no mention of glycogen in the tuberal tissue of the Rhesus monkey. The presence of glycogen, therefore, may possibly vary from species to species. On the other hand, the presence and amount of glycogen may depend upon the physiological state of the cells.

Finally, there is the question of how the tuberal tissue is controlled. In this study, AF, CAH and AB staining techniques were employed to investigate the possible presence of neurosecretory fibres in the PT and in all cases the evidence was negative. 'Colloid' deposits in the PT were observed to stain positively with all of these stains, but unlike neurosecretory material, they continued to react positively when the oxidation step of the technique was omitted, thereby making the two substances histologically distinguishable. In rare instances, a beaded fibre entered the external zone of the ME.

Bodian's protargal impregnation technique was repeatedly attempted without obtaining positive results in the tuberal

tissue, but densely argyrophilic fibres were observed in the neural stalk and in the PI. Although fine fibres were observed in the external ME using this method, it was thought that their numbers were not equal to those observed in the same region under the electron microscope. Szentagothai et al., (1968) remark that fine fibres associated with arterioles are extremely difficult to impregnate, perhaps because the silver available in the section during reduction may be taken up by the larger fibres leaving little for the finer fibres. Also, Wingstrand (1951) noted that in his work, the lower the pH of the silver proteinate, the more selective the impregnation is for nerve fibres, although sensitivity may also be reduced. Increasing the pH, on the other hand, may result in the additional impregnation of glial and reticular fibres. Therefore, a delicate balance must be achieved in order to get an accurate result for nerve fibres alone. In view of these considerations, the evidence for the presence or absence of adrenergic and cholinergic nerve fibres in this study is considered to rest more with the ultrastructural findings.

The examination of approximately 1,000 micrographs provided evidence of nerve fibres in the tuberal tissue only twice. These were observed in the anterio-ventral region of the tissue in a location where the epithelium was observed to be protruding somewhat into the region of the infundibular stem. These are, therefore, considered to be merely invasive nerve fibres from the stalk and not evidence of a general innervation of the tissue. As the different cell profiles were observed to be well distributed in the tissue (except for profile IV cells which were absent in the dorsal areas), there is no reason to think that a particular region may be more

likely innervated than another, It is possible, though not probable, that in the examination of tissue randomly selected from each of five areas of the PT nerve fibres were missed, i.e., the evidence strongly favours the view that there is no innervation of any cell type in the guinea pig PT. This view finds support in the work on other animals both on the light microscopical level (rat, Daikoku et al., 1967; sheep and goat, Beck and Daniel, 1960; mouse, rat, guinea pig, hamster, rabbit and cat, Fuxe, 1964) and on the ultrastructural level (frog, Doerr-Schott, 1971; rat, Stoeckel et al., 1973a & b, Dellmann et al., 1974; hamster, cat, cattle, chicken and newt, Dellmann et al., 1974; mouse, Stoeckel et al., 1973a, Dellmann et al., 1974).

There is, however, a body of evidence in support of the view that tuberal tissue is innervated. Arko and Kivalo (1958), using Gomori's aldehyde fuchsin, reported neurosecretory material around the portal vessels and in the pars tuberalis. Their photographs, however, do not appear to support their observation that neurosecretory material is actually within the parenchyma of the PT. Okomota and Ihara (1960), using Gomori's CAH and AF staining methods, report that there are nerve fibres in the PT of the rat, but their photographs illustrate AF^{+} deposits which are referred to as neurosecretory material but which distinctly appear to be 'colloidal' deposits.

Furthermore, it may be that the frequency of the nerve fibres observed and their precise anatomical position may be extremely relevant to the evaluation of a possible innervation of the cells of the PT. Clearly, some investigators note

that only a small area of the tissue appears to contain nerve fibres (Szentagothai et al., 1968, Beck, et al., 1969). In such cases, the distribution of cell types in the PT of the animals involved would be important for purposes of interpretation. In the goat, cat and dog, this information does not appear to be available, but in general, the literature has not provided evidence of an uneven distribution of cell types except in the immediate vicinity of the PD-PT transition zone and certainly in this study, the evenness of cell type distribution is almost absolute. If one may assume, for the moment, that this is the general pattern in all animals, one would quiry whether or not a few nerve fibres in a small and restricted area could reasonably be considered as evidence of innervation of the cells as a whole.

Others note that only perivascular fibres are observed (Wingstrand, 1951, Barry and Cotte, 1961, Bargmann, 1969), and again, the significance of these could not be clearly understood to mean an innervation of the tissue.

Lastly, one could consider that the control mechanism of the PT may be subject to species variation. In this respect, Dellmann et al. (1974) notes that of eight animals studied, only one (the newt) appeared to have an innervated PT. Furthermore, they note that only in this one animal was the primary capillary plexus seen to be embedded within the tuberal epithelium as opposed to primarily lying between the PT and the neural stalk. They suggest that this may necessitate nerve fibres carrying hypophysiotrophic hormones to the portal vascular system to pass through the tuberal epithelium.

Also, with respect to possible specie specificity, it

may be noted that in the frog, where nerve fibres have also been reported in the tuberal tissue (Dierickx et al., 1971), the PT is situated far from the primary capillary plexus. Therefore, if it were the case that in other animals the primary plexus transported hypophysiotrophic hormones to the PT, this would not be possible in the frog, thereby possibly necessitating a direct innervation of the tissue. Again, the line of reasoning is not conclusive but it is, perhaps, worthy of further speculation.

Clearly, the literature concerning the possible innervation of tuberal tissue reveals much controversy and further experimentation will be necessary to clarify this issue.

The guinea pig PT is thought to be more likely controlled by a blood supply than by a nervous mechanism. The evidence in this study shows that a large number of fenestrated capillaries border the internal length of the PT and because of their location, frequency and tendency to form capillary loops which penetrate the ME, they are considered to be part of the primary capillary plexus of the portal vascular system. Furthermore, a small number of capillaries are seen to be entirely surrounded by tuberal tissue, although most vessels so situated are seen to be arterioles. The arterioles, however, could be considered to represent portal vessels and thus not involved in the regulation of the tissue. Lastly, some fenestrated capillaries are also observed to lie on the external surface of the PT. The origin and nature of the capillaries surrounded by tuberal tissue and those lying external to it cannot be determined from this study. However, the above observations allow, for the present, several speculations as to the possible vascular control of the PT. On the one

hand, the PT may receive from the hypothalmus (via the primary capillary plexus) hypophysiotrophic hormones which regulate its cellular activities. While the weight of the evidence of adenohypophysial control lies with hypothalmic regulation, there are, in addition, recent studies on steroid hormone uptake in some anterior lobe cells, indicating a direct feedback control mechanism (Stumpf et al., 1975, Piacsek and Meites, 1966, Leavitt et al., 1973). Hence, it might also be considered that the tuberal cells are regulated by a direct feedback mechanism which might involve the primary capillary plexus and/or other capillaries. Evidence that the PT has its own capillary blood supply independent of the portal vascular system has been presented in embryologic studies of avians (Wingstrand, 1951) and guinea pigs (Weatherhead, 1968) and in a study of the frog (Dierickx et al., 1971). To carry the speculation a bit further, therefore, one might cautiously put forward the suggestion that the tuberal tissue is controlled by a direct feedback mechanism via tuberal capillaries and that the presumed secretion factors of the granulated PT cells are carried off by the primary capillary plexus into the portal vessels. Needless to say, the actual determination of the role of the blood vessels observed within and immediately adjacent to the PT of the guinea pig and other animals requires much further research.

SUMMARY

1. Profile I and II cells contain small granules averaging 121nm and 114nm respectively. They are considered to be the same cell type in as much as all cytoplasmic features are similar with the exception of the RER which is seen to be more extensive in profile II. While these cells are found in all regions of the PT, they are not recognized to be of any cell type found in the PD. Cytoplasmic granules appear to be packaged in the Golgi zone and are frequently seen to aggregate in groups against the plasma membrane. In addition, the delimiting membrane of some granules is seen to fuse with the plasma membrane.

2. Profile III cells represent the only chromophilic cell of the PT, staining positively with PAS and AB but negatively with AF. They contain granules averaging 229nm and are observed adjacent to capillaries. Although rare, they are seen in all areas of the PT. Granules appear to be packaged in the Golgi apparatus but are never seen to provide evidence of secretion. These cells are considered to be gonadotrophs.

3. Granulated cells are never observed to come into contact with follicular lumina.

4. Profile IV cells are identified by their abundant round RER. They almost always border on follicular lumina and morphological evidence indicates that they are involved with the deposition and/or uptake of 'colloid' materials of these follicles. Cell profile IV

has not been observed in the dorsal region of the PT.

5. Profile V cells are identified by their abundant cytoplasmic filaments which measure approximately 100Å in diameter and have an electron lucent core. These cells are observed in all areas of the PT. Their function is unknown but they may be sustentacular in nature. In rare instances, they are observed to border on the lumen of a 'colloid'-filled follicle.
6. A cell profile is observed which has the primary morphological characteristics common to both cell profile IV and V, suggesting that a morphological transition occurs between these two cell types. The frequency and direction(s) of a possible transformation are not known.
7. The 'colloid' of cysts and follicles have similar staining affinities and ultrastructural morphology.
8. Cysts are most frequently observed in the posterio-ventral PT but may be seen in other areas. The majority of cells of these structures contain electron dense cytoplasm and are unlike those of profiles I-V. Occassionally, a few cells similar to those of profile IV and V are seen to border on the lumen of a cyst.
9. Evidence for the possible presence of glycogen has been observed only in cell profile V.
10. Desmosomes are observed joining cells of all types to each other. Those joining two nongranulated cells are usually observed to be associated with filaments. Elsewhere, their association with filaments is uncommon.
11. Cilia with a 9+2 tubular pattern are seen to extend into

the lumina of cysts from a few cells which contain light cytoplasm. They are never seen to extend into follicular lumina. Cilia are also infrequently seen to originate from granulated cells and in one instance, a cilium was seen to originate from a profile V cell.

12. Centrioles are found in the perinuclear cytoplasm of granulated cells and in the periluminal cytoplasm of profile IV cells.

13. No evidence of innervation for any cell type of the PT was observed in this study.

14. Capillaries of the primary plexus are observed to run adjacent to the internal surface of the PT along its length. Capillaries are also seen on the external surface of the tuberal tissue. Blood vessels seen to be entirely surrounded by tuberal tissue, however, are more often arterioles rather than capillaries.

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MICROGRAPHS

Figures 1 through 9 are light micrographs.

Figures 10 through 63 are electron micrographs taken of tissue from male guinea pigs which was fixed in glutaraldehyde followed by osmium tetroxide unless otherwise stated. Where appropriate, cell profiles are labelled as I, II, III, IV or V to correspond to the profiles described in the text.

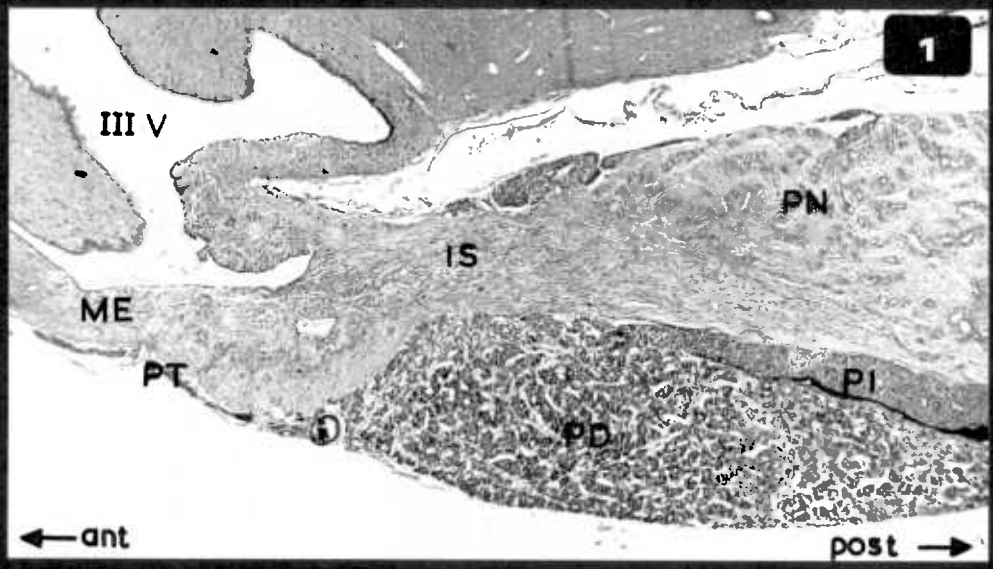
Figure 1. A sagital view of the guinea pig pituitary gland labelled to show the different epithelial and nervous regions. (fixed in FC, stained with APG) X40

Figure 2. A transverse cut through the hypophysial stalk of the pituitary gland. The primary capillary plexus is seen to be situated between the median eminence and the pars tuberalis. Neurosecretory material can be observed in the median eminence (arrows). (fixed in Bouin solution, stained with CAH) X60

Figure 3. A horizontal view of the pars tuberalis which is attached to the neural stalk. Note the longitudinal arrangement of the blood vessels in the pars tuberalis. (fixed in FC, stained with H & E) X60

Figure 4. Vertical view of the pituitary gland in the region of the pars tuberalis to show the continuous morphology of the pars tuberalis to the pars distalis ventrally and the close proximity of the pars intermedia to the pars tuberalis in the dorsal regions. (fixed in FCC, stained with H & E) X90

Abbreviations for Figures 1 through 4: PT - pars tuberalis, PI - pars intermedia, PD - pars distalis, PN - pars nervosa, IS - infundibular stem, ME - median eminence, IR - infundibular recess, III V - third ventricle, d - dorsal, v - ventral, ant - anterior, post - posterior.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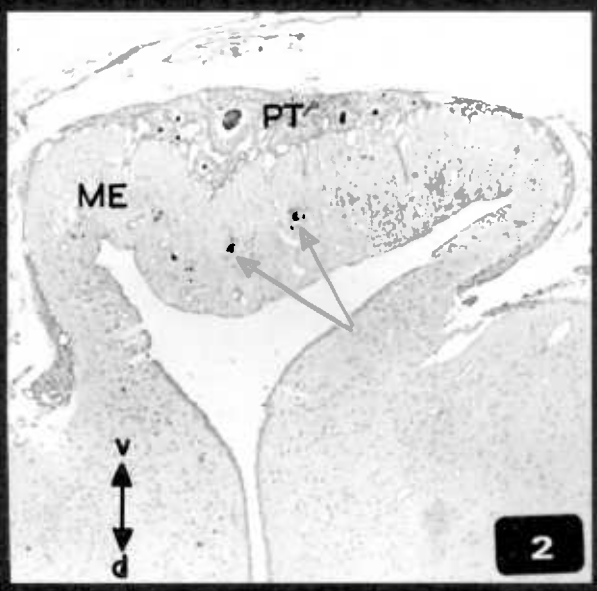
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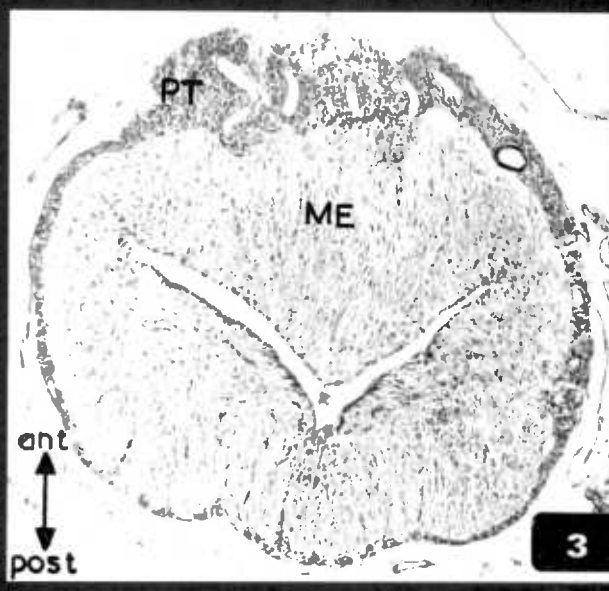
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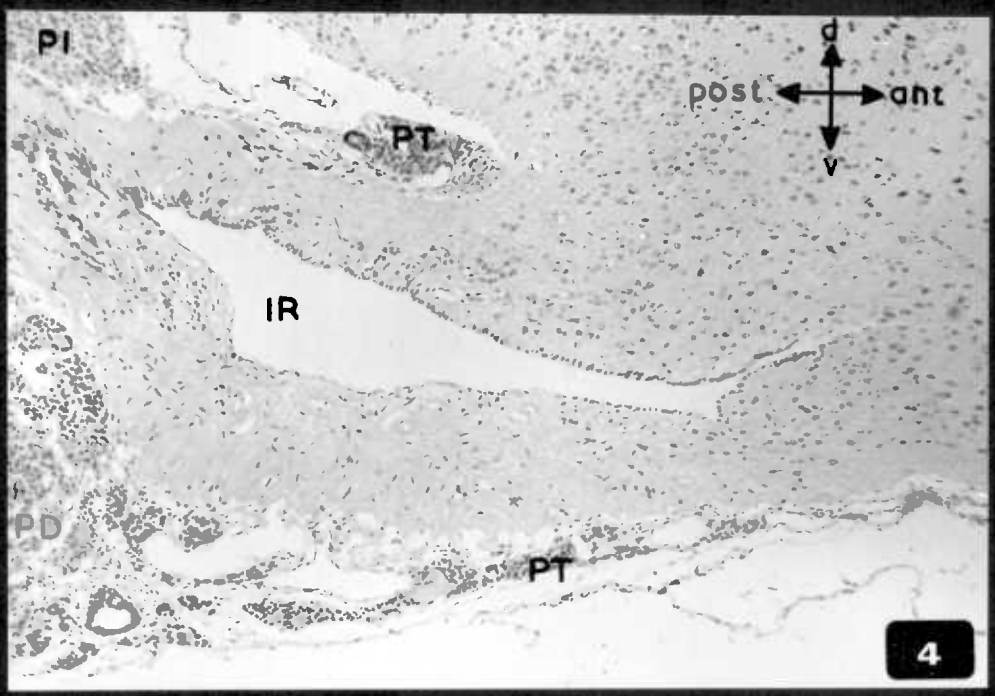
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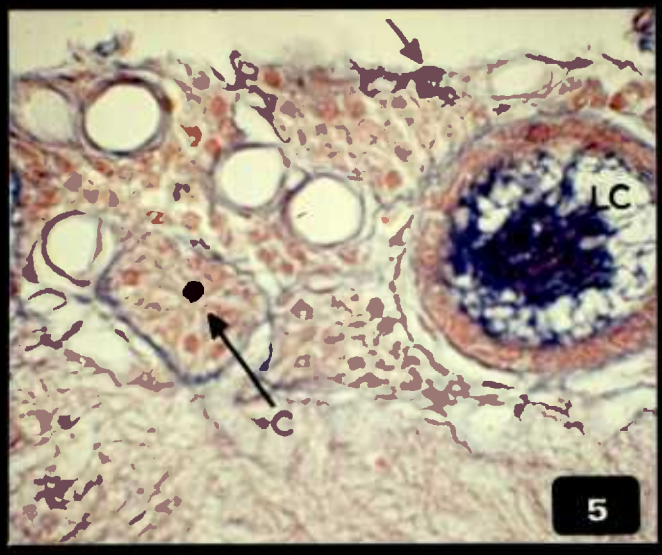
Figure 5. Vertical section of the dorsal pars tuberalis in which only one chromophile is demonstrated (arrow). Also note a small 'colloid'deposit (c) within one cell cord and elsewhere a large cyst (the lumen of which is labelled LC). (fixed in FC, stained with APG) X 465

Figure 6. Vertical view of part of the ventral pars tuberalis demonstrating a single PAS positive cell (arrow). (fixed in FC, stained with PAS/OG) X90

Figure 7. Transverse view of part of the pars tuberalis. Note two heavily granulated cells at the periphery of the cord (arrows) and the 'colloid' deposit centrally situated. (fixed in glutaraldehyde followed by osmium tetroxide, stained with Azur II) X1400.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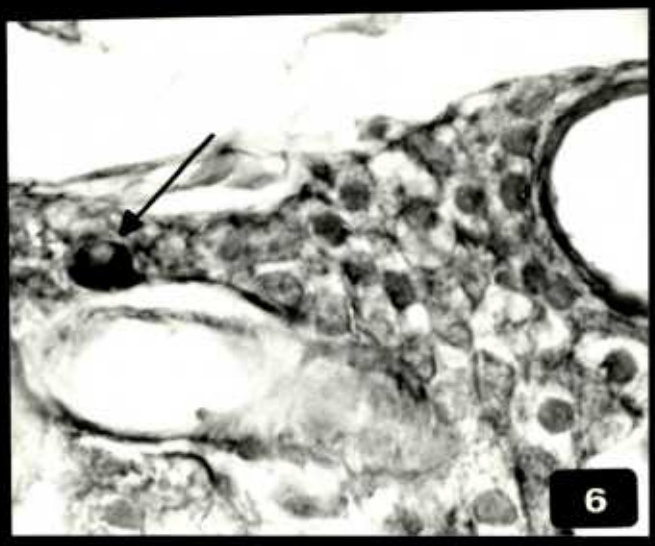
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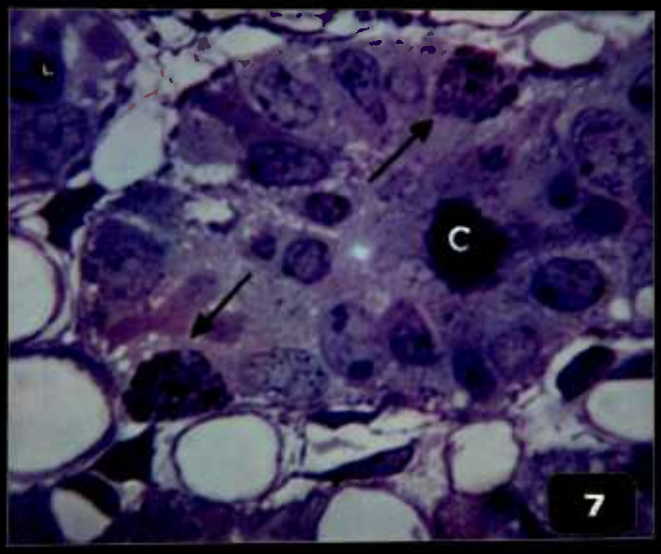
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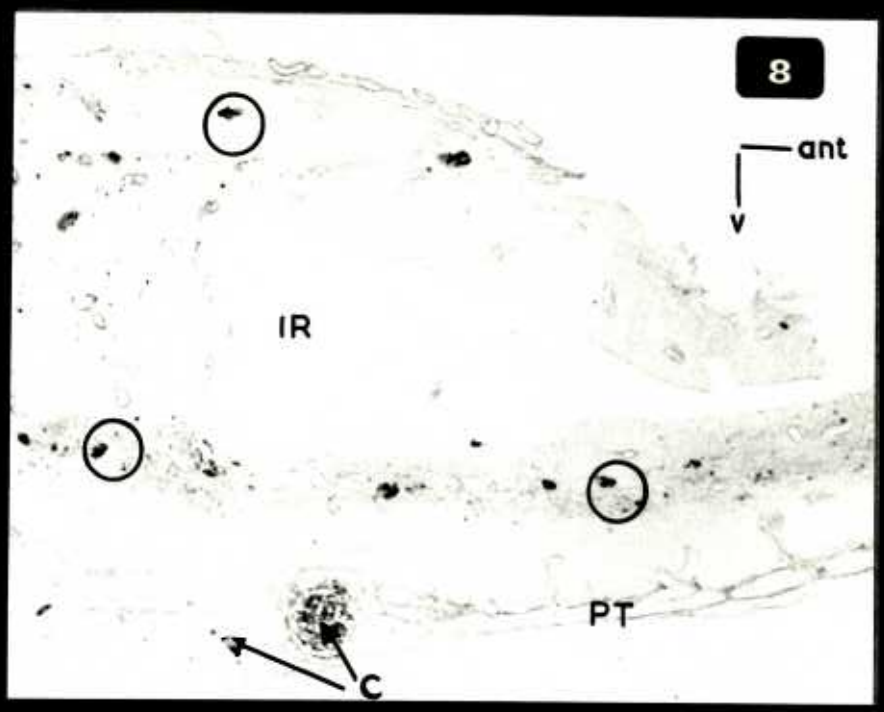
Figure 8. Vertical view of the hypophysial stalk. Positively stained neurosecretory material is seen in the internal median eminence and in the infundibular stem (some of which is circled). Positively stained 'colloid' (c) is seen in small follicules and in one cyst of the pars tuberalis. v - ventral; ant - anterior. (fixed in Bouin solution, stained with AF) X120

Figure 9. A cyst and adjacent epithelial cells in the posterior pars tuberalis. Note the extensive metachromasia of the 'colloid' in the lumen of the cyst (LC) and the multiciliated cell (c). (fixed in glutaraldehyde followed by osmium tetroxide, stained with Azur II) 1440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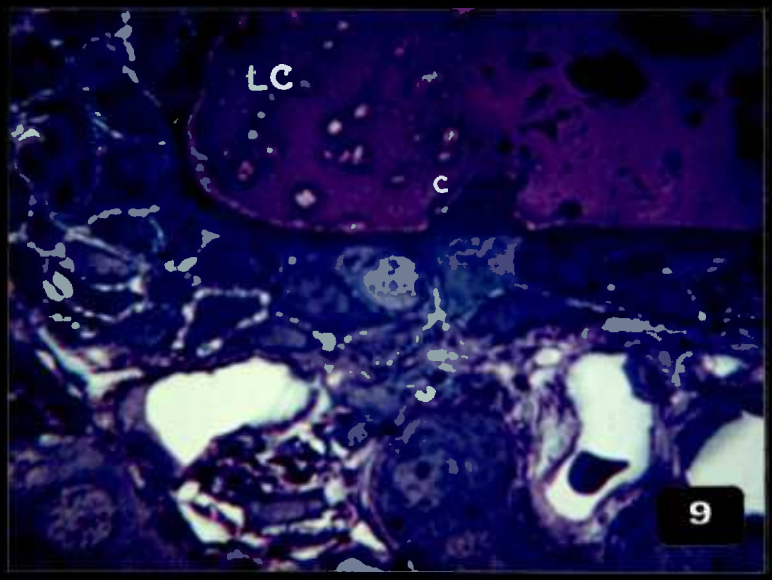
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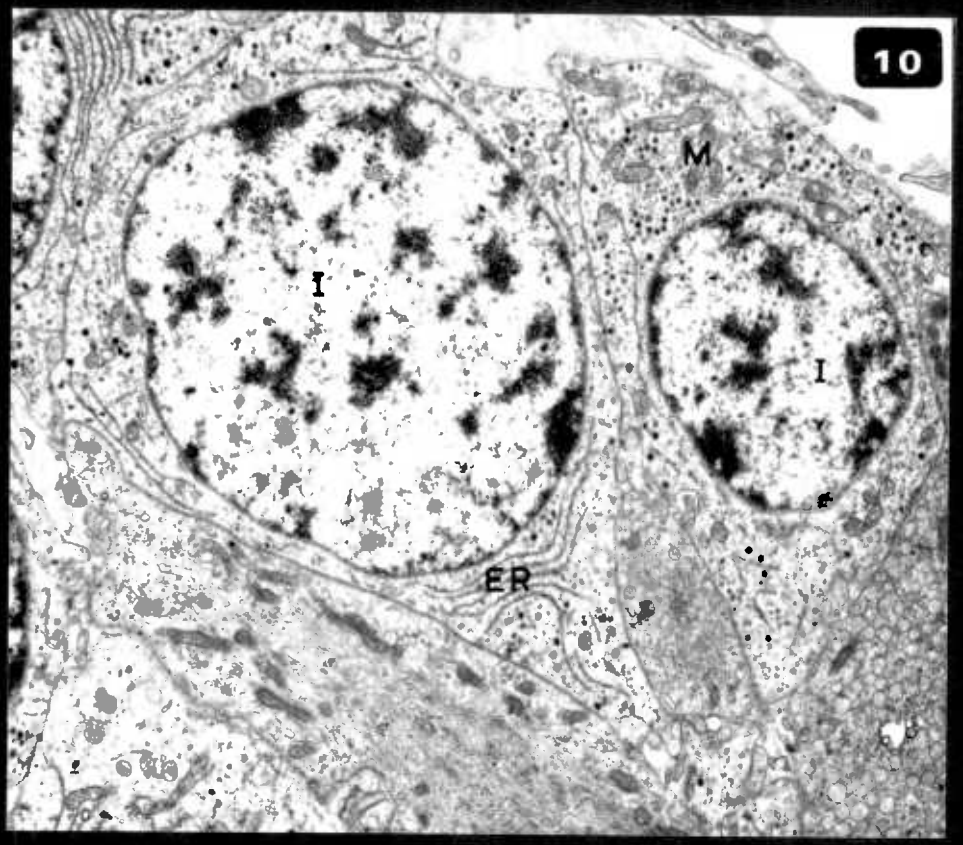
Figure 10. Two cells of profile I showing typical rough endoplasmic reticulum (ER), mitochondria (M) and numerous scattered granules in the cytoplasm. X10,000

Figure 11. A process of a cell of profile I containing abundant cytoplasmic granules with variable amounts of electron lucent space between the dense cores and the granule membranes. Also note the scattered, long rough endoplasmic reticulum (ER). X11,000

Figure 12. Enlargement of an area of the preceding photograph showing a small foot-like appendage of a cell of profile I, at the end of which is seen an electron dense substance (arrow). Also note the coated vesicle (v) which appears to be attached to the plasma membrane X25,000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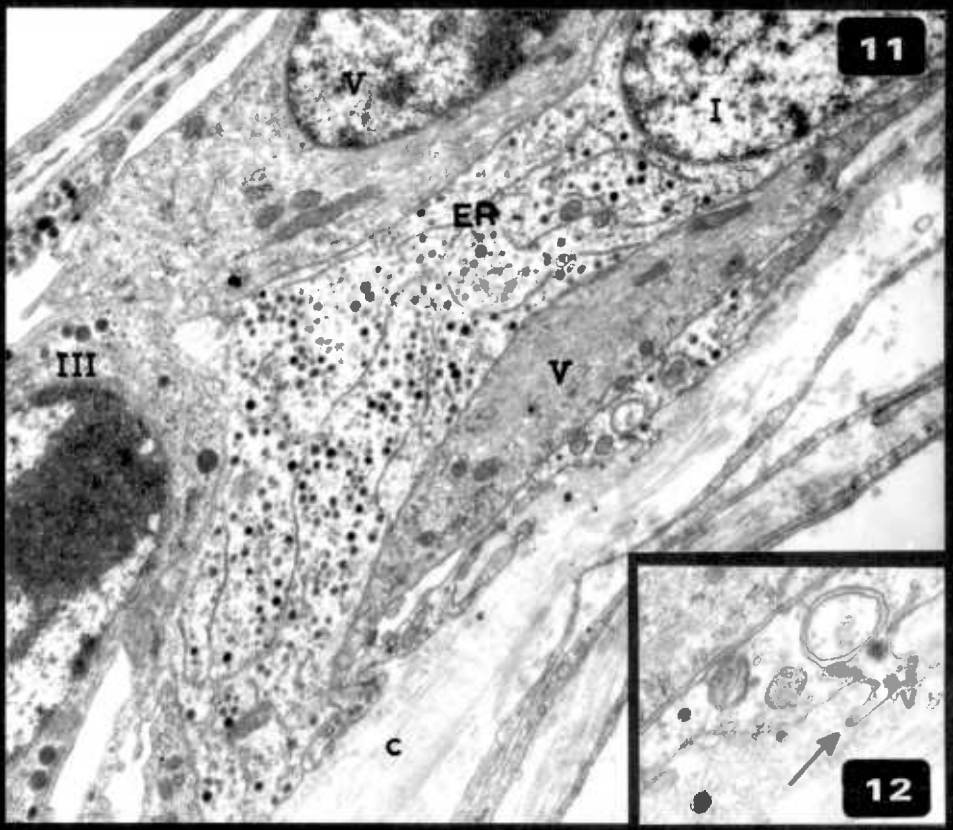
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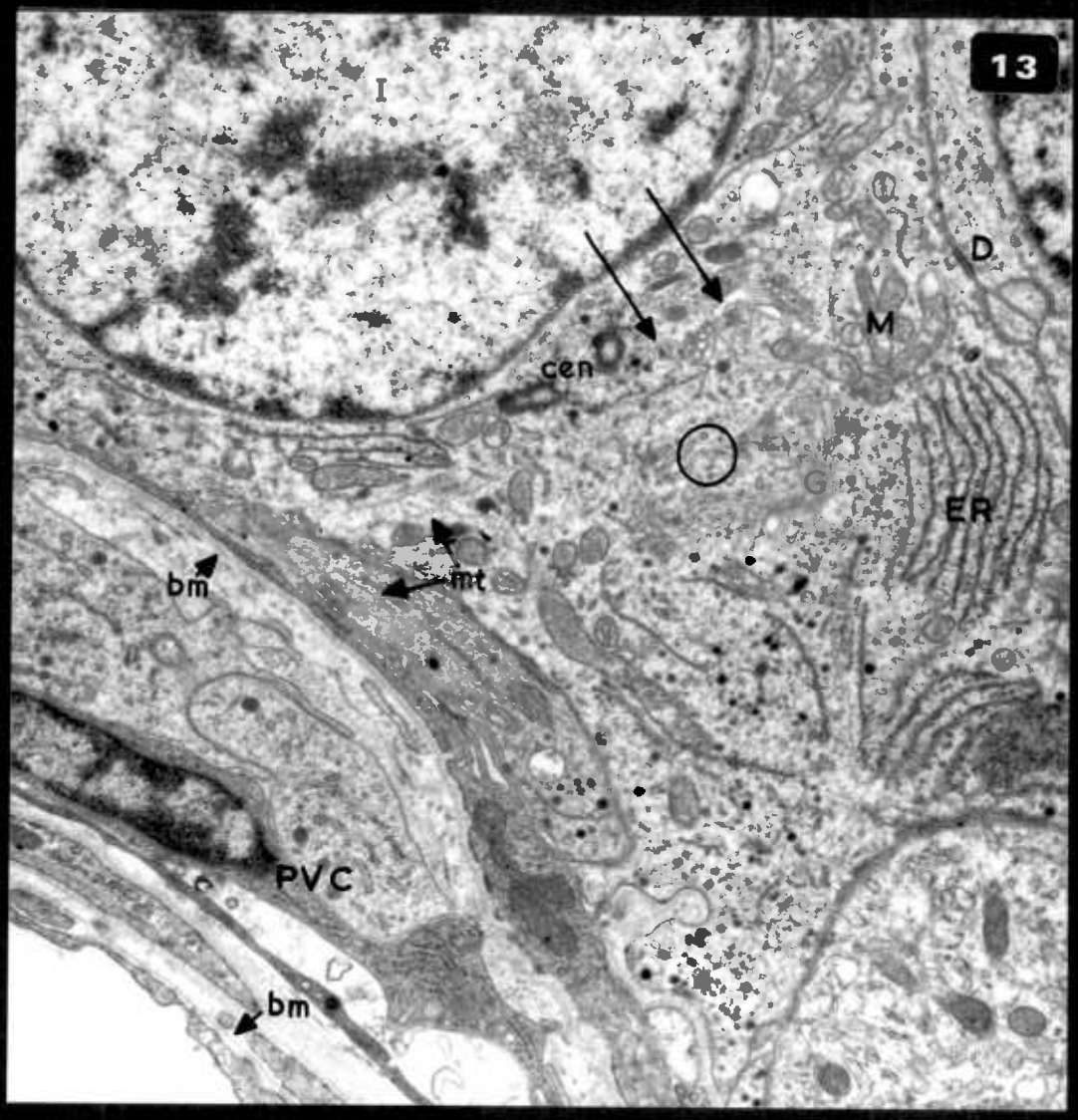
Figure 13. The synthetic pole of a cell of profile I. Immature appearing granules (arrows) and coated vesicles (some of which are circled) are seen in the Golgi zone (G). D - desmosome; ER - rough endoplasmic reticulum; M - mitochondria; cen - paired centrioles; mt - microtubules; bm - basement membrane; PVC- perivascular cells. X15,000

Figure 14. Perivascular region of a cell of profile I showing aggregation of granules at the plasma membrane (arrow). g - granule; mt - microtubule; c - collagen; * - fenestrations in endothelium of capillary. X40,000

Figure 15. Exocytotic-like figure in a profile I cell. X37,500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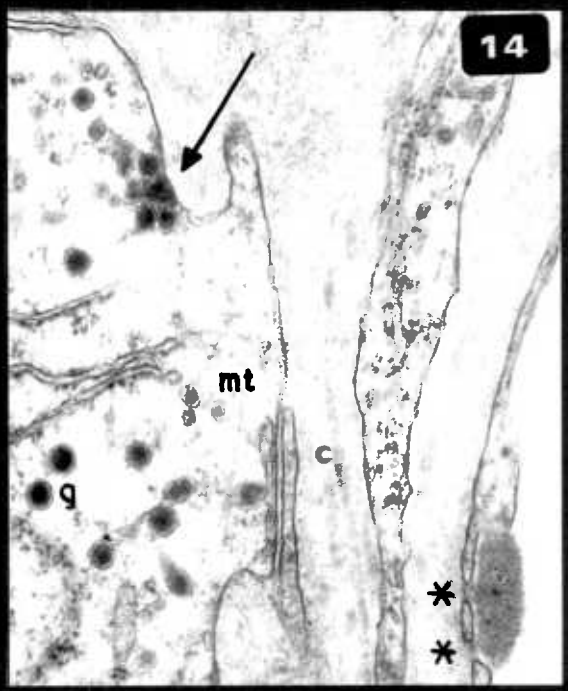
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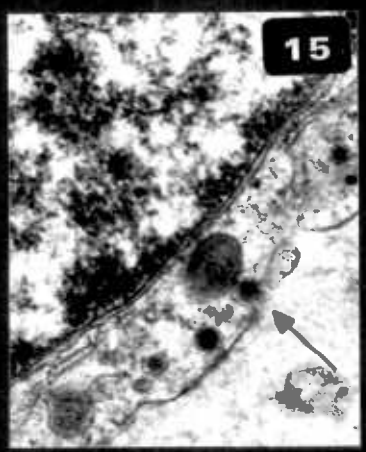
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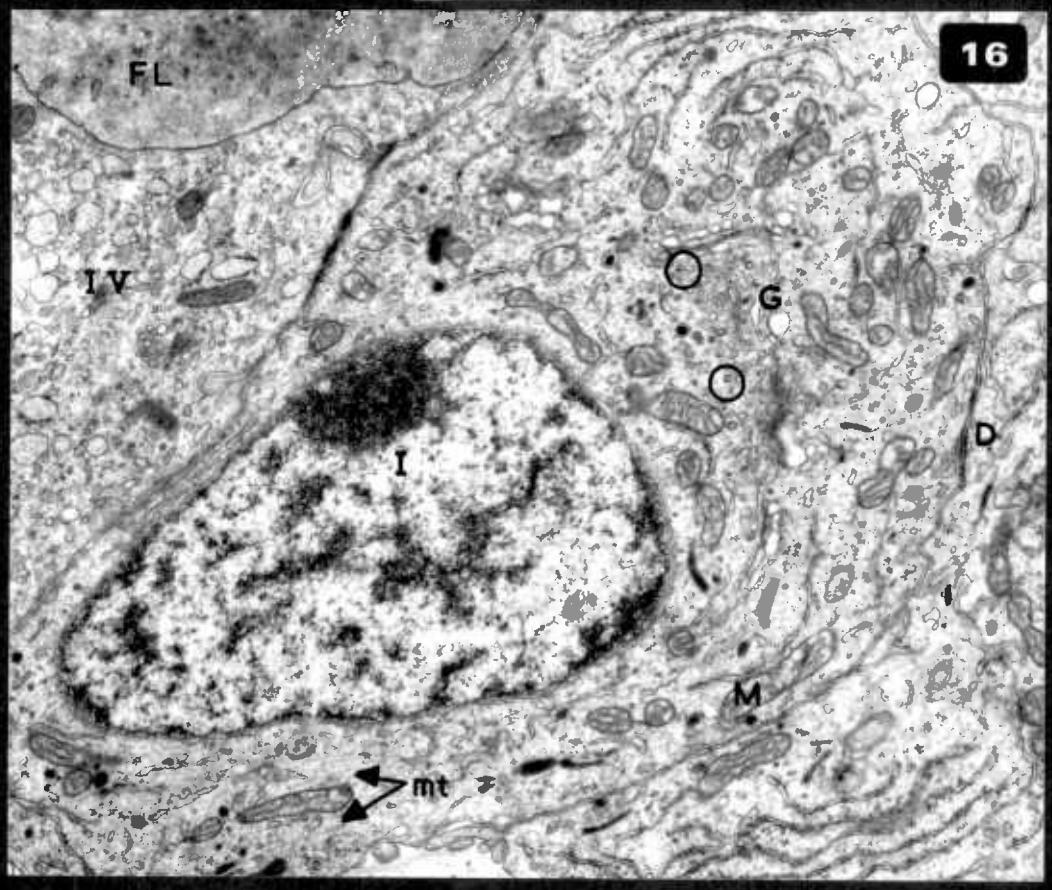
Figure 16. Micrograph of a profile I cell from a female guinea pig in diestrus. Fewer cytoplasmic granules are seen than in male specimens (compare with Figures 10 through 13). The Golgi apparatus (G) is seen to contain numerous vesicles and some lamellae which appear to dilate into vesicles at their ends (circled). Part of a cell of profile IV is also seen bordering on a follicular lumen (FL). mt - microtubules; M - mitochondria; D - desmosomes. X15,000

Figure 17. A profile II cell showing extensive rough endoplasmic reticulum (ER), some of which is flat, and some of which is dilated and rounded. X15,000

Figure 18. An enlargement of a portion of Figure 17 showing the aggregation of granules at the plasma membrane. The granules in some instances appear unbounded and adhere closely to the plasmalemma (arrows). X37,500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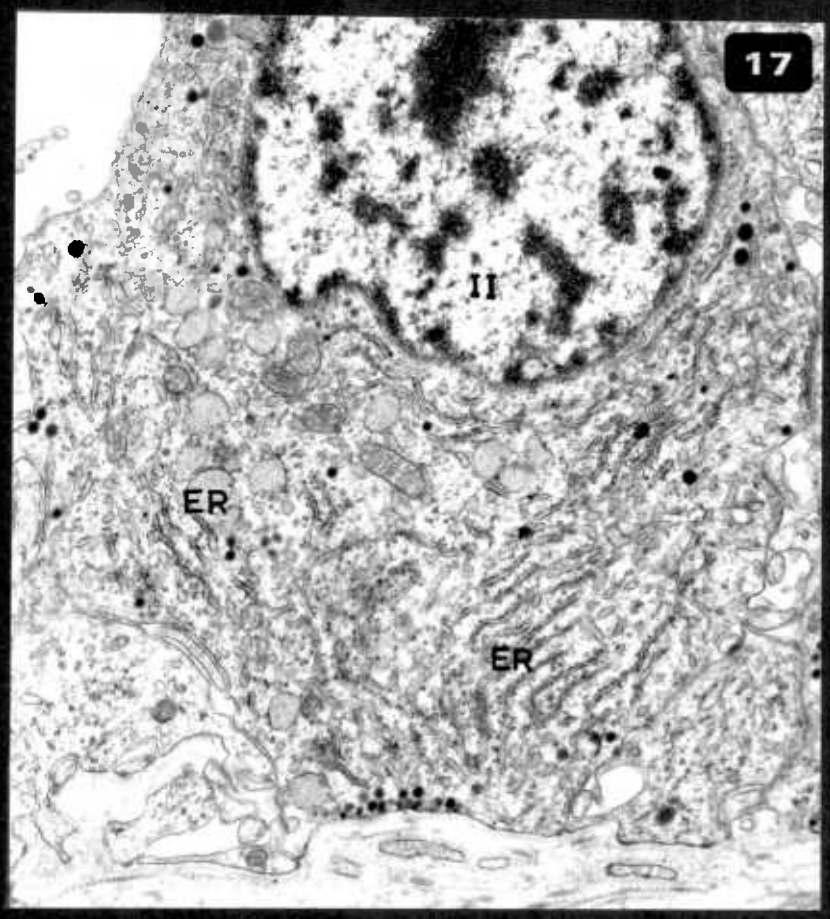
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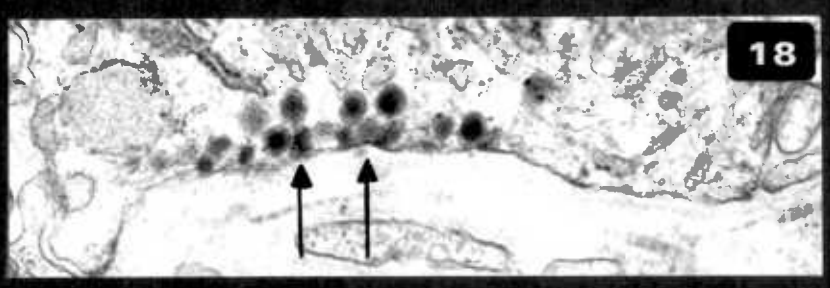
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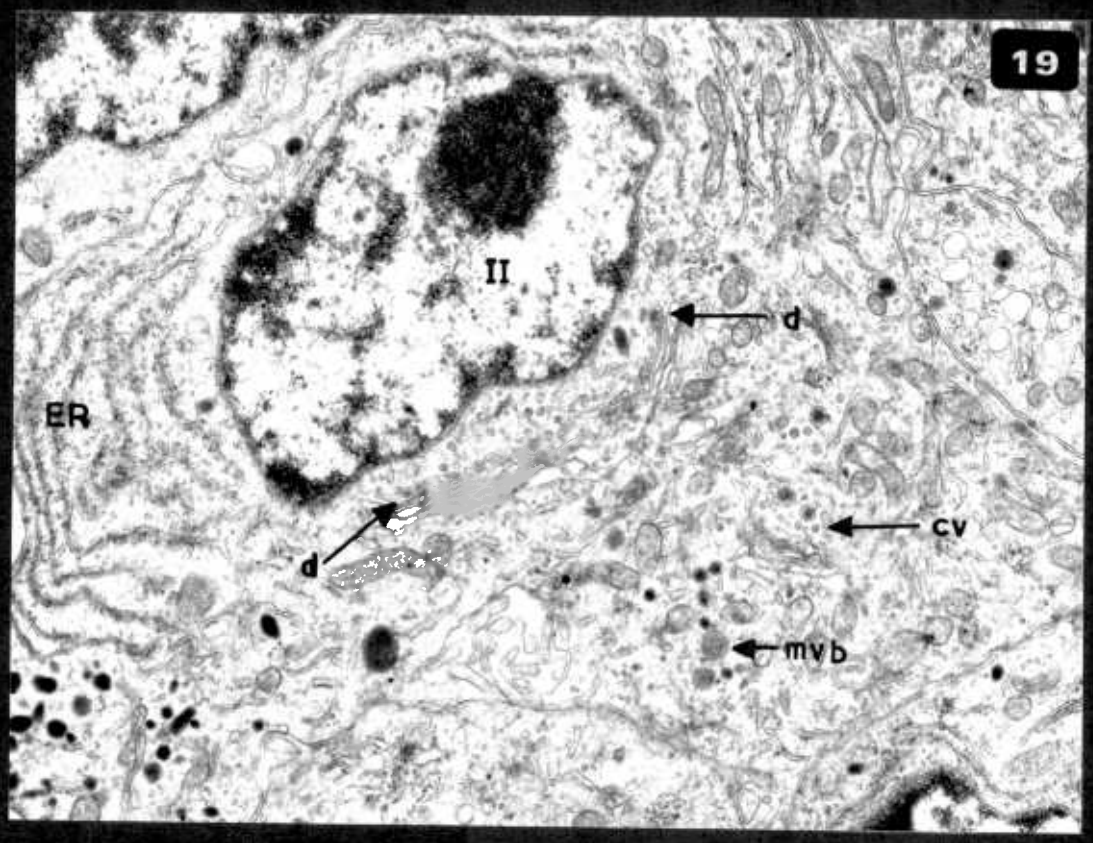
Figure 19. A synthetic region of a cell resembling a profile II cell. Note the electron dense substances (d) in the Golgi apparatus. cv - coated vesicles; mvb - multivesicular body; ER - rough endoplasmic reticulum. X 15,000.

Figure 20. A profile II cell showing extensive rough endoplasmic reticulum (ER) separated by the nucleus from a larger Golgi region (G) in which numerous coated vesicles can be seen. D - desmosomes. X15,000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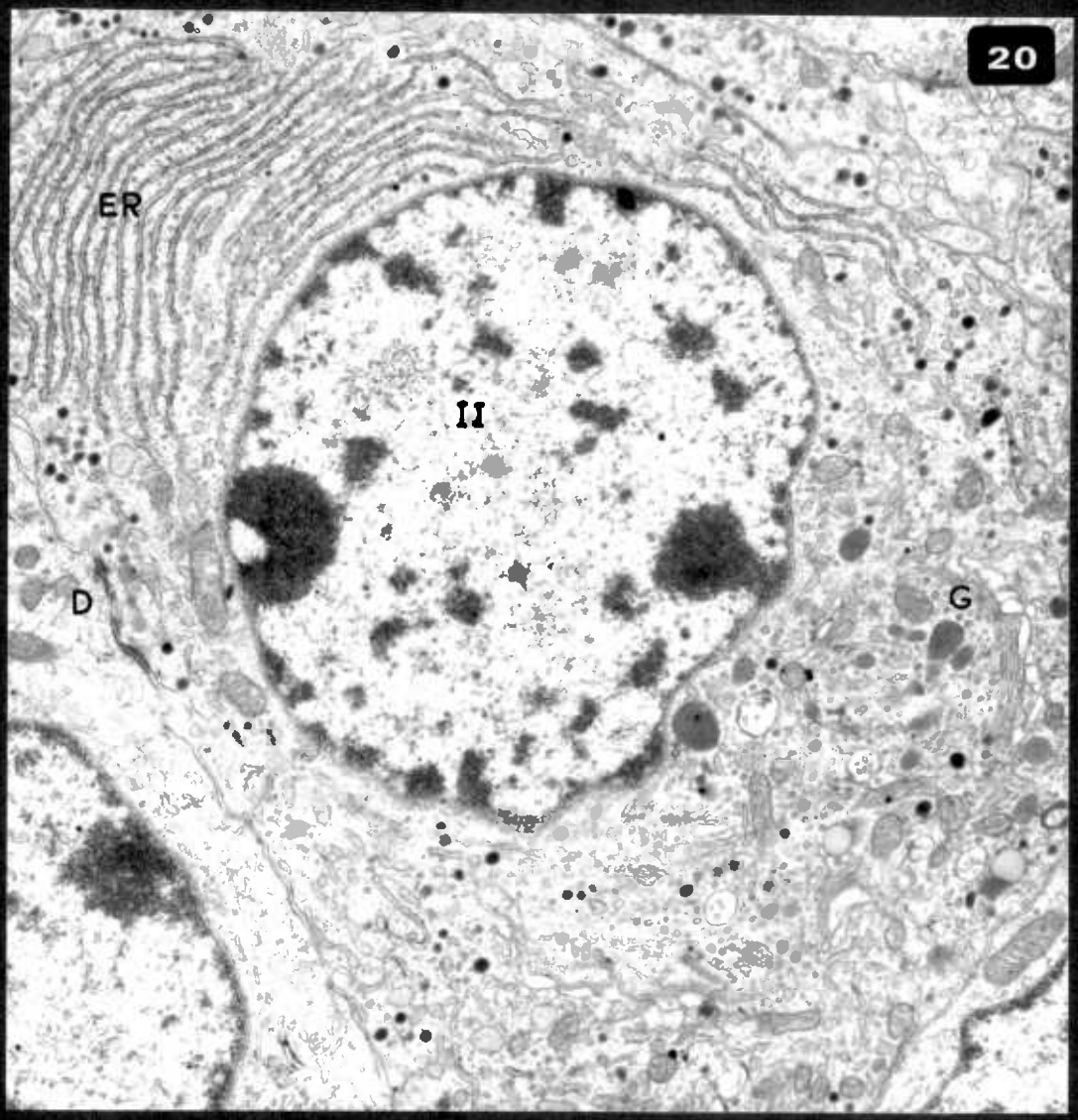
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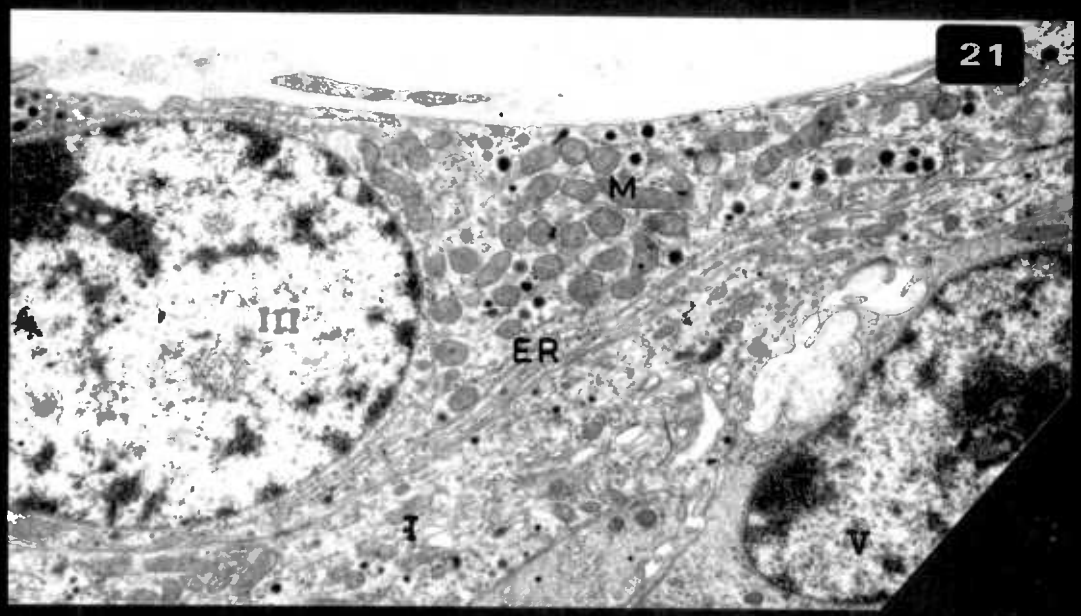
Figure 21. An elongated profile III cell showing rough endoplasmic reticulum (ER), much of which is near the plasma membrane, and abundant mitochondria (M). X10,000

Figure 22. Cell of profile III near a capillary (cap). Note the variable density of the substance within the membrane of some granules (*), the electron dense substance within one cisterna of the rough endoplasmic reticulum (d) and the morphology of some Golgi lamellae which appear to dilate into vesicles at their ends (dl). cv - coated vesicles; M - mitochondria; G - Golgi apparatus; ER - rough endoplasmic reticulum; c - collagen in the perivascular spaces; f - transverse view of endothelial fenestrations. X15,000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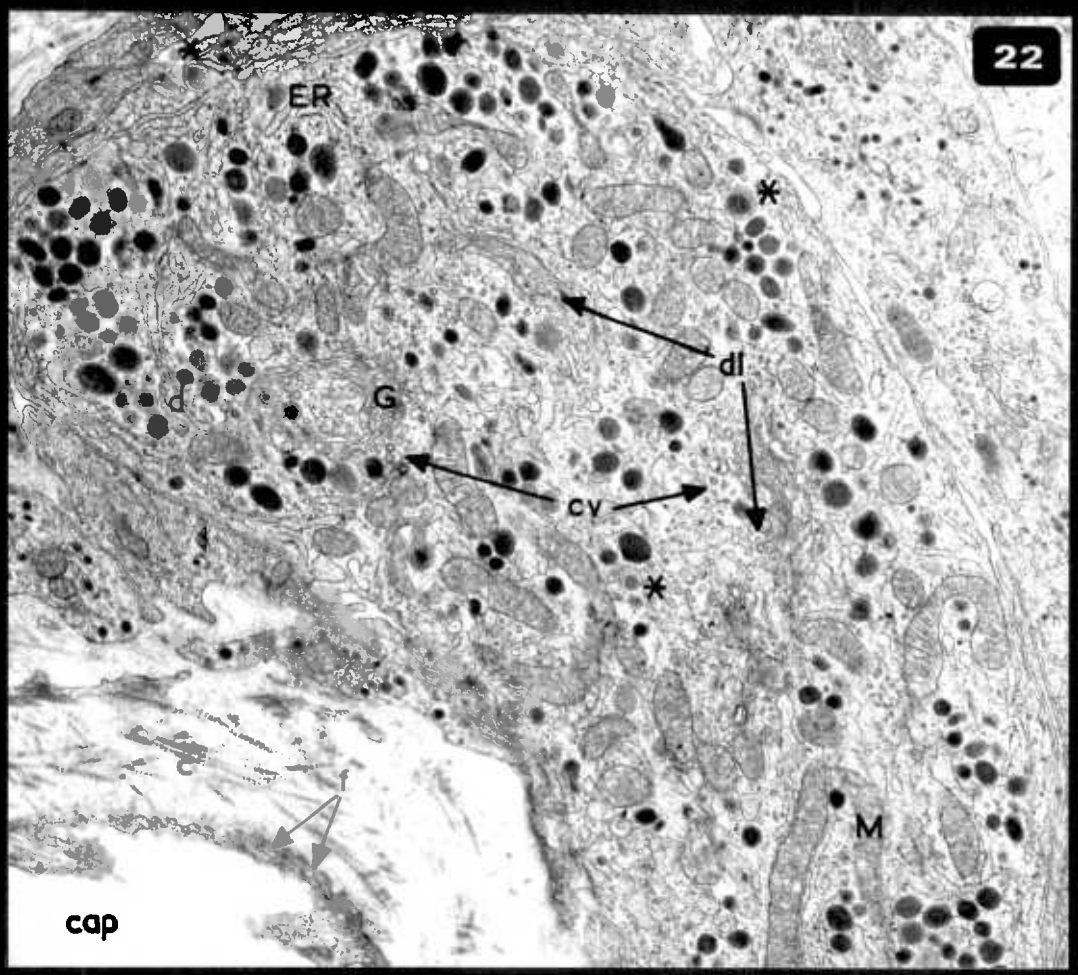
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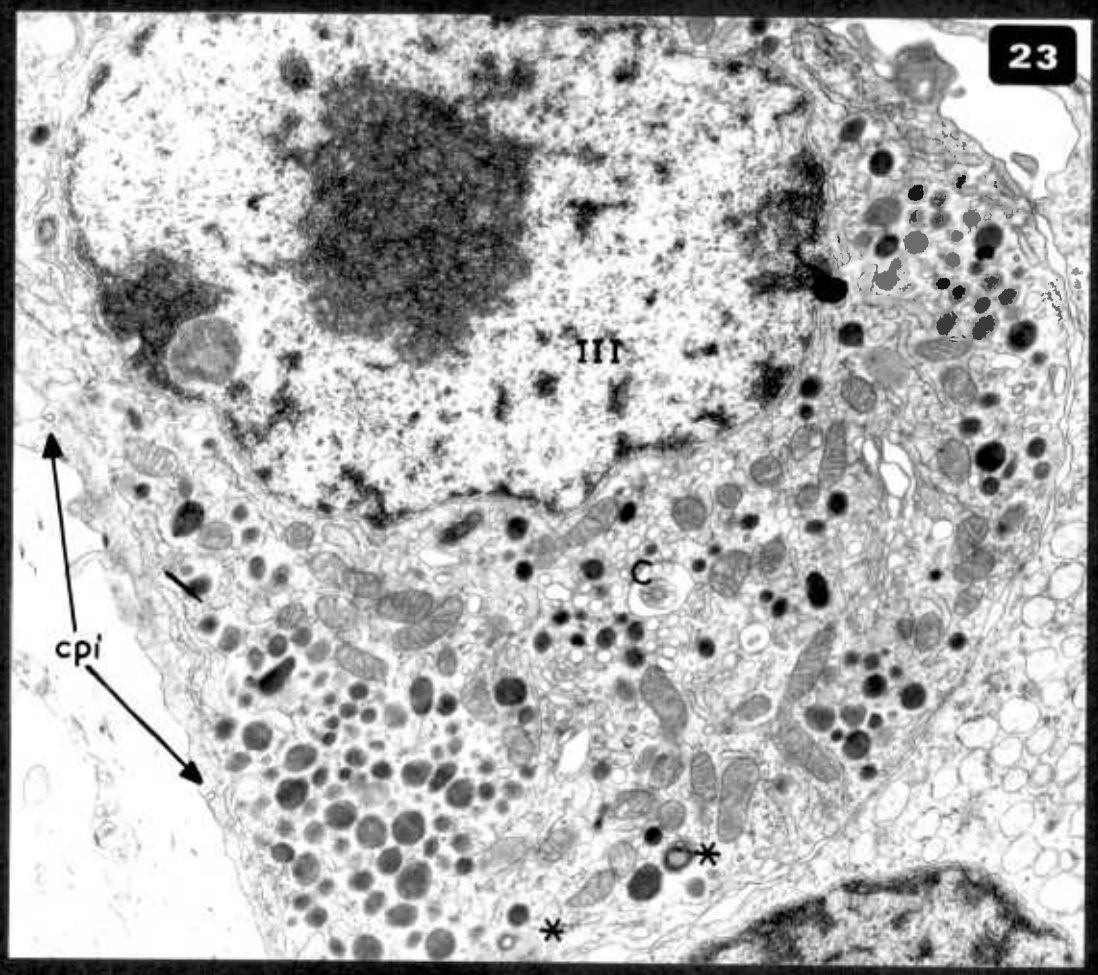
Figure 23. A cell of profile III from a female animal in diestrus. Note the coated pinocytotic-like invaginations (cpi) and the dense bodies containing myelin-like substances (*). C - transverse section of a cilium. X 15,000

Figures 24 and 25. Serial sections of a Profile III cell which appears to be liberating a myelin-like substance (arrows). Both at X15,000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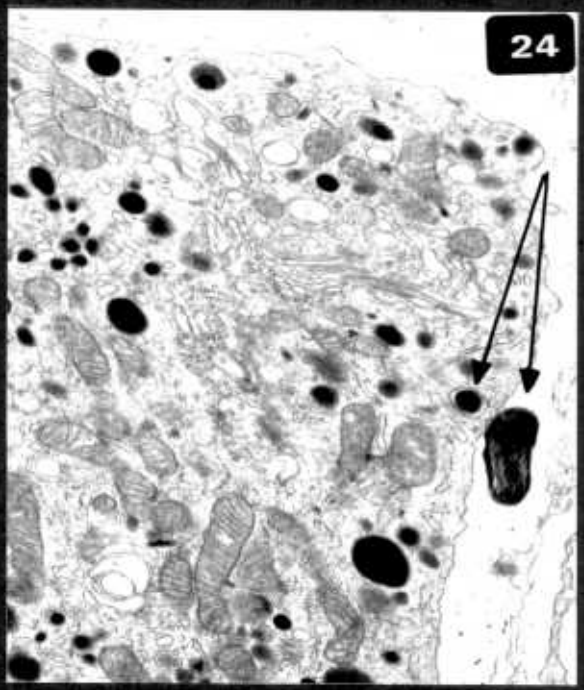
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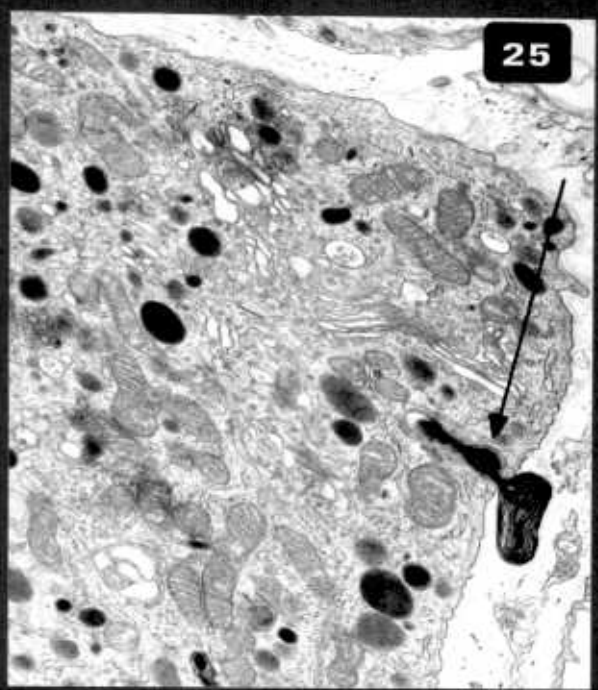
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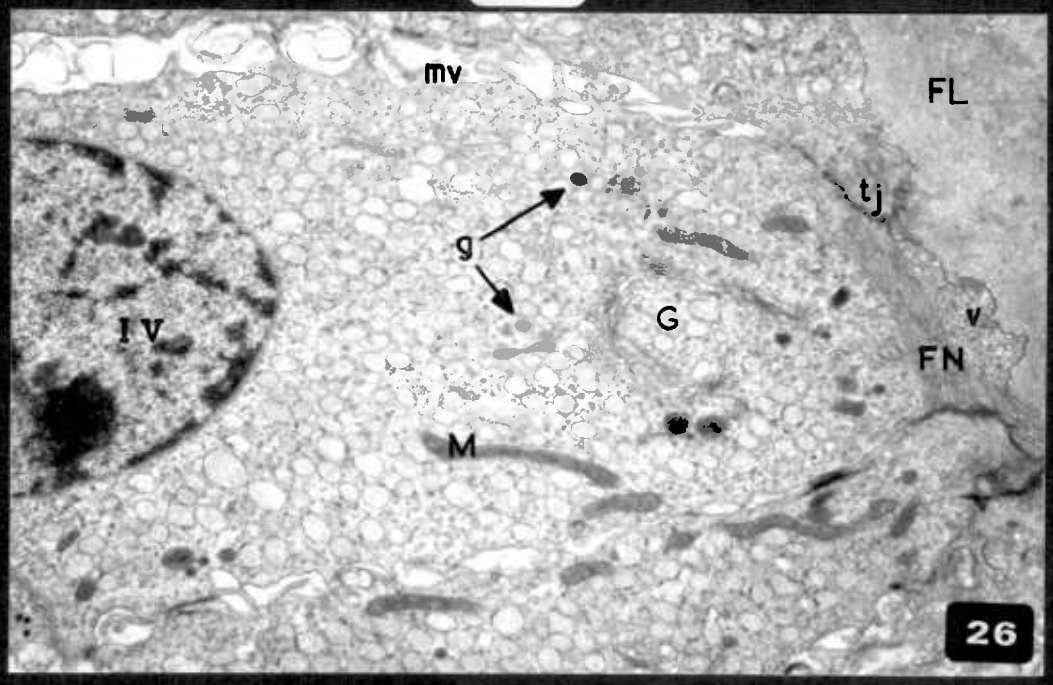
Figure 26. A typical cell of profile IV bordering on a follicular lumen (FL) filled with 'colloid'. The polarity of the cell is characterized by a basely located nucleus, a more central Golgi apparatus (G) and an apical pole containing a filamentous network (FN) extending between the periluminal tight junctions (tj). Also, small vesicles (v) are seen to predominate in the apical cytoplasm. Microvillous-like processes characterize the lateral plasma membrane (mv). Dilated rough endoplasmic reticulum (unlabelled) is seen to fill the cytoplasm. M - mitochondria; g - granules. X10,000

Figure 27. Higher magnification to show the granulated morphology of two cytoplasmic granules (g) seen in a cell of profile IV and the filaments associated with a desmosome (D) joining two cells of profile IV . M - mitochondria; FL - follicular lumen; ER - rough endoplasmic reticulum. X60,000

Figure 28. Perivascular region of a profile IV cell which appears to contain vesicles attached to or penetrating through the plasmalemma (*). ER - rough endoplasmic reticulum; g - granule; bm - basement membrane. X60,000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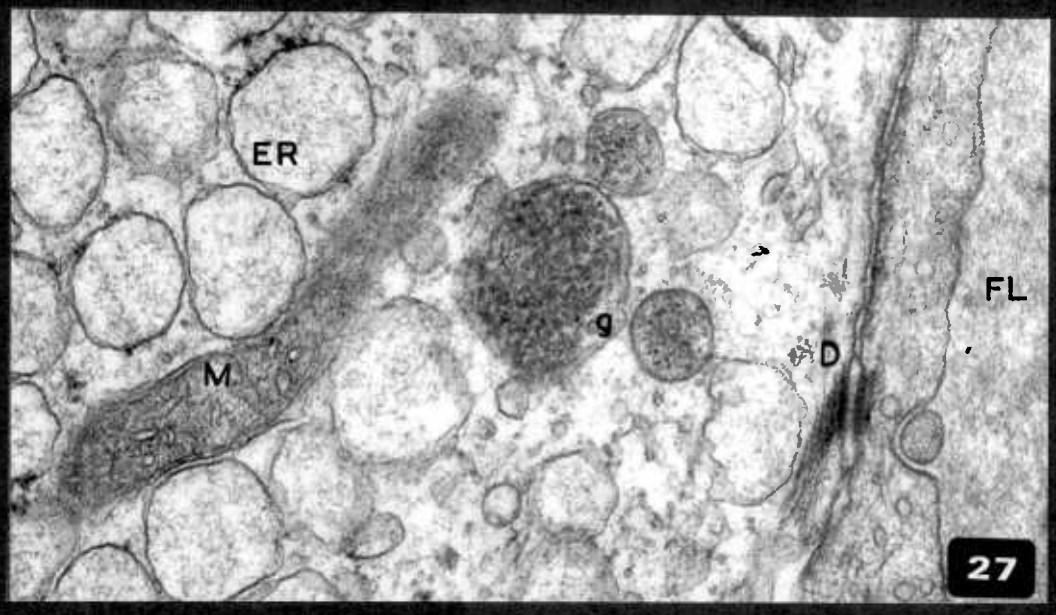
FL
tj
g
G
v
IV
FN
M
26


ER
FL
g
M
D
27

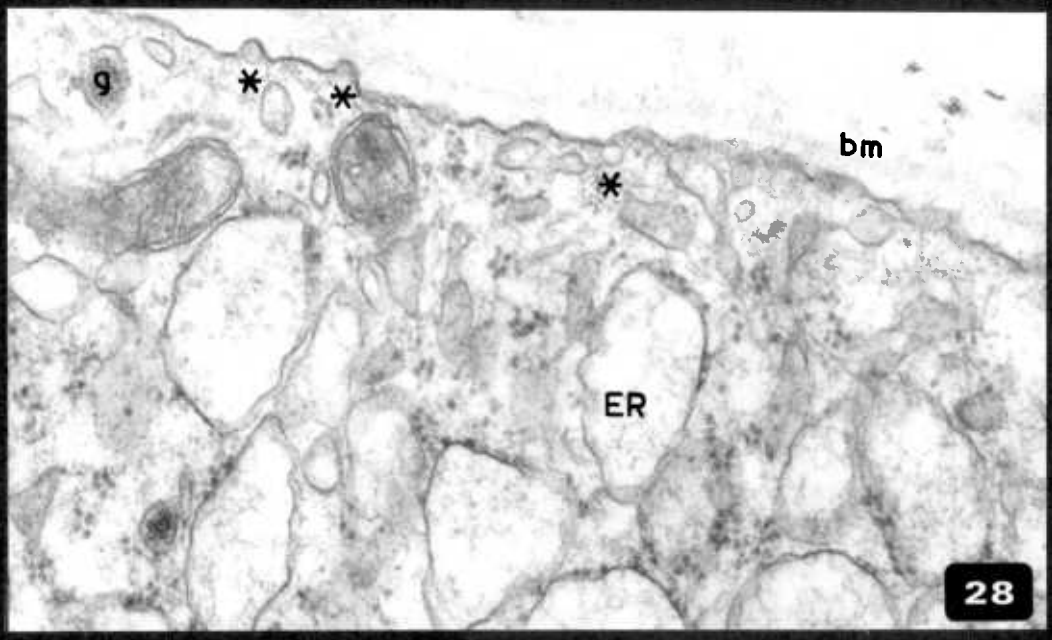
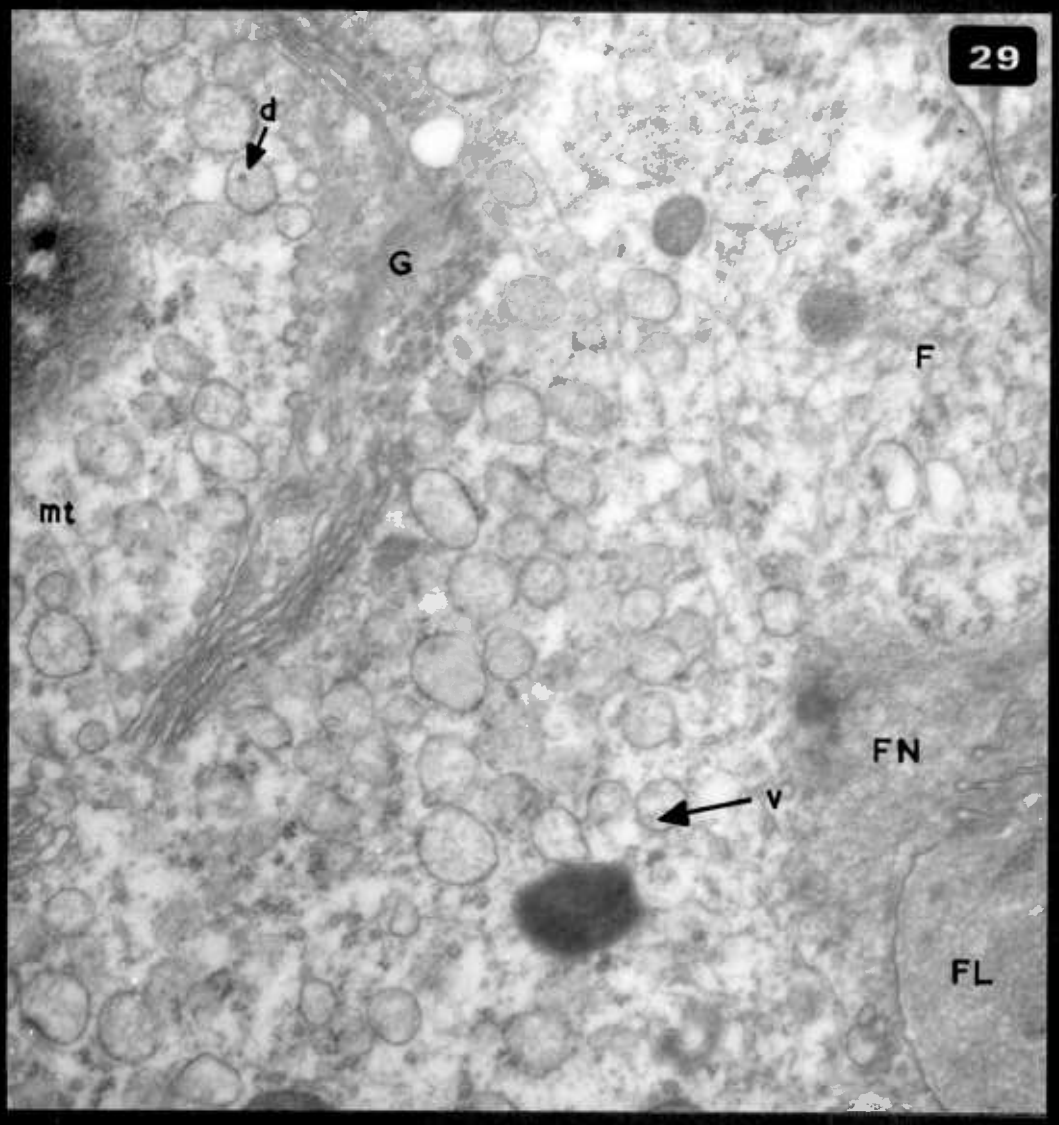

g
bm
ER
28

Figure 29. The cytoplasm of a profile IV cell. Note the presence of a dense particle (d) and a vesicle (v) in the cisternae of the endoplasmic reticulum. mt - microtubules; FN - filamentous network; FL - follicular lumen; F cytoplasmic filaments; G - Golgi apparatus. X30,000

Figure 30. A profile IV cell. Many ribosomes (r) are seen to be attached to the endoplasmic reticulum. The mitochondria (M) are moderately electron dense and the Golgi lamellae again appear to dilate into vesicles at their ends (arrow). X10,000


29
d
G
F
mt
FN
v
FL

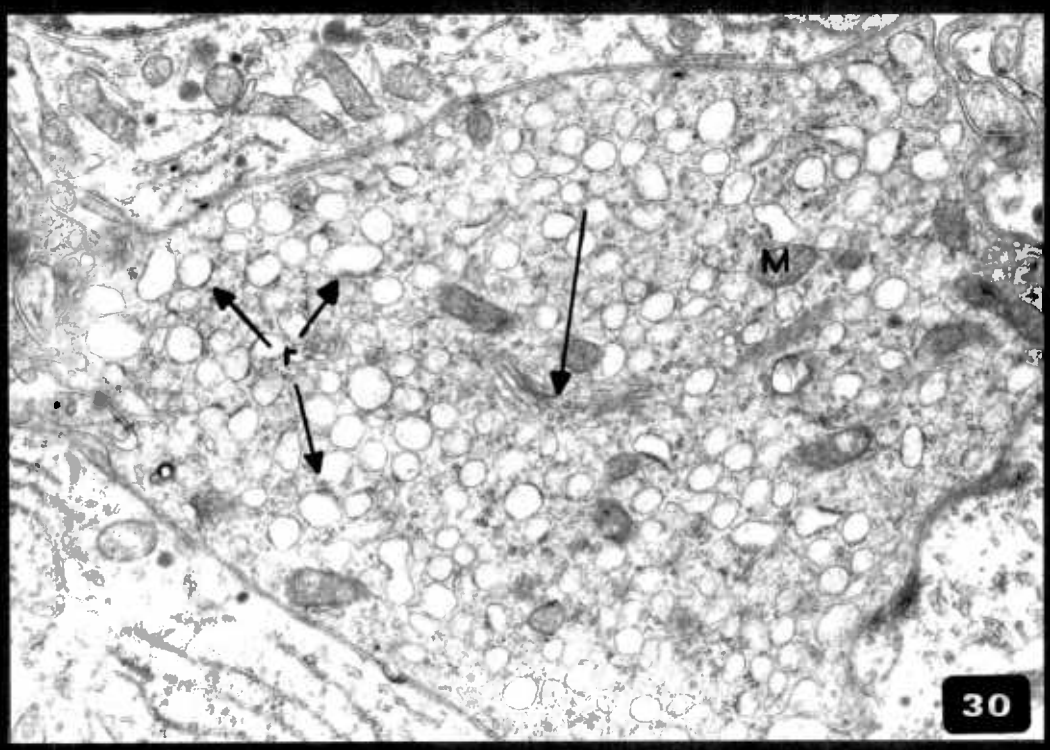
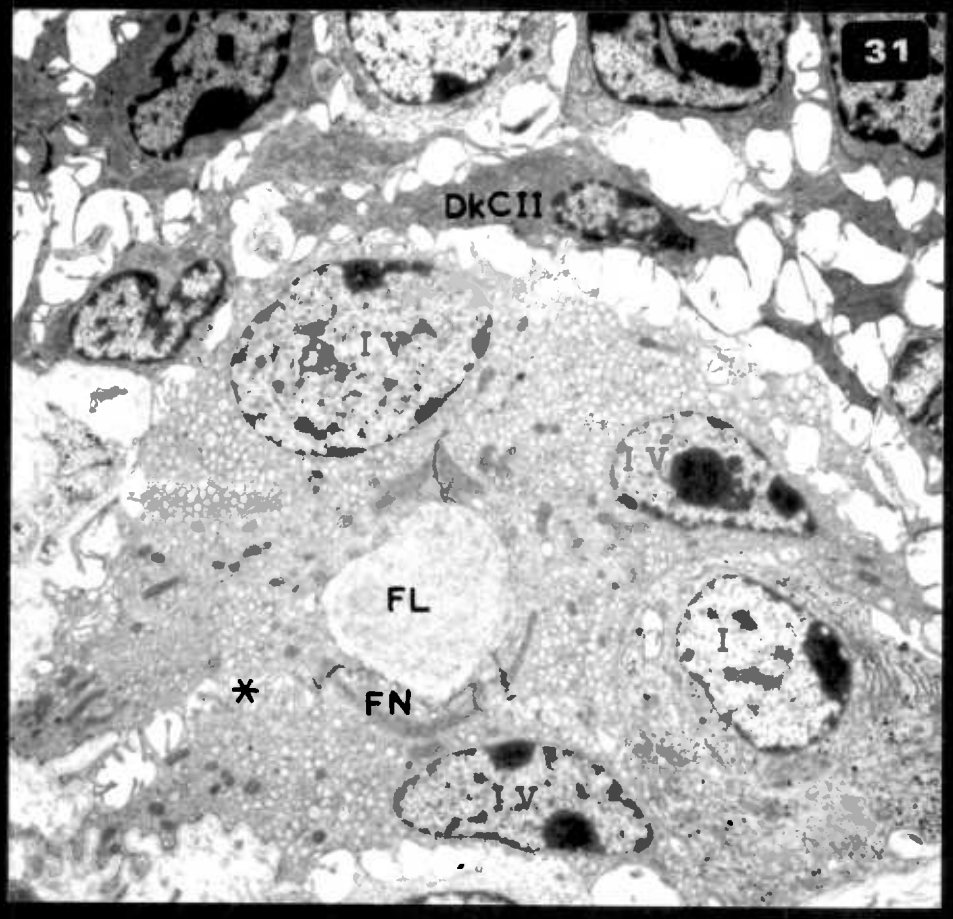
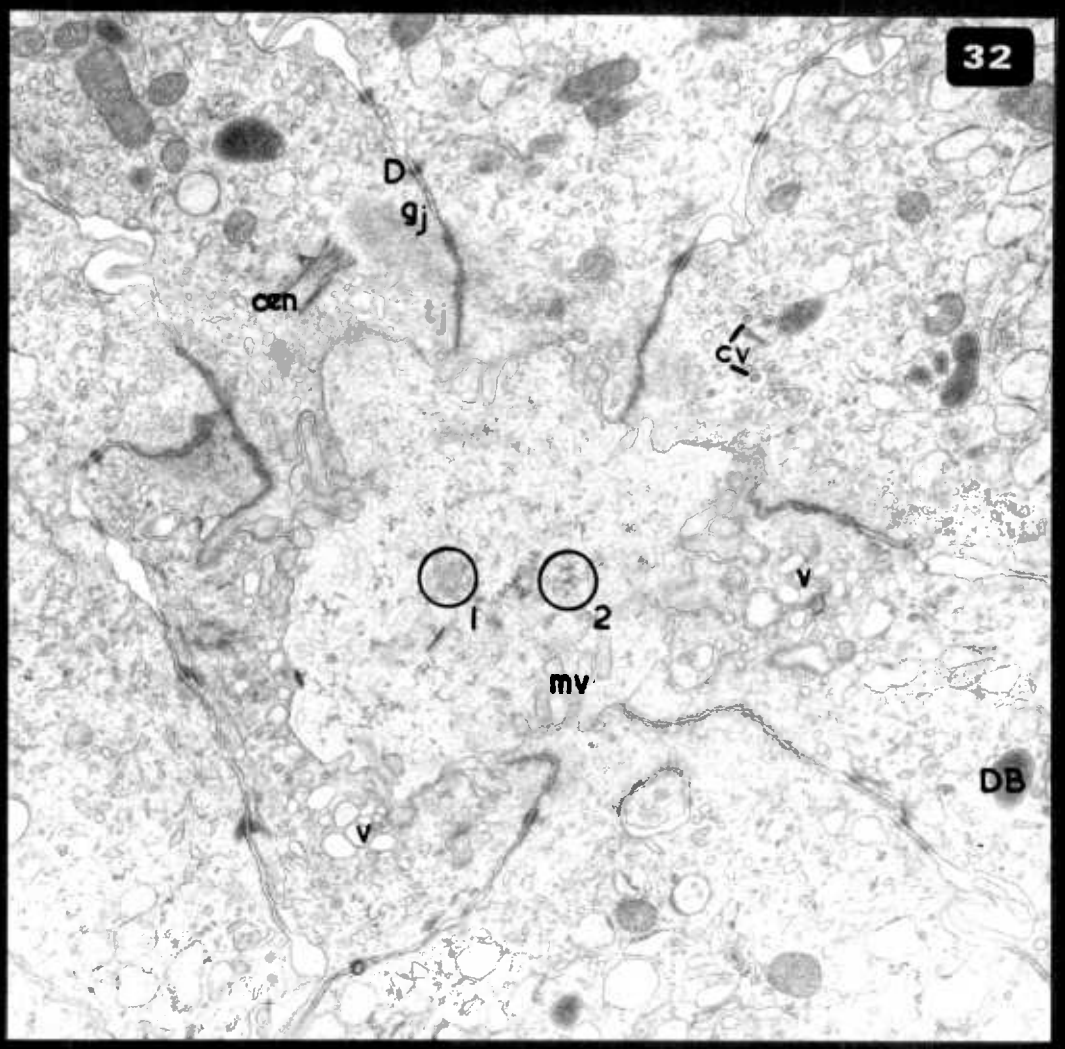

M
r
30

Figure 31. A follicle surrounded by profile IV cells. Note that a nearby profile I cell does not come into contact with the follicular lumen (FL). Dark cells (Dk CII) of the type frequently seen on the external surface of cysts are seen here in contact with cells of profile IV. * - marks the interdigitation of villous structures of the adjacent plasma membranes. FN - filamentous network. X6,000

Figure 32. Higher magnification of a 'colloid'-filled follicular lumen and the apical cytoplasm of bordering profile IV cells. The 'colloid' in this follicle is heterogeneous, circles 1 and 2 enclosing morphologically different particulate substances. Junctional complexes can be seen between adjacent cells at the apical pole (tj - tight junction, gp - gap junction, D - desmosome). Note the dense bodies (DB) and coated vesicles (cv), larger cytoplasmic vesicles (v), microvilli (mv) and longitudinal view of a centriole (cen). X20,000


31
DkCII
IV
IV
FL
I
*
FN
IV


32
D
gj
cen
cv
1
2
v
mv
DB
v

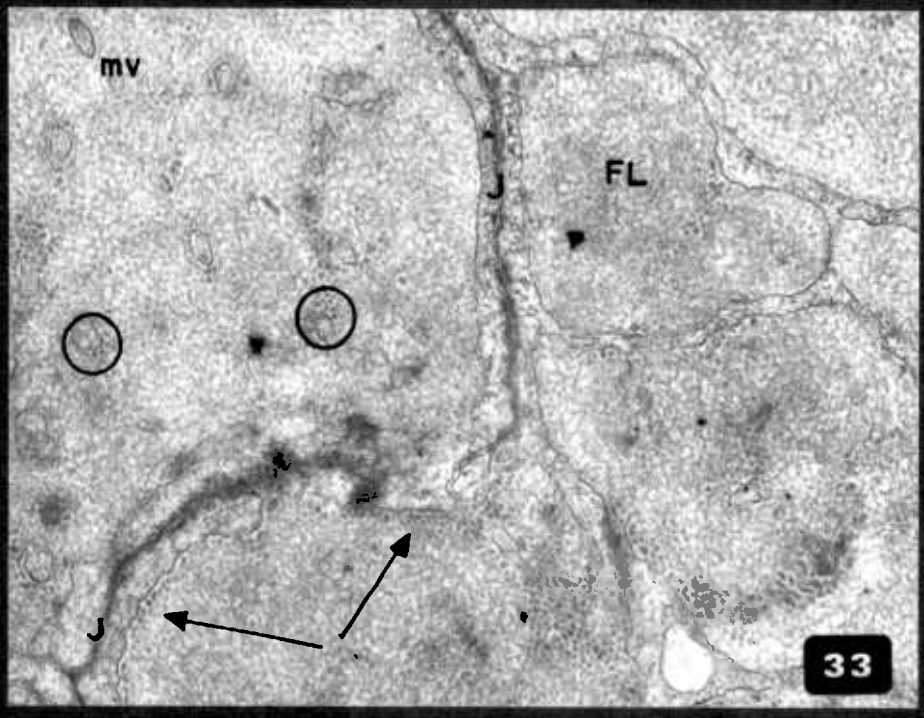
Figures 33, 34 and 35. 'Colloid'-filled follicular lumena.

(Fig. 33. Routine fixation, X30,000)
(Fig. 34. Fixed in potassium permanganate, X30,000)
(Fig. 35. Fixed in potassium permanganate, X27,500)

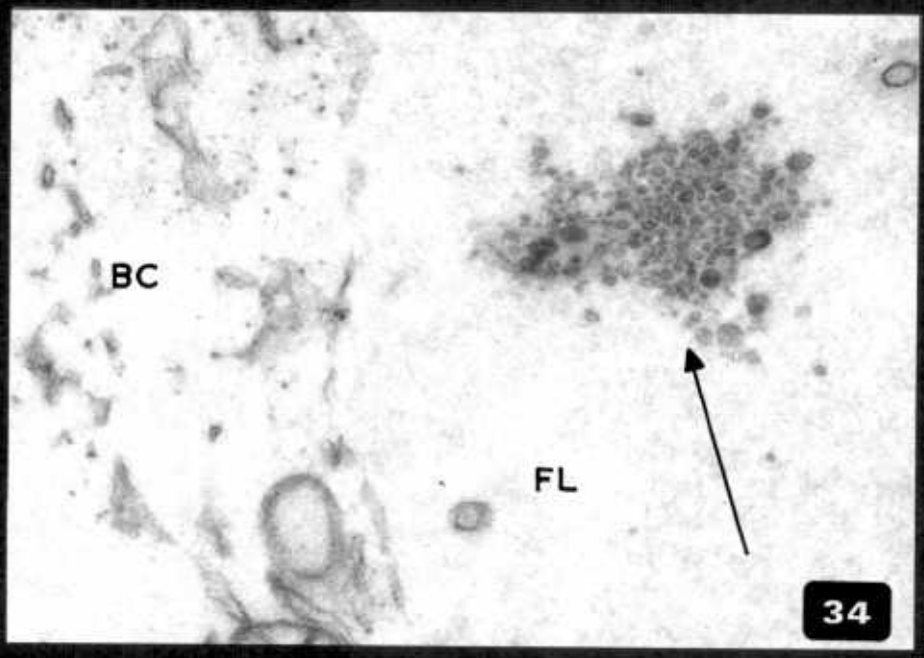
Figure 33. Note the dense particulate substances (arrows) which tend to line up next to the plasmalemma of bordering cells or aggregate near microvilli (circled). mv - microvilli in cross section; FL - follicular lumen; J - junctional complex.

Figure 34. Note aggregation of vesicles (arrow) in the follicular lumen (FL). BC - bordering cell.

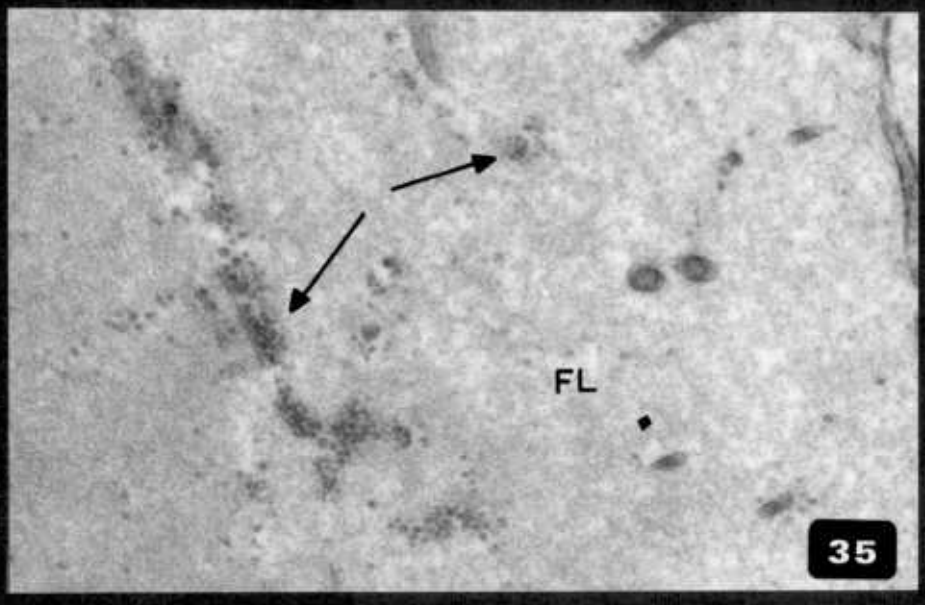
Figure 35. Note dense particles (arrows) in the follicular lumen (FL) which could possibly be membrane substances.



33



34

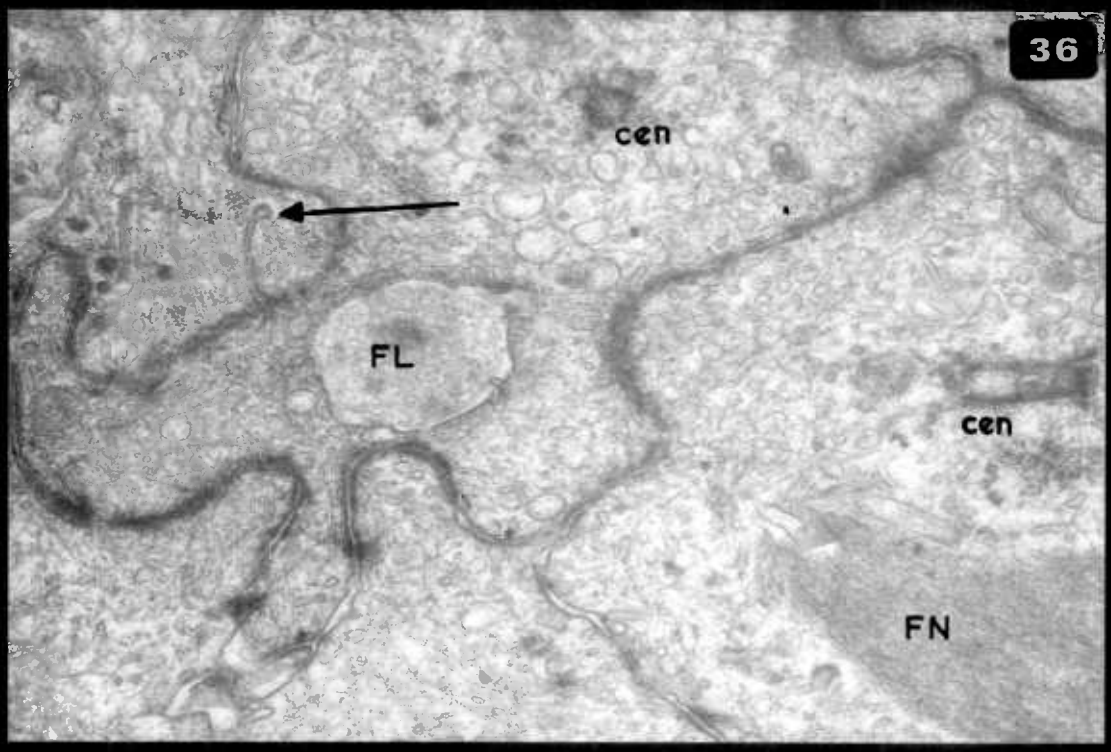


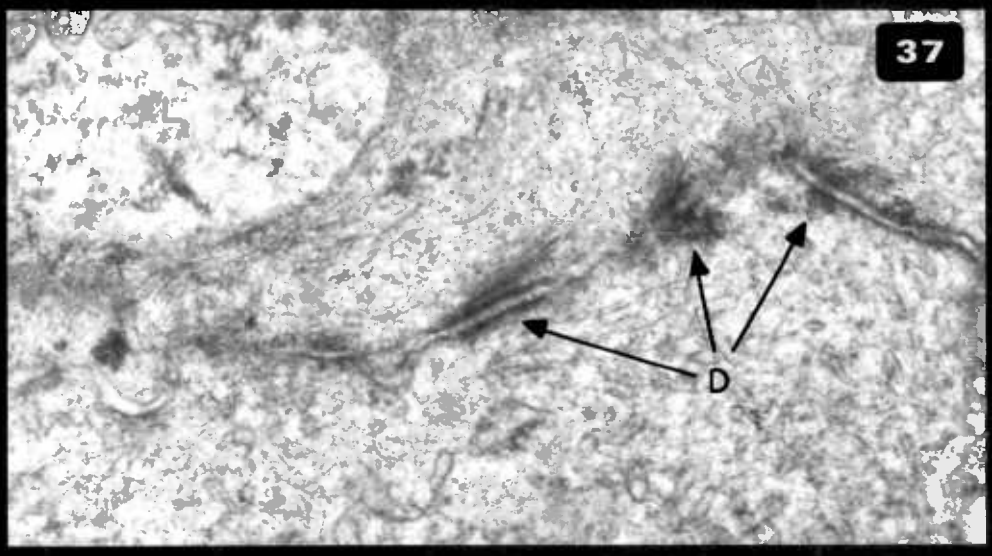
35

Figure 36. A possible projection of a follicular lumen (FL) into the cytoplasm of a cell. FN - filamentous network; cen - centriole. Arrow points to coated dilation at the end of what appears to be a cisterna in a neighboring granulated cell. X30,000

Figure 37. Series of desmosomes connecting two follicular cells to which filaments appear to be attached. The follicular lumen contains dense particulate substances. X20,000

Figure 38. A transverse cut through a centriole (cen). Nine sets of tubules in triplicate are easily seen. X60,000


36
cen
FL
cen
FN


37
D

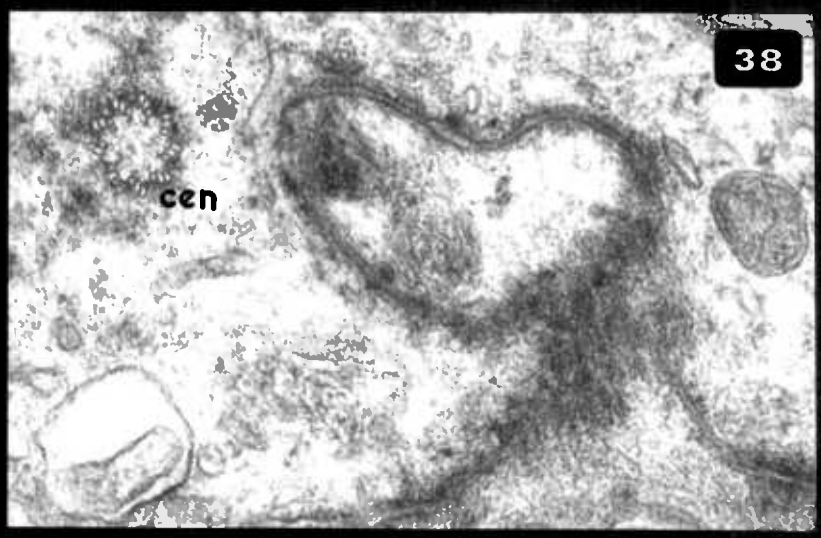

38
cen

Figure 39. A profile V cell identified by its abundant content of filaments. The short scattered rough endoplasmic reticulum (ER), Golgi apparatus (G) and a few dense mitochondria (M) are typical of this type of cell. X10,000

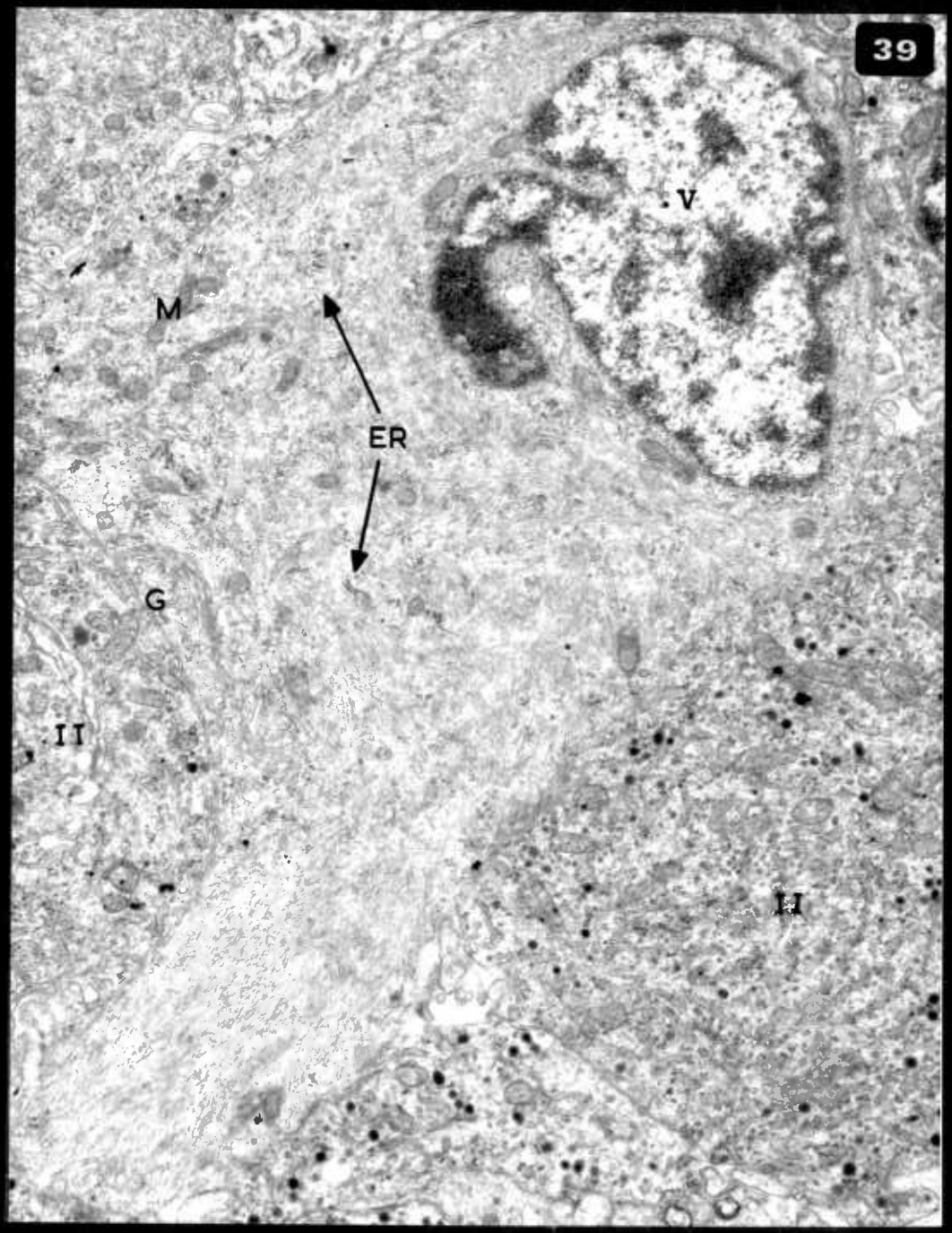
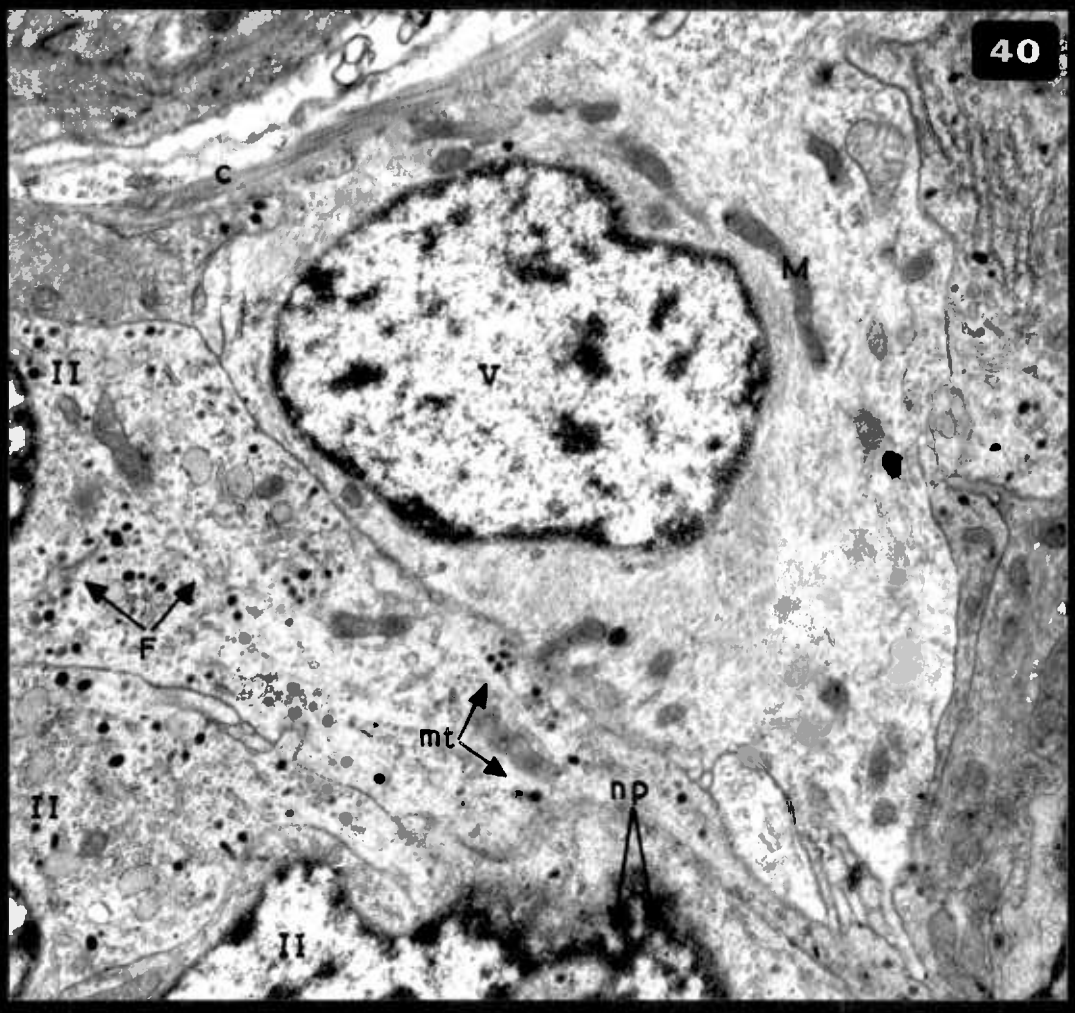

39
V
M
ER
G
II
II

Figure 40. A profile V cell in which the cytoplasmic filaments are seen to be more abundant nearer the nucleus. Several adjacent profile II cells can be seen in one of which cytoplasmic filaments (F) are observed. C - collagen; mt - microtubules; np - nuclear pores; M - mitochondria. X15,000

Figure 41. Region of contact between three adjacent profile V cells. Desmosomes (D) and numerous microvillous-like extensions (mv) are seen. Also note disruptions of the cytoplasm which appear as though some substance may have been extracted (circled). M - mitochondria. X30,000

Figure 42. High magnification of cytoplasmic intermediate-sized filaments from a cell of profile V. Note the tubular morphology of a filament cut in cross section (arrow) and what appears to be radiating spikes from the electron dense wall of another filament cut transversly (circled). X165,000


40
C
M
V
II
F
mt
np
II
II

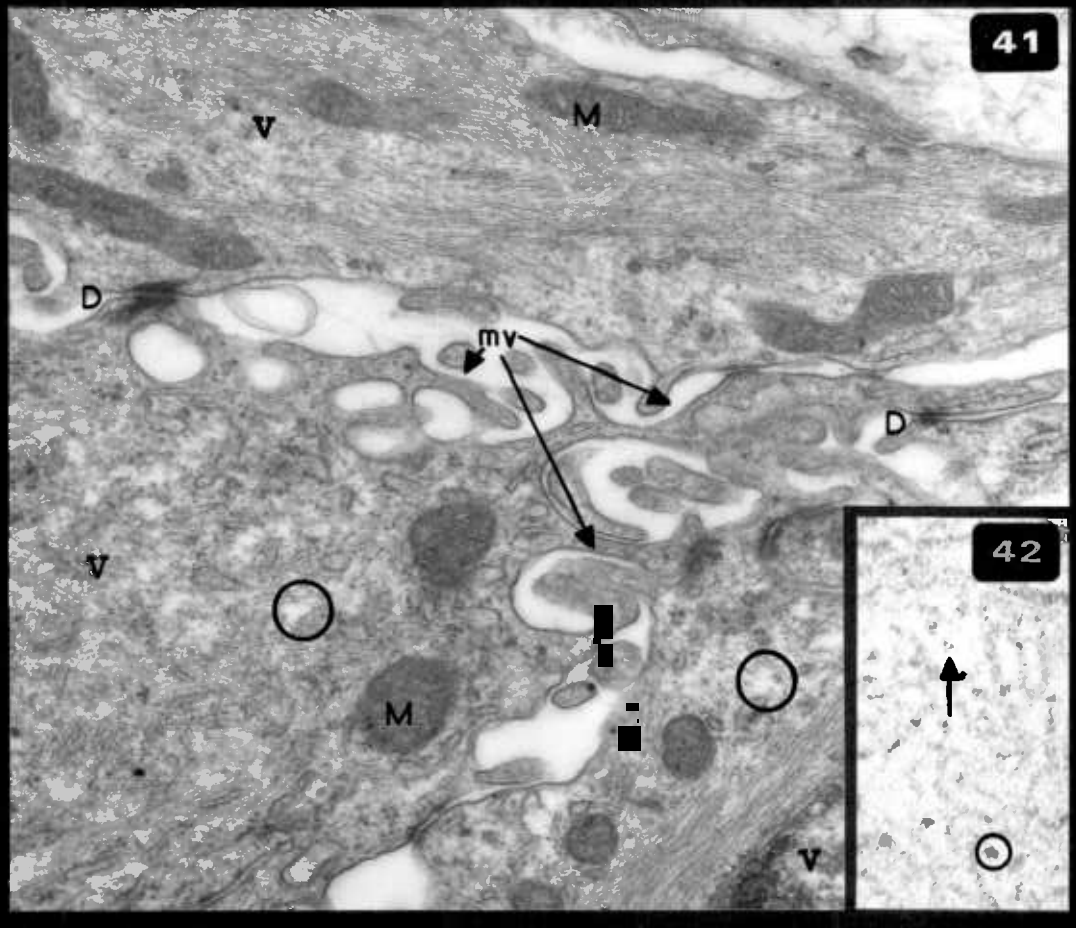
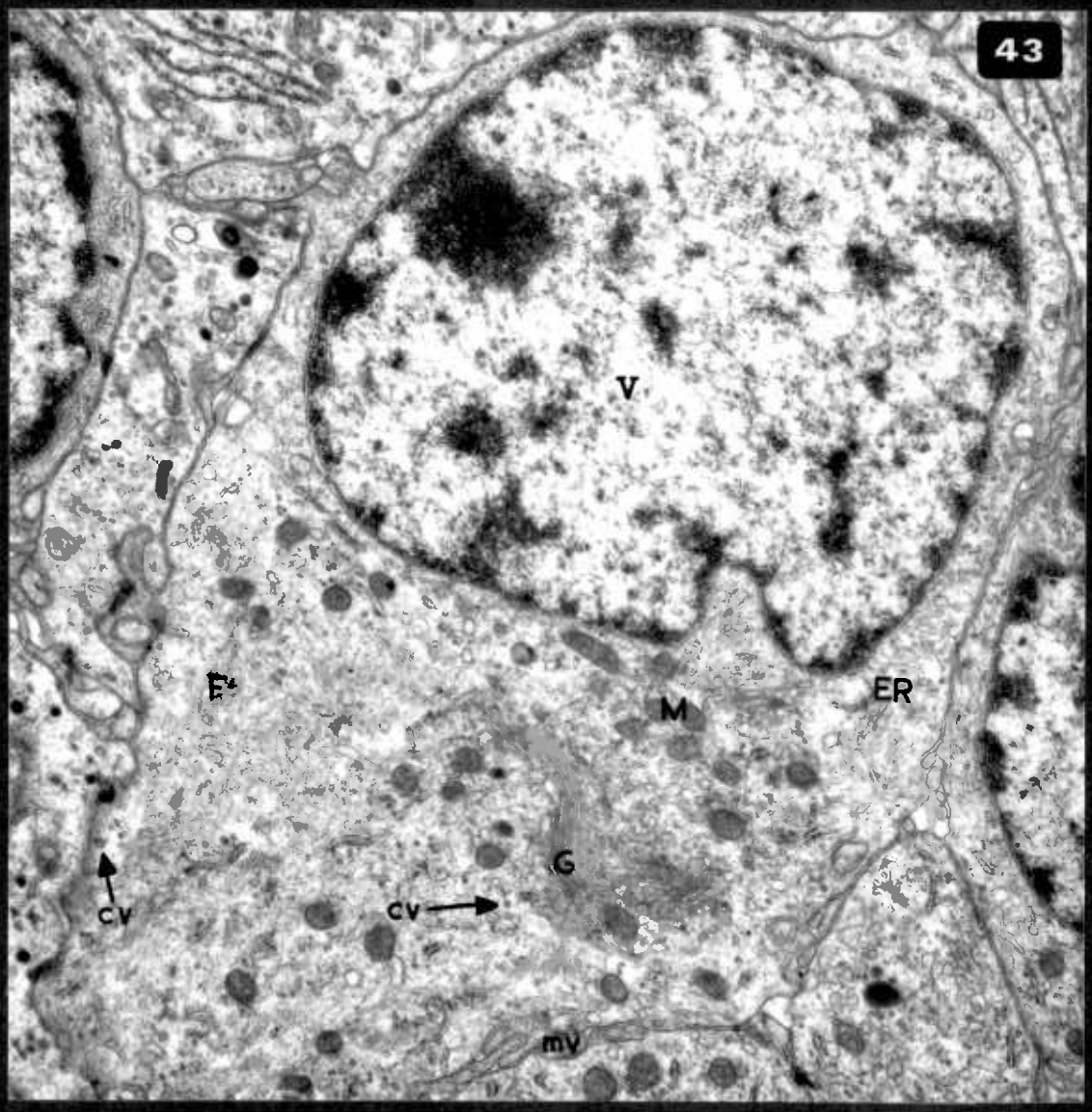

41
V
M
D
mv
D
V
M
V
42

Figure 43. A profile V cell in which filaments (F) are less abundant than usual and in which there is a perinuclear Golgi apparatus (G). ER - rough endoplasmic reticulum; cv - coated vesicles; mv - microvillous-like processes; M - mitochondria. X15,000

Figure 44. A follicle bordered in this section by two cells of profile V. FL - follicular lumen. X6,000


43
V
F
M
ER
G
cv
cv
mv

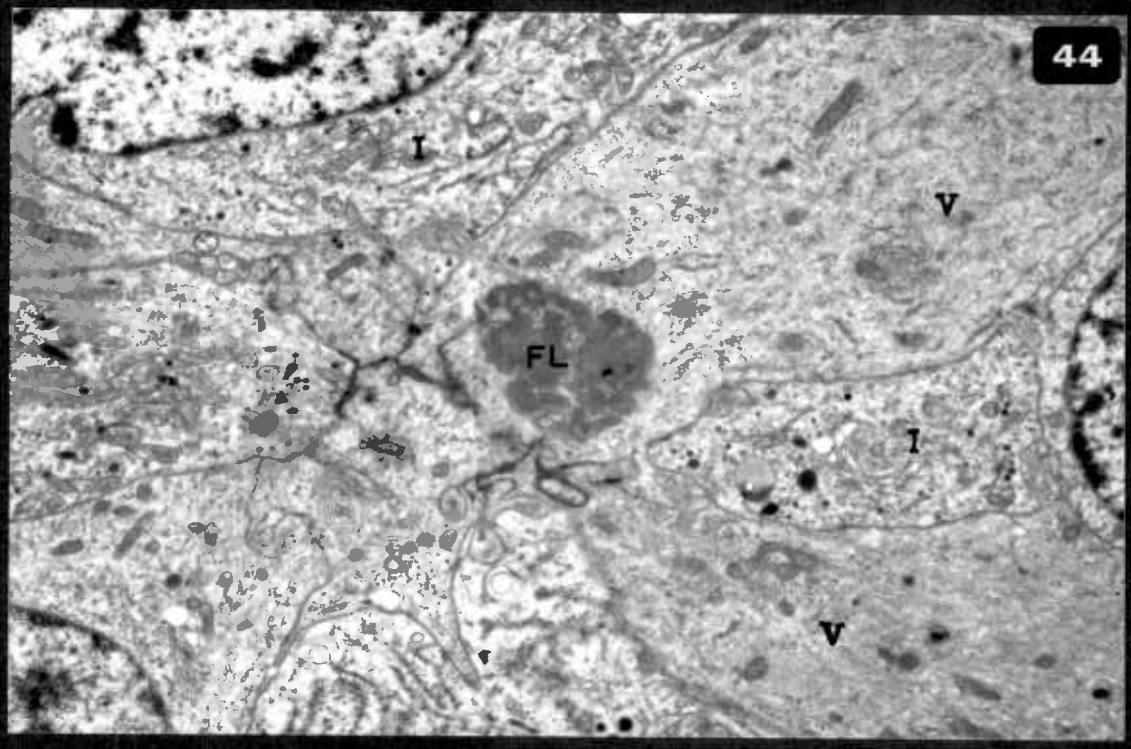
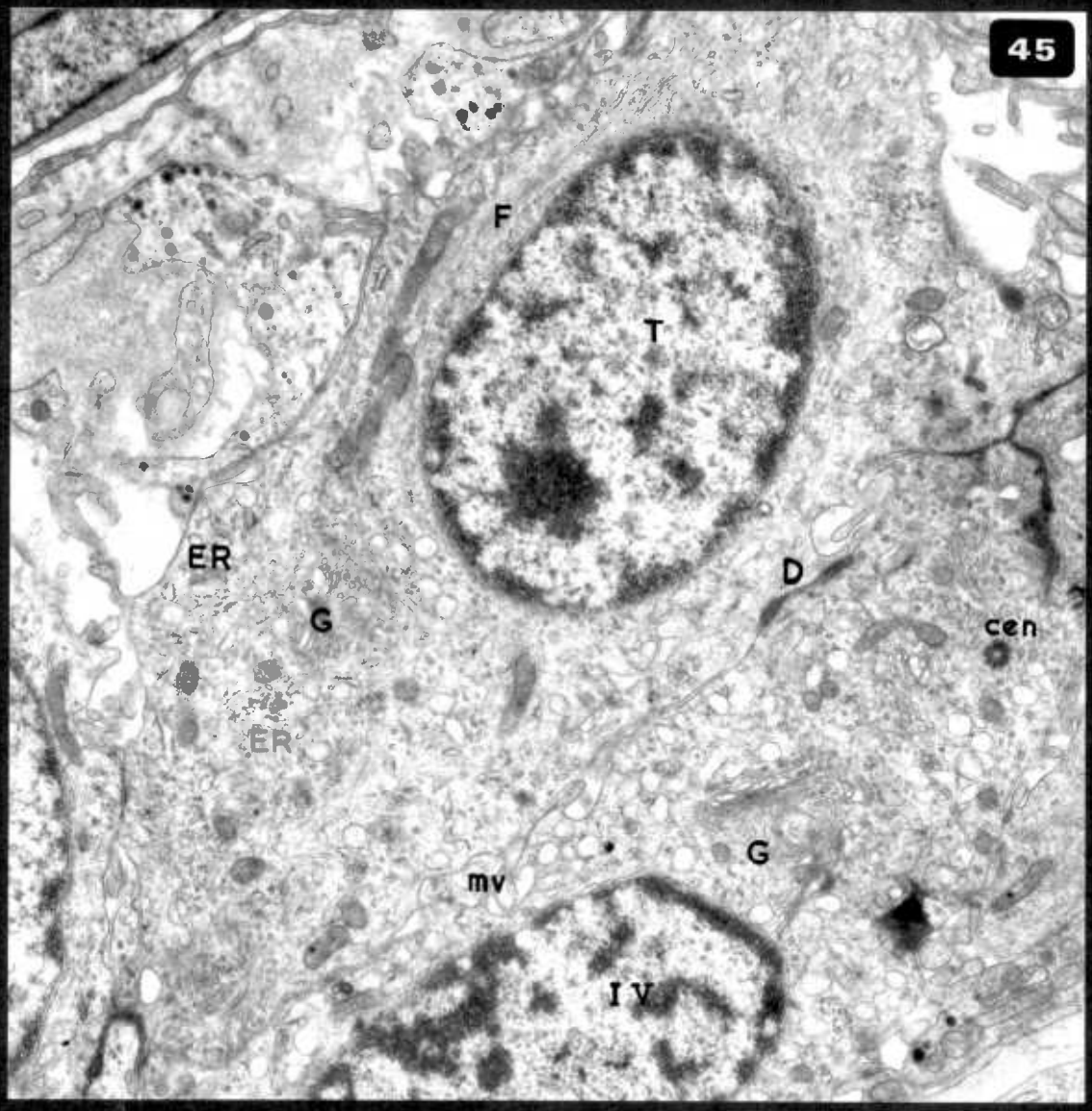

44
I
V
FL
I
V

Figure 45. A transitional cell profile (T) in which both filaments (F) and dilated rough endoplasmic reticulum (ER) can be seen in the cytoplasm. Part of an adjacent profile IV cell is also seen. cen - centriole: G - Golgi apparatus; D - desmosome; mv - microvillous-like extensions. X15,000

Figure 46. Another profile of a transitional cell (T) which is adjacent to a fenestrated capillary (arrows). ER - rough endoplasmic reticulum; M - mitochondria; np - nuclear pores; F - filaments; bm - basement membrane. X15,000


45
F
T
ER
G
D
cen
ER
mv
G
IV

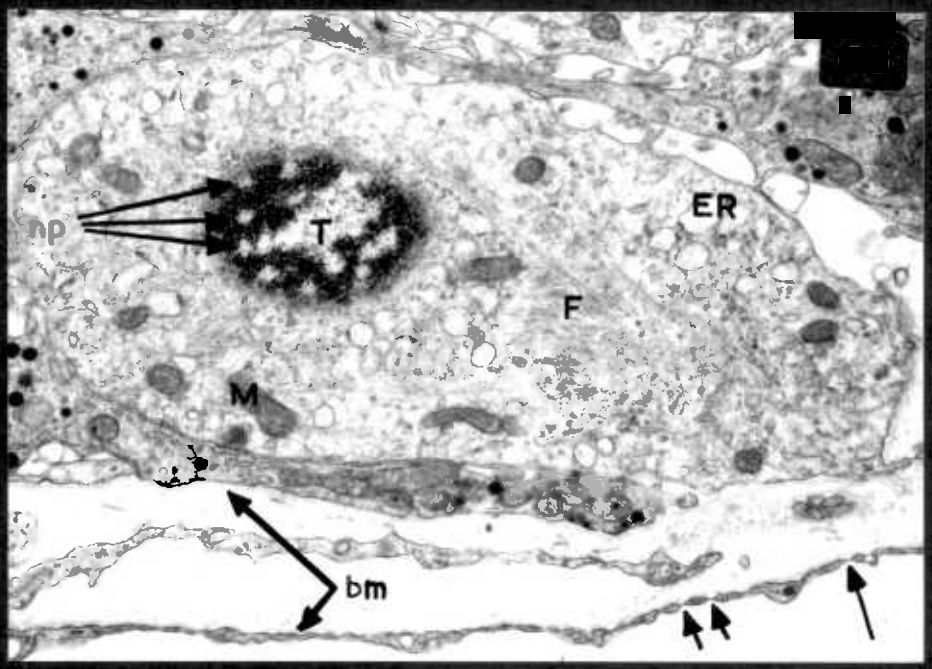
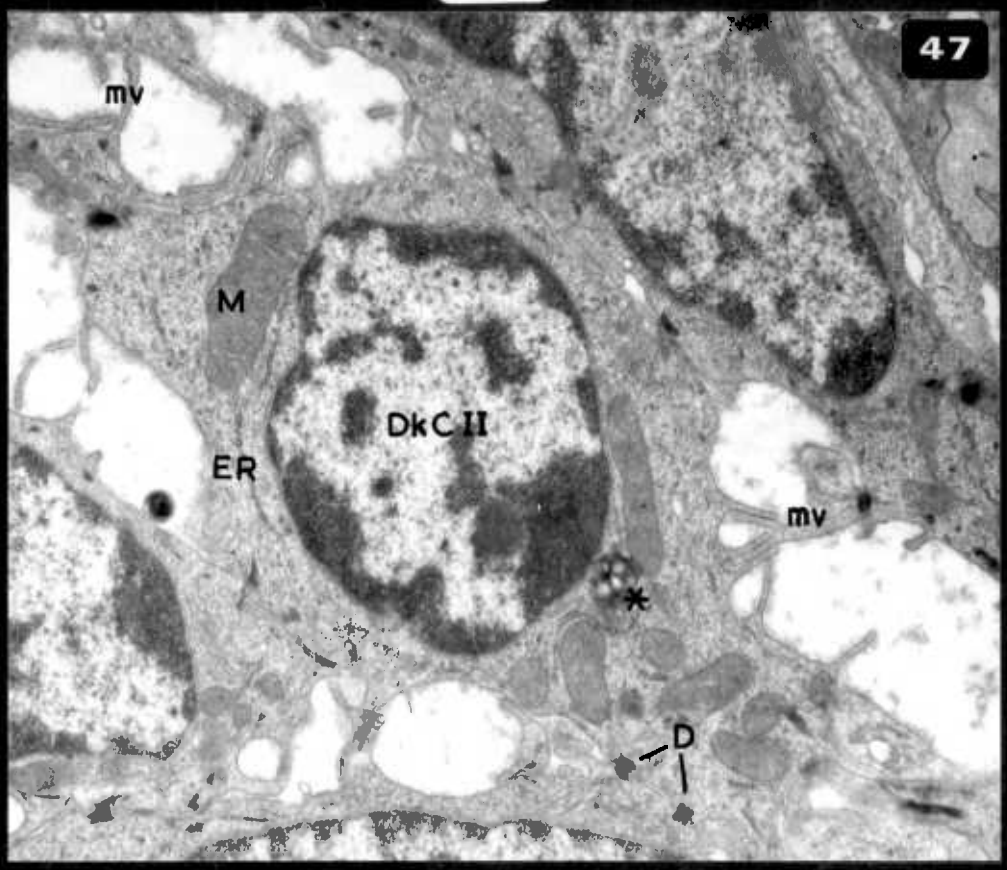

np
T
ER
F
M
bm

Figure 47. Dark cell II (Dk CII) seen in the external region of a cyst and showing typical features of this type of cell: numerous large mitochondria (M), desmosomal contacts (D) with adjacent cells, a few long, flat cisternae of rough endoplasmic reticulum (ER), a lipofuscin-like inclusion (*) and microvilli extending from the plasmalemma (mv). Broad intercellular spaces typically are present between these cells. X15,000

Figure 48. A cell containing abundant intermediate-sized filaments bordering on the lumen of a cyst. This cell appears much like a profile V cell except that here a definite cellular polarity is evident with the nucleus located at the base of the cell, the Golgi apparatus in a perinuclear position and many small vesicles and microvilli (mv) at the apical pole. This cell is bordered by dark cells (Dk CI and Dk CII). ER - rough endoplasmic reticulum; tj - tight junction; LC - lumen of the cyst; M - mitochondria; db - dark bodies. X15,000


47
mv
M
DkC II
ER
mv
*
D

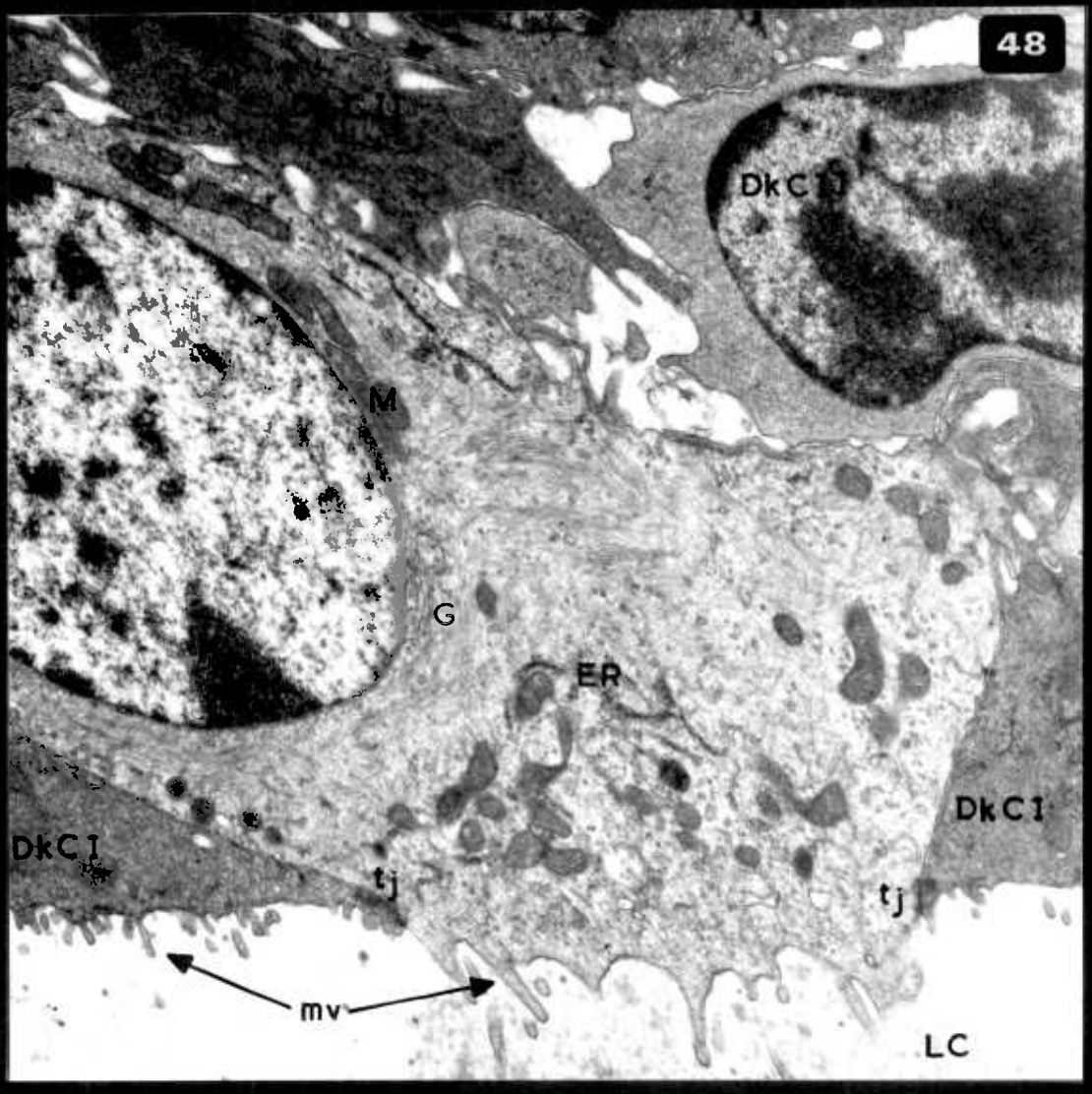
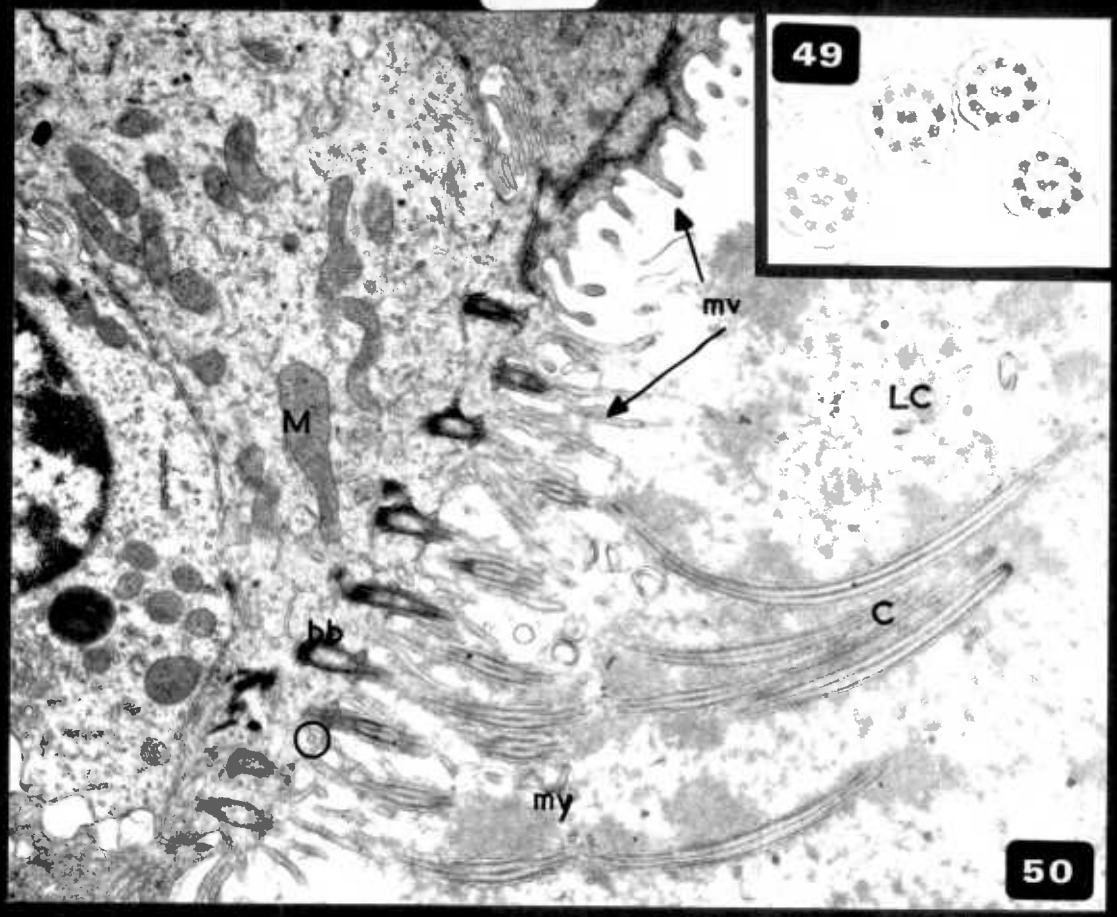

48
DkC I
M
G
ER
DkC I
DkC I
tj
tj
mv
LC

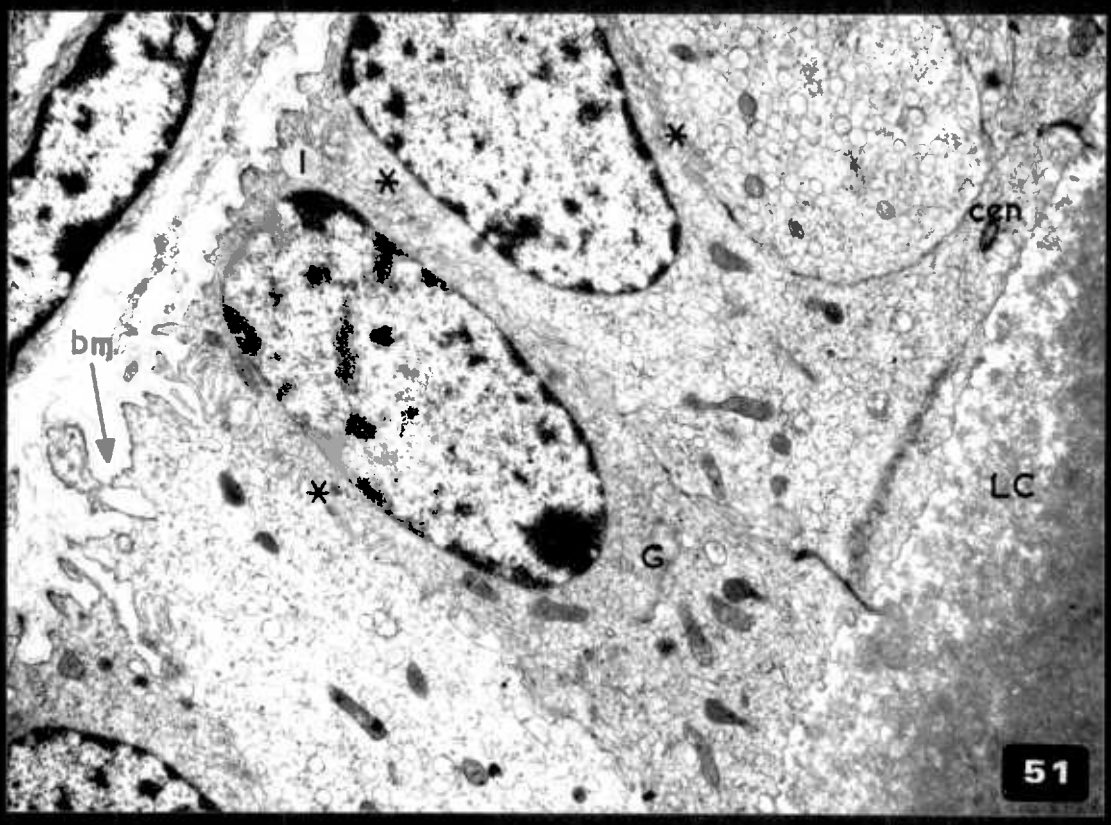
Figure 49. A transverse view of four cilia extending into the lumen of a cyst. These appear to have a 9+2 tubular formation. X30,000

Figure 50. A multiciliated epithelial cell bordering on the lumen of a cyst (LC). A coated vesicle is circled. C - cilia; my - myelin-like substance; mv - microvilli; bb - basal body. X15,000

Figure 51. Cells of profile IV bordering on the lumen of a cyst (LC). bm - basement membrane; G - Golgi apparatus; cen - centriole; l - lipid-like body; * - interdigitating microvillous-like extensions of adjacent plasma membranes. X10,000

Figure 52. An enlargement of the apical plasma membrane of several cells in the previous photograph. Note the pinocytotic-like profile (arrow) and the small vesicles closely associated with either side of the plasmalemma (*). tj - tight junction; FN - filamentous network. X25,000


49
mv
LC
M
C
bb
my
50


l
cen
bm
LC
G
51

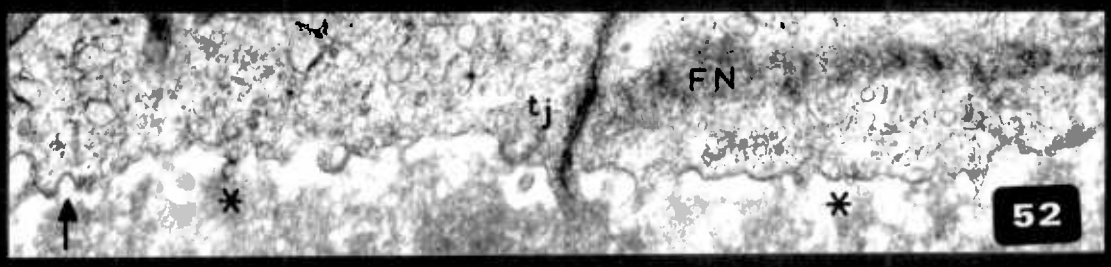
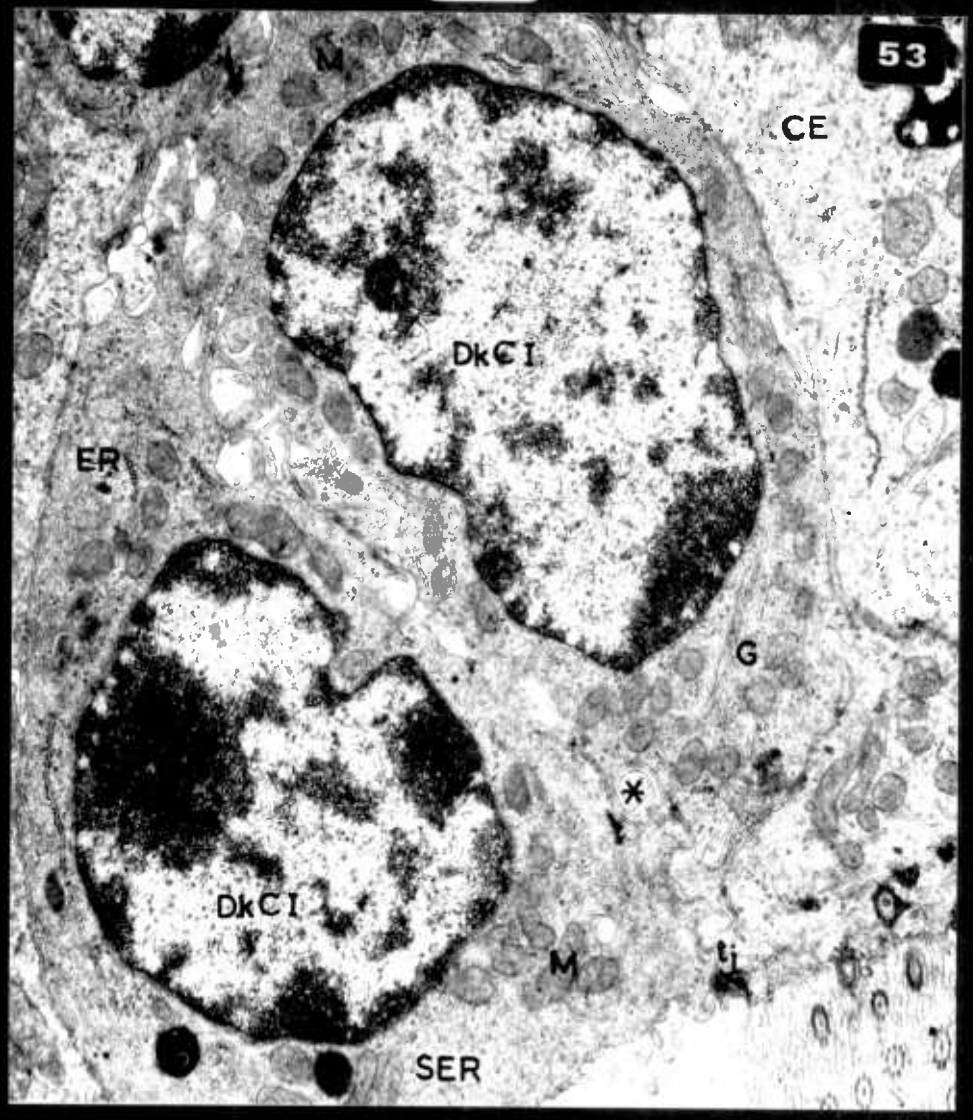
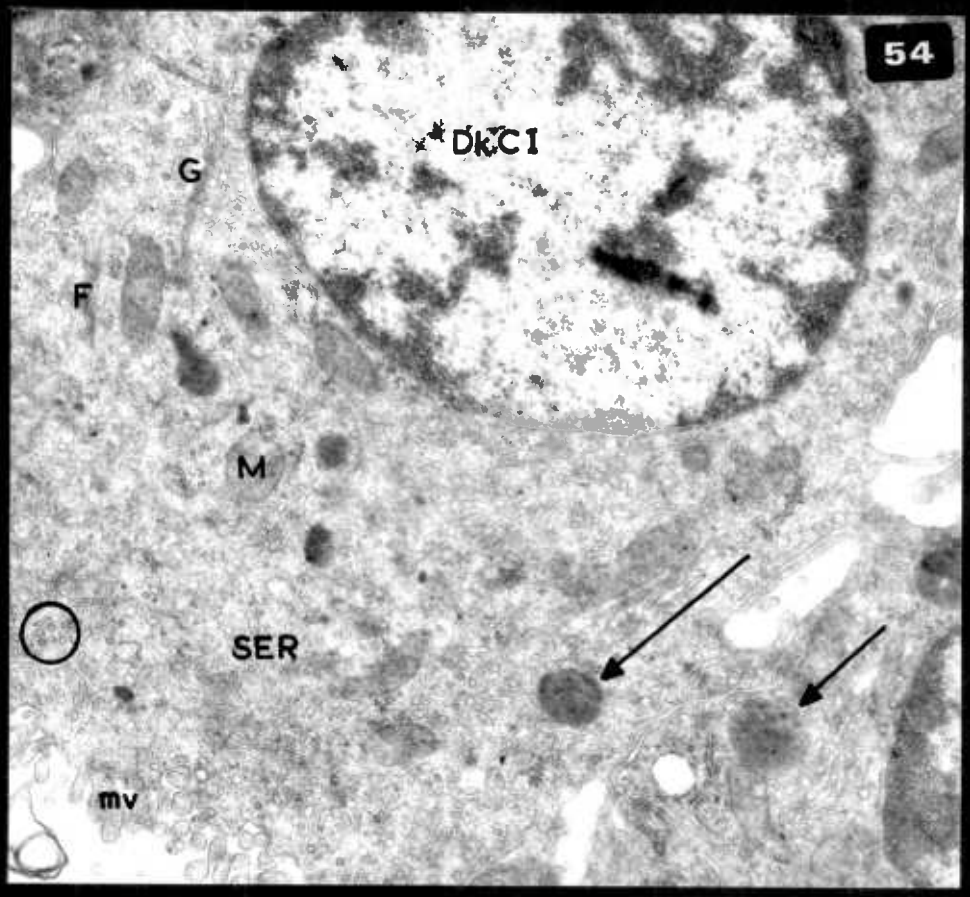

tj
FN
52

Figure 53. Several type I dark cells (Dk CI) and part of a ciliated epithelial cell (CE) bordering on the lumen of a cyst. The dark cells are seen to contain numerous mitochondria (M), some strands of rough endoplasmic reticulum (ER) and what appears to be smooth endoplasmic reticulum (SER). G - Golgi apparatus; tj - tight junction; * - interdigitating microvillous-like structures of the lateral plasma membrane. X15,000

Figure 54. Dark cell I (Dk CI) bordering on a cyst in which the smooth endoplasmic reticulum (SER) is abundant. Of particular note, coated vesicles (circled), large heterogeneous lysosome-like dark bodies (arrows) and a few filaments (F) can be seen in the cytoplasm. G - Golgi apparatus; mv - microvilli at the apical plasma membrane. X20,000

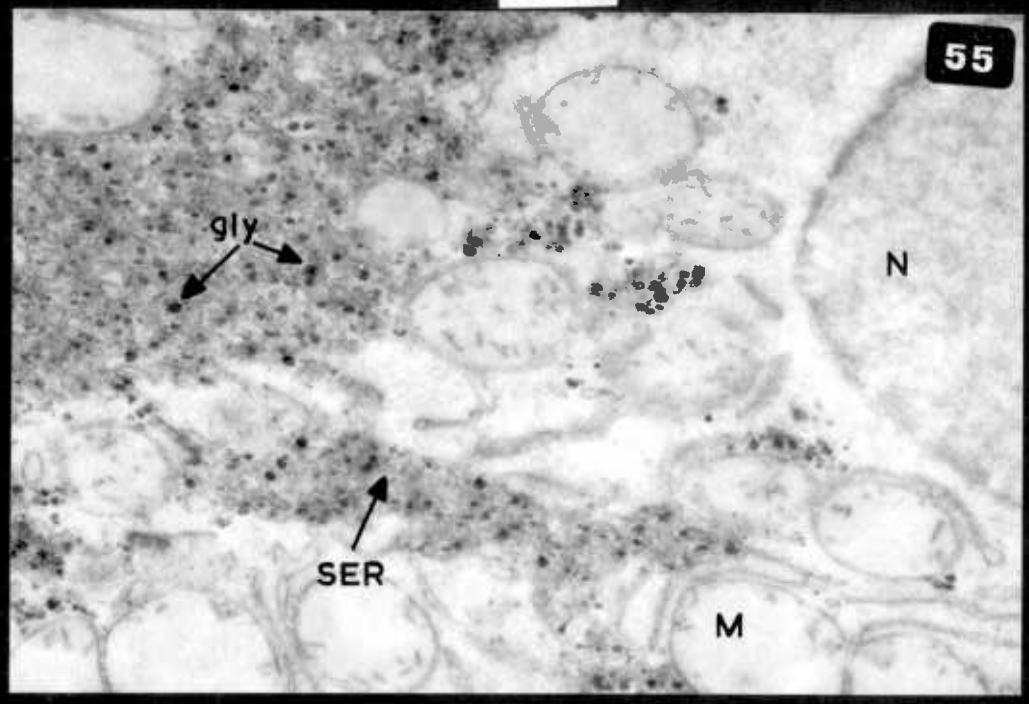

53
CE
DkCI
ER
G
DkCI
M
SER

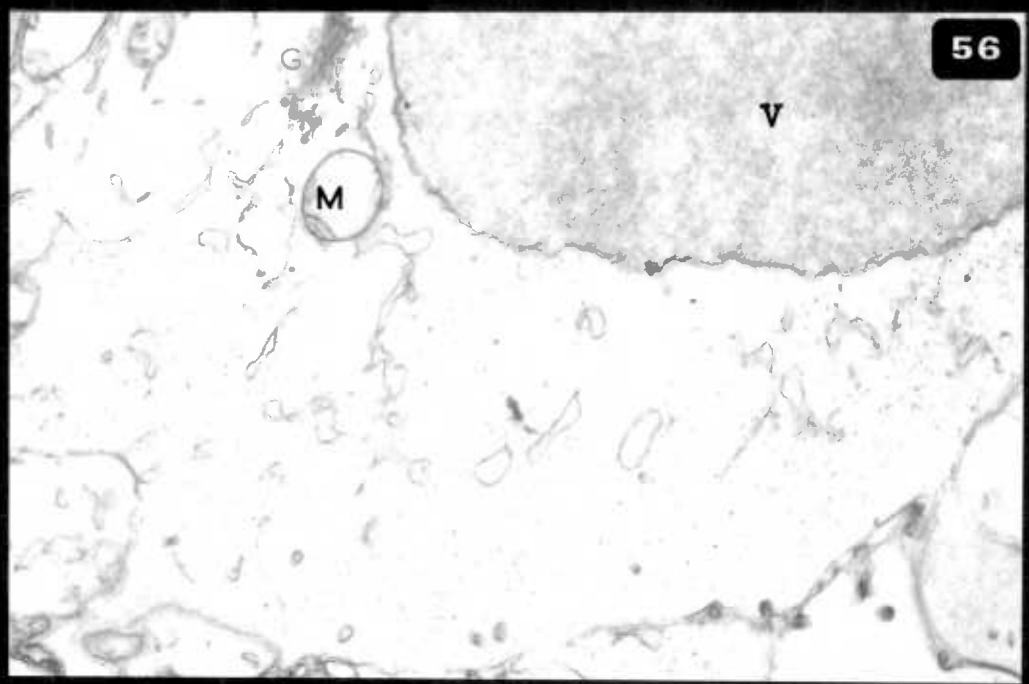

54
DkCI
G
F
M
SER
mv

Figures 55,56 and 57. Tissue fixed in potassium permanganate and printed at a magnification of X15,000. N - nucleus; M - mitochondria; FL - follicular lumen; ER - probable membranes of endoplasmic reticulum in a profile IV cell.

Fig. 55. A guinea pig hepatocyte showing alpha glycogen (gly)

Figs. 56 and 57. Sections of cells of the guinea pig pars tuberalis. Probable cell types are indicated. Fig. 56, in particular, shows a small number of fine, scattered particles in the cytoplasm which could possibly represent beta glycogen.


55
gly
N
SER
M


56
G
V
M

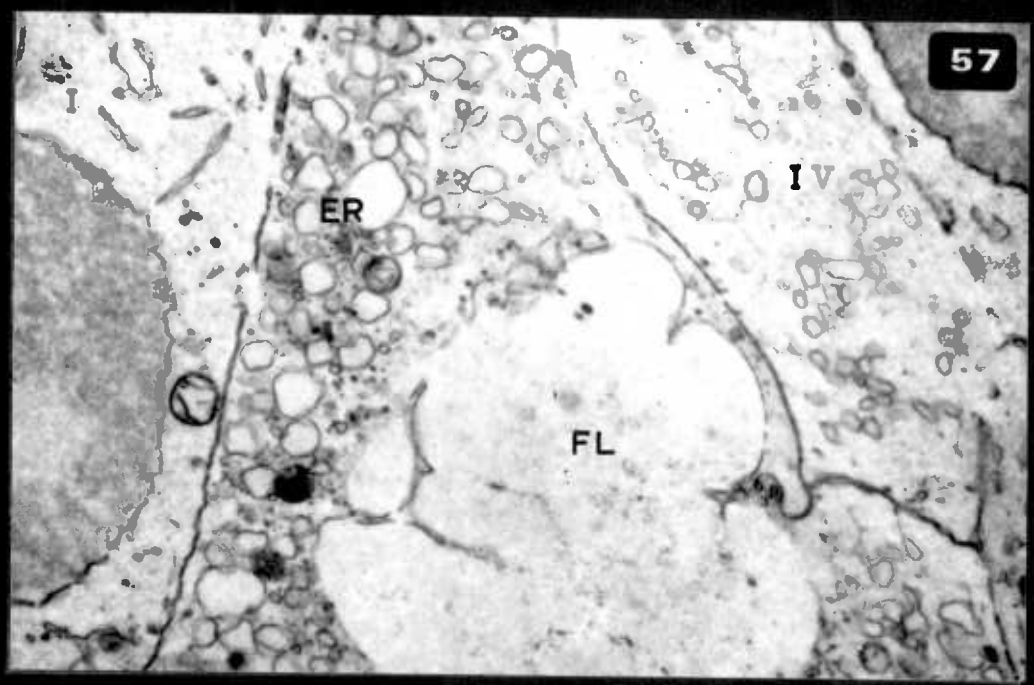
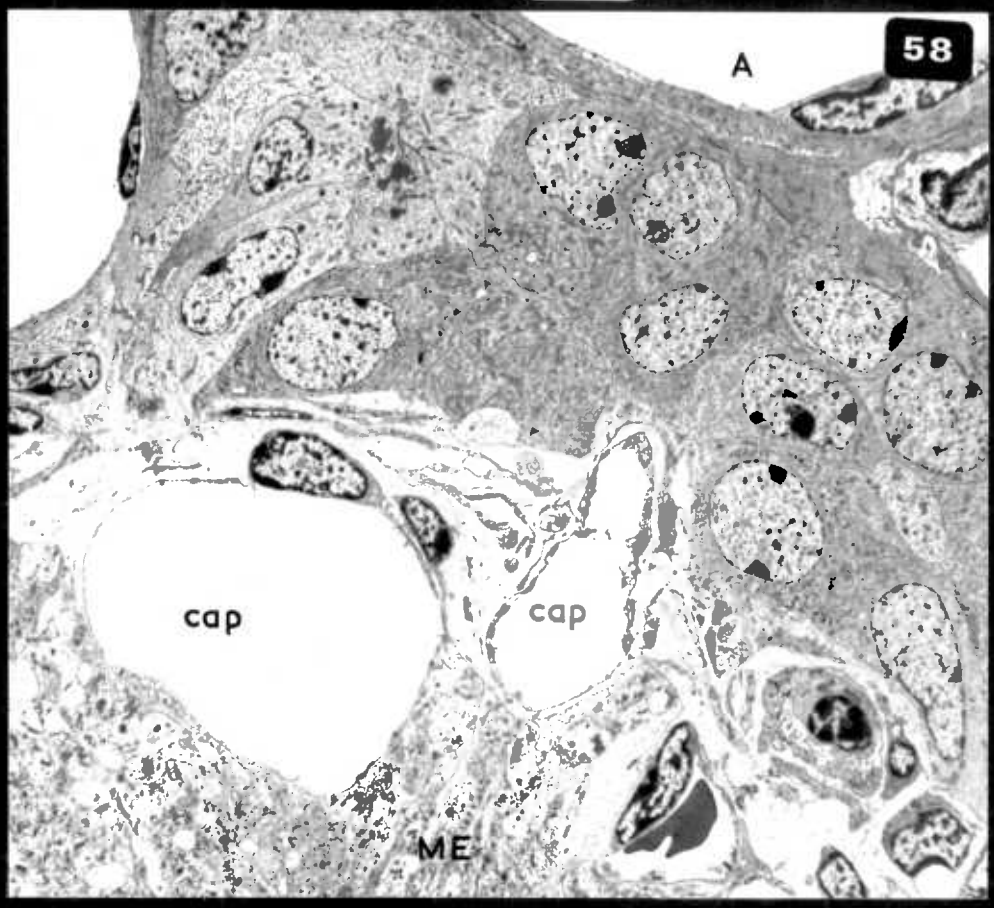

57
I
I V
ER
FL

Figure 58. Low magnification micrograph of cells in the dorsal pars tuberalis. cap - capillary; ME - median eminence; A - arteriole. X3,000

Figure 59. Survey micrograph of cells in the anterioventral pars tuberalis showing cells of profiles I and IV, some of which are labelled. Note the large perivascular space (PVS). X6,000


58
A
cap
cap
ME

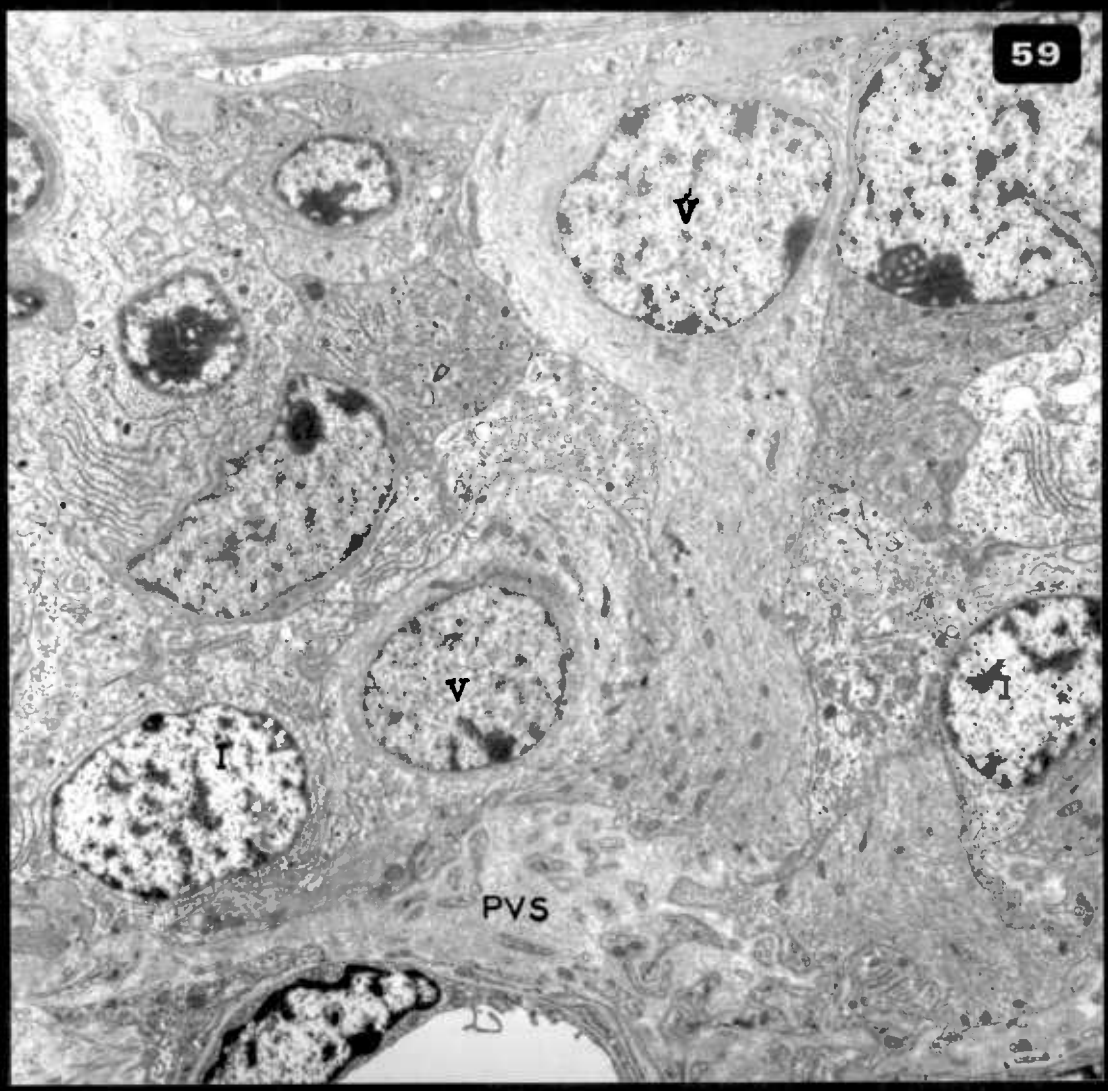
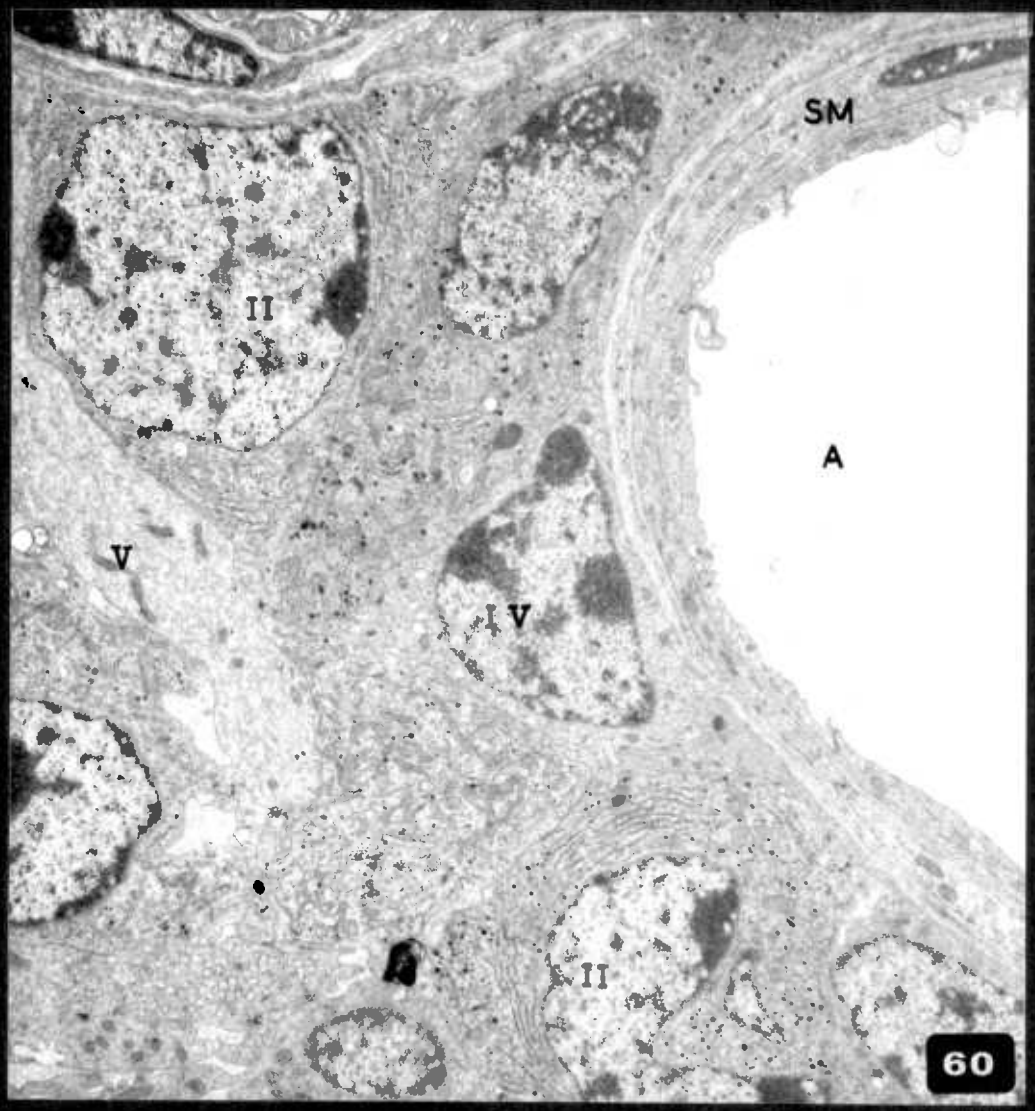

59
V
V
I
PVS

Figure 60. Survey micrograph of cells in the anterio-ventral pars tuberalis showing three cell profiles, II, IV and V, some of which are labelled. An arteriole (A) with surrounding smooth muscle cells (SM) is seen within the tuberal tissue. X7,500

Figure 61. A small portion of an arteriole (A) bordered by a smooth muscle cell process (SM) and nearby nerve fibres (nf) containing many electron lucent vesicles. Pinocytotic invaginations (circled) are seen in the plasma membrane of the endothelial cell and smooth muscle cell. X15,000


SM
II
A
V
IV
II
60

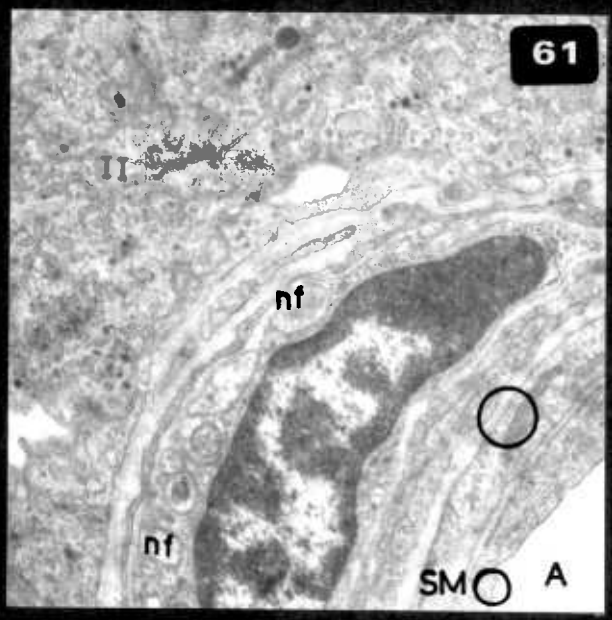
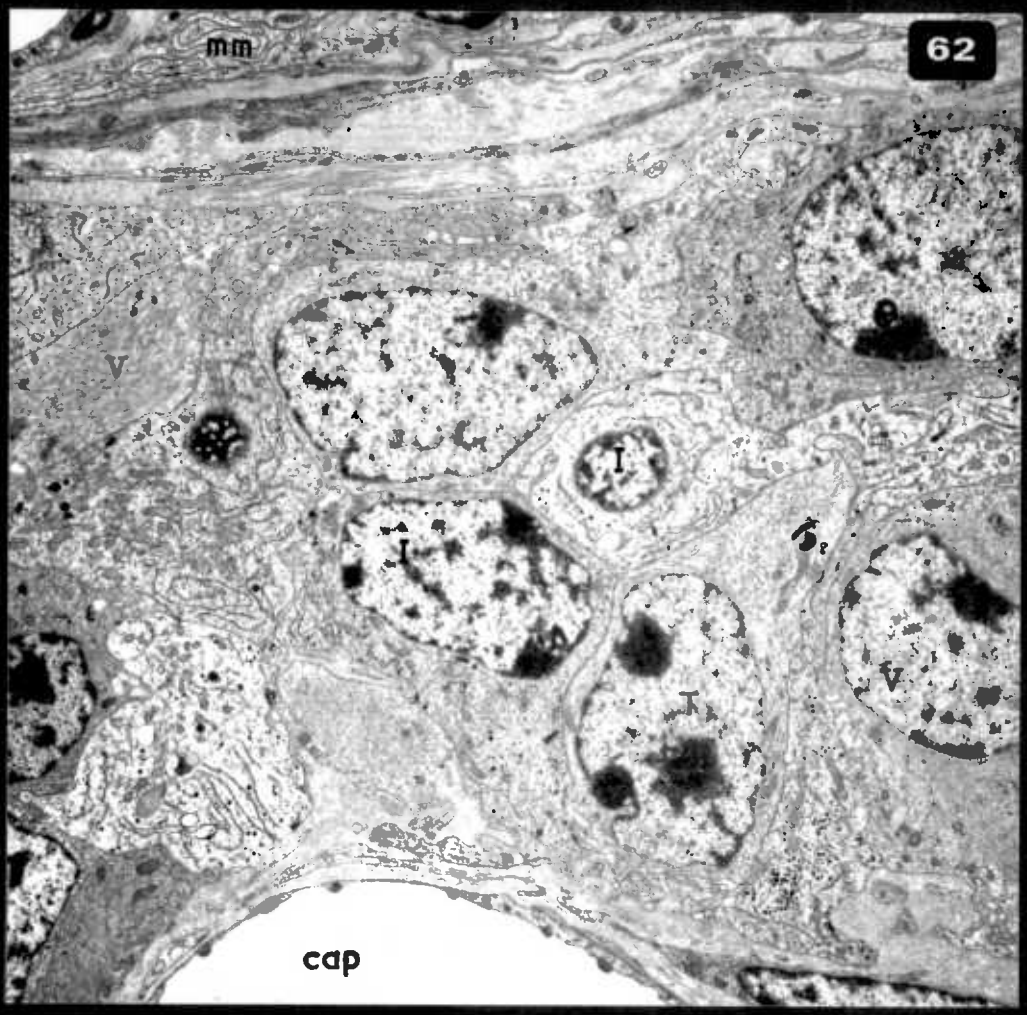
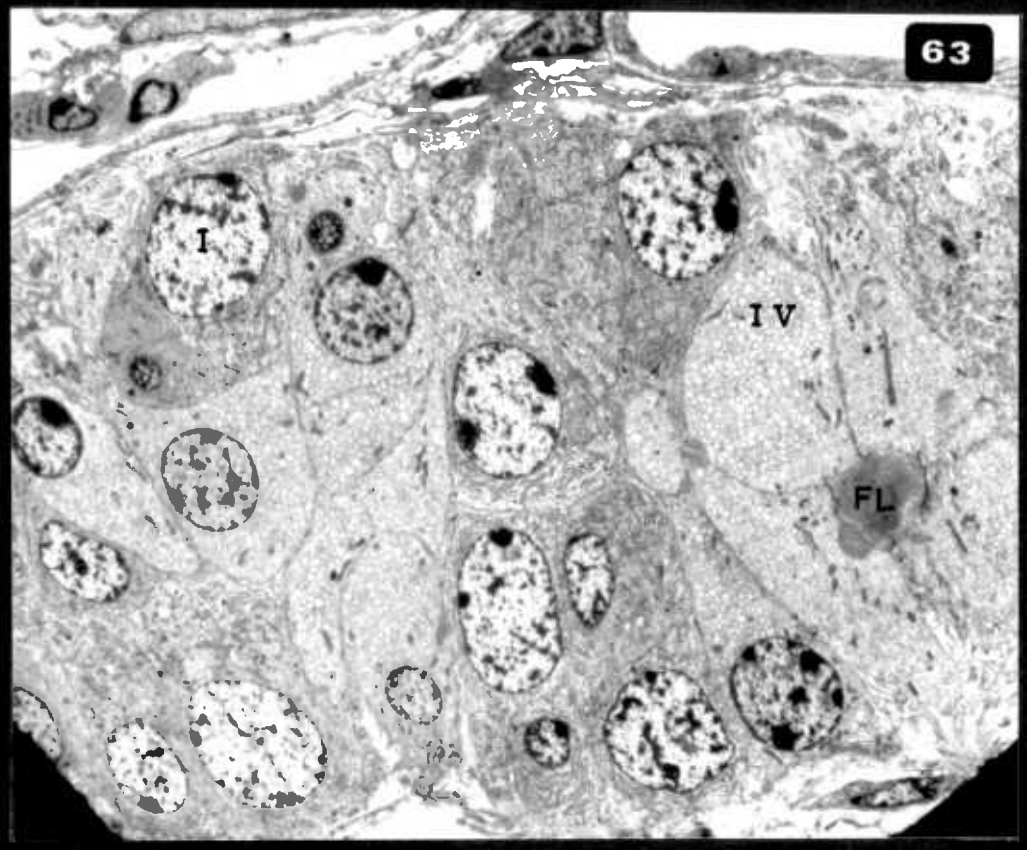

61
II
nf
nf
SM
A

Figure 62. Survey micrograph of the centro-ventral pars tuberalis showing part of a large capillary (cap) at the internal surface of the tissue and the thin cellular processes of the meningeal membrane (mm) on the external surface. Cells of profiles I and V are seen to be present as well as a cell which appears to have a transitional profile (T). X6,000

Figure 63. Survey micrograph of cells in the posterio-ventral pars tuberalis in an area containing many profile I and IV cells. FL - follicular lumen. X3,000


62
mm
V
I
I
I
V
cap


63
I
IV
FL