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ELECTRON MICROSCOPIC STUDIES OF CALCITONIN PRODUCING CELLS IN A VARIETY OF VERTEBRATE SPECIES AND THE EFFECTS OF HORMONAL AND NUTRITIONAL VARIATIONS

BY

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PART I

GENERAL INTRODUCTION

A. Introduction

The calcitonin concept, introduced by Copp and his co-workers in 1961 (Copp et al., 1961) has completely revamped the concept of calcium homeostasis and the metabolism of bone. It led to a new era of active investigations. The demonstration that calcitonin originates from the mammalian thyroid gland (Hirsch et al., 1963), in the parafollicular cells (Foster et al., 1964) and from the ultimobranchial glands in the non-mammalian vertebrates (Copp et al., 1967) gave a new impetus to morphological studies on these structures, which have hitherto been of unknown function. Purified preparations of calcitonin were obtained and allowed studies of its chemical and biological properties.

B. Nature and Origin of Calcitonin

1. Chemistry

Porcine calcitonin has been isolated by various investigators (Putter et al., 1967; Hawker et al., 1967; MacIntyre, 1967; Gudmundsson et al., 1968; Kahnt et al., 1968; Frantz et al., 1968; Bell, 1968).

Four separate active fractions may exist (Putter et al., 1967; Neher and Kahnt, 1968; Gudmundsson et al., 1968; Bell, 1968). Gudmundsson et al. (1968) designated the fractions as alpha, beta, gamma and

delta calcitonin. Alpha and beta calcitonin have been isolated and characterized. The beta form is the oxidized form of alpha calcitonin (Kahnt et al., 1968).

Porcine calcitonin is a polypeptide (Baghdiantz et al., 1964; Hirsch et al., 1964). It is composed of thirty-two to thirty-five amino acid residues (Table I). The molecular weight is approximately 4000 (Neher et al., 1968; Gudmundsson et al., 1968; Potts et al., 1968). Recently, calcitonin has been synthesized (Rittel et al., 1968).

Elution studies show that calcitonin from the ultimobranchial glands in a variety of vertebrates is less retarded, suggesting a larger size (Copp et al., 1968).

2. Sites of Origin of Calcitonin

In the earlier experiments of Copp et al. (1961; 1962) and Kumar et al. (1963) in the dogs, it was difficult to discriminate between the thyroid and the parathyroid origin of calcitonin on account of the close anatomical relationship and the common blood supply of the glands.

However, Copp et al. (1961; 1962) suggested that calcitonin was secreted by the parathyroid gland. They observed that calcitonin did not appear to be released when the thyroid gland alone was perfused

TABLE I

Amino acid composition of calcitonin*

Amino acid residues	Calcitonin	
	(Potts, 1968)	(Gudmundsson et al., 1968)
Alanine	1	1
Glycine	3	4
Leucine	3	3
Serine	4	4
Threonine	2	2
Valine	1	1
Aspartic acid	4	5
Glutamic acid	1	2
Arginine	2	2
Histidine	1	1
Tryptophane	1	1
Proline	3	3
Thyrosine	1	1
Phenylalanine	3	3
Cystine	1	1
Methionine	1	1
Residues	32	35
Molecular weight	3,600	4,100

*Data adapted from Potts, 1968 and Gudmundsson et al., 1968.

with the hypercalcemic blood.

Hirsch et al. (1963) were the first to suggest the thyroid origin of the hypocalcemic principle. They observed that the serum calcium level of rats parathyroidectomized by cautery fell to lower levels than that of rats parathyroidectomized by surgery. It was shown that the serum calcium level of the surgically parathyroidectomized rats could be further depressed by cautery of the thyroid gland or by injection of a small amount of rat thyroid extract. They concluded that the fall in the plasma calcium after surgical parathyroidectomy was due to the loss of the parathyroid hormone alone, while unavoidable thermal stimulation of the thyroid gland provoked the release of the hypocalcemic substance which was responsible for the excess fall in serum calcium after parathyroidectomy by cautery. They proposed the name "Thyrocalcitonin" for the active substance to indicate the thyroid origin and the possible relation to calcitonin.

However, the problem of whether the active principle originated from the thyroid (thyrocalcitonin), from the parathyroid (calcitonin) or from both, remained unsettled. In an attempt to resolve this problem, <u>in situ</u> perfusion experiments were carried

out in the sheep, goat and pig because in these animals, the thyroid and the parathyroid glands are situated in different locations. Copp and Henze (1964) observed that in the sheep, hypercalcemic perfusion of the isolated parathyroid with the thyroid intact produced significant hypocalcemic response, whereas perfusion of the thyroid alone yielded negative results. Nevertheless, Foster et al. (1964) demonstrated that in the goat, perfusion of thyro-parathyroid complex resulted in the lowering of the systemic calcium, whereas perfusion of the isolated superior parathyroid after thyroidectomy did not. Further evidence came from Care(1965) who showed that in the pig, hypercalcemic perfusion of the thyroid after parathyroidectomy resulted in the lowering of the systemic calcium.

Other studies have confirmed the thyroid origin of calcitonin. Administration of acid extract of goat thyroid into the donor animal produces a definite hypocalcemia (Foster et al., 1964). Pig thyroids have been used for the preparation of more highly purified material, which, when injected consistently produced significant hypocalcemic effects in the rats(Baghdiantz et al., 1964; Hirsch et al., 1964; Tenenhouse et al., 1965).

Talmage et al. (1965) studied the effect of calcium loading in rats subjected to parathyroidectomy, thyroparathyroidectomy, and thyroidectomy with parathyroid replacement by transplants. They concluded that the thyroid gland must release the hypocalcemic principle.

However, in some mammals, particularly rabbit, dog and man, the internal parathyroid glands seem to contain some calcitonin (Copp, 1969).

In the non-mammalian vertebrates, the thyroid gland contains no calcitonin (Copp et al., 1967a; Kraintz and Puil, 1967; Clark, 1968). However, high concentrations of calcitonin have been obtained from the ultimobranchial glands of chickens and turkeys (Copp et al., 1967a; Tauber, 1967) and dogfish (Copp et al., 1967b). Extracts of ultimobranchial glands of reptiles (Mosely et al., 1968; Clark, 1968), amphibians and teleost fishes (Copp and Parkes, 1968) also contain calcitonin.

There is now substantial agreement that calcitonin and thyrocalcitonin are identical (Munson and Hirsch, 1965; MacIntyre, 1967; Copp, 1967).

3. Cellular Origin of Calcitonin

The mammalian thyroid gland contains a

second type of epithelial cell called parafollicular cell (Fig. 1) which is histologically and histochemically different from the follicular cell (Nonidez, 1932). Foster et al. (1964) observed that the levels of certain enzymes in the parafollicular cells of the dog thyroid were affected by changes in the calcium concentration in the blood. On the basis of this observation, they postulated that these cells might be responsible for the production of calcitonin. Pearse (1966a) renamed these cells C cells to indicate their possible role in calcitonin production.

Hargis et al. (1966) applied conjugated rabbit antiserum against porcine calcitonin to sections of pig thyroid and by fluorescent techniques, observed specific fluorescence in all the thyroid epithelial cells. Bussolati and Pearse (1967) using guinea pig antiserum to purified porcine calcitonin, demonstrated the presence of calcitonin in the parafollicular cells of the dog thyroid. However, it is known that the polypeptide used in preparing the fluorescent antibody in Hargis's experiment is impure (Hawker et al., 1967).

In the non-mammalian vertebrates, the ultimobranchial gland is retained as a separate entity and not incorporated into the thyroid during

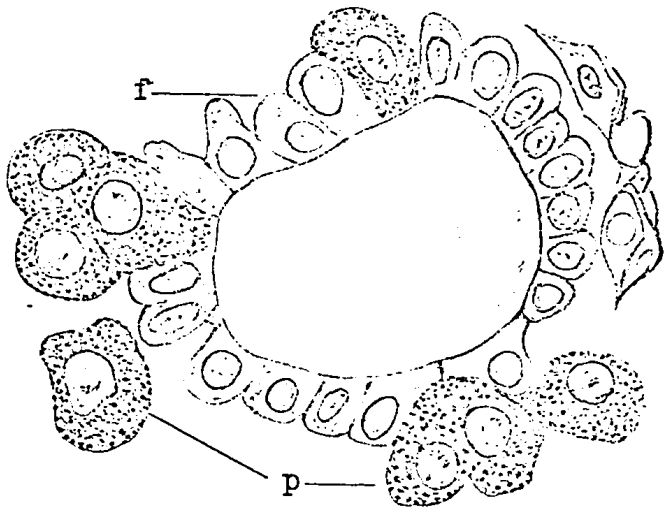

Fig.1. A follicle from the dog thyroid gland showing follicular cells (f) and the parafollicular cells (p) which show argyrophil granules.

Adapted from Nonidez, J.F. Amer. J. Anat. 49:501,1931.

embryonic development. In the ultimobranchial gland of the chick, light cells which appear morphologically similar to those in the mammalian thyroid gland have been observed (Chan et al., 1969).

C. Morphology and Embryology of Calcitonin Producing Cells

1. The Ultimobranchial Body

The ultimobranchial body is a small, usually paired glandular structure derived from the pharyngeal endoderm. These bodies are present in all vertebrates except the cyclostomes. In the non-mammalian vertebrates, it exists as a distinct structure. However, in the mammals, the ultimobranchial body is incorporated into the thyroid gland during embryonic development.

Greil (1905) suggested the term "ultimobranchial body" which means, by definition, structures derived from the last pair of pharyngeal pouches, even though the number of pouches is reduced along the phylogenetic scale. They were also known as suprapericardial and postbranchial bodies in the various genera (Maurer, 1888; Camp, 1917).

In the elasmobranch fishes, the ultimobranchial body arises from the outpocketings of the sixth pharyngeal pouch (Camp, 1917). In the dogfish, the ultimobranchial body is situated beneath the

floor of the pharynx at the level of the pericardium. It consists of variable number of vesicles containing mucous-like material. In the teleost fishes, the ultimobranchial body is situated on the ventral surface of the esophagus at the level of the sinus venosus. The ultimobranchial body varies from a collection of vesicles to compact glandular structures in the various species of fishes (Giacomini, 1908; Watzka, 1933; Krawarik, 1936; Rasquin and Rosenbloom, 1954; Sehe, 1960; Bertmar, 1961).

In the amphibians, the ultimobranchial body develops from the ventral wall of the sixth pouch (Maurer, 1888; Saxén and Toivonen, 1955). The ultimobranchial bodies in the anurans are paired follicular glands situated on either side of the glottis. In urodeles, the ultimobranchial body is unpaired and it is usually the left one that is developed. It invariably consists of a number of follicles which are variable in size, form and location (Wilder, 1929; Watzka, 1933).

Of the reptiles, the ultimobranchial body of snakes (Van Bemmelen, 1893), lizards (Peter, 1901), and turtles (Van Bemmelen, 1893; Shaner, 1921; Johnson, 1922) have been investigated. The ultimobranchial body here develops as outpocketing on the posterior

wall of the fourth pharyngeal pouch. In lizards and turtles, the development of the ultimobranchial body continues on the left side only and that on the right remains vestigial. The ultimobranchial body in lizards and turtles consists of a collection of vesicles. In the turtle, the ultimobranchial body is situated between the left aortic and pulmonary arteries in close association with the left posterior parathyroid gland. In the lizard, the ultimobranchial body is located to the left of the tracheal margin at the level of the superior cardiac margin (Fig. 2).

In the aves, Johnson (1918a; 1918b) observed the ultimobranchial body in the chick and several other birds to arise from the fourth, fifth and sixth pouches. However, other studies have shown the ultimobranchial body of the chick to develop from the sixth pharyngeal pouch (Dudley, 1942; Nagy and Swartz, 1966). In the chick, the ultimobranchial body is located near the bifurcation of the subclavian and common carotid arteries (Fig. 3).

In the mammals, the origin of the ultimobranchial body is from the outpocketing of the fourth pharyngeal pouch which forms a "caudal pharyngeal complex" (Kingsbury, 1914). Born (1883) showed that the thyroid of the pig was formed from three anlagen,

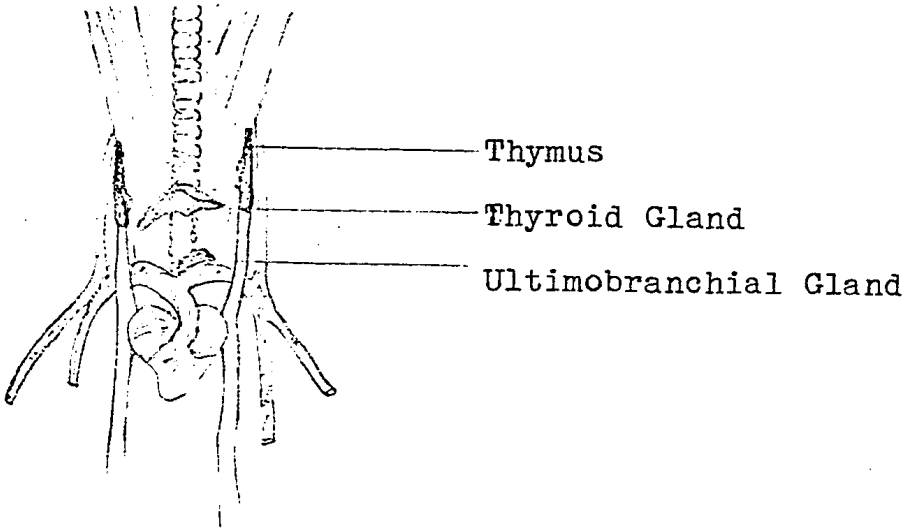

Fig.2. Illustration showing the anatomical relationship of the pharyngeal glands in the lizard.

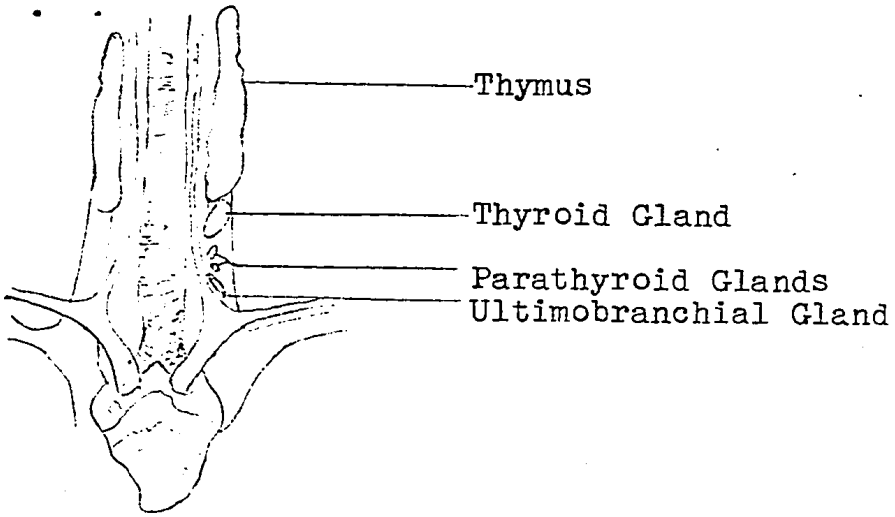

Fig.3. Illustration showing the anatomical relationship of the pharyngeal glands in the chick.

one medial from the pharyngeal floor and a pair of lateral outgrowths from the fourth pouch.
The ultimobranchial body differentiates as an inherent "lateral thyroid" component and contributes to the general thyroid parenchyma.

Other studies show the degeneration and the complete disappearance of the ultimobranchial body in mammals (Rabl, 1907; Klapper, 1946). Furthermore, Kingsbury (1915; 1935; 1939) reported the degeneration of the structure completely or resulting in the formation of epithelial cysts.

Politzer and Hann (1935) believed that the ultimobranchial body did not degenerate in the developing human thyroid gland. They were not sure whether the remnants of the ultimobranchial body were incorporated into the thyroid parenchyma or into a functionally different tissue within the thyroid. Godwin (1937) suggested that the ultimobranchial tissue might become scattered to form the parafollicular cells in the thyroid.

2. Parafollicular Cell

Baber (1876) first reported the presence of a distinct group of parenchymatous cells in the dog thyroid. He described them as "large rounded cells, each provided with an oval nucleus, found either

singly or in groups, amongst the epithelial cells". Hürthle (1894) reported an interfollicular epithelial cells in the dog thyroid which he called "protoplasma-reichen Zellen". In the thyroid gland of the opossum, Bensley (1914) observed the numerous "ovoid cells" which contained numerous small granules. He believed that the granules of the "ovoid cells" represented a new secretory product. Takagi (1922) investigated the cytology of the dog thyroid gland using special technique to demonstrate mitochondria. He observed that the "interfollicular epithelial cells" differed from the follicular cells and concluded that the cells represented a special variety of glandular cells. Nevertheless, Wilson (1927) disputed the presence of the interfollicular cells. He believed that the apparent cell mass was tangentially cut sections of follicles.

The most important work on the second epithelial cell of the thyroid was carried out by Nonidez (1932). Using silver nitrate method of Cajal, he demonstrated the presence of the second epithelial cell which showed argyrophilia. He named them "para-follicular cells" because at one stage of their life cycle they forsook their close association with the follicular cells to migrate to the adjacent connective

Dempsey (1954) considered the parafollicular cells to be argyrophilic connective tissue cells which migrated in between the follicular cells.

Godwin (1937) suggested the ultimobranchial origin of the parafollicular cells. In the dog thyroid gland, he identified the "gray cells", an unorganized cell mass of ultimobranchial origin with the parafollicular cells. Van Dyke (1945) found two kinds of interfollicular cells in young sheep. One type developed from the follicular cells of broken follicles and the other from the ultimobranchial body. Dumont (1956) observed that the parafollicular cells were distributed around the remnants of the thyroglossal duct from which they took their origin. Similarly, Sato (1959) observed more parafollicular cells in and near the residual cysts of ultimobranchial origin in the thyroid gland of hamster.

Electron microscopic studies have added further support to Godwin's hypothesis. Tashiro (1963; 1964) studied the parafollicular cells and the epithelial cells of the ultimobranchial cyst in the thyroid gland of dogs with the electron microscope and suggested a common origin for these cells. This observation has since been confirmed by Sato (1966).

Pearse and Carvalheira (1967) provided

cytochemical evidence for the ultimobranchial origin of the rodent thyroid parafollicular cells. Mouse thyroid parafollicular cells have shown an ability to take up injected 5-hydroxytryptophan (5-HTP) (Ritzen et al., 1965; Gershon and Ross, 1966; Pearse, 1966a; Pearse and Ocumpaugh, 1966). In addition, Larsen et al. (1966) showed that dopamine and dihydroxyphenylalanine (DOPA) were taken up by the mouse thyroid parafollicular cells. The amines can be demonstrated using the fluorescent amine technique. Pearse and Carvalheira (1967) injected 5-hydroxytryptophan (5-HTP) and dihydroxyphenylalanine (DOPA) into mice on the eleventh to the eighteenth and twentieth days of gestation. They observed fluorescent cells on the ventral wall of the fourth pouch which could be traced to the definitive thyroid parafollicular C cells in later stages of development.

4. Cytochemistry of Calcitonin Producing Cells

Parafollicular cells are stained with the Mallory's aniline blue (Pearse, 1966a) and Cajal's silver nitrate (Nonidez, 1932). However, the silver nitrate reaction is not specific in other species of animals.

Parafollicular cells contain high levels of

(C-terminal) carboxyl groups (Solcia and Sampietro, 1967). The presence of high phospholipid content in the cell has been demonstrated by the Sudan black B and Baker's acid haematein methods (Sandritter et al., 1956) and Luxol fast blue (Pearse, 1966a).

Parafollicular cells are characterized by the presence of cholinesterase (Dejardin, 1955; Dumont, 1956; Pearse, 1966a). Esterase is also present in the parafollicular cells of several mammalian species (Carvalheira and Pearse, 1967). Parafollicular cells contain relatively high concentration of alpha-glycerophosphate dehydrogenase (Foster et al., 1964b).

In goat and sheep, 5-hydroxytryptamine (5-HT) has been shown to be a normal constituent of the parafollicular cells (Flack et al., 1964; Flack and Owman, 1967). However, in other species, the parafollicular cells have shown an ability to concentrate and store injected amine precursors such as 5-hydroxytryptophan (5-HTP) and dihydroxyphenylalanine (DOPA) (Ritzen et al., 1965; Gershon and Ross, 1966; Pearse, 1966b; Larson et al., 1966; Owman and Sundler, 1968).

In the mouse embryo, the cells on the ventral wall of the fourth pharyngeal pouch have shown an ability to take up amine precursors such as 5-hydroxytryptophan (5-HTP) and dihydroxyphenylalanine

(DOPA) when administered to the pregnant mouse (Pearse and Carvalheira, 1967).

In the ultimobranchial gland of the chick, the oval cells have shown an ability to store monoamines (Hachmeister et al., 1967). The oval cells in the ultimobranchial gland show specific fluorescence following administration of 5-hydroxytryptophan (5-HTP).

D. Biological Properties of Calcitonin

In most of these studies, porcine calcitonin has been used; it is not known whether calcitonin from other species and sources would exhibit identical biological properties. However, calcitonin from chicken ultimobranchial glands has been shown to have hypocalcemic effect on rats (Copp et al., 1967a), and in acute partially parathyroidectomized cockerals (Kraintz and Intscher, 1969).

1. Effects of Exogenous Calcitonin on Plasma Composition

Calcitonin causes a fall in both plasma calcium and phosphate. Hirsch et al. (1963) demonstrated that an acid extracts of rat thyroid produced hypocalcemia in the recipient animals. Similar consistent fall in plasma calcium and phosphate have been confirmed by various investigators (Hirsch et al., 1965; Kenny and Heiskell, 1965; Gudmundsson et al., 1966). The rate of fall in plasma calcium and the duration of hypocalcemia

are dose-dependent (Aliapoulios and Munson, 1965). The hypocalcemic effect of the hormone has been observed in parathyroidectomized (Hirsch et al., 1964; Aliapoulios et al., 1965; Tashjian, 1965), nephrectomized (Hirsch et al., 1964; Gudmundsson et al., 1966) and eviscerated (Aliapoulios and Munson, 1965) rats.

Calcitonin administration has little or no effect on plasma magnesium concentration in rats (Gudmundsson et al., 1966; Care et al. 1967).

2. Effects on Bone

Milhaud et al. (1965) studied the turnover of calcium in the plasma pool in both control and calcitonin-treated rats. On the basis of the changes in the specific activity of blood calcium, they postulate that the hormone inhibits resorption of bone. Calcitonin prevents the release of radioisotopes of calcium and strontium from prelabeled rat bone *in vivo* (Johnston and Deiss, 1966; Robinson et al., 1967; Wallach et al., 1967; Hirsch, 1967). Furthermore, Martin et al. (1968) observed significant reduction in the urinary excretion of hydroxyproline after calcitonin administration. Diminished excretion of hydroxyproline is a presumptive evidence of inhibition of bone resorption.

In bone perfusion studies, measurement of the arterio-venous differences in the isolated cat tibia has shown calcitonin to increase net calcium retention (MacIntyre et al., 1967).

In bone culture studies calcitonin acts directly on bone by inhibiting resorption. Friedman and Raisz (1965) demonstrated calcitonin in vitro inhibited release of calcium-45 both from control bones and from those treated with parathyroid hormone. Similarly, Aliapoulios et al. (1966) showed that calcitonin inhibited parathyroid hormone stimulated bone resorption in mouse bone in vitro. Inhibition is greater when resorption is stimulated by either parathyroid hormone (Friedman and Raisz, 1965; Aliapoulios et al., 1966) or vitamin A (Aliapoulios et al., 1966; Reynolds, 1968).

Foster et al. (1966) demonstrated that the trabecular bone in the metaphysis of the parathyroidectomized rats was greatly increased when chronically treated with calcitonin. Furthermore, there is a relative increase in the number of osteoblasts and a decrease in the number of osteoclasts in bones of the calcitonin-treated animals (Matrajt et al., 1968). Similarly, Gaillard (1967) observed the hormone to cause "cellular shift" in bone culture studies and

suggested the hormone to increase bone formation rate. Other studies have shown the relative percentage of osteoid, the precursor of mineralized bone, is increased in calcitonin-treated animals (Foster et al., 1966; Matrajt et al., 1968). Recently, Bélanger and Rasmussen (1968) observed that osteocytes under the combined influence of parathyroid hormone and calcitonin produced an inadequate amount of mucopolysaccharides and showed decreased protease activity. Baxter et al. (1968) reported that the synthesis of glycosaminoglycan, a mucopolysaccharide constituent of bone matrix, was increased by calcitonin in vitro. Furthermore, in the chronically thyroidectomized rats maintained on thyroxine, bone formation surfaces at the periosteal and endosteal regions of the femur are reduced (Kumar et al., 1968).

3. Effects on Kidney

Calcitonin causes an increased excretion of phosphate in the rat, but the output of urinary calcium or creatinine is not affected (Kenny and Heiskell, 1965). They regarded the phosphaturic effect of calcitonin to be due to the secondary stimulation of the parathyroid hormone released by hypocalcemia. However, the phosphaturic effect of calcitonin is

present in parathyroidectomized and thyroidectomized rats respectively (Robinson et al., 1966; Milhaud and Moukhtar, 1966).

4. Relation to Parathyroid Hormone

Calcitonin inhibits many of the actions of the parathyroid hormone: calcium release, lactate production, stimulation of ribonucleic acid (RNA) synthesis, hydroxyproline excretion (Friedman and Raisz, 1965; Johnston and Deiss, 1966; Raisz and Niemann, 1967; Martin et al., 1966). Tissue culture studies of bone resorption indicate that the action of calcitonin is not directly antagonistic to parathyroid hormone. The net response is dependent on the relative concentrations of the two hormones present in the medium (Aliapoulios et al., 1966; Friedman and Raisz, 1965). Calcitonin is effective in the absence of parathyroid hormone both *in vivo* and *in vitro*. Furthermore, calcitonin is active in vitamin D deficiency (Evanson et al., 1967; Morii and DeLuca, 1967), and in the presence of actinomysin D (Tashjian, 1965).

5. Mechanism of Action of Calcitonin

The exact mechanism of the action of calcitonin is not known. However, several possible mechanisms have

been suggested for its action. A brief description of some of the current views is included.

Calcitonin may affect the bone cells directly. In vivo and in vitro experiments have shown the ability of the hormone to cause an increase in the number of osteoblasts and a decrease in the number of osteoclasts (Foster et al., 1966; Gaillard, 1967; Matrajt et al., 1968).

Wells and Lloyd (1968) proposed a hypothesis that calcitonin produced hypocalcemia by activating phosphodiesterase. Activation of phosphodiesterase increases the degradation of cyclic 3'5'-adenosinemonophosphate, which is thought to act directly on bone to promote resorption. Theophylline, which inhibits phosphodiesterase, raises the blood calcium in parathyroidectomized rats. Imidazole, which stimulates the enzyme, produces a hypocalcemic and hypophosphatemic effect.

The possibility that calcitonin affects the bone cells through acid phosphatase activity has been suggested by Doty et al. (1968). They observed a decrease in the enzyme level in the calcitonin-treated animals, and an increase in the parathyroid hormone-treated animals.

E. Nomenclature

In the text, calcitonin will be used to refer to the hypocalcemic hormone, since there is general agreement that calcitonin and thyrocalcitonin are identical (Munson and Hirsch, 1966; MacIntyre, 1967; Copp, 1967).

In the succeeding section of the thesis, the term "light cell" (Axelrad and Leblond, 1955) is used to refer to the second epithelial cell type in the thyroid gland in preference to "parafollicular cell" (Nonidez, 1932) in order not to imply any topographical localization. "C" cell (Pearse, 1966a) is not used because the term has been used to refer to follicular cell (Yoshimura et al., 1962).

In the non-mammalian vertebrates, the term "ultimobranchial gland" will be used rather than ultimobranchial body since the ultimobranchial tissue can be demonstrated to have a secretory parenchyma.

F. Background of Problem and Object of the Present Study

Present evidence suggests that calcitonin is produced by the light cells of the thyroid gland. Foster et al. (1964) observed changes in the alpha-glycerophosphate dehydrogenase and acid phosphatase activity of the light cells when the thyroid glands

were perfused with blood having a high calcium concentration and concluded that these cells were responsible for secretion of calcitonin. The hypothesis has been confirmed by the results of improved immunofluorescent studies on pig and dog thyroid which showed calcitonin to be present in the light cells (Bussolati and Pearse, 1967).

Ultrastructurally, light cells have been shown to be sensitive to changes in blood calcium level (Pearse, 1966a; Matsuzawa and Kurosumi, 1967; Young et al., 1968). However, in these studies, the results are at variance as both a diminished (Pearse, 1966a; Matsuzawa and Kurosumi, 1967) and an increased (Young et al., 1968) number of secretory granules have been reported. Similarly, opinions differ regarding the decreased (Pearse, 1966a) and unchanged (Young et al., 1968) number of light cells in such conditions. The perfusion studies are transient in nature and as such the results are valid only for short-term hypercalcemia. Reports on the effects of chronic hypercalcemia are few and brief (Cameron, 1968; Rohr and Hasler, 1968; Ericson, 1968).

Ultrastructural studies on the effect of hypocalcemia on the light cells are usually obtained from parathyroidectomized animals (Wetzel and Gittes, 1966). The effect of a low dietary calcium intake

on the light cell is not known. The value of this type of study lies in demonstrating the changes that may apply to those instances where calcium deprivation occurs, whether by dietary, malabsorption, renal or other means.

Little information is available on the effect of hypophysectomy on the fine structure of the light cell. It is hoped that the ultrastructural changes observed will provide more information on the effect of the pituitary and the thyroid glands on the light cell.

Recent cytochemical studies have provided evidence for the ultimobranchial origin for the thyroid light cells in the mouse (Pearse and Carvalheira, 1967). This observation has been confirmed by the studies of Copp and Parkes (1968) which show the high calcitonin content in the ultimobranchial glands in the various species of non-mammalian vertebrates.

However, the significance of calcitonin in the lower vertebrates is not known. In order to obtain more information regarding the functional role of the ultimobranchial gland in the chick, the effects of high and low dietary calcium on the ultimobranchial gland were evaluated. The ultimobranchial glands in the frogs and fish in the normal conditions were

studied. It is hoped that the ultrastructural characteristics observed will provide further insight regarding the functional role of the gland.

There are indications that calcitonin may play a significant role in the pathogenesis of certain metabolic diseases. Thyroid extracts from patients with pseudohypoparathyroidism have contained approximately one hundred times as much calcitonin as normal glands (Aliapoulios et al., 1966b). In such condition, the thyroid gland appears to contain an increased number of light cells (Mazzouli et al., 1968a). Tashjian and Voelkel (1967) have reported that less calcitonin is extractable from thyroids of patients with hyperparathyroidism than from normal glands. There are also indirect evidence that calcitonin may be responsible for osteopetrosis. Thyroid glands of affected animals contain more light cells than those of their unaffected litter mates (Walker, 1966). Similarly, Krook et al. (1969) propose that osteopetrosis in the bull is the result of ultimobranchial hyperplasia which, in turn is caused by excessive dietary calcium. Capen and Young (1967) suggest that calcitonin is responsible for parturient paresis, a spontaneous metabolic disease of cattle.

Furthermore, histological and other evidence

show the medullary carcinoma of the thyroid gland to consist of light cells and produce calcitonin (Milhaud et al., 1968; Melvin and Tashjian, 1968; Meyer and Abdel-Bari, 1968; Cunliffe et al., 1968).

The present study was designed to investigate the ultrastructural differences of the light cells in rats under the influence of excess parathyroid hormone, on a low calcium diet and under the influence of hypophysectomy and low phosphate diet. The ultimobranchial glands of chick in a high and a low calcium diet and those of normal frogs and trouts were also examined.

PART II

EXPERIMENTAL SECTIONS

A. Materials and Methods

1. Animals

Experiment 1

Forty male Sprague-Dawley rats of average weight 100-125 gm. were used. In all the experiments, feed or special diet and water were supplied ad libitum.

1. Controls

   Twenty rats were used as controls.

2. Parathyroid Hormone Treated Rat.

   Ten rats were given Parathyroid hormone extracts (Parathormone, Eli Lilly and Co., Indianapolis), 100 units per injection by intraperitoneal route for three days and sacrificed three hours after the last injection.

3. Low Calcium Diet

   Ten rats were placed on a Low Calcium Diet (General Biochemicals, Chagrin Falls, Ohio; Calcium content 0.03 gm. per 100 gm.) for thirty days. The control diet consisted of the same supplemented with calcium.

4. Hypophysectomy

   Hypophysectomy was performed by the Selye's pharyngeal approach on twenty male rats of average weight 100-125 gm. at the

Charles River Breeding Laboratories. The feed was supplemented by 5% Dextrose in the drinking water.

Ten hypophysectomized rats were placed on a Low Phosphate Diet (General Biochemicals, Chagrin Falls, Ohio; P content 0.0012 gm. per 100 gm.) for twenty days. The diet was initiated ten days after hypophysectomy.

Experiment 2

Eighty 2-week-old male, White Plymouth Rock chicks were divided randomly into three equal groups. The control group was continued on a commercial diet (6011, Chick Startena, Ralston Purina, Calcium content 1.3%). The second group was started on a high calcium diet which contained 2.9% calcium content. The third group was started on a low calcium diet with a calcium content of 0.63%. Feed and water were available ad libitum. The chicks were sacrificed at the end of the fourth week. Some chicks from each representative group were fasted twelve to twenty-four hours and subsequently sacrificed.

Experiment 3

Twenty specimens of Rana pipiens (35-40 gm.) were obtained from a commercial source in Wisconsin in April. They were kept at 13°C until sacrificed.

Rainbow trouts, Salmo gairdnerii, were used.

2. Techniques for Morphological Studies

Electron Microscopy

Fixation

The rats were anesthetized with ether. The thyroid gland was quickly excised and cut into small pieces with razor blades. The fishes, frogs and chicks were killed by decapitation and the ultimobranchial glands were removed and cut into small pieces. The tissues were fixed by immersion in a 6.25% glutaraldehyde in 0.2 M cacodylate buffer at pH 7.4 for two hours at 4°C and postfixed in 1% osmium tetroxide in Veronal acetate buffer, pH 7.4 containing 0.25 M sucrose (Caulfield, 1957) for ninety minutes at 4°C.

Some tissues were fixed directly in 1% osmium tetroxide in Veronal acetate buffer, pH 7.4 for ninety minutes at 4°C.

In some frogs and chicks, fixation was

accomplished by perfusion through the ventricle with 6.25% glutaraldehyde in 0.2 M cacodylate buffer at pH 7.4 for ten minutes. The tissues were excised and fixed for two hours in the same perfusing fluid, and postfixed in osmium tetroxide as described above.

Embedding and Sectioning

The tissues were dehydrated in a graded series of ethanol solutions. After two changes of propylene oxide (15 minutes each), the tissues were transferred to a mixture of propylene oxide and epoxy resin mixture (Luft, 1961) and rotated overnight to ensure better infiltration of the tissues.

Pieces of tissue were transferred to individual gelatin capsule partially filled with resin mixture, and incubated overnight at 35°C., twenty-four hours in 45°C. and forty-eight hours in 60°C.

The ratio of the resin mixture of Epon with Dodecenyl-succinic Anhydride (DDSA) and Epon with Nadic Methyl Anhydride (NMA) was 50:50. A 1.2% of 2, 4, 6-Tridimethylaminomethyl-phenol (DMP-30) was used as an accelerator.

Silver to gold sections (500-1000 Å) were cut on a Porter Blum MT1 or a Reichert OmU-2 microtome using glass knives. Sections were mounted on uncoated copper grids and stained with uranyl acetate (Watson, 1958)

and/or lead citrate (Reynold, 1963).

Microscopy

Sections were examined in a Philips EM 100 and EM 200 at an accelerating voltage of 60 kV. Micrographs were taken at initial magnification of 1500-12,000 and subsequently enlarged photographically to the desired size.

Light Microscopy

Tissues were fixed in Acid-Formaldehyde-Ethanol (1-4-15 parts, AFA) for twenty-four hours. 7 μ thick sections were stained with periodic acid-Schiff, Haematoxylin-Orange G (PAS-H.O.), and Masson's Trichrome stain. In addition, 1-2 μ Epon-embedded sections, stained with 1% toluidine blue were examined. Micrographs were taken with a Zeiss Photo-microscope.

3. Serum Calcium Determination

Blood was collected by cardiac puncture. It was allowed to stand for two hours and centrifuged. Serum was removed by pipetting. Calcium was estimated, in 0.1 ml. aliquot of serum, by semi-automatic titration with ethylene-diaminetetraacetate (EDTA) using murexide as the indicator (Copp, 1963).

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primate (Aoi, 1966). However, in most of these studies, the tissues were usually fixed in osmium tetroxide and the secretory granules were not adequately preserved.

Recent advances in the calcitonin concept have given new impetus to the morphological study of the light cell. The most rewarding line of work is probably the electron microscopic studies of light cell after exposure to hypercalcemic perfusion (Pearse, 1966a; Matsuzawa and Kurosumi, 1967; Young et al., 1968). However, the transient nature of the perfusion experiments makes it impossible to access the cellular changes in various secretory phases. On the other hand, reports on the effects of chronic hypercalcemia on the light cells are few and brief (Cameron, 1968; Rohr and Hasler, 1968; Ericson, 1968), while the ultrastructural changes in the light cells maintained on a low calcium diet do not seem to have been investigated.

The effects of hypophysectomy on the light cells has been studied exclusively by light microscopy (Saito and Shibata, 1957; Thompson et al., 1962; Yoshimura et al., 1962; Sarker and Isler, 1963). However, little information is available on the effects of hypophysectomy on the ultrastructure of the light cells. Furthermore, the functional integrity of the light cells in the absence of the pituitary control

was tested by maintaining the hypophysectomized rats on a phosphate deficient diet, which is known to raise the serum calcium level (Copp et al., 1965).

The present study of the ultrastructure of light cells in the rat thyroid gland under the influence of excess parathyroid hormone, a low calcium diet, hypophysectomy and low phosphate diet was undertaken to provide more information on the sequential changes of the light cells in these syndromes.

2. Observations

Controls

General Organization

The thyroid gland is composed of an organization of follicles of different sizes which usually vary from circular to spheroidal in shape.

Each follicle is composed of a single layer of follicular cells which line the central lumen filled with a homogeneous, moderately dense colloid. However, in addition to the follicular cells, a second type called the light cell is present in some follicles.

Follicular Cell

In the rat thyroid gland, the follicular cells were generally variable in shape, ranging from

cuboidal to pyramidal. The round or oval nucleus was usually centrally located.

On the apical surface, the cell membrane formed microvilli which protruded into the colloid. The microvilli appeared as finger-like projections, measuring approximately 0.1 to 0.2 μ in diameter (Fig. 5). The number of microvilli in an individual cell was variable. No organelles were present in the cytoplasm of the microvilli.

The lateral surface of the cell (Fig. 5) was relatively straight, although at the basal half of the cell it usually showed irregular dilations. The cell membranes between the adjacent cells were separated by an intercellular space of about 150 Å in width. Near the apical surface, the cell membranes formed the junctional complex and more basally along the lateral cell membrane, one or more desmosome could be observed (Fig. 6).

The basal surface of the cell membrane was relatively straight. In places, however, the cell membrane formed irregular invaginations. A basement membrane consisting of a moderately dense material was closely apposed to the cell membrane (Fig. 5).

The endoplasmic reticulum was invariably well developed (Figs. 5 and 6). In the basal region

DESCRIPTION OF FIGURES

Tissues for electron microscopy were fixed in glutaraldehyde and postfixed in osmium tetroxide, unless otherwise stated.

Fig. 5 Portion of a rat thyroid follicle showing the follicular cells surrounding the central colloid (C). Follicular cells are characterized by the presence of microvilli (Mv), prominent endoplasmic reticulum (Rer), Golgi complex (G) and numerous mitochondria (M). A basement membrane (Bm) is closely apposed to the outer limiting cell membrane. Osmium tetroxide fixation. Lead. X 19,500.

Rer
M
M
C
Mv
5

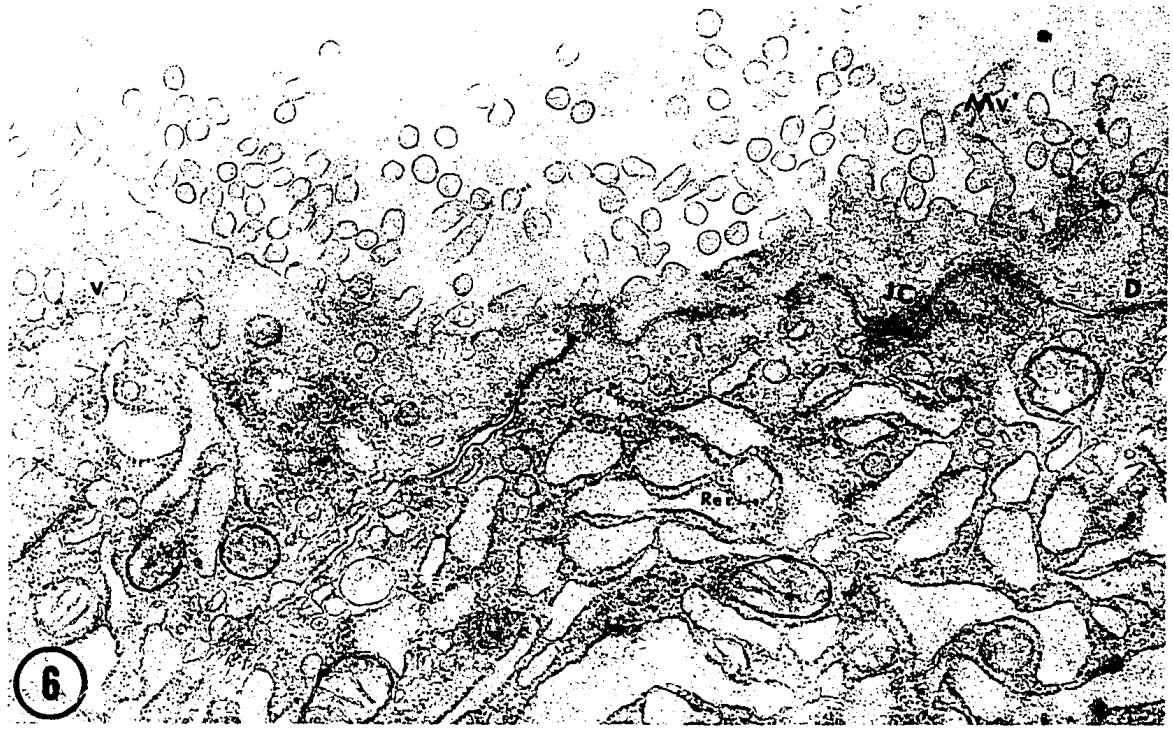
of the cell, the rough-surfaced endoplasmic reticulum consisted of an elaborate network of large, oval or irregularly-formed cisternae. In the apical region, the cisternae were small and more rounded. The lumen of the cisternae usually contained some amorphous material of low electron density (Figs. 5 and 6). Free ribosomes were present in moderate numbers in the cytoplasm.

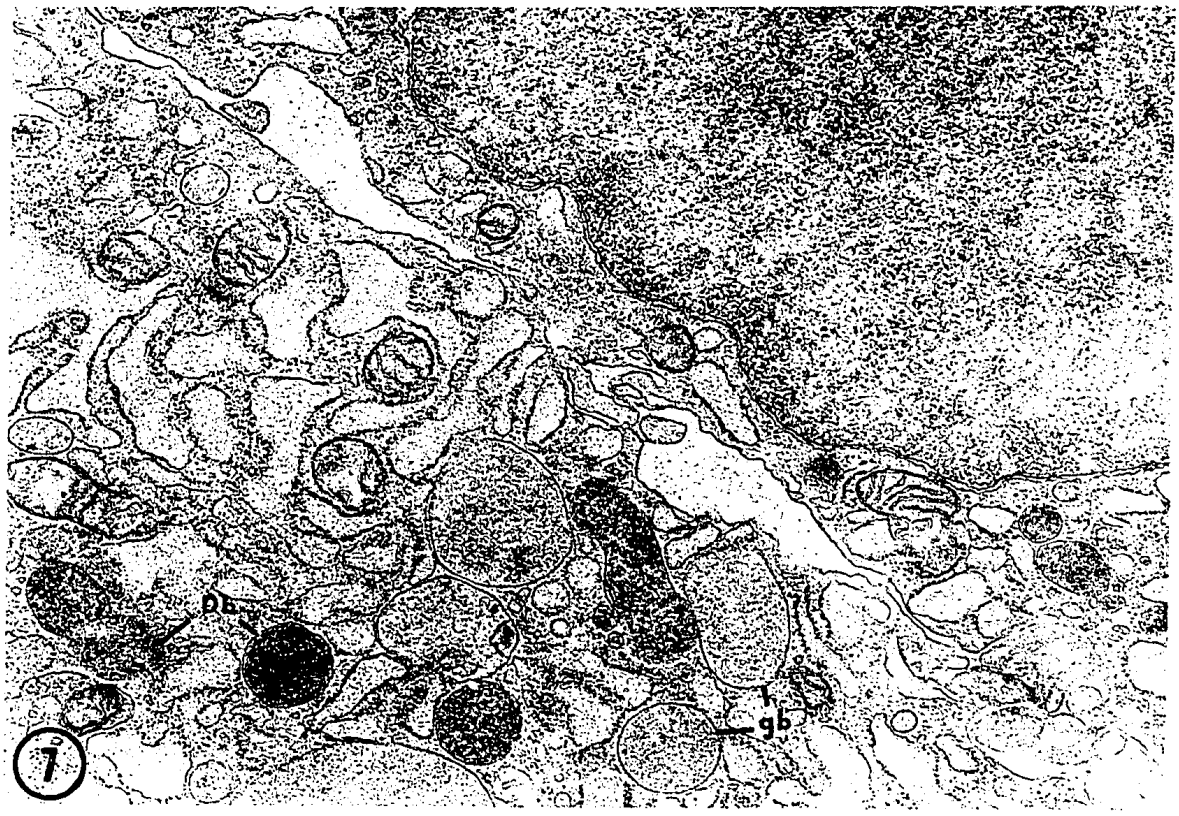
The Golgi complex was prominent (Fig. 5). It consisted of stacks of closely spaced, flattened saccules of smooth-surfaced membranes arranged in concentric layers. Variable number of vacuoles, which contained material of low electron density were present in the Golgi region. In addition, clusters of smooth-surfaced vesicles, which measured approximately 300 to 800 Å in diameter were observed (Fig. 5).

The cytoplasm was characterized by the presence of variable number of structures which differed in size and appearance. At the apical border, numerous vesicles measuring about 0.02 to 0.2 μ in diameter were present. These were delimited by a smooth membrane and contained material of moderate density (Fig. 6). In addition, the apical half of the cell usually contained variable number of globules, which measured from 0.5 to 3 μ in diameter. They

Fig. 6 Apical region of the follicular cell showing the presence of vesicles (v) and microvilli (Mv). The endoplasmic reticulum assumes a more rounded appearance in this region (Rer). A junctional complex (JC) and desmosomes (D) are observed. Osmium tetroxide fixation. Lead. X 24,750.

Fig. 7 Portions of two adjacent follicular cells showing the presence of globules (gb) and dense bodies (Db). Globules (gb) contain a dense homogeneous material, while the dense bodies (Db) usually have a dense, finely particulate matrix. Osmium tetroxide fixation. Lead. X 24,750.


Mv
V
JC
D
Ret
6


DB
gb
7

were lined by a smooth limiting membrane and contained a homogeneous granular material similar to that of the luminal colloid (Fig. 7). Dense bodies were also observed in the cytoplasm of the follicular cells. They measured from 0.1 to 1 μ in diameter. Their content was often granular and moderatly dense. Fragments of membranes or cellular debris were frequently observed in these bodies (Fig. 7).

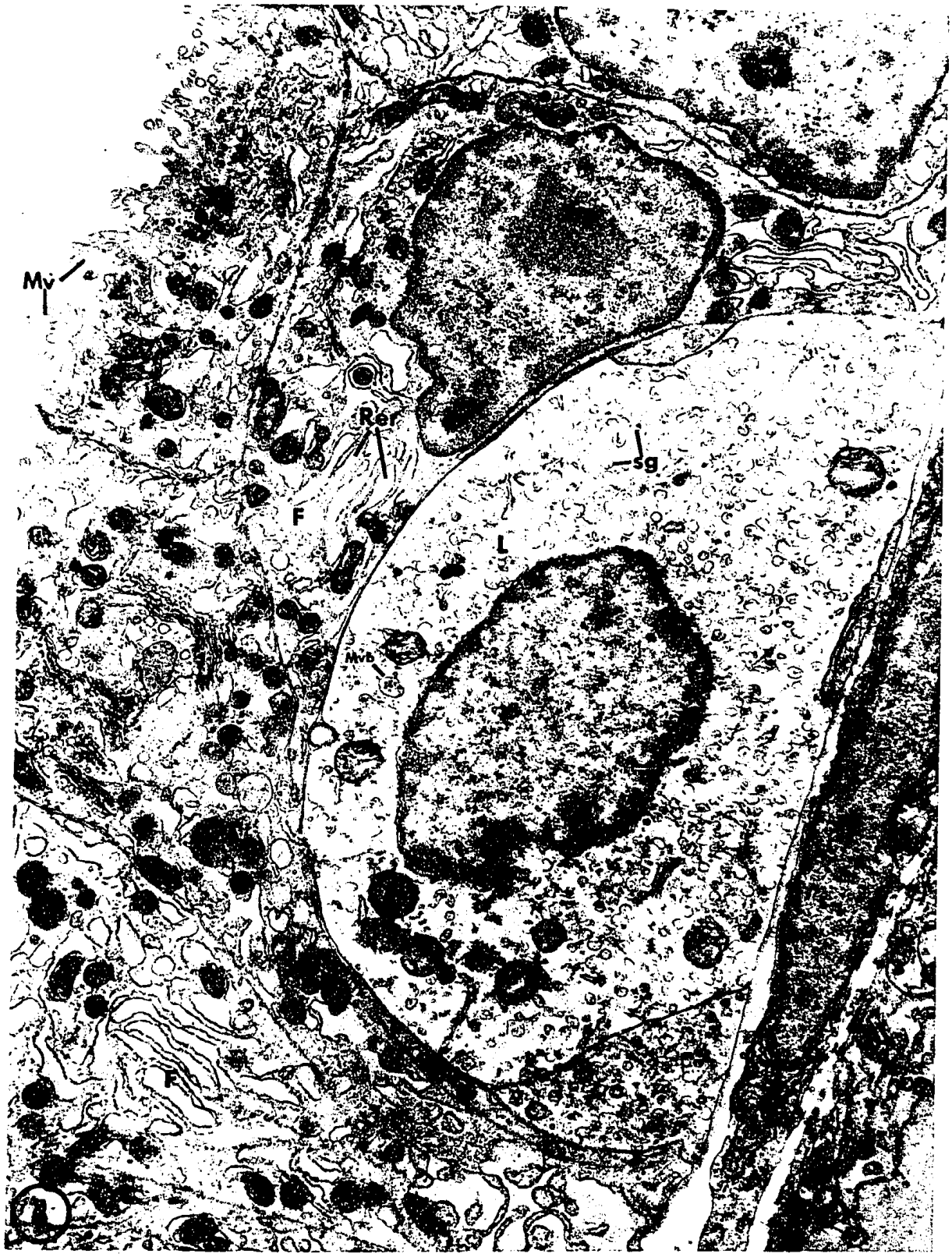
Mitochondria were relatively numerous and were distributed throughout the cytoplasm, particularly in the middle and the basal regions of the cell. The mitochondria were oval or rod-shaped. The matrix was dense and the cristae were transversely oriented.

Light Cell

Light cells were generally oval or ellipsoidal in shape (Figs. 8, 9 and 10). They were usually located between the follicular cells and within the confines of the follicular basement membrane. Light cells were never in direct contact with the colloid in the lumen. A layer of follicular cells, which at times could be extremely attenuated, always intervened between the light cell and the colloid (Fig. 13). In the young rat, light cell usually occurred singly or in aggregates of two or three cells.

The cell membranes were relatively straight

Fig. 8 Light (L) and follicular (F) cells in the rat thyroid gland. Follicular cells contain well developed endoplasmic reticulum (Rer) and microvilli (Mv). Light cell (L) is characterized by the pale cytoplasm. In osmium tetroxide fixation, the secretory granules (sg) appear as vesicles. Occasional multivesicular bodies (Mvb) are observed. Osmium tetroxide fixation. Lead. X 11,700.


Mv
Rer
sg
F
L
Mvb
F

but in certain areas, the adjacent cell membranes were dilated to form invaginations. The cell membranes between the adjacent light or follicular cells were separated by an intercellular space of about 130 Å in width. An occasional desmosome was observed linking the cell membranes of the light and follicular cells.

The endoplasmic reticulum was invariably developed in the light cells. In some cells, the rough-surfaced endoplasmic reticulum consisted of cisternae arranged in parallel arrays whereas in others, it consisted mainly of isolated cisternae distributed randomly throughout the cytoplasm (Fig. 13). However, in many light cells, the endoplasmic reticulum was poorly developed (Figs. 8 and 10). Varying amount of free ribosomes were usually present in the cytoplasm.

The Golgi complex varied in the degree of development among the light cells. In some cells, the Golgi complex was prominent and voluminous. It consisted of three to four closely spaced, flattened saccules arranged in concentric layers. Clusters of smooth-surfaced vesicles, measuring from 300 to 800 Å in diameter and some vacuoles were present in the Golgi region. In addition, some vesicles which contained varying amount of granular material and some secretory granules were usually observed in the vicinity. In

Fig. 9 Light cell showing the secretory granules (sg) which contain finely homogeneous material. The Golgi complex (G), endoplasmic reticulum (Rer) are poorly developed. Mitochondria (M) and multivesicular bodies (Mvb) are observed. Osmium tetroxide fixation. Lead. X 9,900.

Fig. 10 Light cell (L) of the rat thyroid fixed in glutaraldehyde and postfixed in osmium tetroxide. The contents of the secretory granules (sg) appear as electron dense material. Variable number of mitochondria (M) are observed. Uranyl and lead. X 6,500.

9

10

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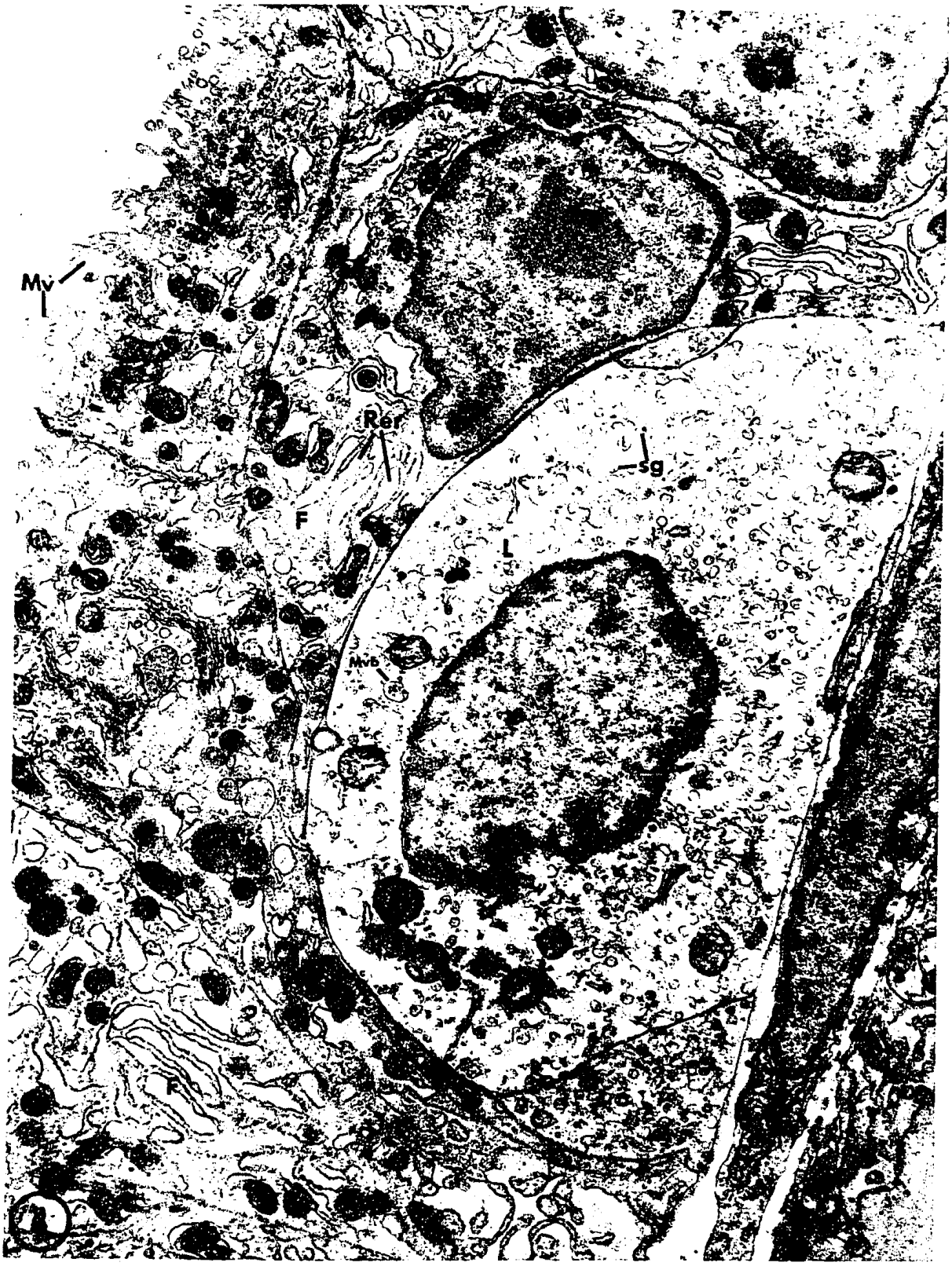
Mv
RER
sg
F
L
MVB

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Fig. 10 Light cell (L) of the rat thyroid fixed in glutaraldehyde and postfixed in osmium tetroxide. The contents of the secretory granules (sg) appear as electron dense material. Variable number of mitochondria (M) are observed. Uranyl and lead. X 6,500.

sg
9

10

other cells, the Golgi complex was poorly developed. It consisted of a few saccules and some smooth-surfaced vesicles (Fig. 9).

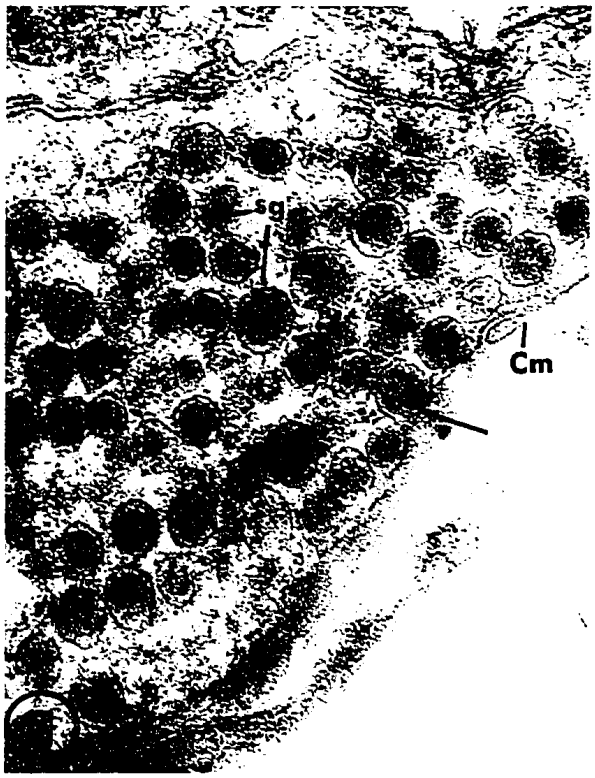
The light cell was characterized by a pale cytoplasm, which contained numerous secretory granules, measuring from 150 to 300 mμ in diameter. In osmium tetroxide-fixed tissues, the secretory granules appeared as round to oval vesicles. Each vesicle was delimited by a smooth membrane. The content of the vesicles varied from a finely, granular to a slightly electron dense material (Figs. 8 and 9). In glutaraldehyde-fixed tissues, the secretory granule appeared as electron dense material delineated by a smooth membrane (Figs. 10 and 11). The secretory granules were distributed randomly in the cytoplasm. However, in some cells, a greater concentration of the secretory granules was usually observed in the basal region of the cell adjacent to the capillaries. Some secretory granules were lined near the cell membrane and fusions of the limiting membranes of the secretory granules and the cell membrane were observed (Fig. 11).

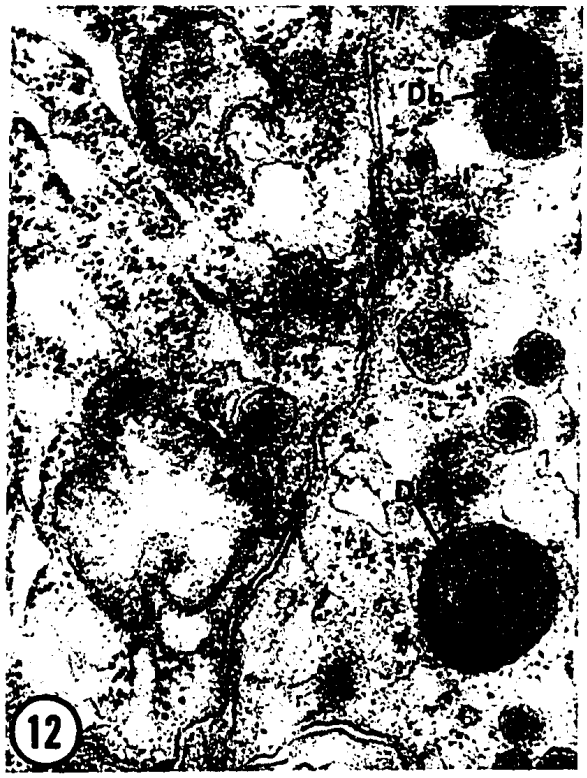
Occasional dense bodies were observed in the cytoplasm. The size of these bodies varied from 0.1 to 1 μ in diameter. They were lined by a distinct

Fig. 11 Portion of a light cell showing the close association of the secretory granules (sg) and the cell membrane (Cm). Secretory granules are located in direct contact with the cell membrane (arrow). Uranyl and lead. X 35,640.

Fig. 12 Dense bodies (Db) are present in the cytoplasm of the light cell. They are delineated by a fine limiting membrane and contain a finely, granular, evenly distributed material which is often separated from the surface membrane by a less dense zone. Uranyl and lead. X 41,250.

Fig. 13 Light cell (L) is always separated from the luminal colloid (C) by follicular cells (F) which may be extremely attenuated. In the light cell (L) cisternae of the rough-surfaced endoplasmic reticulum (Rer), free ribosomes (r), mitochondria (M) and some secretory granules (sg) are observed. Uranyl and lead. X 13,650.


sg
Cm


Db
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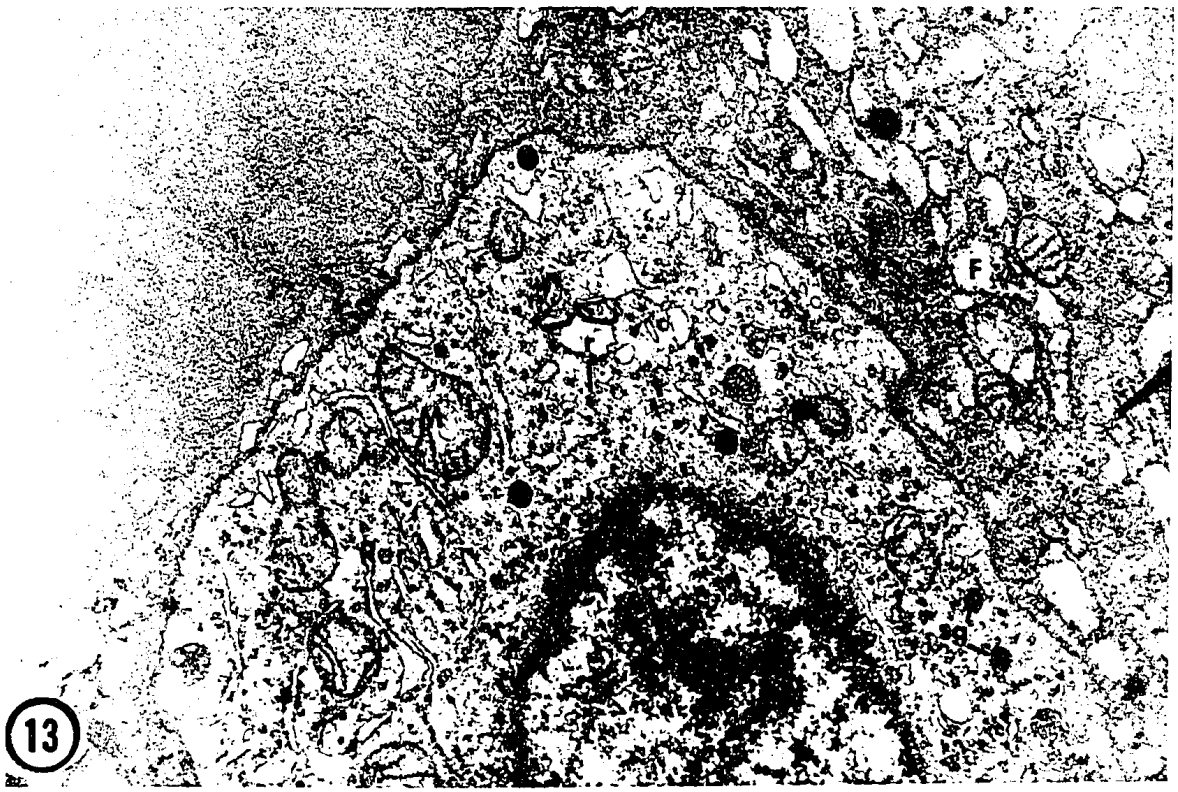

F
Ner
sg
13

membrane and usually contained a finely, granular material (Fig. 12).

Mitochondria were present in moderate number. In general, they varied from round to oval. However, sometimes long, slender forms of mitochondria were also encountered. The matrix was moderately dense and the cristae were transversely oriented.

In addition, the cytoplasm contained occasional multivesicular bodies. The single-membrane limited structure measured approximately 450 mμ in diameter. It contained multiple small vesicles of varying sizes in a moderately dense matrix (Figs. 8 and 9).

Parathyroid Hormone Treated Rat

There was an increase in the number of the light cells in the thyroid gland of the parathyroid hormone treated rats. The light cells were hyperplastic and groups of two to five cells were frequently observed. In addition, many light cells were hypertrophied. In these cells, the cytoplasm bulged into the connective tissue. However, other light cells, characterized by the reduced cytoplasm were also observed.

The most conspicuous change in the light cells was the general decrease in the number of the

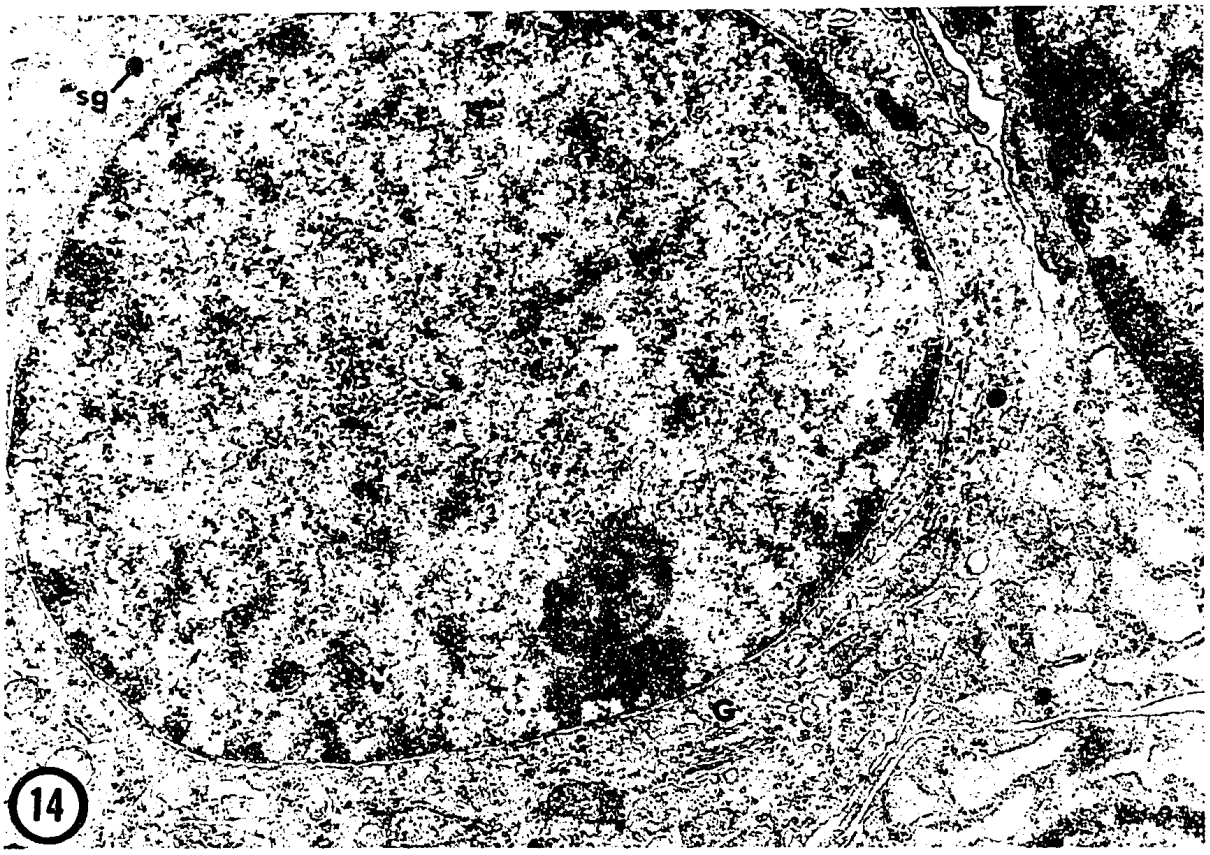
secretory granules. In many light cells the cytoplasm was totally depleted of the secretory granules (Figs. 14 and 15). However, in other light cells, variable number of secretory granules were observed located near the cell membrane (Fig. 19) or in the vicinity of the Golgi complex (Fig. 15).

The endoplasmic reticulum showed prominent changes. In many cells, the rough-surfaced endoplasmic reticulum was well developed and hypertrophied. In places, the cisternae of the rough-surfaced endoplasmic reticulum were organized in parallel arrays (Figs. 15, 16 and 18). In other cells, the endoplasmic reticulum consisted mainly of isolated cisternae which ramified throughout the cytoplasm (Fig. 14). The lumen of the cisternae usually contained some finely floccular material (Fig. 18). Free ribosomes were also observed in the cytoplasm (Fig. 15).

The Golgi complex was prominent and markedly enlarged in many light cells. In some cells, three to four groups of the Golgi complex were dispersed throughout the cytoplasm (Figs. 16 and 17). The Golgi complex invariably consisted of stacks of five to eight saccules arranged in concentric layers. Saccules with other configurations were frequently observed. In some cases, the saccules were dilated at their extremeties and at points along their length (Figs. 16 and 17),

Fig. 14 A discharged light cell from the parathyroid hormone treated rat. The reduced cytoplasm contains occasional secretory granules (sg). The Golgi complex (G) is small and appears inactive. Uranyl and lead. X 13,650.

Fig. 15 Light cell of the parathyroid hormone treated rat showing the decrease in the number of secretory granules (sg). The endoplasmic reticulum (Rer) is well developed. Mitochondria (M), free ribosomes (r) and occasional secretory granules (sg) are present. Uranyl and lead. X 13,650.



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15

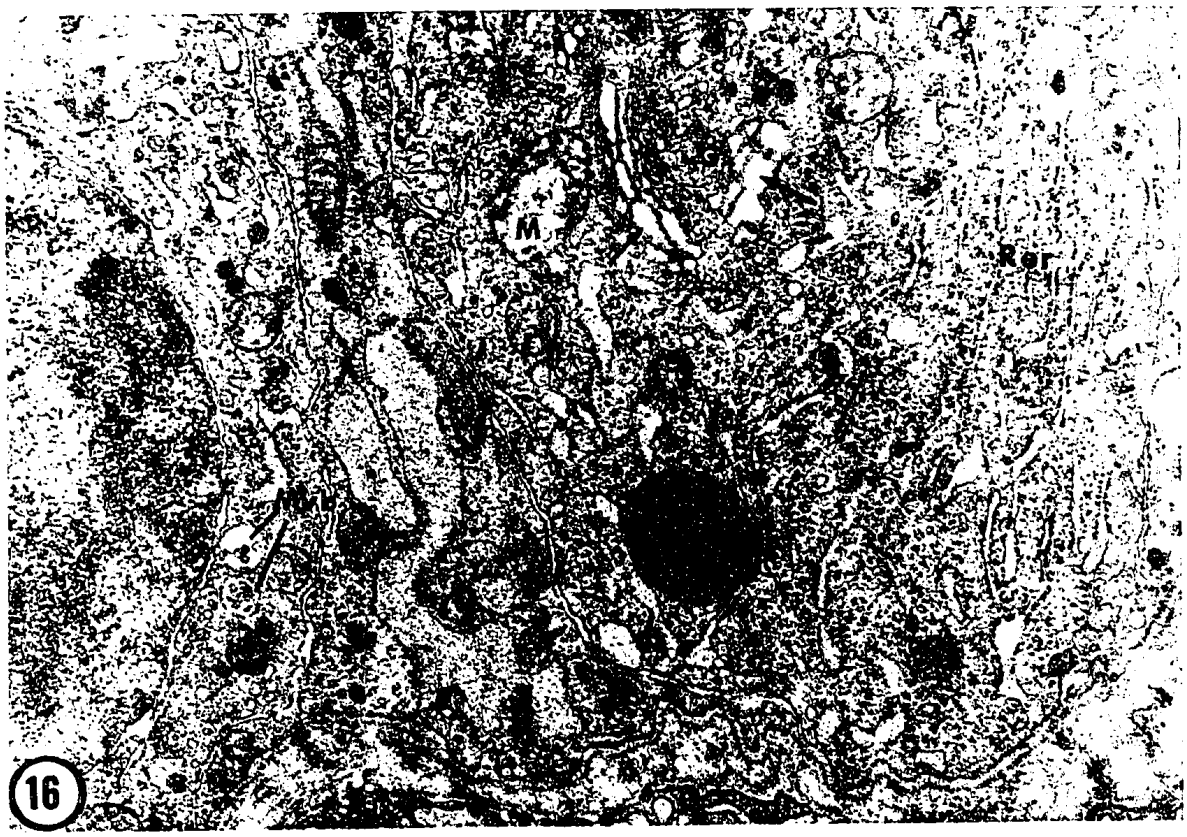
whereas in others, the entire cisternae of the saccules were dilated. Clusters of smooth-surfaced vesicles, which measured from 300 to 800 Å in diameter and some vacuoles were observed in the Golgi region (Fig. 18). In addition, varying masses of condensing secretory material and some secretory granules were present.

There was a general increase in the number of mitochondria in the light cells of the parathyroid hormone treated rats. Although mitochondria of normal size were seen in many cells, other mitochondria showed changes in size and configuration (Fig. 17). Profiles of mitochondria varied from round to elongated form. The enlarged mitochondria showed changes in morphology which varied from club-shaped to other bizarre configurations. The internal matrix of the mitochondria was dense and homogeneous and showed changes from those in the controls. Mitochondria were distributed randomly throughout the cytoplasm, although a greater concentration was usually found in the Golgi region (Fig. 17).

Multivesicular bodies were more numerous than in the controls. Often, two to four multivesicular bodies were present in an individual cell (Fig. 16). They contained multiple small vesicles of varying sizes. The background matrix was usually dense and

Fig. 16 Active light cells in the hypercalcemic rat. The endoplasmic reticulum (Rer) is well developed and consists of a network of cisternae. The saccules of the Golgi complex (G) are dilated in places (arrow). Some mitochondria (M) show loss of cristae. Multivesicular bodies (Mvb) are also seen. Uranyl and lead. X 13,650.

Fig. 17 Golgi region in the actively secreting light cells. Several groups of Golgi complex are present throughout the cell. Saccules are dilated. Mitochondria are numerous (M) and some are enlarged and show loss of cristae. Uranyl and lead. X 13,650.



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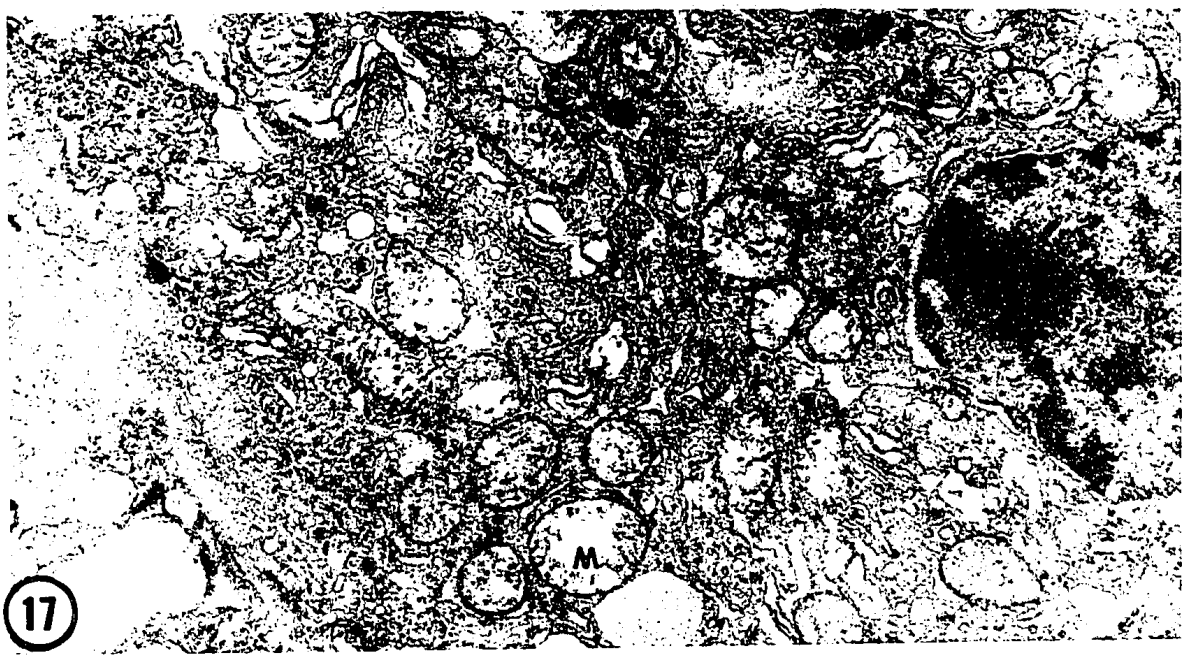

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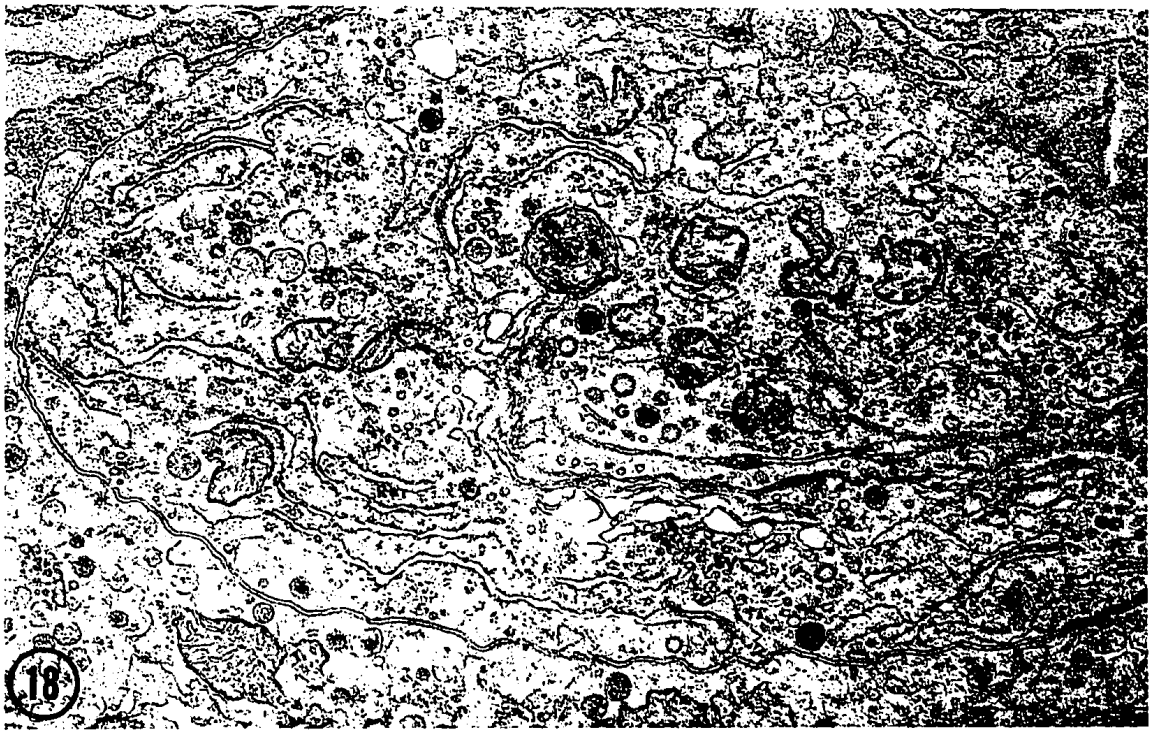
Fig. 18 Light cells in the parathyroid hormone treated rat showing the development of the endoplasmic reticulum (Rer) and the Golgi complex (G). Numerous smooth-surfaced vesicles (Sv) are present in the Golgi region. Osmium tetroxide fixation. Lead. X 19,500.

Fig. 19 Light cell in the parathyroid hormone treated rat showing the presence of variable number of secretory granules (sg). Secretory granules (sg) are lined along the cell membrane (Cm). Uranyl and lead. X 13,650.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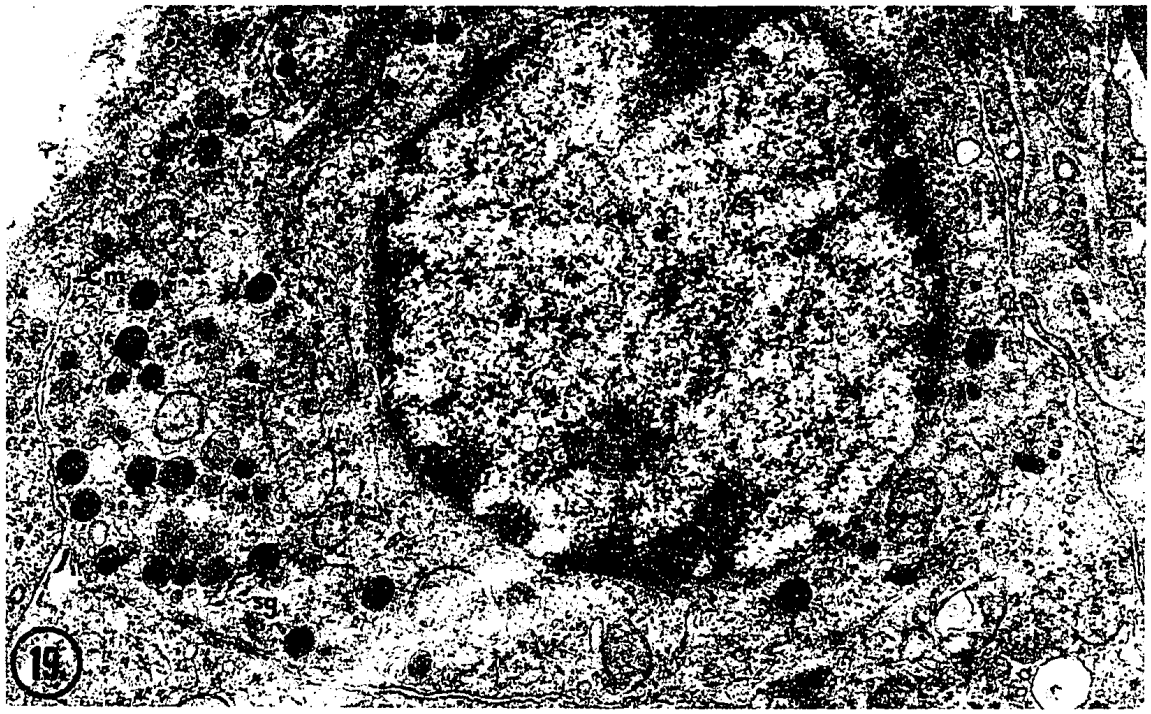
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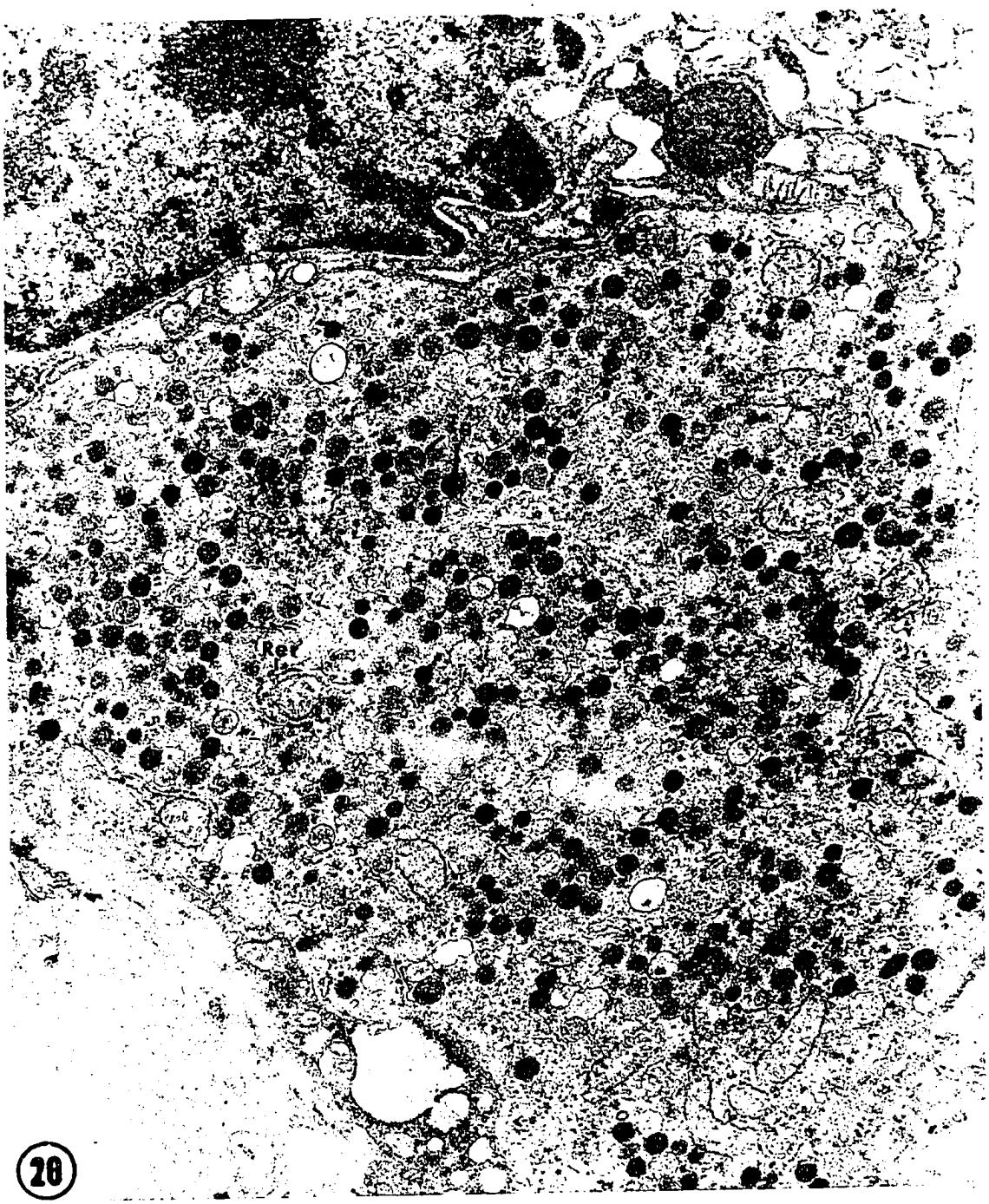
homogeneous. In addition, some multivesicular bodies were partially emptied of their vesicles and varying number of small vesicles were observed just outside the outer limiting membrane of the multivesicular bodies.

Low Calcium Diet

In the rats fed a low calcium diet, the light cells of the thyroid gland were easily distinguishable (Fig. 20). They usually occurred individually although occasionally small groups of cells were observed.

In the hypocalcemic state, the majority of the light cells were generally small. The cytoplasm was characterized by the presence of numerous secretory granules. The secretory granules varied from round to ovoid in shape and measured approximately 150 to 300 mμ in diameter. Each individual secretory granule was delineated by a delicate, closely-applied limiting membrane. The contents of the secretory granules varied in electron density. In many secretory granules, the contents were extremely electron dense, whereas in others, various degrees of opacity were observed (Figs. 20 and 21). Nevertheless, in occasional secretory granules, the contents appeared partially empty (Figs. 20 and 21). The secretory granules were distributed

Fig. 20 Light cell from the thyroid of the rat on a low calcium diet. The cytoplasm contains numerous secretory granules (sg) which vary in density. The endoplasmic reticulum (Rer) is poorly developed. Uranyl and lead. X 13,650.


Rer
28

randomly throughout the cytoplasm.

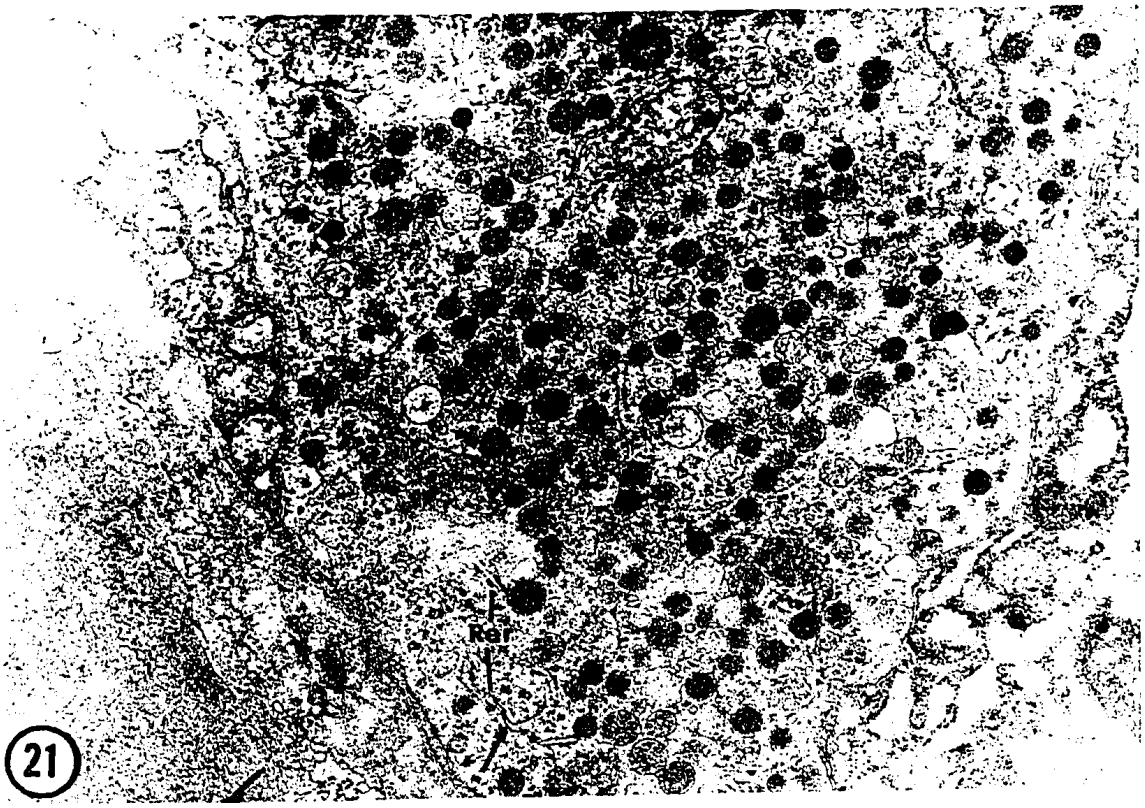
The endoplasmic reticulum was poorly developed. In many light cells, the rough-surfaced endoplasmic reticulum consisted of short, isolated cisternae distributed randomly throughout the cytoplasm (Figs. 20 and 21). The lumen of the cisternae was narrow and appeared constricted. In certain areas, the ribosomes on the membrane of the cisternae were detached (Fig. 21). However, in occasional light cells, the endoplasmic reticulum was moderately developed. In such cells, the cisternae were more organized (Fig. 22).

The Golgi complex was inconspicuous and infrequently seen. It usually consisted of a few membranous saccules and some smooth surfaced vesicles. No secretory materials in various stages of condensation were observed.

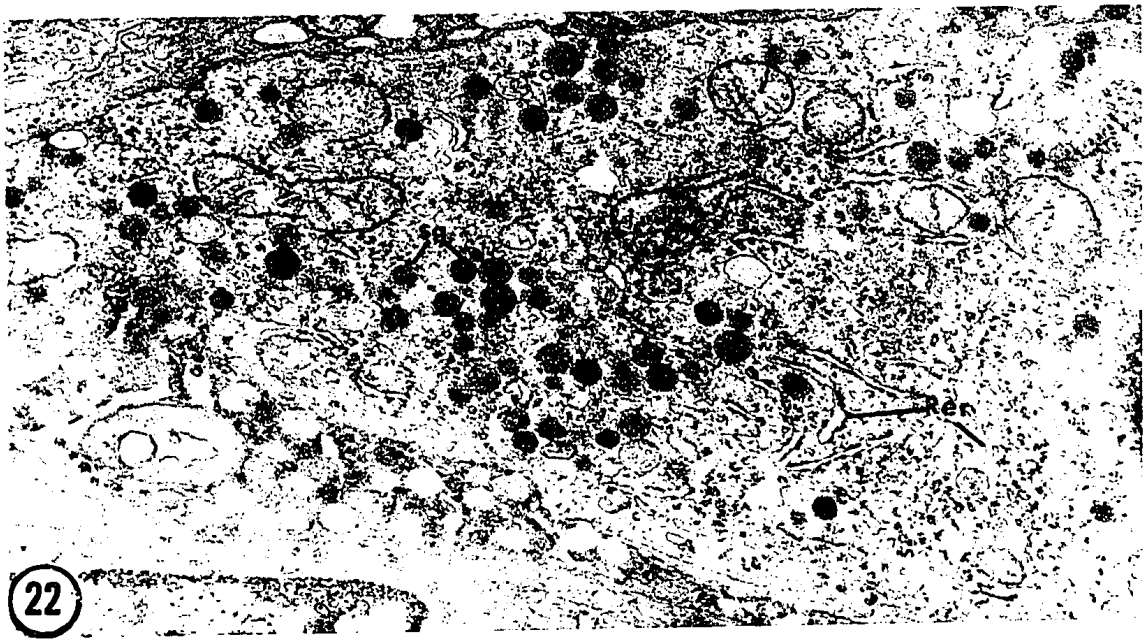
Variable number of mitochondria were present in the light cells of the hypocalcemic rats. The size and shape of the mitochondria varied, but some mitochondria were reduced in size. The mitochondrial matrix was homogeneous and dense. The cristae in some mitochondria appeared normal but in others, there seemed to be a reduction in number.

Fig. 21 Portion of the light cell from the thyroid of the rat on a low calcium diet showing the dense cytoplasmic matrix. The endoplasmic reticulum consists of isolated strands of cisternae (Rer) which in places show loss of ribosomes (arrow). Uranyl and lead. X 15,400.

Fig. 22 Light cell of the rat on low calcium diet showing the moderately developed endoplasmic reticulum (Rer) and the secretory granules (sg). Uranyl and lead. X 13,650.



21



22

Hypophysectomized Rat

Follicular Cell

Conspicuous changes occurred in the follicular cells after hypophysectomy. The cytoplasm of the follicular cells was greatly reduced in volume. In some areas, the cytoplasm was extremely attenuated (Fig. 23). Concomittant with the reduction in the cytoplasmic volume, there was a marked reduction in the development of the endoplasmic reticulum and Golgi complex. Similarly, there was a decrease in the number of globules and dense bodies in the follicular cells.

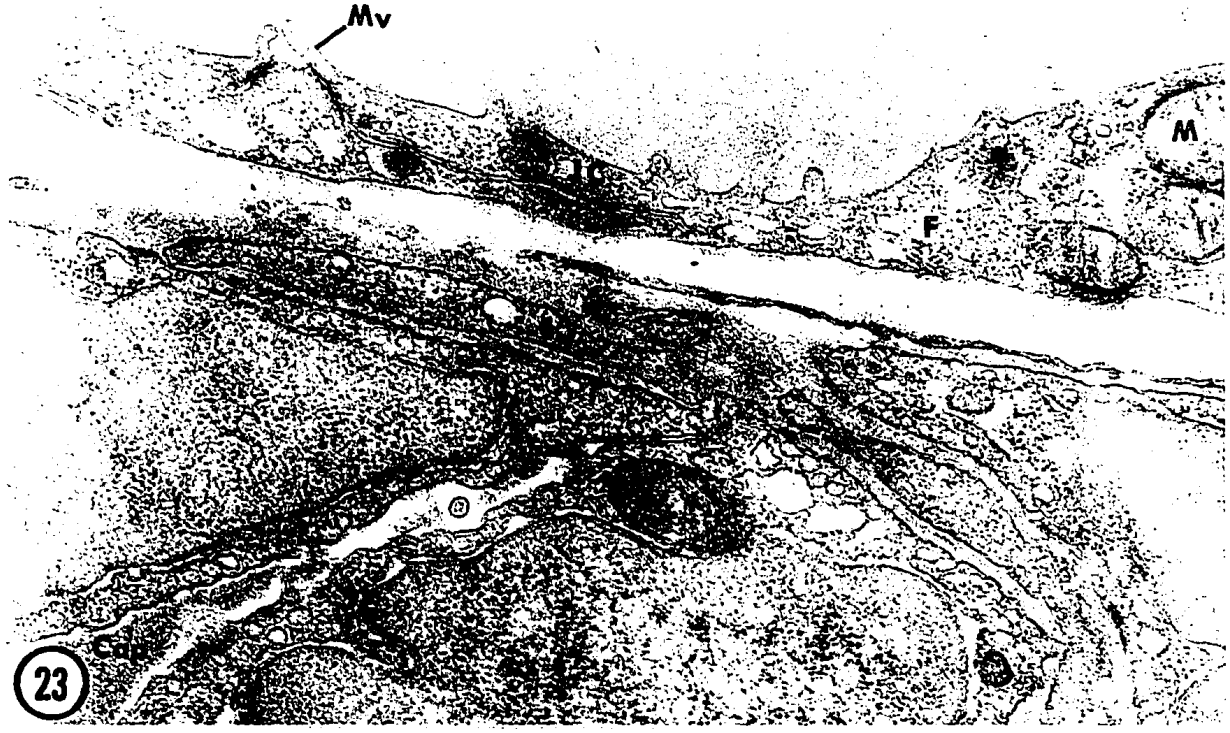
Light Cell

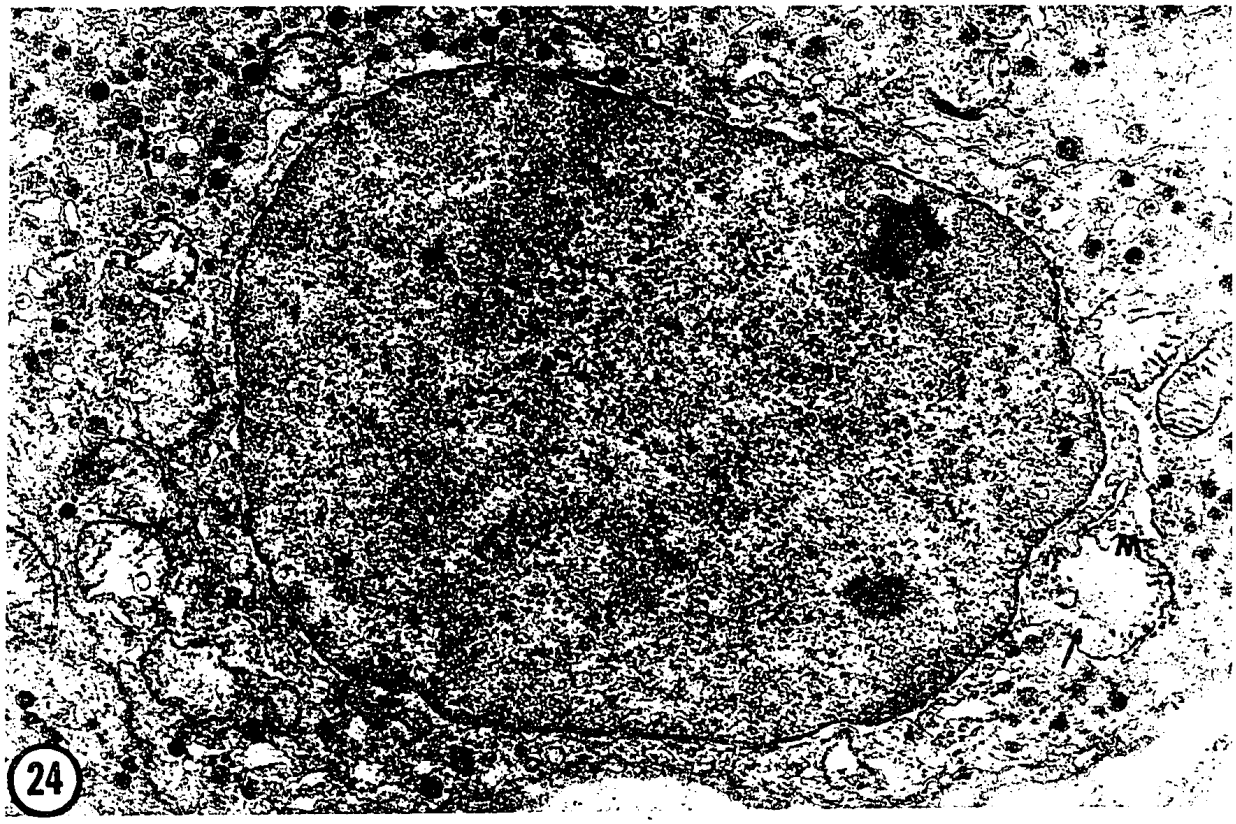
There was an increase in the number of the light cells as a result of the hypophysectomy. As a result of the atrophy of the follicular cells, light cells appeared more prominent. Groups of light cells were frequently observed. They occupied epi-and parafollicular locations.

Ultrastructurally, the light cells were characterized by the pale cytoplasm which contained numerous secretory granules. The secretory granules varied from round to ovoid and measured approximately 150 to 300 mμ in diameter (Fig. 25). The secretory granule was lined by a smooth limiting membrane. The contents of the secretory granules varied in degree

Fig. 23 Follicular cells (F) in hypophysectomized rat showing the great reduction in cytoplasmic volume. Microvilli (Mv) are reduced in number. Junctional complex (JC) and mitochondria (M) are observed. The bottom left shows a capillary (Cap). Osmium tetroxide fixation. Lead. X 29,700.

Fig. 24 Portion of the light cell in hypophysectomized rat showing the general distribution of secretory granules (sg). Mitochondria (M) show contoured outer surface and loss of cristae (arrow). The rough-surfaced endoplasmic reticulum consists of isolated cisternae (Rer). Osmium tetroxide fixation. Lead. X 13,500.


Mv
M
F
23


M
24

of opacity (Fig. 25).

There was a similar variance in the development of the endoplasmic reticulum in the various light cells. In the cells which contained numerous secretory granules, the endoplasmic reticulum was usually poorly developed. In such condition, the endoplasmic reticulum consisted of an occasional cisternae distributed randomly throughout the cytoplasm (Fig. 24). In other cells, the cisternae were organized into parallel arrays (Fig. 27).

The Golgi complex seemed to be prominent in some light cells. It consisted of three to four slightly curved smooth-surfaced saccules arranged in concentric layers. In some areas, the saccules were dilated. Smooth-surfaced vesicles and variable number of vacuoles were observed in the Golgi region (Fig. 26).

Mitochondria were present in moderate number. Mitochondria with the normal morphology were observed. In addition, enlarged mitochondria with contoured outer membrane were also encountered and in some cases, cristae were damaged (Figs. 24 and 27).

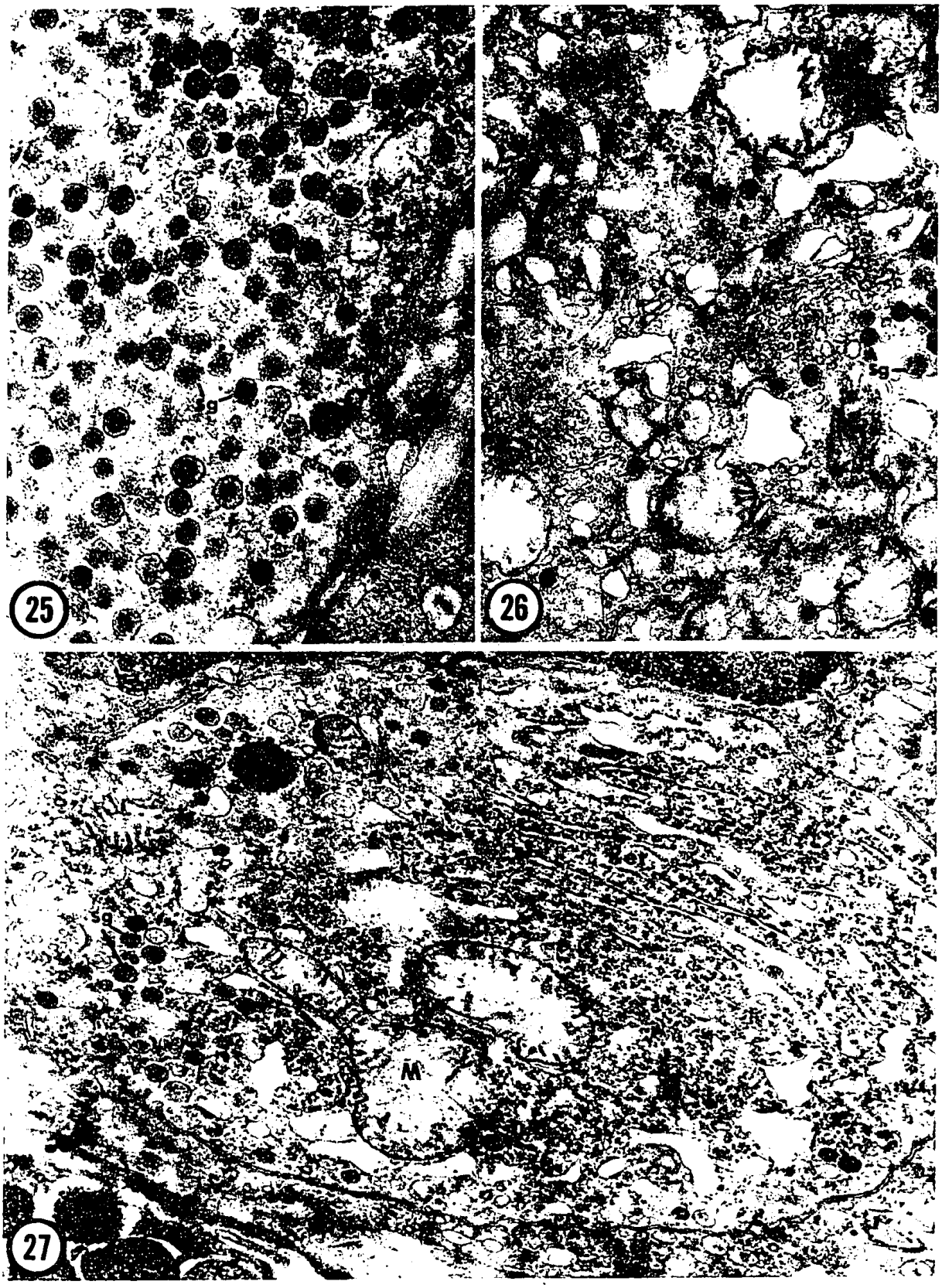
Hypophysectomized Rat on a Low Phosphate Diet

Under the combined effect of hypophysectomy and a phosphate deficient diet, there was a great

Fig. 25 Secretory granules (sg) of a light cell in the hypophysectomized rat. Secretory granules (sg) are lined by a smooth limiting membrane and contain dense, granular material. Uranyl and lead. X 35,640.

Fig. 26 Portion of the Golgi complex in the light cell of the hypophysectomized rat. Saccules are extensive and dilated in places. Numerous smooth-surfaced vesicles (sv) and some secretory granules (sg) are present. Uranyl and lead. X 30,690.

Fig. 27 Light cell of the hypophysectomized rat which shows a well developed endoplasmic reticulum (Rer). Mitochondria (M) are enlarged and some show loss of cristae. Free ribosomes and variable number of secretory granules (sg) are observed. Uranyl and lead. X 35,000.


sg
25
sg
26
sg
M
27

increase in the number of light cells. Light cells occurred singly or in groups which contained up to twenty-five cells or more (Figs. 28 and 29). The hyperplastic cells usually occupied inter- or parafollicular sites between the follicles (Fig. 28).

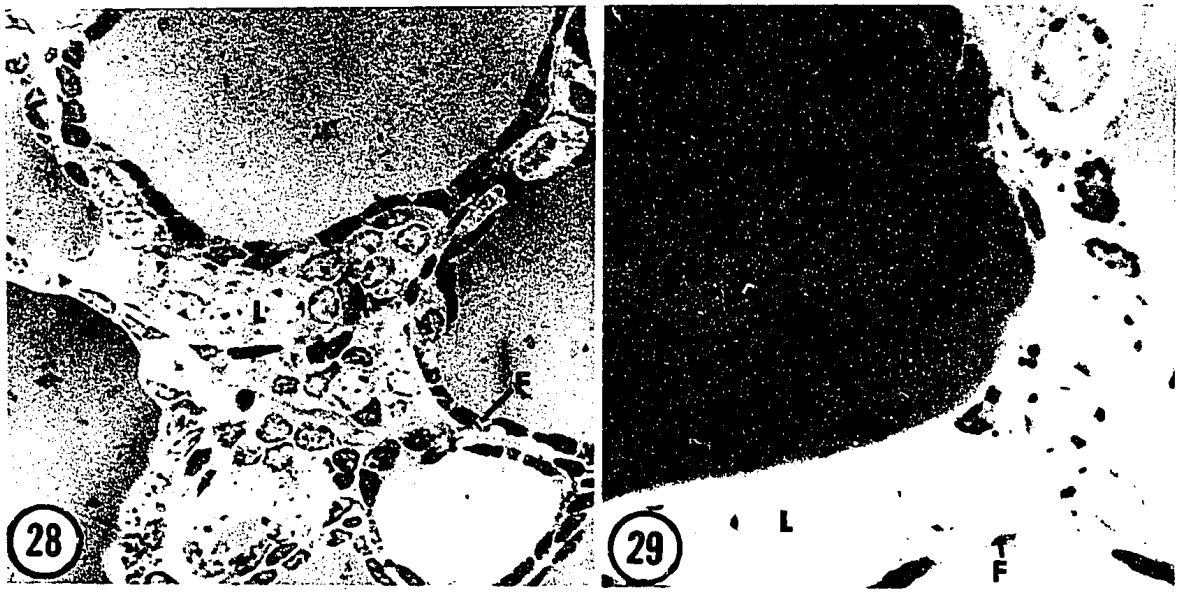
Ultrastructural observation confirmed the hyperplastic areas to consist exclusively of light cells. Although the light cells were generally variable in size, many hypertrophied cells were observed (Fig. 31). The cytoplasm of the light cells was characterized by the presence of variable number of secretory granules which measured approximately 150 to 300 mμ in diameter. The distribution of the secretory granules appeared to be random. However, in some cells, many secretory granules were observed to be located along the cell membrane (Fig. 33) whereas in others, there seemed to be a decrease in the number of secretory granules (Figs. 32 and 36).

The endoplasmic reticulum was invariably developed in the light cells. In many cells, the endoplasmic reticulum was extensive and consisted of cisternae arranged in parallel arrays (Figs. 32 and 35). In other cells, the cisternae were distributed randomly throughout the cytoplasm (Fig. 30). Free ribosomes were abundant in the cytoplasm of

Fig. 28 Thyroid tissue from a hypophysectomized rat on a low phosphate diet showing an area of hyperplastic light cells (L). The follicular cells (F) are atrophied. Epon-embedded section. Toluidine blue. X 425.

Fig. 29 High power view of some light cells (L) and follicular cells (F). Light cell (L) contains a pale cytoplasm. Epon-embedded section. Toluidine blue. X 1,800.

Fig. 30 Portions of several light cells in hypophysectomized rat and fed a low phosphate diet. The light cells contain numerous secretory granules (sg) which vary in electron density. Endoplasmic reticulum (Rer), mitochondria (M) and free ribosomes (r) can be observed. Uranyl and lead. X 15,340.


L
E
28
L
29
F

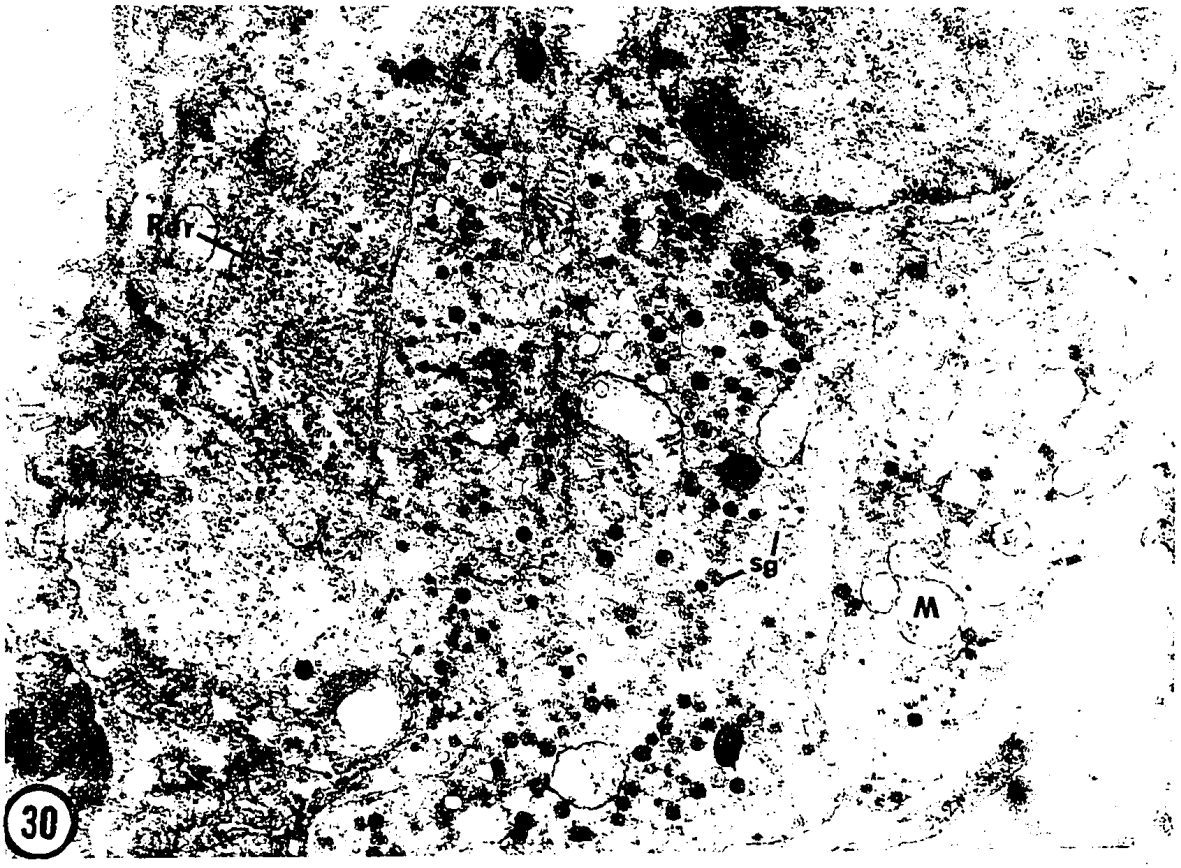
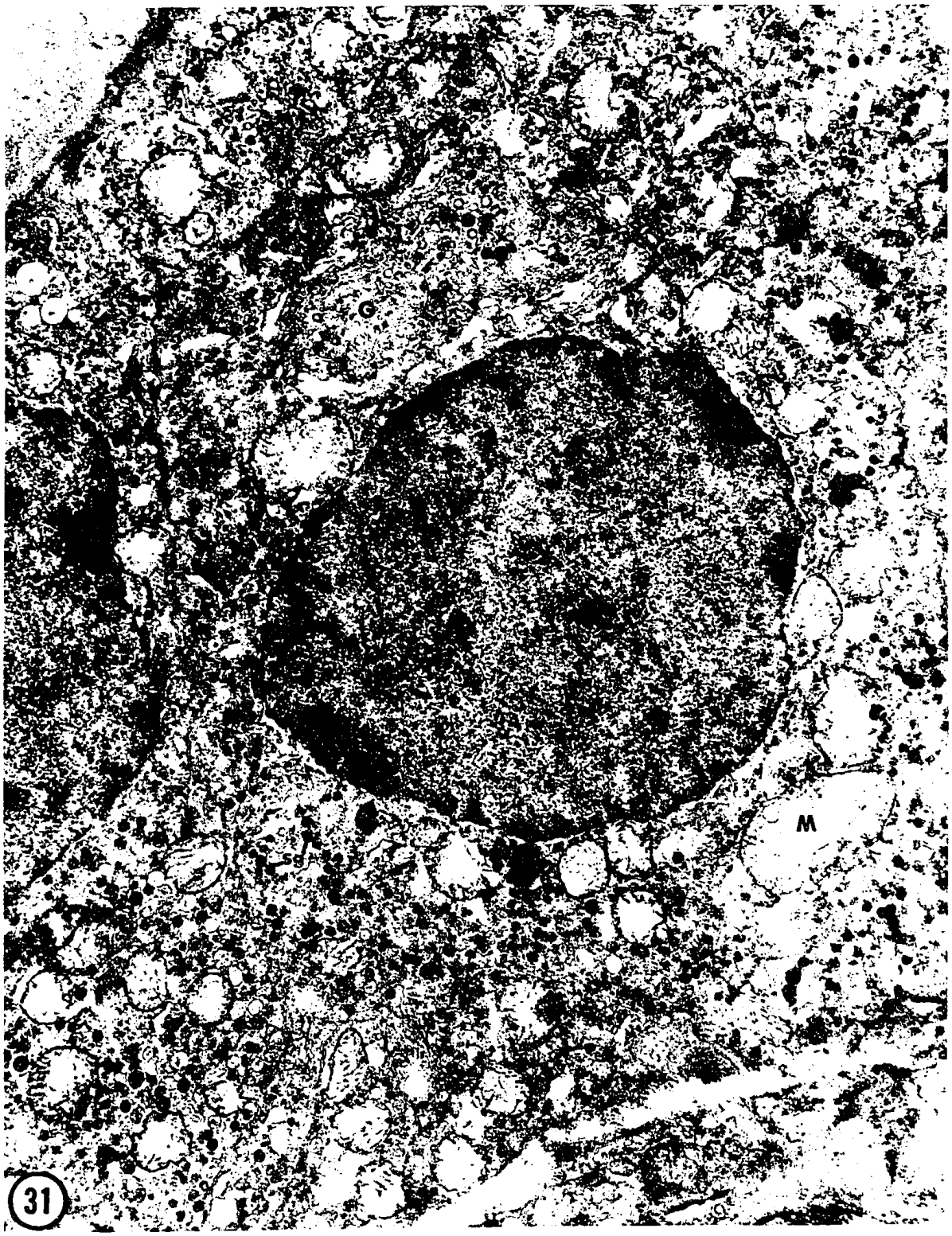

RER
sg
M
30

Fig. 31 Portions of the hyperplastic light cells showing the presence of secretory granules (sg), free ribosomes (r) and the extensive Golgi complex (G). Mitochondria (M) are numerous and some are enlarged and show loss of cristae. Uranyl and lead. X 14,750.


G
M
31

many light cells (Figs. 30 and 34).

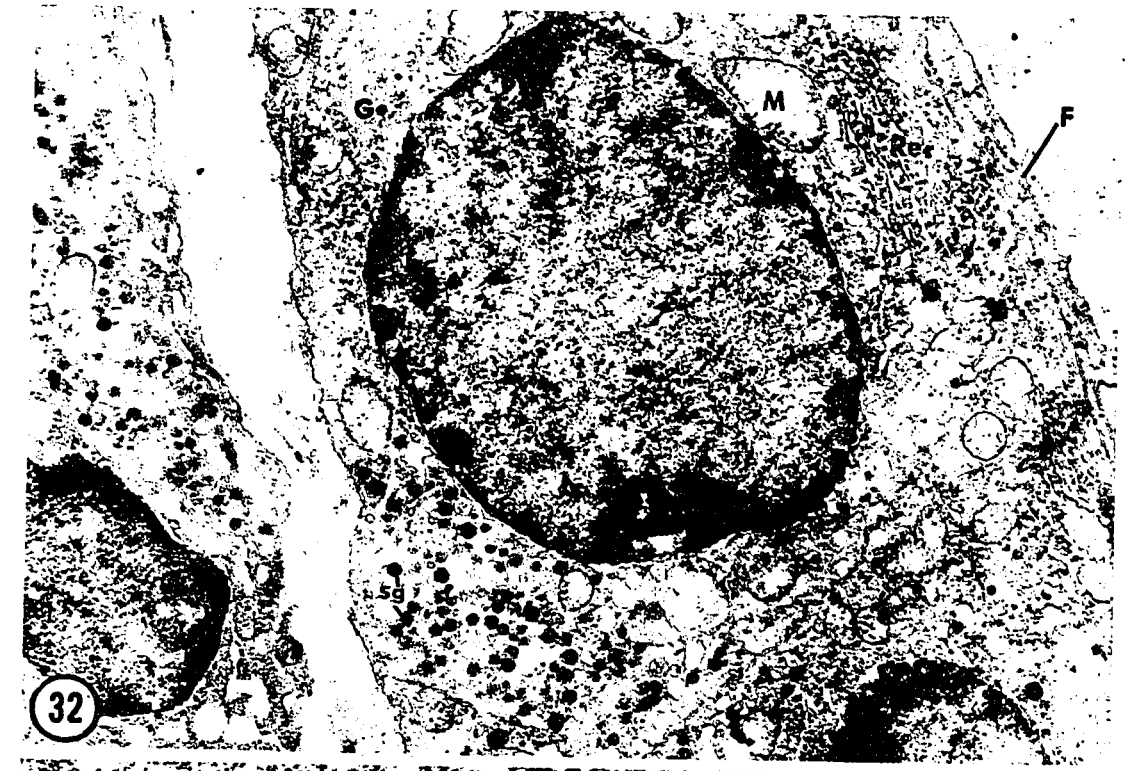
The Golgi complex seemed to be more prominent. In some cells, two to three groups of the Golgi complex were present in an individual cell. The saccules were more extensive. Some saccules were dilated at points along the length while others assumed irregular profiles (Fig. 34). Numerous smooth-surfaced vesicles, which measured 300 to 800 Å in diameter were present in close association with the cisternae (Fig. 35).

Mitochondria seemed to be increased in number. Some mitochondria were dilated and possessed a contoured outer membrane. In such cases, some cristae were usually damaged (Fig. 31). Enlarged mitochondria were frequently observed.

There was an increase in the number of multivesicular bodies in the cells. Dense bodies were frequently observed (Fig. 33).

Fig. 32 Light cells in the hypophysectomized rat fed a low phosphate diet. The endoplasmic reticulum (Rer) is well developed, Golgi complex (G) and mitochondria can be observed. The secretory granules (sg) seem to decrease in number. The follicular cell (F) is atrophied. Uranyl and lead. X 14,750.

Fig. 33 Light cell in hypophysectomized rat on low phosphate diet. The secretory granules (sg) and some dense bodies (Db) are observed. Uranyl and lead. X 35,000.

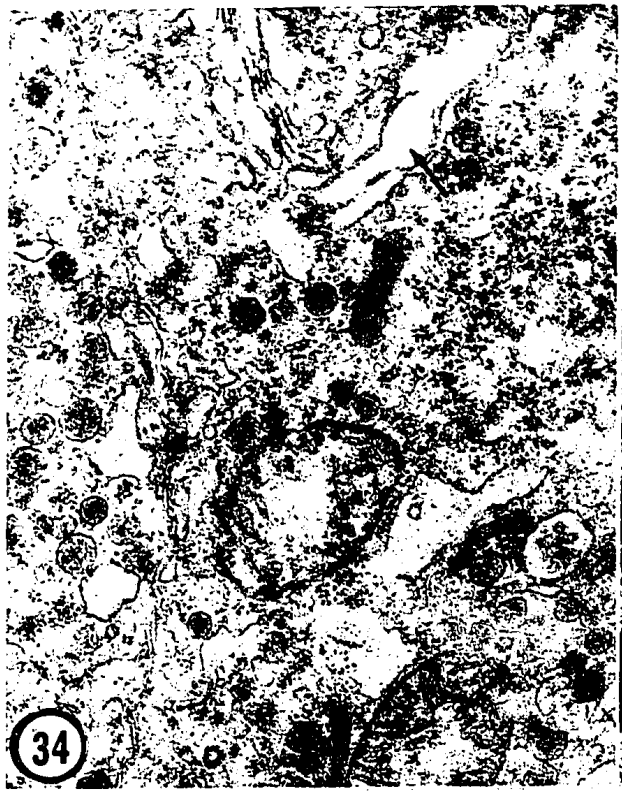

G
M
Re
F
Sg
32


M
Gl
33

Fig. 34 An area of the Golgi complex from the light cell of a hypophysectomized rat on a low phosphate diet showing the dilated saccules (arrow). Numerous free ribosomes (r) are present. Uranyl and lead. X 29,000.

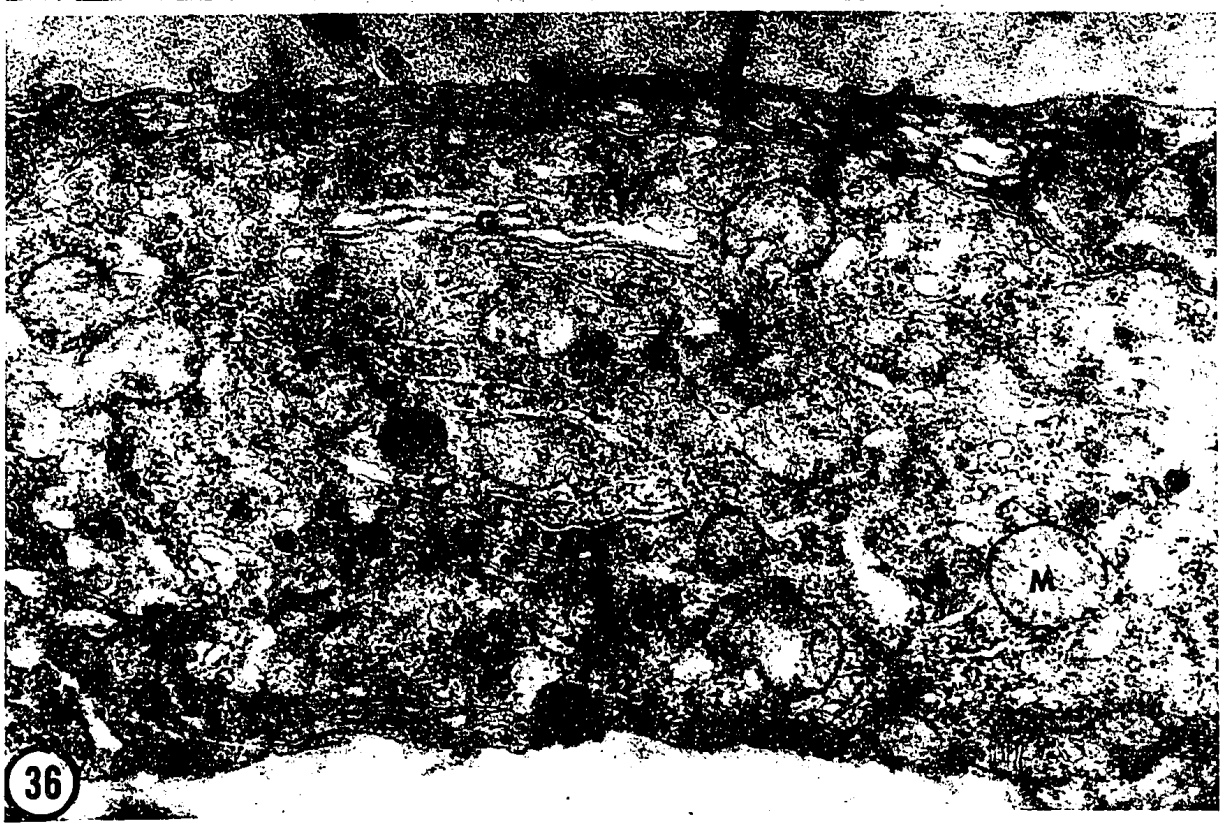
Fig. 35 Portion of a well developed endoplasmic reticulum (Rer) in the light cell of the hypophysectomized rat on a low phosphate diet. The cisternae are arranged in parallel arrays. The lumen of the cisternae contain a finely floccular material. Numerous smooth-surfaced vesicles (sv) are observed. Uranyl and lead. X 35,000.

Fig. 36 A light cell of the hypophysectomized rat on a low phosphate diet showing the development of the endoplasmic reticulum (Rer) and Golgi complex (G). Mitochondria (M) and some secretory granules (sg) are present. Uranyl and lead. X 27,720.



34

35



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3. Discussion

Light Cell in Normocalcemia

Although the presence of the light cell has been recognized by light microscopy in the various mammalian species under different names, its existence has not gained universal acceptance. A minority of opinions regard them as tangential sections of follicles and "artefacts of the histological technique" (Wilson, 1927; Ludwig, 1953).

With the electron microscope, the light cells can be easily identified. In the present study, the ultrastructural differences between the light and the follicular cells are in agreement with those reported by Wissig (1962), Young and Leblond (1963) in rat and by Tashiro (1964) in dog. Furthermore, no intermediate forms between the light and the follicular cells are observed.

Numerous papers have been published speculating on the function of the light cell. Wissig (1962) considered them to be "a second independent class of endocrine epithelial cells" but he failed to cite any specific function. Young and Leblond (1963) regarded them as follicular cells which lost contact with the colloid and were unable to discharge their secretion due to the degeneration of the secretory

vesicles. Similarly, Azzali (1964) believed they were a functional stage of the follicular cells. On the other hand, Luciano and Reale (1964) suggested the possibility of the reversible development between the two cell types because of the similarity in cytoplasmic components.

At the ultrastructural level, the morphologic evidence seems to suggest a secretory function for the light cells. The cytoplasm of these cells is characterized by the presence of variable number of membrane-limited granules. In osmium tetroxide fixation, the granules appear as vesicles which contain finely, granular materials which vary in density from light to moderate and have been referred to as vesicles (Wissig, 1962; Young and Leblond, 1963; Chan and Bélanger, 1968). However, in glutaraldehyde fixation followed by postfixation in osmium tetroxide, the contents of the granules presumably form complexes with the glutaraldehyde and render the material fairly dense. Combined cell fractionation and electron microscopy studies have shown the membrane-limited granules to be associated with hypocalcemic activity (Bauer and Teitelbaum, 1966; Cooper and Tashjian, 1966). Obviously, the evidence indicates that the contents of the membrane-limited

granules contain calcitonin and thus they may be called secretory granules.

The varied development of the rough-surfaced endoplasmic reticulum and the Golgi complex is presumably related to the synthesizing activity of the cells. The role of ribosomes and the rough-surfaced endoplasmic reticulum in protein synthesis is well established (Siekevitz and Palade, 1960; Caro and Palade, 1964). From the present study, it appears that the secretory material is synthesized at the level of the ribosomes, transferred into the cavities of the rough-surfaced cisternae and then concentrated in the Golgi complex. The presence of immature secretory granules in various stages of maturation in the vicinity of the Golgi complex seems to support the generally accepted view of the dehydrating and concentrating function of the Golgi complex (Zeigel and Dalton, 1962).

The mechanism whereby secretory granules are released from the light cells appears similar to that described in the anterior pituitary cells by Farquhar (1961) and Pasteels (1963). The secretory granules approach the cell membrane and the fusion ensues and this releases the secretory product. No trace of the products can be seen outside the cytoplasm.

Probably the products dissolve rapidly upon leaving the cytoplasm and undergo some physicochemical changes so that they are no longer visible.

The presence of secretory granules in close proximity with the cell membrane and images of the fusion of the limiting membranes of the secretory granules with the cell membranes in the light cells in normocalcemic conditions would seem to indicate that the secretory products are being released. This is in agreement with the results of Minkin and Talmage (1968) which show by the peritoneal lavage procedure that calcitonin is being secreted throughout the normal range of plasma calcium values (9 mg% to 11 mg%) in the rat. Furthermore, radioimmunoassay studies show that calcitonin is continuously being secreted in normocalcemia (Defto et al., 1968).

However, the presence of numerous secretory granules in many light cells in normocalcemia seems to indicate the ability of the cells to accumulate and store excess secretory products. The results correlate well with the observations of Care (1967) which show the resting thyroid gland to contain a large reserve of calcitonin. In this respect, the light cells in the thyroid gland differ from the chief cells in the parathyroid gland in their ability to store excess secretory products.

These findings lend considerable support to the hypothesis that calcitonin plays an important metabolic role in mammals by acting as a continuous influence on the rate of bone remodeling and by rapidly suppressing bone resorption in response to hypercalcemic challenge.

Effects of Parathyroid Hormone Stimulation

The parathyroid hormone has been shown to have its primary effect on bone. The hormone stimulates the release of calcium from bone by increased osteolysis around osteocytes (Bélanger et al., 1963) and by increased osteoclast activity (Toft and Talmage, 1960). An extract of the parathyroid hormone when injected into the experimental animals causes a rise in the serum calcium level.

The light cells are affected by the elevation of the calcium level in the blood. The initial reaction of the light cells to the hypercalcemia appears to be the degranulation of the secretory granules. This is reflected in the significant decrease in the number of the secretory granules in the light cells. Furthermore, images of secretory granules which lined closely along the cell membrane seem to suggest that they are in the process of being secreted. The presence of many depleted light cells demonstrates

that in conditions of hypercalcemic stress, most of the secretory granules can be rapidly utilized. This may probably be related to the secretion rate, which has been shown to be increased in the hypercalcemic conditions (Care, 1968).

The degranulation phenomena of the light cells is similar to those observed in chronic hypercalcemia induced by vitamin D_2 (Ericson, 1968) and in acute exogenous hypercalcemia induced by calcium perfusion (Matsuzawa and Kurosumi, 1967).

The increase in the number of light cells in the parathyroid hormone treated rat is significant. The hyperplasia of the light cells is possibly related to the increased demand of calcitonin. There is a four fold increase in the light cells of the calcium acetate-vitamin D_3 treated rats (Rohr and Hasler, 1968). However, in hypercalcemic perfusion studies, Pearse (1966a) claimed that the light cells were more difficult to find, whereas Young et al. (1968) observed no changes at all. It is not known whether the discrepancy is related to the transient nature of the perfusion experiments.

Other compensatory changes include augmentation of synthesizing activity in the light cells. This is reflected in the increased number of light

cells which seem to be in active synthesis. The hypertrophy of the endoplasmic reticulum, the prominent and extensive Golgi complex and the increased number of free ribosomes are morphologic parameters which seem to indicate increased metabolic and synthesizing activity of the cells. The elevated serum calcium level seems to have a direct effect on the synthesizing activity of the light cells.

The increased endogenous secretion of calcitonin in the parathyroid hormone treated rats demonstrates the importance of the light cells in the maintainance of calcium homeostasis. The major effect of calcitonin is to inhibit bone resorption (Friedman and Raisz, 1965; Milhaud et al., 1965; Aliapoulios et al., 1966). Calcitonin has been shown to block the parathyroid stimulation of calcium release and the subsequent increase in uridine incorporation into RNA (Raisz and Niemann, 1967). Rasmussen and Tenenhouse (1967) observed that simultaneous administration of calcitonin and excess parathormone prevented the development of renal calcinosis and soft-tissue calcification. Furthermore, Bélanger and Rasmussen (1968) demonstrated that osteocytes under the combined influence of parathyroid hormone and calcitonin produced an inadequate

amount of mucopolysaccharides and that their protease activity was reduced. Therefore, the increased secretion of calcitonin may presumably act to inhibit osteolysis caused by the excess parathyroid hormone and thus affect a homeostatic control. It is conceivable that the severity of the clinical manifestations of hyperparathyroidism might be related, in part at least, to the extent to which the light cells respond to hypercalcemia by increasing the production of calcitonin.

Nevertheless, with the persistent hypercalcemia, it is evident that the endogenous secretion of calcitonin is not sufficient to lower the serum calcium to normal values. The prolonged stimulus eventually lead to the depletion of the reserve calcitonin. This is in agreement with the bioassay studies which show reduced calcitonin content in the thyroid glands of hypercalcemic rats (Gittes et al., 1968) and patients with hyperparathyroidism (Tashjian and Voelke, 1967). In such conditions, the animals can be said to be in a calcitonin deficient state. Administration of calcitonin could possibly serve as a therapeutic agent in the management of hypercalcemia.

Effects of a Low Calcium Diet

The serum calcium levels seem to have a direct effect on the light cells. High serum calcium

level is a stimulus for the release of calcitonin (Copp et al., 1962; Kumar et al., 1963). Morphologically, this is manifested by the degranulation of the secretory granules as discussed in the previous section. However, in the hypocalcemic states induced by the low dietary calcium intake, the secretion of the calcitonin seems to be inhibited. This is reflected in the presence of many light cells whose cytoplasm is packed with secretory granules. The increase in density and number of the secretory granules is probably related to the increase in the amount of calcitonin which accumulated as the result of the absence of stimulus for secretion.

Other cytological changes in the light cells of the chronically hypocalcemic rats include the atrophy of the organelles involved in protein or polypeptide synthesis. The endoplasmic reticulum is poorly developed and in places, loss of ribosomes on the membrane of the cisternae seems to suggest signs of disorganization. The Golgi complex is seldom encountered. The morphological evidence seems to suggest a suppression of metabolic and synthesizing activity in the light cells. However, the exact manner by which the light cell function is regulated by changes in the serum calcium level is not known.

Dietary levels of calcium affect bone metabolism and therefore also affect the response to calcitonin. It is known that as the calcium content of the diet is decreased, bone resorption is increased (Bonner and Aubert, 1965). The increased resorption is presumably the result of the increased parathyroid hormone production stimulated by the persistent low serum calcium value. Possibly, this represents the attempts of the animal to increase the serum calcium level to normocalcemia. However, because the dietary regimen is low in calcium, the serum level tended to remain low, causing continued stimulation of the parathyroid hormone secretion.

In such conditions of high bone resorption, the action of calcitonin can be anticipated to be more enhanced. The low serum calcium level inhibits the release of endogenous calcitonin. Therefore, the administration of calcitonin tends to give a maximum response. This probably explains why rats on the low calcium diet give a better response to calcitonin (Copp and Kuczerpa, 1966a).

Effects of Hypophysectomy

The atrophy of the follicular cells subsequent to hypophysectomy is in accord with the concept of the pituitary control of the cell. On the contrary, the increase in the number of light cells in the syndrome demonstrates clearly that the light cell is independent of pituitary control. The hyperplasia of the light cells has been reported by Saito and Shibata (1957); Thompson et al. (1962) and Yoshimura et al. (1962). Furthermore, the possibility that the change in ratio of light cells over follicular cells might be an artifact due to atrophy of follicular cells is discounted by the statistical studies of Sarker and Isler (1963).

The light cells seem to be functional in the absence of the pituitary control. This is reflected in the presence of light cells which appear morphologically similar to those in the controls. Other light cells endowed with well developed endoplasmic reticulum and Golgi complex seem to suggest evidence of metabolic and synthesizing activity. However, with the great increase in the light cell population, the possibility exists that these cells might represent younger cells which are in various stages of maturation.

The changes in the mitochondria in some of the light cells are of interest. The enlargement of the mitochondria increases considerably the effective

surface area of the inner mitochondrial membranes. Presumably, this may be a compensatory response of the light cells to the changes in the levels of precursor substance. However, the loss of cristae of the enlarged mitochondria would seem to suggest that the phenomena may be short-lived, terminating with the degeneration of the organelles.

The functional significance for the hyperplasia of the light cells is not known. The removal of the pituitary gland diminishes the secretion of the thyroid hormone. In the hypothyroid states, the rate of bone turnover is reduced. Furthermore, hypophysectomy, as a growth depressing factor, is known to inhibit bone resorption responses to parathyroid hormone (Copp and Kuczerpa, 1967). In such conditions, the requirement of calcitonin can be anticipated to be considerably reduced. However, the possibility exists that a greater release of the secretory product is not taking place. The serum calcium level is not much affected by the hypophysectomy. Therefore, in the absence of the stimulus for secretion, the secretory granules can accumulate in the cells. Although the secretory granules appear morphologically similar to those in the controls, it is not known whether there is any biochemical or immunological changes in the secretory

granules as a result of the adaptation of the light cells to the reduced turnover of skeleton present in hypothyroidism. However, it has been shown that the amount of calcitonin in the rat thyroid gland is not appreciably affected by hypophysectomy (Milhaud and Moukhtar, 1965). This is significant in view of the increase in number of the light cells in the hypophysectomized animal. Therefore, the possibility of degeneration of the secretory granules should not be discounted.

Effects of Hypophysectomy and a Phosphate Deficient Diet

A diet deficient in phosphate causes a prompt rise in the serum calcium level in the rat (Copp et al., 1965). The hyperplasia of the light cells in the phosphate deficient state presumably is related to the compensatory response of the cells to the hypercalcemic stimuli (Chan and Bélanger, 1968).

In the hypophysectomized rats maintained in the phosphate deficient diet, the light cells seem to increase further in number. Morphologically, the light cells seem to be functionally active. The decrease in the secretory granules of some of the light cells seems to suggest secretory activity. Furthermore, other changes in the light cells seem

to indicate increased metabolic and synthesizing activity. The increase, in many light cells of organelles that are involved in protein synthesis, such as well developed endoplasmic reticulum, prominent Golgi complex, free ribosomes and mitochondria are morphological evidence suggesting increased metabolism of the light cells. These changes are probably related to the manifestation of the light cells to the hypercalcemic stimuli. It provides further evidence that the light cells of the hypophysectomized rat are still responsive to the normal stimuli. Baghdiantz et al. (1965) demonstrated the ability of the hypophysectomized rat to withstand hypercalcemic perfusion. On the other hand, Care et al. (1968) found that in the pig, the ability to withstand hypercalcemia was impaired by hypophysectomy. However, the reason for the discrepancy is not known.

C. Experiment 2. Effects of Dietary Calcium on the Ultimobranchial Gland in the Chick

1. Introduction

Investigations of the ultimobranchial gland of the chick have been chiefly concerned with the embryonic development and description of the location of the glands, and therefore little attention was given to the morphology of the gland (Johnson, 1918a; Terni, 1924, 1927; Dudley, 1942; Nagy and Swartz, 1966). The histology of the gland has been briefly described in some species of birds (Watzka, 1933; Sehe, 1965) and chick (Michelucci, 1961).

Various functions have been proposed for the ultimobranchial gland in the birds. Terni (1927) and Watzka (1933) suggested an endocrine function for the gland. Dudley (1942) concluded that the gland had no secretory function of endocrine importance. However, the presence of follicles in the ultimobranchial gland has been interpreted as thyroid-like "colloid" in appearance and function. Radioautographic studies in chicks and several species of birds demonstrate that the ultimobranchial tissue does not bind I^{131} (Michelucci, 1961; Sehe, 1965). Moreover, histochemical studies fail to show any functional similarity between the thyroid and the ultimobranchial gland in the

chick embryo (Nagy and Swartz, 1966).

Recent studies have shown the high calcitonin content in the ultimobranchial glands of the chickens, pigeons and turkeys (Copp et al., 1967b; Tauber, 1967; Mosely et al., 1968; Copp and Parkes, 1968; Matthew et al., 1968). Moreover, the thyroid gland of the chick has been shown to contain no hypocalcemic activity (Copp et al., 1967a; Tauber, 1967; Kraintz and Puil, 1967).

Little information is available on the role of the ultimobranchial gland in the avian species. However, recent brief reports on the effect of dietary calcium on the ultimobranchial gland in cockerels (Copp et al., 1968) and chicks (Chan et al., 1968) seem to suggest a possible functional role for the gland.

Knowledge of the role of the ultimobranchial glands in the avian species is of particular interest for a variety of reasons. Calcium metabolism in the avian species is more intense than those in other vertebrates. This is due to the need for a buoyant skeleton and the need for an adequate calcium reservoir for the egg shell formation. In this regard, it might reasonably be expected that special mineral problems to either affect or be affected by the parathyroid

and the ultimobranchial glands.

The present study was designed to investigate the effects of a dietary regimen containing a high and a low content of calcium on the ultimobranchial gland of the chick. It was hoped that the ultrastructural characteristics of the ultimobranchial gland observed would provide some clues as to the functional significance of the gland.

2. Observations

Anatomical Relationships

The ultimobranchial gland is located near the origin of the subclavian and the common carotid arteries (Fig. 3). It is embedded in the adipose tissue and lined along the carotid artery, more posteriorly to the thyroid and the parathyroid glands (Fig. 37).

Serum Calcium Levels

The mean serum calcium of chicks are shown in Fig. 4. In the control chicks, the mean ± S.E. level of serum calcium was 10.39± 0.185 mg%. In the high calcium group, the value was 13.89± 0.386 mg% and in the low calcium group, the value was 5.78 mg%±0.237 mg%.

Controls: Light Microscopy

The parenchyma of the ultimobranchial gland consisted of clusters and strands of polygonal

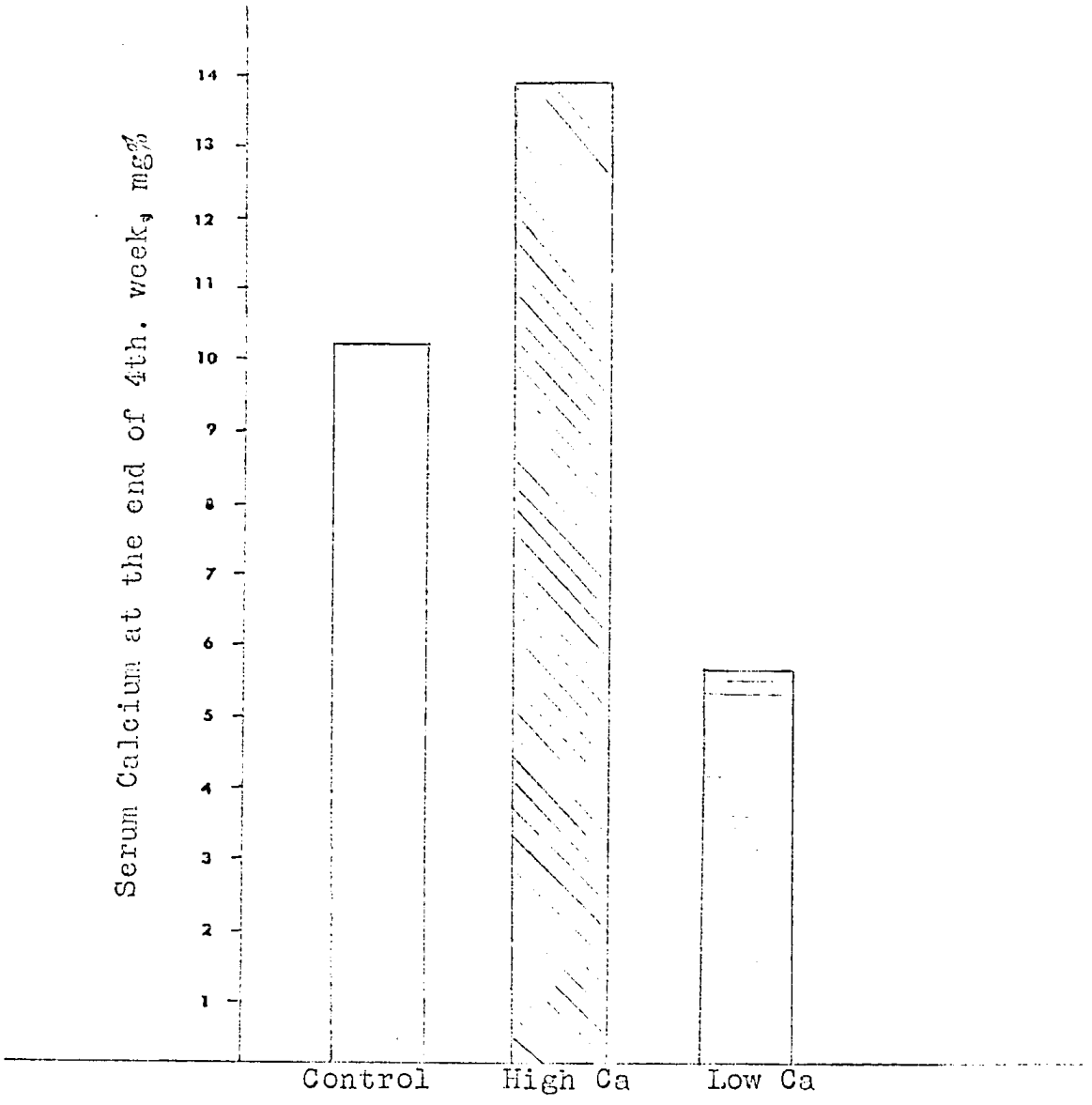
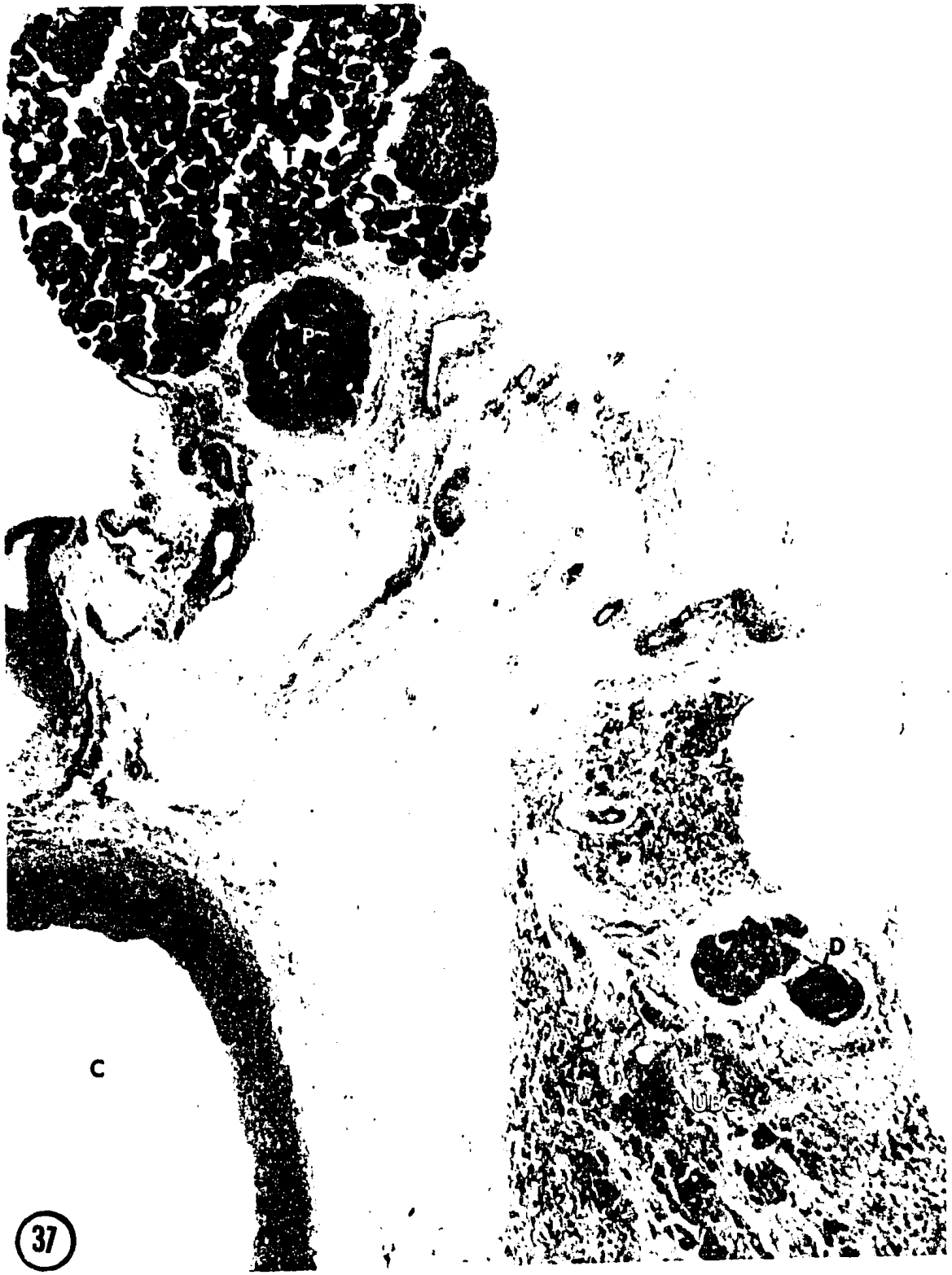


Fig.4. Effect of the dietary level of calcium on the level of the serum calcium in chicks.

Fig. 37 Para-median cross section of the pharyngeal region of the chick showing the ultimobranchial gland (UBG) and the adjacent tissue, carotid artery (C), thyroid (T), parathyroid (P) and cystic duct-like structure (D). P.A. - Schiff - Haematoxylin - Orange G. X 120.



37

cells in a connective tissue framework. Two cell types, namely the light and dark cells, were found in the ultimobranchial tissue. The light cell was characterized by the pale cytoplasm. In the dark cell, the cytoplasm appeared granular and stained more densely (Fig. 38).

The ultimobranchial tissue contained a variable number of blind ducts of various sizes (Fig. 37). The ducts were lined by an epithelium which varied from cuboidal to squamous. P.A.S.-positive materials and fragments of degenerated cells were frequently observed in the lumen of the ducts. In addition, P.A.S.-positive materials were also present in the epithelial cells.

The connective tissue contained an abundant capillary network. In addition, lymphocytes , extravascular granulocytes in various stages of maturation and nerves were also present in the ultimobranchial tissue.

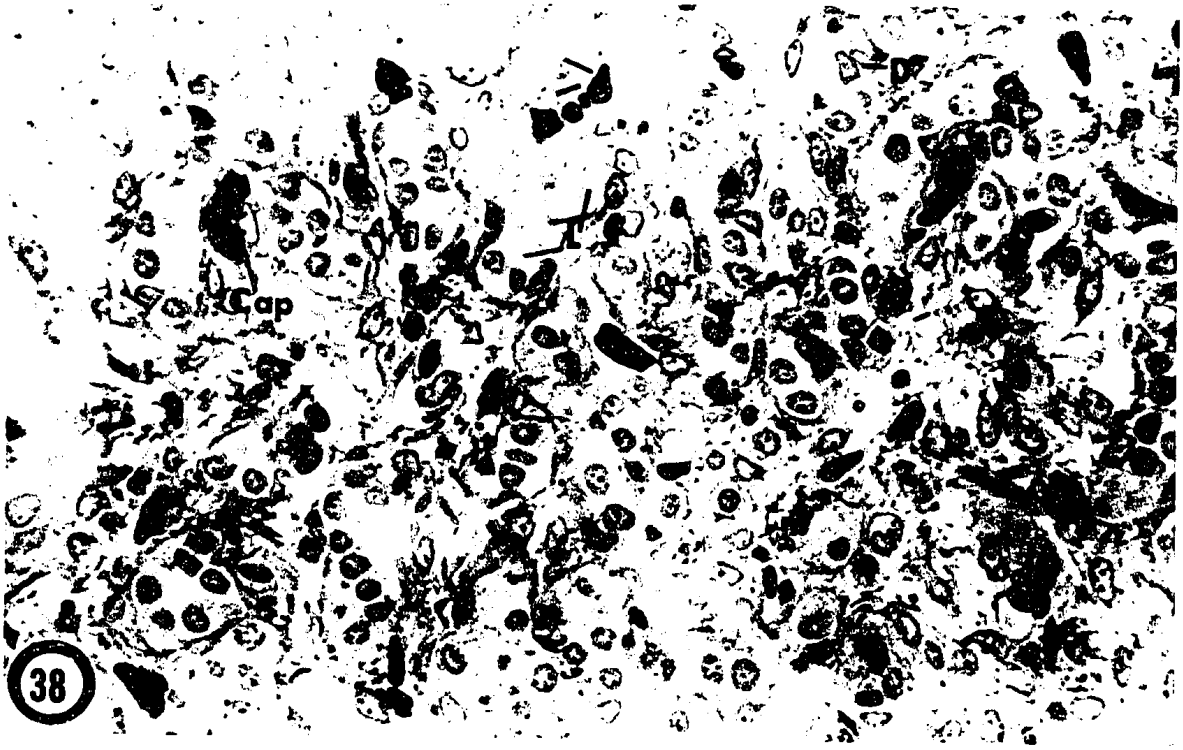
Electron Microscopy

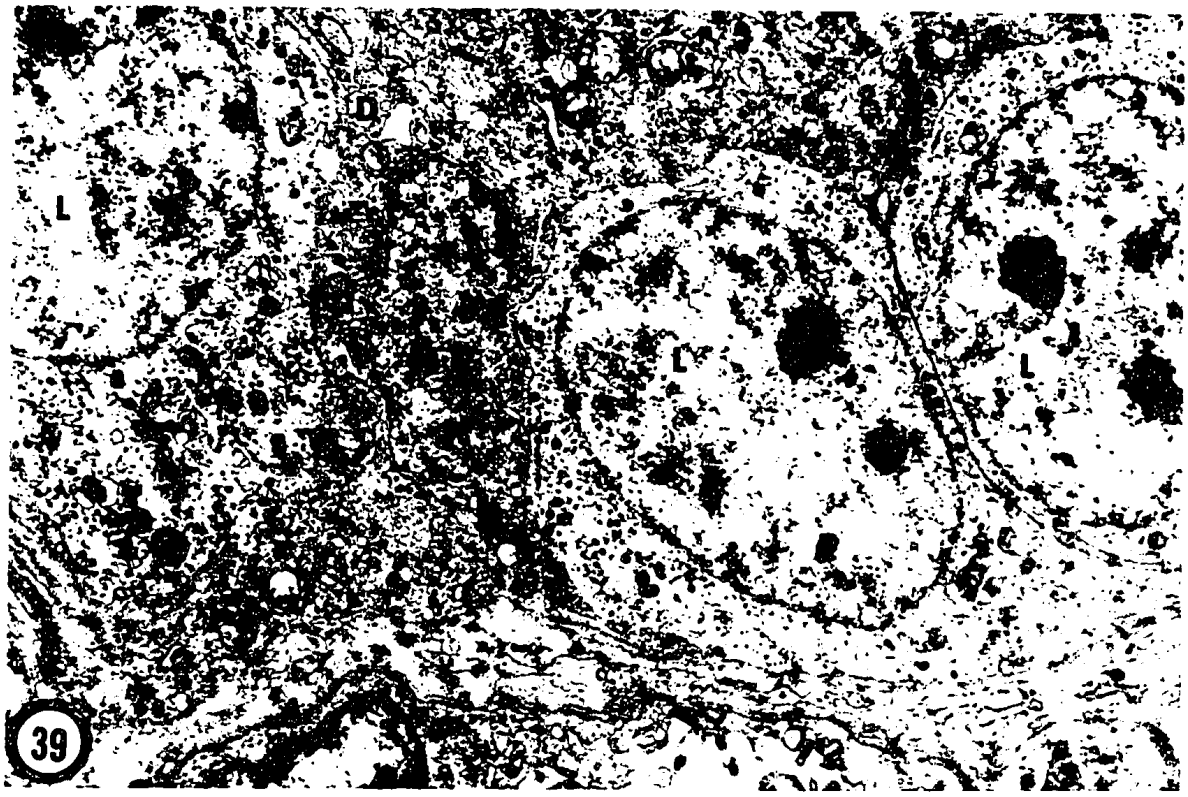
Light Cell

The light cell formed the majority of the cell type. They varied from ovoid to polygonal in shape. The opposing cell membranes in areas were relatively straight but at times they interdigitated.

Fig. 38 Parenchyma of the ultimobranchial gland in the chick showing the general distribution of the light (L) and dark (D) cells and their relationship to the capillaries (Cap). Epon-embedded section. Toluidine blue stain. X 590.

Fig. 39 Light (L) and dark (D) cells from the ultimobranchial gland of the chick. The dark cell is characterized by the closely packed cytoplasmic organelles which gives it a somewhat denser appearance. The light cell is characterized by the pale cytoplasm. Portion of the adjacent capillary (Cap) is shown. Uranyl and lead. X 8,500.


Cap
38


L
L
L
39

The limiting cell membranes of the light cells subjacent to the connective tissue were bound by a basement membrane (Fig. 42). The cells were separated from the endothelium by the perivascular space which contained collagen and occasional fibroblasts (Fig. 39).

The light cell was characterized by the pale cytoplasm which contained a variable number of granules. In tissues fixed with glutaraldehyde and postfixed with osmium tetroxide, two morphologically different types of granules were observed. These two types were referred to as secretory granules and light granules. In most instances, these two types of granules were easily distinguished from each other by their different size, electron density and texture (Fig. 40).

Secretory granules varied from round to rod-shaped and measured from 100 to 250 mμ in diameter. The secretory granule was lined by a smooth limiting membrane. The matrix of the secretory granules was composed of a homogeneous, dense material (Fig. 42). The secretory granules formed the majority of the granules in the light cells. They were distributed randomly throughout the cytoplasm, although some were usually lined close to the cell membrane. In some cells, the cytoplasm was packed with the secretory granules (Fig. 44). In other cells with a greatly reduced

Fig. 40 Light cell showing the presence of light granules (lg) and secretory granules (sg). Uranyl and lead. X 13,650.

Fig. 41 Portion of a light cell in the control chick showing an array of well developed rough-surfaced endoplasmic reticulum (Rer). Part of a Golgi complex (G), mitochondria (M), free ribosomes (r) and some smooth-surfaced vesicles can be observed. Uranyl and lead. X 14,700.

Fig. 42 Portions of some light cells showing the Golgi complex (G) é Smooth-surfaced vesicles (sv), vacuoles (va) and secretory granules (sg) can be observed. Several dense bodies (Db) show the dense, finely particulate matrix. Some cisternae of the rough-surfaced endoplasmic reticulum (Rer), numerous free ribosomes (r) and a centriole (C) are present in this section. Uranyl and lead. X 23,700.

cytoplasm, few secretory granules were observed (Fig. 43).

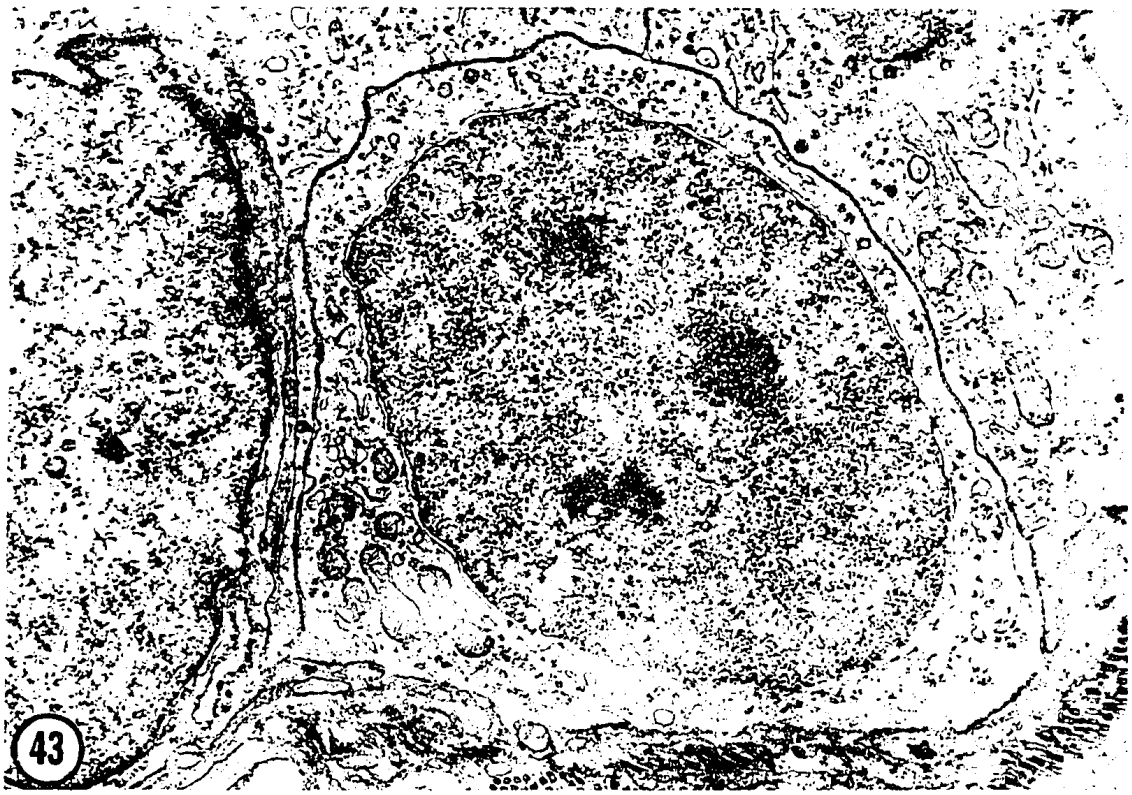
The light granules appeared as round or ovoid shape, measuring approximately 300 to 600 mμ or more. They contained floccular material of low electron density. The periphery of the core was so close to the limiting membrane that no space between was observed between these structures (Fig. 40). The light granules were usually found in close association with the endoplasmic reticulum and the Golgi complex.

The endoplasmic reticulum consisted of the rough-surfaced type. In some cells, the cisternae of the endoplasmic reticulum were arranged in parallel arrays (Fig. 41), whereas in others, the cisternae assumed the forms of membranous sacs distributed irregularly throughout the cytoplasm (Fig. 42). The lumen of the cisternae usually contained some finely, granular material (Figs. 41 and 42).

The Golgi complex in the light cells invariably consisted of three to four slightly curved saccules arranged in concentric layers. Clusters of small, smooth-surfaced vesicles, vacuoles and secretory

Fig. 43 Portions of some inactive light cells in the control chick. The cell is characterized by the reduced cytoplasmic volume containing few organelles and occasional secretory granules. Uranyl and lead. X 13,650.

Fig. 44 Portion of light cells which contain numerous secretory granules (sg). There is a reduction in other cytoplasmic organelles. Uranyl and lead. X 14,200.



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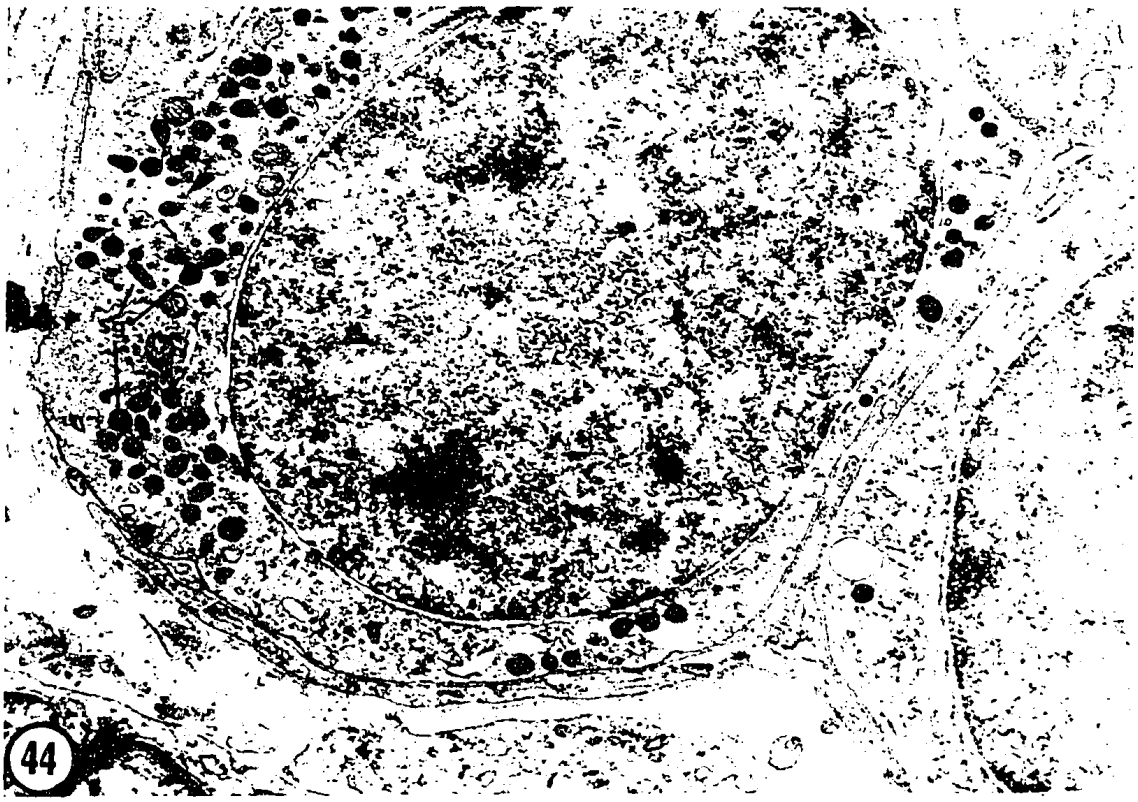


44

granules were present in the Golgi region (Fig. 42). However, the endoplasmic reticulum and the Golgi complex were usually poorly developed in cells which contained numerous secretory granules (Fig. 44) or in cells which contained a greatly reduced cytoplasm (Fig. 43).

Mitochondria were present in moderate number. In general, they appeared as round to rod-shaped. The mitochondrial matrix was dense and homogeneous. The internal cristae were distinct. The mitochondria were present throughout the cytoplasm although a greater number was usually found in the vicinity of the Golgi region (Fig. 42). Profiles of the endoplasmic reticulum in very close topographical relation to the mitochondria were frequently observed.

In addition, the cytoplasm also contained occasional multivesicular bodies and dense bodies. Multivesicular bodies measured from 300 to 450 mμ in diameter. They contained variable number of small vesicles in a matrix of low density. Dense bodies of variable sizes, averaging about 450 mμ in diameter, were observed. Most of them contained dense, finely granular material (Fig. 42).

High Calcium Diet

In the high calcium diet, the ultimobranchial

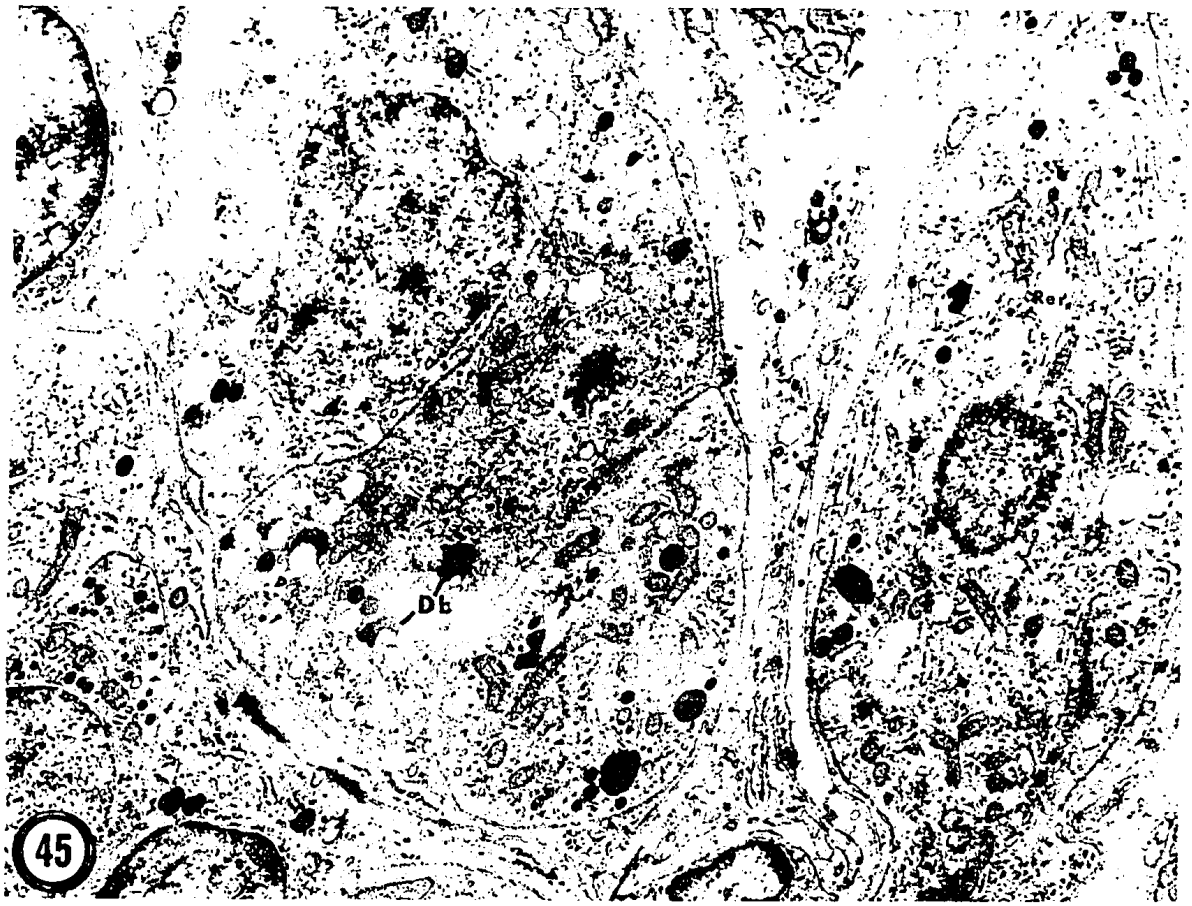
glands of the chick were grossly enlarged and hyperemic. There was a hypertrophy and hyperplasia of the light cells in the hypercalcemic conditions.

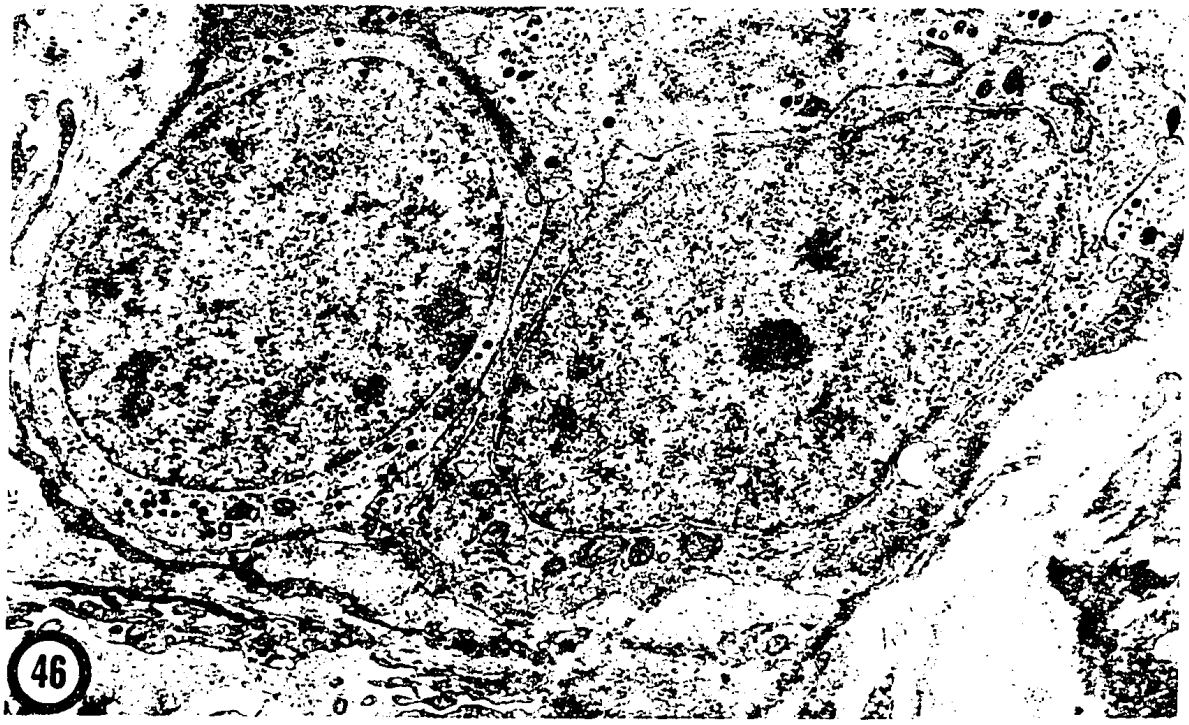
Ultrastructural observations revealed a general decrease of the secretory granules in many light cells (Fig. 45). In some cells, most of the secretory granules were discharged and the cells appeared to be reduced in size (Fig. 46). However, although degranulation was extensive, individual cells containing variable number of secretory granules were also observed. In these cells, the secretory granules were usually located along the cell membrane Figs. 47 and 50) whereas in others, they were frequently observed in the Golgi region (Figs. 48 and 49). Variable number of light granules were observed in the light cells. In some cells, these granules were aggregated closely (Fig. 51) whereas in other cells, they seemed to be more dispersed (Fig. 49). In addition, some empty vacuoles containing some finely granular material were also present in the cells (Figs. 47, 48 and 49).

There were conspicuous changes in the endoplasmic reticulum of many light cells in the hypercalcemic conditions. The endoplasmic reticulum was markedly hypertrophied in many light cells. In some cells, the cisternae of the rough-surfaced endoplasmic

Fig. 45 Groups of light cells from a hypercalcemic chick showing the general decrease in the secretory granules. Tne prominent endoplasmic reticulum (Rer) and variable number of dense bodies (Db) are observed. Uranyl and lead. X 9,650.

Fig. 46 A group of three light cells from a hypercalcemic chick. There is a reduction in cytoplasmic volume. Occasional secretory granules (sg) are seen. Uranyl and lead. X 7,800.


Db
Rer
45


g
46

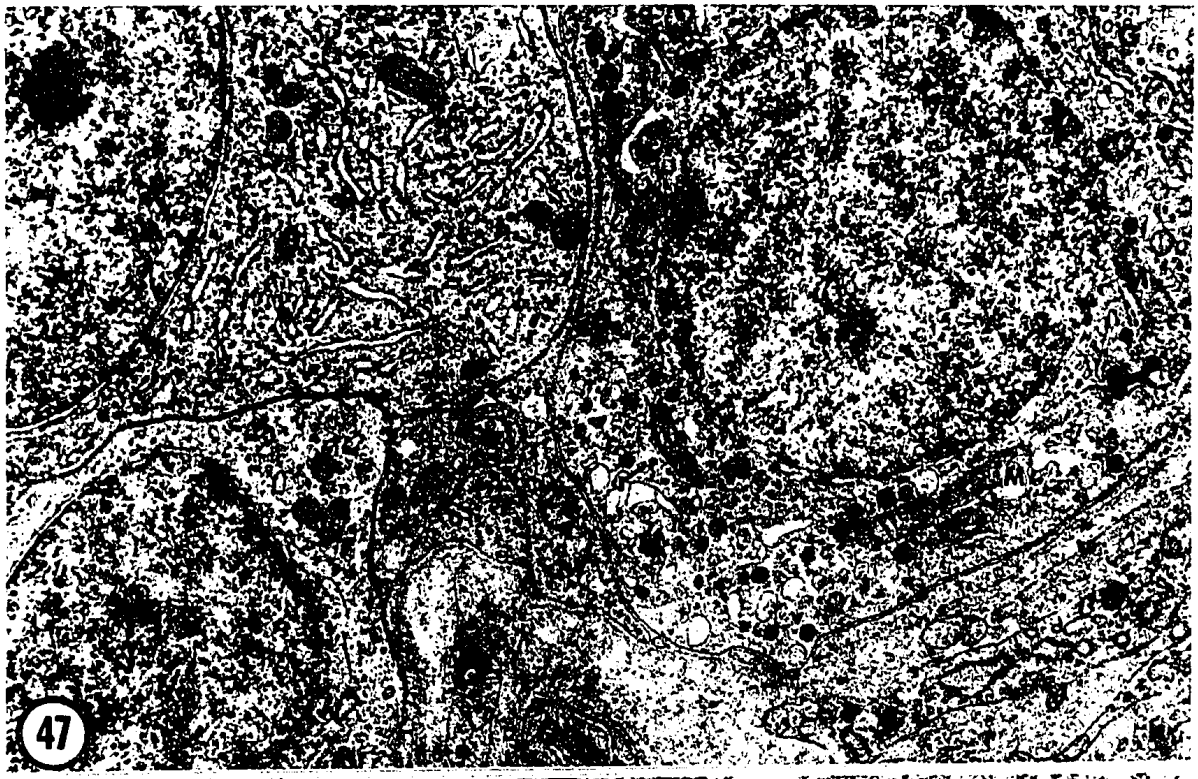
reticulum were organized into a well-ordered pattern (Fig. 47), while in others, the cisternae were distributed at random throughout the cytoplasm (Fig. 50). In some cells, the cisternae of the endoplasmic reticulum were dilated (Fig. 51). The lumen of the cisternae usually contained amorphous material of low density. Numerous free ribosomes were present in the cytoplasm.

The Golgi complex was more prominent in the light cells of the hypercalcemic chicks. In some cells, as many as four Golgi areas could be observed. The large Golgi complex was concentric or S-shaped and consisted of stacks of four to eight slightly curved saccules arranged in concentric layers. Clusters of smooth-surfaced vesicles and some vacuoles were present in the Golgi region (Figs. 48 and 49). Some saccules were dilated along the length. Electron dense granules could be observed at the dilatations of the saccules (Figs. 48 and 49). Immature secretory granules containing material in varying states of aggregation and condensation were present in the Golgi region (Fig. 48). Mature secretory granules were frequently seen in the vicinity.

There seemed to be an increase in the number of dense bodies (Fig. 45) and multivesicular bodies in some light cells (Fig. 50). Dense bodies

Fig. 47 Portions of hypertrophied light cell showing the general distribution of the secretory granules (sg). Numerous mitochondria (M), Golgi complex (G) and free ribosomes (r). The adjacent light cell has a prominent endoplasmic reticulum (Rer). Variable number of empty vacuoles (v) are observed. Uranyl and lead. X 13,570.

Fig. 48 The Golgi region of a light cell from a hypercalcemic chick showing several groups of saccules. The cisternae of the saccule are dilated in many areas and condensing dense secretory material is seen in some dilations (arrows). Light granules (lg) and empty vacuoles (v) are observed. Uranyl and lead. X 25,000.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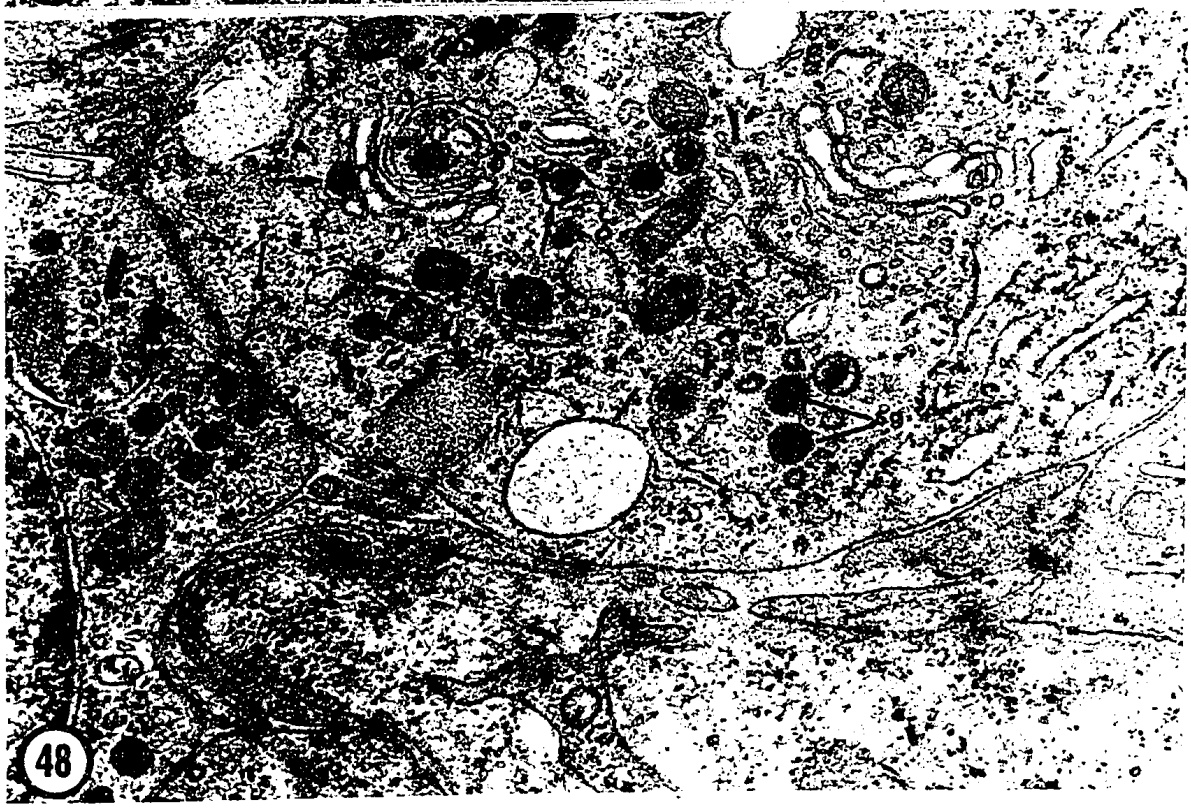

48

varied somewhat in size, averaging 450 mμ in diameter. Most of them contained a finely particulate material of high density, but a few contained some vacuoles of low density (Fig. 50). Multivesicular bodies were more numerous than in the controls. Some were dilated and assumed irregular outlines. The matrix was of low density and contained a variable number of small vesicles (Fig. 50).

Mitochondria were more numerous in the hypertrophied cells. Morphologically, they appeared similar to those in the control, but enlarged mitochondria were also observed. The mitochondria were present throughout the cytoplasm, although a greater concentration was found in the vicinity of the Golgi region.

Centriole was frequently observed in the light cells of the hypercalcemic chicks. Longitudinal or cross sections through centrioles were encountered close to the nucleus. The centriolar cylinder consisted of a hollow cylinder 300 to 500 mμ in length and about 100 mμ in diameter. Its wall was composed of nine evenly-spaced, triplet, hollow fibrils or tubules embedded in dense amorphous matrix (Fig. 51). In some cells, fine filamentous microtubules were associated with the centriole (Fig. 51).

In addition to the many hypertrophied light

Fig. 49 Portions of some light cells in a hypercalcemic chick showing the extensive and voluminous Golgi complex. Saccules are dilated at places (arrow). Variable number of light granules (lg) and empty vacuoles (v) are observed. Uranyl and lead. X 16,000.

Fig. 50 Portion of the Golgi area of the light cell of a hypercalcemic chick. Multivesicular bodies (Mvb) are frequently seen and some assuming irregular form (arrow). Several dense bodies (Db) show the dense, finely particulate matrix. Secretory granules (sg) are localized near the plasma membrane. A centriole (C) and some mitochondria (M) can also be observed. Uranyl and lead. X 25,300.


49

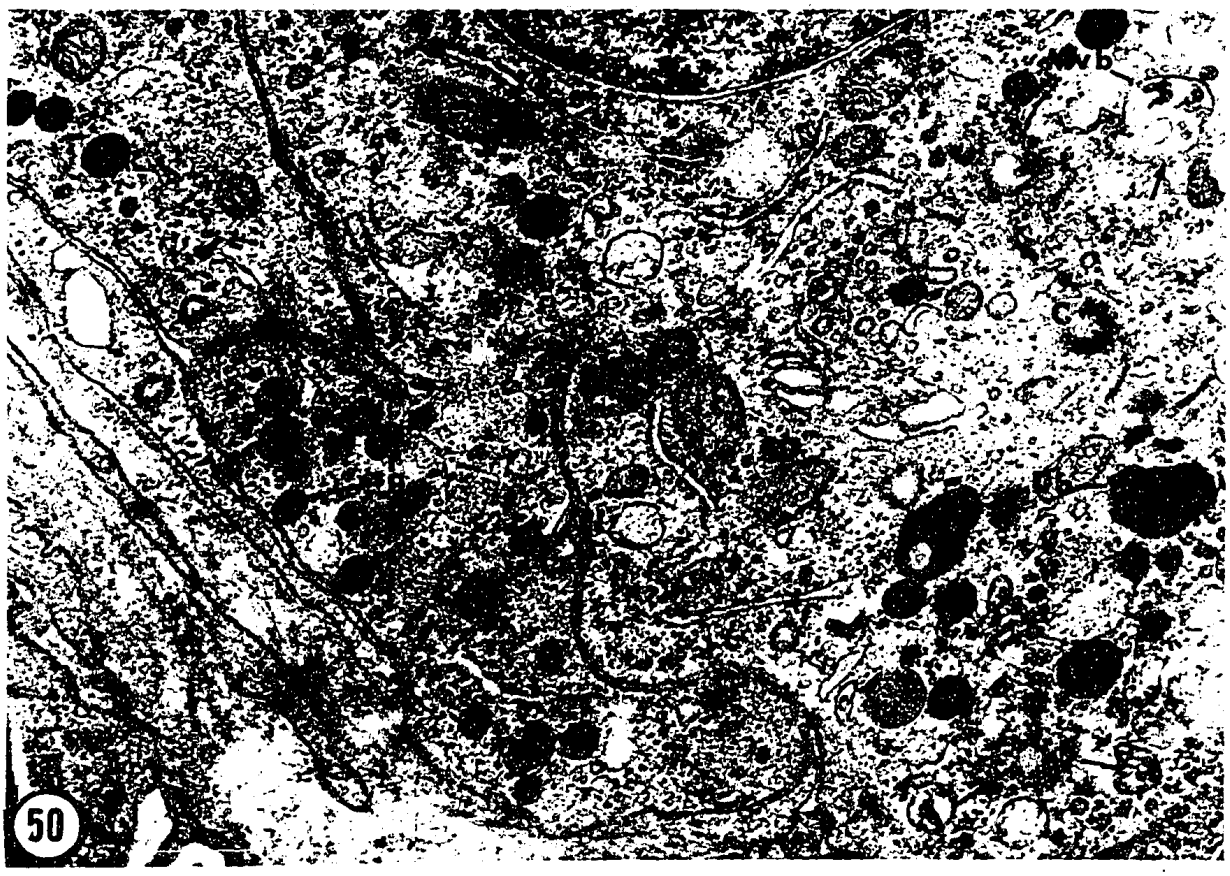
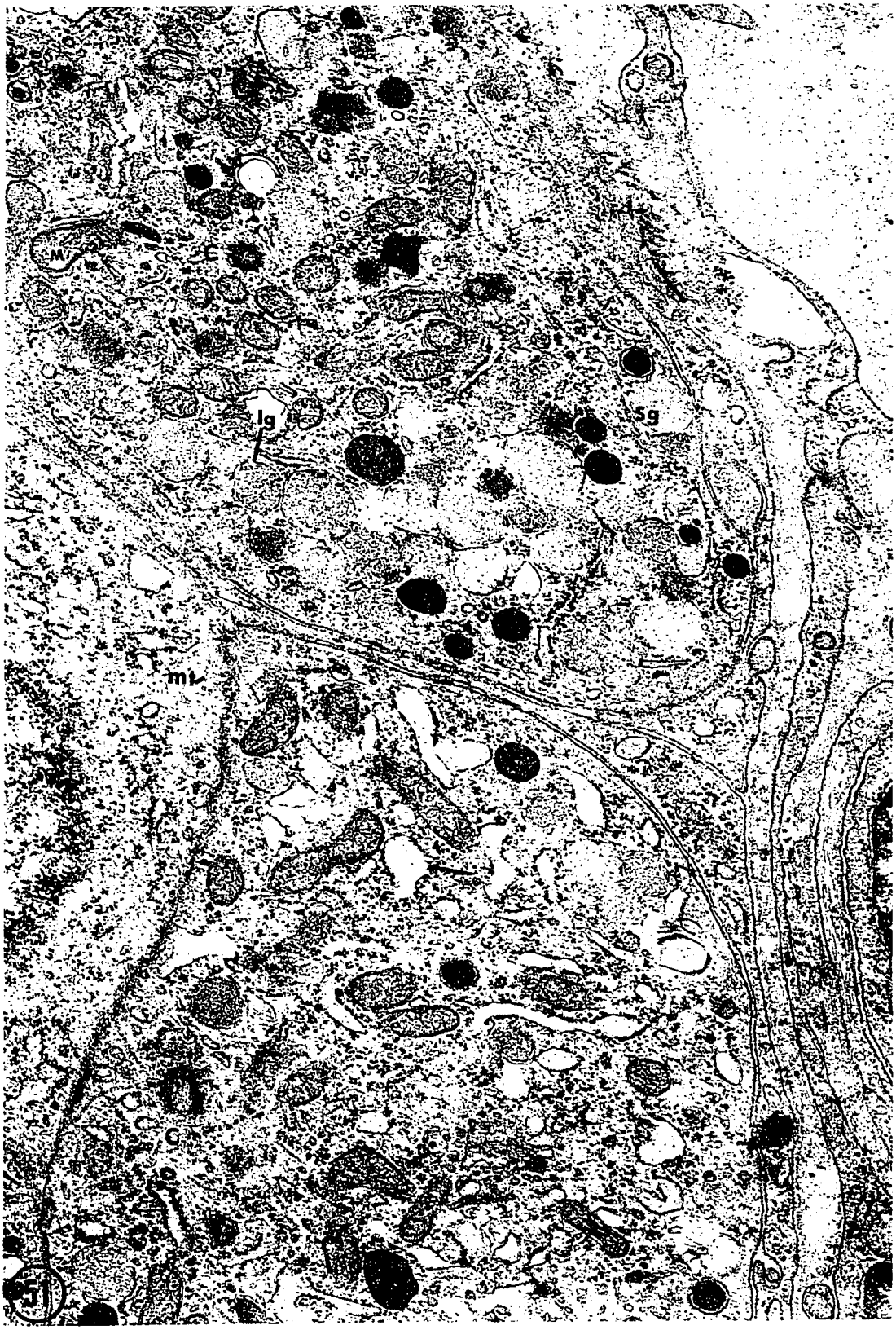

mvb
50

Fig. 51 Portion of some light cells of a hypercalcemic chick. The rough-surfaced endoplasmic reticulum (Rer) is dilated at places (arrows). Many mitochondria (M), centriole (C) and some microtubules (mt) are observed. Light granules (lg) are located close together. Variable number of secretory granules (sg) are also present. Uranyl and lead. X 25,000.


M
lg
sg
mt

cells, other light cells characterized by the reduced cytoplasmic volume were present. These cells contained few cytoplasmic organelles and occasional secretory granules (Fig. 46).

Low Calcium Diet

In the hypocalcemic conditions, the ultimobranchial glands were markedly reduced in size, and appeared grossly to be more compact.

The majority of the light cells were small and appeared to be reduced in size (Figs. 52 and 53). In these cells, the cytoplasm invariably contained secretory granules which seemed to vary in size and density (Fig. 53). Similarly, a variable number of light granules were observed in some cells (Figs. 52 and 53).

Other light cells were characterized by the greatly reduced cytoplasm (Fig. 53). These cells usually contained a few secretory granules and a reduced number of cytoplasmic organelles.

The endoplasmic reticulum in the majority of the light cells was poorly developed. In many cells, the endoplasmic reticulum consisted of isolated strands of cisternae which were distributed throughout the cytoplasm (Figs. 52, 53 and 55).

The Golgi complex was poorly developed. It

Fig. 52 Group of light cells in the hypocalcemic chick showing the distribution of secretory granules (sg) and light granules (lg). The endoplasmic reticulum (Rer) and Golgi complex (G) are poorly developed. Myelin figures (mf) are observed. Uranyl and lead. X 13,650.

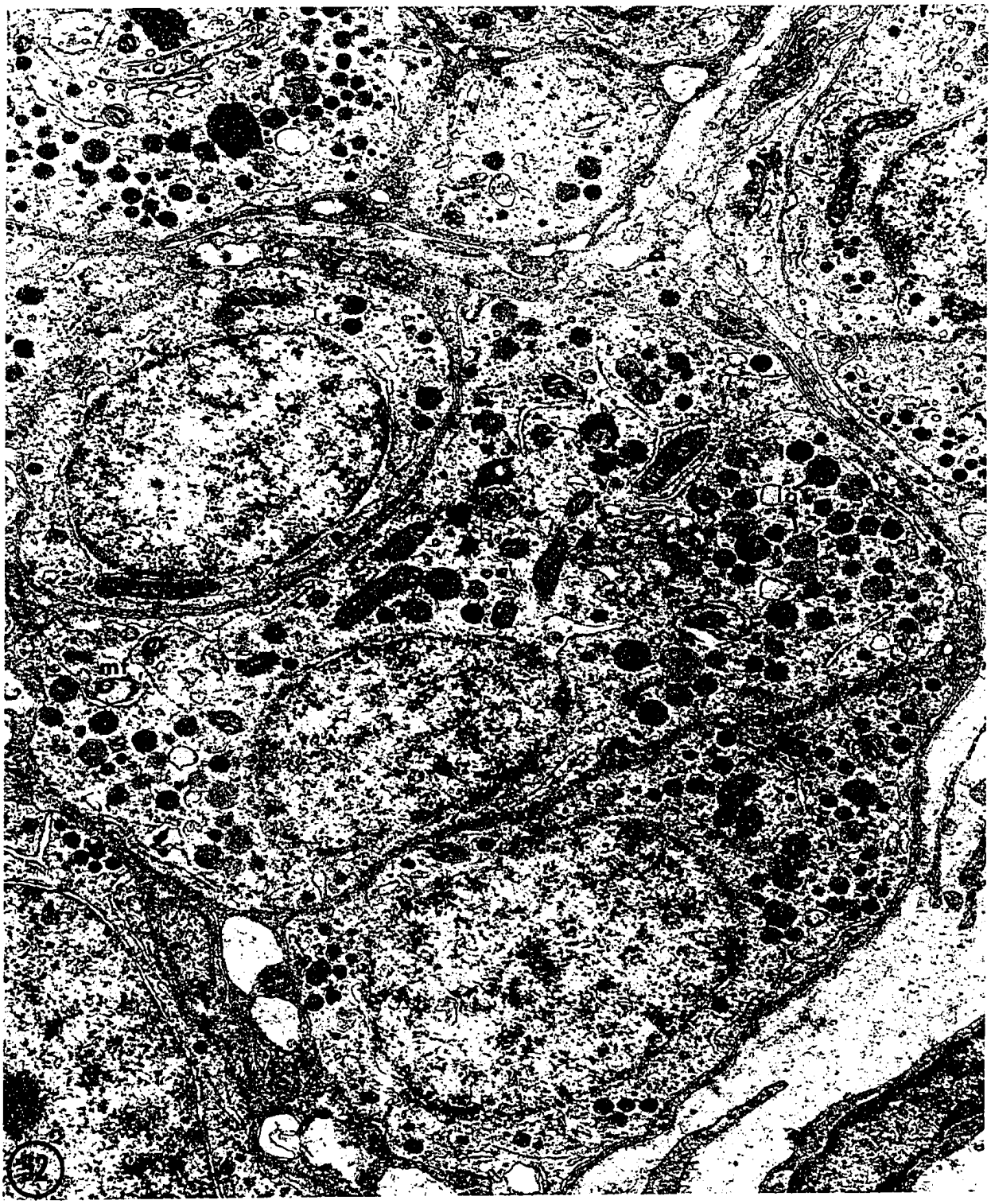

mf
12

Fig. 53 A group of light cells from the ultimobranchial gland in the hypocalcemic chick. Light cells are small in size. Variable number of secretory granules (sg), light granules (lg) and mitochondria (M) are seen. The endoplasmic reticulum (Rer) and the Golgi complex (G) are poorly developed. Uranyl and lead. X 13,650.

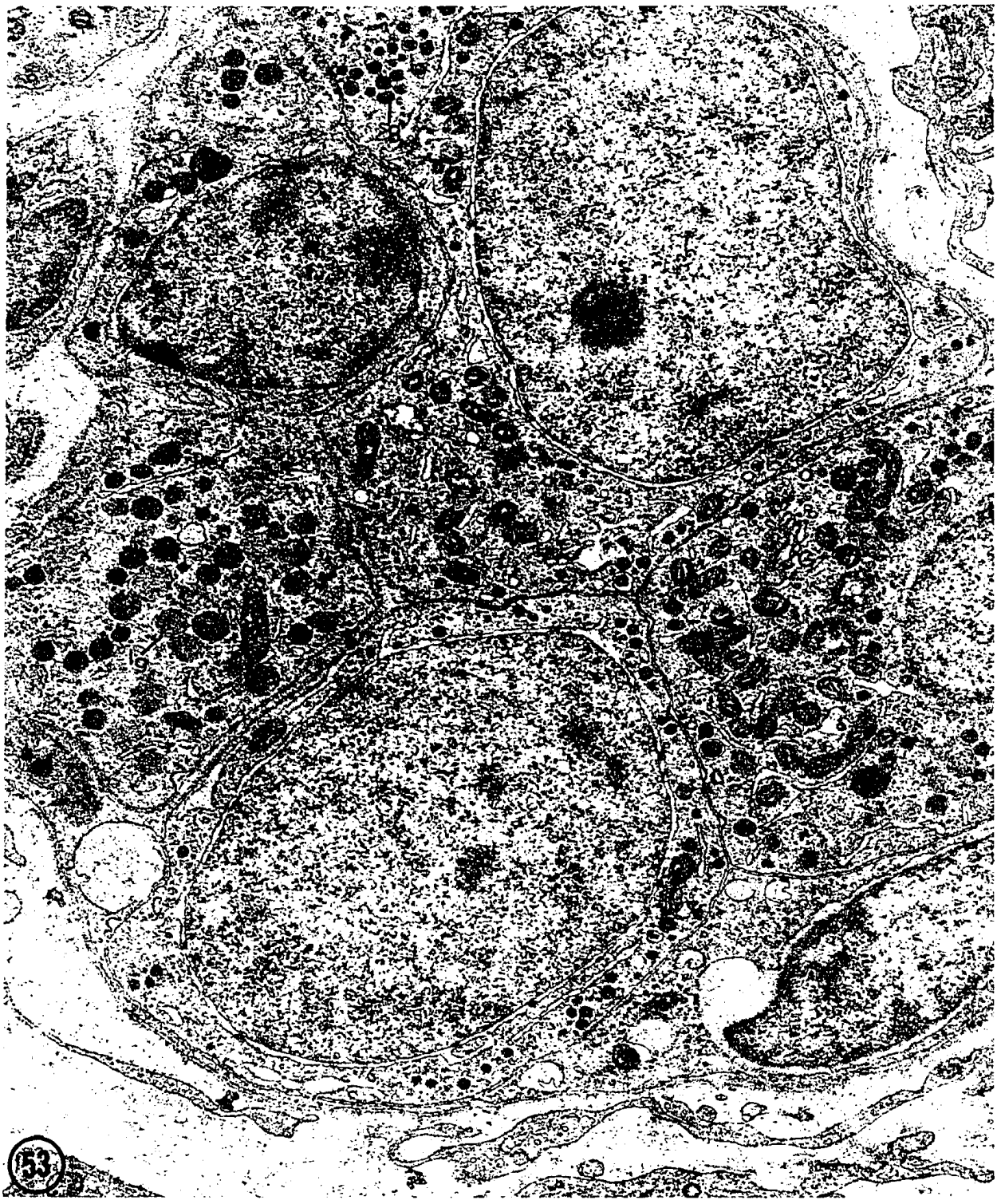
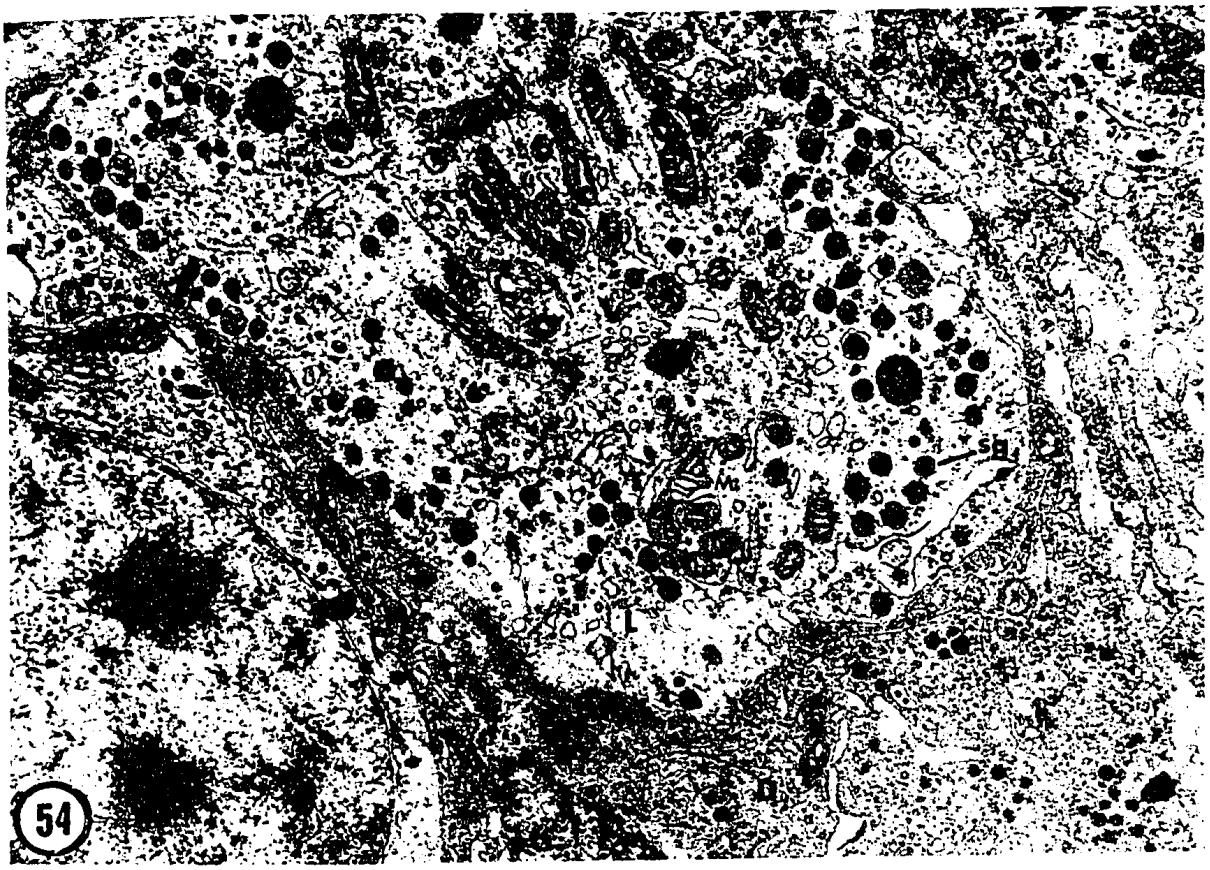

53

Fig. 54 Light (L) cell of the ultimobranchial gland in the hypocalcemic chick showing the loss of cytoplasmic matrix in the cell. Variable number of secretory granules (sg) and some mitochondria (M) which show dilatation of cristae are observed. Uranyl and lead. X 13,650.

Fig. 55 Portions of three light cells in the hypocalcemic chick showing the distribution of secretory granules (sg) and mitochondria (M). Golgi complex (G) is present in a light cell. Uranyl and lead. X 13,650.


SR
M
I
54


55

usually consisted of three to four saccules and some smooth-surfaced vesicles (Fig. 55). No evidence of secretory materials were present in the saccules. However, in many cells, the Golgi complex was not encountered.

Variable number of mitochondria were present in the light cells of the hypocalcemic chicks (Figs. 52, 53 and 54). They varied from round to elongate in shape. The mitochondrial matrix appeared dense and in some cases, the cristae spaces were dilated (Fig. 54).

In addition, some light cells appeared considerably paler than other cells (Figs. 52 and 54). There seemed to be a loss of the cytoplasmic matrix in these cells (Fig. 54) and in other cells, myelin figures were observed in the cytoplasm (Fig. 52).

3. Discussion

The Ultimobranchial Gland in Normocalcemia

In the chick, the ultimobranchial gland is a distinct glandular structure. The organization of the cell types in cords and clusters and the rich vascular network appears to be principally very similar to that of other endocrine glands.

The light cell is the dominant cell type

in the ultimobranchial tissue. Morphologically, the light cells show features relevant to the functional role of the gland. The cytoplasm of the light cell is characterized by the presence of variable number of secretory granules. The secretory granules appear similar to those in the light cells of the mammalian thyroid gland in either osmium tetroxide or glutaraldehyde fixation. The presence of the light granules in the light cell is of interest. The exact nature and function of the light granules is not known. However, the close relationship of the light granules to the endoplasmic reticulum and Golgi complex seems to suggest a possible involvement of the light granules as a transport mechanism for the precursor substance.

In normocalcemia, light cells in various functional states are observed. Ultrastructurally, the light cells can be classified into three functional stages; active cells, resting cells and storage cells. Active cells are characterized by the presence of well developed endoplasmic reticulum and Golgi complex and presumably are in active synthesizing phase. Cells which are packed with secretory granules and devoid of other organelles are probably in a storage phase. Other cells which contain a poorly developed endoplasmic reticulum and few secretory granules

may probably represent resting cells, having discharged most of their secretory granules. The light cells presumably undergo periods of active synthesis, followed by secretion and periods of physiologic rest or storage. Evidently, the relative proportion of the different functional types present probably reflects the state of the physiologic activity of the gland at any given time.

The synthetic pathway of the secretory granules is probably similar to those in other protein secreting cells. Presumably, the secretory material is synthesized at the level of the ribosomes, transferred into the cisternae of the endoplasmic reticulum and then concentrated in the Golgi complex. This mode of secretory granule formation is similar to that suggested for the light cells in the thyroid gland (Nunez et al., 1967; Azzali, 1968; Chan and Bélanger, 1968). The mechanism of release of the secretory granules appears similar to that described for the light cells in the thyroid gland as discussed in the earlier section.

Ultrastructurally, the light cells of the ultimobranchial gland share many common features with the light cells of the thyroid gland in the various mammalian species. In addition, the light cells in

the chick ultimobranchial gland have shown an ability to take up and store amine precursors such as 5-hydroxytryptophan (5-HTP) (Hachmeister et al., 1967). Therefore, morphological and cytochemical evidence seem to support Godwin's hypothesis of the ultimobranchial origin of the light cells in the dog thyroid. The light cells in the ultimobranchial gland seem to be homologous to the light cells in the mammalian thyroid gland. Presumably, in the non-mammalian vertebrates, the ultimobranchial gland is not incorporated into the thyroid gland during embryonic development but retained as a separate entity.

The physiological significance of the ultimobranchial gland is not known. However, the morphological evidence shows the gland to be functionally active, involving in the synthesis, secretion and storage of the secretory product. This correlates well with the biological assay studies which show the high calcitonin content in the ultimobranchial gland of the chick (Copp et al., 1967; Tauber, 1967). Recently, avian calcitonin has been shown to produce hypocalcemia in the acute partially parathyroidectomized cockerals (Kraintz and Intscher, 1969).

Calcium metabolism in the avian species is more intense than in other vertebrates. This is

related to the turnover of large amounts of calcium involved in the process of calcification of the egg shell. In the female, the level of the blood calcium rises from a normal value of 10 mg% to over 20 mg% at the time of ovulation. It is generally agreed that the excess calcium comes from the medullary bone. Resorption of the medullary bone by osteocytic osteolysis during the egg-laying cycle has been reported by Bélanger and Taylor (1967). It is conceivable that calcitonin could possibly play a regulatory role between period of changing physiological states. In this regard, the observation of Urist (1967) regarding the hypertrophy of the ultimobranchial gland in the egg laying hens is significant.

Effects of a High Calcium Diet

In order to stimulate metabolic activities related to the hypocalcemic hormone synthesis and/or release of the hormone from the light cells, the serum calcium level was raised in the experimental chicks by increased supply of calcium from diet.

The initial response of the light cells to the hypercalcemic stress appears to be the degranulation of the secretory granules. This is supported by the general decrease in the number of secretory granules in many light cells and indirectly

by the predominantly peripheral localization of the secretory granules which seem to suggest that they are in the process of being secreted. The significant decrease of the secretory granules may possibly be related to the increased secretion rate. The greater blood flow through the gland involving increased movement of fluids as a result of hyperemia may probably enhance the secretion rate.

However, in spite of the significant degranulation of the secretory granules in many cells, individual cells which contain variable number of secretory granules are still encountered. The difference in response is consistent with the hypothesis that the light cells individually pass through phases of the secretory cycle. As such, only cells which are in a particular phase are capable of releasing their secretory products.

The changes observed in the light granules and the presence of many empty vacuoles seem to add further support to the suggested role for these granules. Presumably, the changes involving greater movement of material may be related to the increased metabolic activity of the cells in hypercalcemia.

The results indicate clearly that degranulation of the secretory granules as a specific effect of the

induced hypercalcemia. The degree of degranulation is a cytological characteristic indicative of secretory activity. Therefore, the close parallel between the degree of degranulation and the level of hypercalcemia suggests a possible functional role for the light cells in the maintenance of the calcium homeostasis.

The hypertrophy and hyperplasia of the light cells in the hypercalcemic conditions seem to be causally related. The presence of well developed endoplasmic reticulum, prominent Golgi complex and the increased number of mitochondria in many light cells seems to reflect enhanced metabolic and synthesizing activity in these cells. The changes in the light cells probably reflect the ability of the ultimobranchial gland to react to the increased demand of the hormone. The importance of this compensatory response is demonstrated in the perfusion studies in turkey, whereby ultimobranchialectomy impairs the ability of the bird to control the hypercalcemia induced by calcium infusion (Walker et al., 1969).

Biochemical results provide further evidence of increased hypocalcemic activity in the hypercalcemic chicks (Cipera et al., 1969). When the hypercalcemic chicks were deprived of their food (Stimulant which increases their serum calcium levels) their

serum calcium levels were consistently lower than those of the fasted controls. This demonstrates an increase hypocalcemic activity in the hypercalcemic chicks which could be related to the hypertrophy and hyperplasia of the light cells.

The present study shows that hypercalcemia, induced by nutritional factors, can have a stimulating effect on the secretory and synthesizing activity of the light cells in the ultimobranchial gland in chick.

Effect of a Low Calcium Diet

The chicks seem to be very susceptible to the low calcium diet. The persistent hypocalcemia observed seems to suggest a greater dependence of the chicks on the diet for their nutritional supply of calcium.

The atrophy of the ultimobranchial gland in hypocalcemia seems to be causally related. The presence of many light cells in resting or storage stage is probably related to the decrease in metabolic activity of the cells. In the storage cell, the cytoplasm contains numerous secretory granules of varying sizes and densities. Presumably, these secretory granules must have accumulated in the absence of the stimulus for secretion. The morphological evidence correlates well with the biological assay studies which show an

increase calcitonin content in the ultimobranchial glands of the hypocalcemic chicks (Copp, 1968).

The reduced secretory activity of the ultimobranchial gland in the hypocalcemic chick is further reflected in the presence of the light cells in the resting stage. The poor development of the endoplasmic reticulum and the Golgi complex seems to suggest decreased metabolic and synthesizing activity of the cells. Presumably, these cells must have discharged most of their secretory granules prior to the onset of hypocalcemia and have since remained in the resting stage. However, the fact that these cells are inactive is a presumptive evidence that there is little need for protein synthesis normally required for the formation and regeneration of enzymes and organelles.

In other light cells, ultrastructural evidence is indicative of cytoplasmic degeneration and autophagia. These degenerative cellular changes are probably related to the prolonged lack of stimulation for secretion of the secretory granules, as a result of the persistent hypocalcemia.

The ultrastructural changes in the light cells are probably related to the decreased need of calcitonin in the hypocalcemic state. In such conditions, the problem is one of attempting to increase serum

calcium level to normal value. The hypertrophy of the parathyroid glands is probably related to the increased endogenous secretion of parathyroid hormone. This is further supported by the studies of Bélanger and Cipera (1969) which show increased osteocytic osteolysis in the tibia of the calcium deficient chicks.

The results of the present series of experiments show that the light cells in the ultimobranchial gland of the chick are sensitive to changes in the serum calcium level. Therefore, the regulation of the serum calcium in the chick may be explained, as in the mammals, on the basis of the dual action of the parathyroid hormone and calcitonin, in such a way as to maintain constant serum calcium level. This is supported by the reciprocal reactions of the parathyroid and the ultimobranchial glands to the hyper- and hypocalcemic stimuli. It provides further support to the concept of a dual hormonal feedback mechanism involved in the regulation of the calcium metabolism.

Experiment 3. The Ultimobranchial Glands in the Frog and Fish

1. Introduction

Numerous reports have been published on the morphology of the ultimobranchial gland in the various species of anurans (Maurer, 1888; Watzka, 1933; Saxén and Toivonen, 1955; Boschwitz, 1960; Robertson and Swartz, 1964). Similarly, the ultrastructure of the ultimobranchial gland of Rana pipiens has been studied (Robertson and Bell, 1965; Robertson, 1968b). Therefore, in the present study, the ultrastructural features of the frog ultimobranchial gland previously unnoted will be described.

Similarly, there have been numerous reports on the ultimobranchial gland in the fishes (Van Bemmelen, 1885; Giacomini, 1908; Camp, 1917; Watzka, 1933; Krawarik, 1936; Sehe, 1960). In spite of the numerous reports, there was no consensus as to the function of the gland in the fishes. However, the lack of parathyroid glands in the fishes (Bern, 1967; Fleming, 1967; Urist, 1967) has led some investigators (Rasquin and Rosenbloom, 1954) to suggest a possible parathyroid-like function for the ultimobranchial gland.

Recent studies showing the high calcitonin content in the ultimobranchial gland in the fishes (Copp et al; 1967b; Copp and Parkes, 1968) which

effects hypocalcemia in the rats have provided evidence suggesting a possible functional role for the gland.

The aim of the present study was to examine the ultrastructural characteristics of the cells which appear to be the producer of calcitonin.

2. Observations

The Ultimobranchial Gland in the Frog

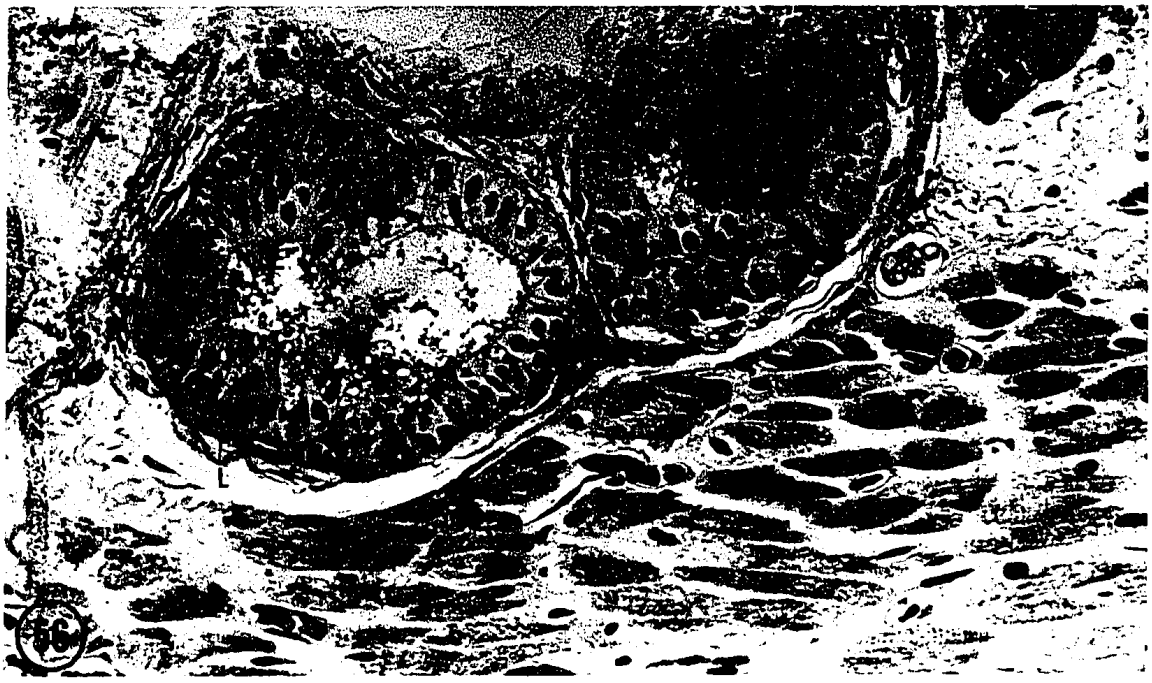
Light Microscopy

The ultimobranchial gland invariably consisted of two to three follicles (Fig. 56). The central lumen of the follicle was lined by a pseudostratified epithelium. The follicle was enclosed by a connective tissue capsule which contained fibroblasts and capillaries.

The parenchyma of the ultimobranchial tissue consisted of distinct cell types, namely epithelial and light cells. The epithelial cells were smaller and contained dense staining nuclei. P.A.S.-positive materials were observed in the cytoplasm. The epithelial cells invariably occupied the apical area of the pseudostratified epithelium (Fig. 56).

The light cell was characterized by the elongated shape and the finely, granular nucleus. Furthermore, the cytoplasm of the light cell appeared

Fig. 56 Light micrograph showing two follicles of the ultimobranchial gland of the frog. The lumen of the follicle contain some granular material. Pseudostratified epithelium contains epithelial cell (E) and light cell (L). Toluidine Blue Stain. X 550.



paler than those of the epithelial cell.

P.A.S.-positive materials were invariably observed in the lumen of the follicles. Portions of cell fragments were frequently observed in association with the granular material.

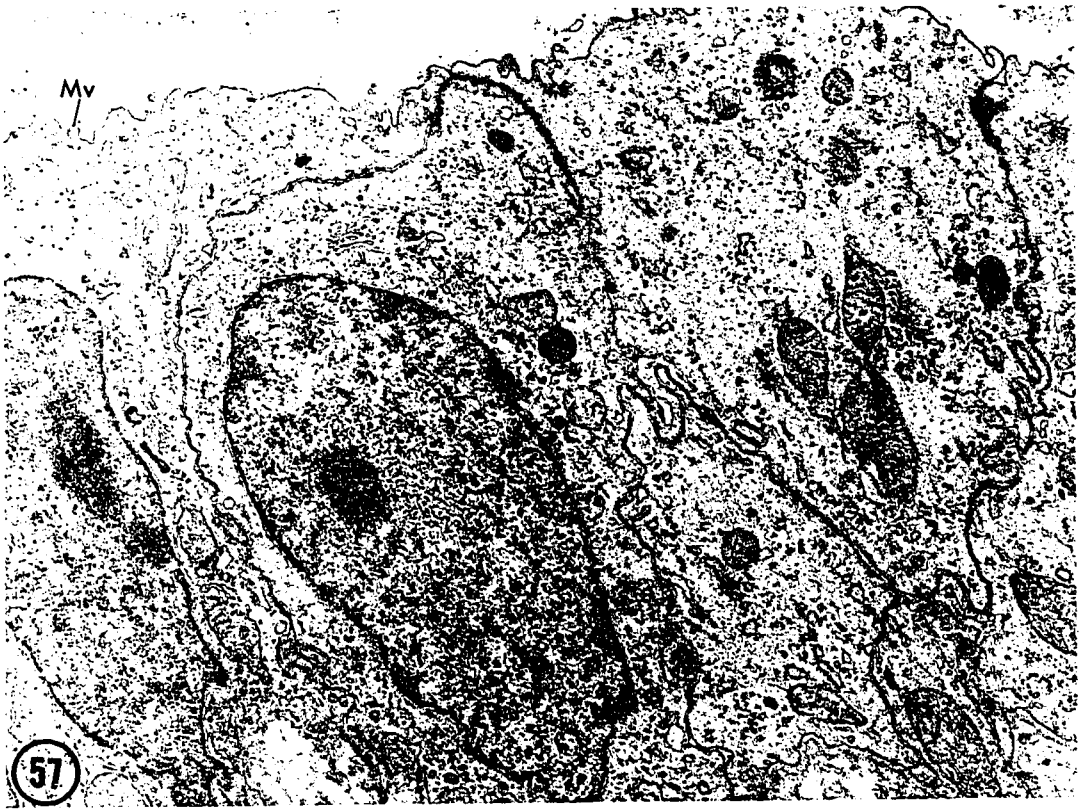
Electron Microscopy

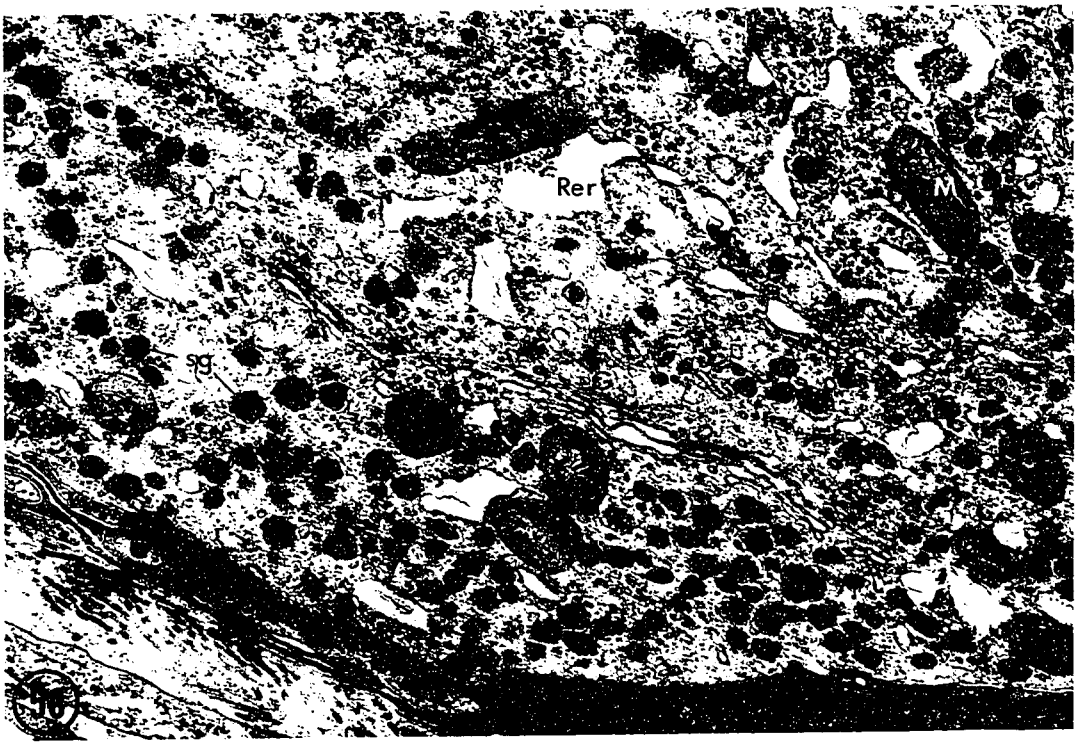
Electron microscopic observations of the ultimobranchial gland confirmed the light microscopic classification of the different cell types.

The epithelial cells invariably formed the inner layer of cells which lined the lumen. The apical surface of the cell formed microvilli which protruded into the lumen (Fig. 57). The cytoplasm of the epithelial cell was characterized by the presence of filaments which were distributed randomly in the cytoplasm. The endoplasmic reticulum was invariably developed. The rough-surfaced endoplasmic reticulum consisted of cisternae which were distributed in the cytoplasm. The Golgi complex consisted of three to four saccules arranged in concentric layers. Clusters of smooth-surfaced vesicles were also present in the vicinity. The cytoplasm contained variable number of dense bodies which were aggregated near the apical surface. Mitochondria were distributed randomly in the cytoplasm. They varied from spherical to elongate

Fig. 57 Apical region of the ultimobranchial tissue of the frog showing the epithelial cells with the microvilli (Mv). Dense granular materials were present in the lumen. Uranyl and lead. X 14,000.

Fig. 58 Portion of the basal region of the light cell showing the presence of numerous secretory granules (sg). The presence of mitochondria (M), endoplasmic reticulum (Rer) and Golgi complex (G) is well shown. Uranyl and lead. X 13,650.


Mv
57


Rer
M
sg
56

and contained cristae surrounded by a dense matrix.

The light cell was characterized by the pale cytoplasm which contained numerous secretory granules. The secretory granules measured from 150 to 300 mμ in diameter. They were lined by a smooth limiting membrane. The contents of the secretory granules varied in degrees of densities (Fig. 58). The endoplasmic reticulum of the light cell were usually well developed. However, in those cells which were packed with the secretory granules the endoplasmic reticulum was less well developed. The Golgi complex showed similar variation in the light cells. The prominent Golgi complex usually consisted of three to four saccules arranged in concentric layers. Clusters of smooth surfaced vesicles were also observed in the vicinity of the Golgi complex (Fig. 58).

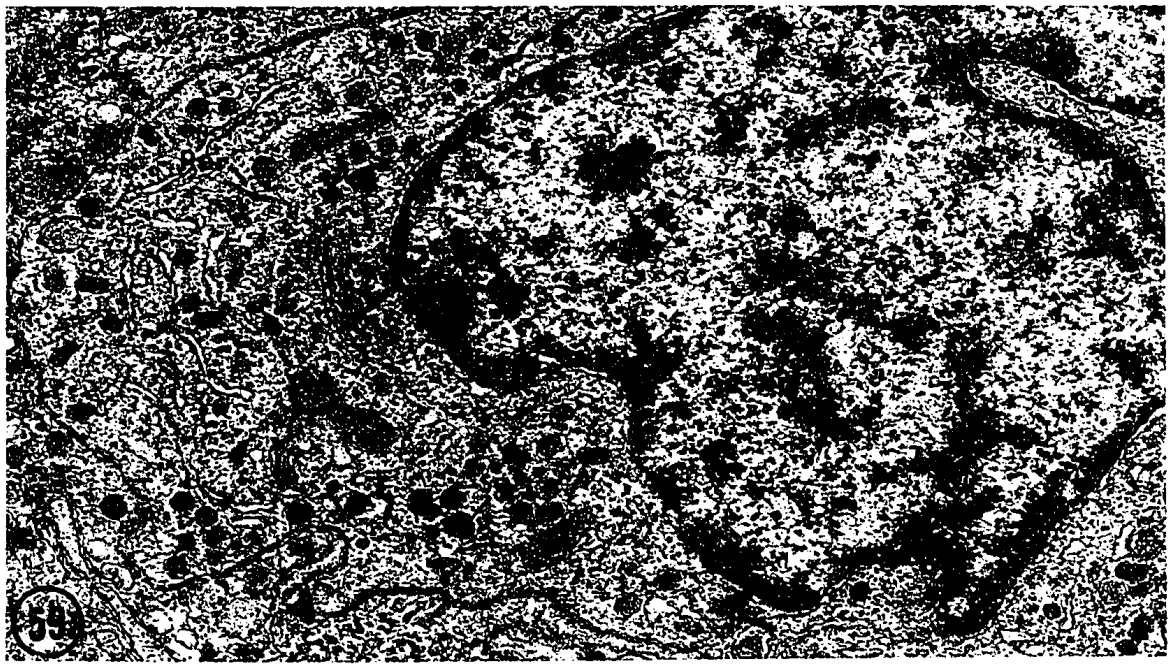
Variable number of mitochondria and an occasional multivesicular bodies were usually present in the cytoplasm of the light cells.

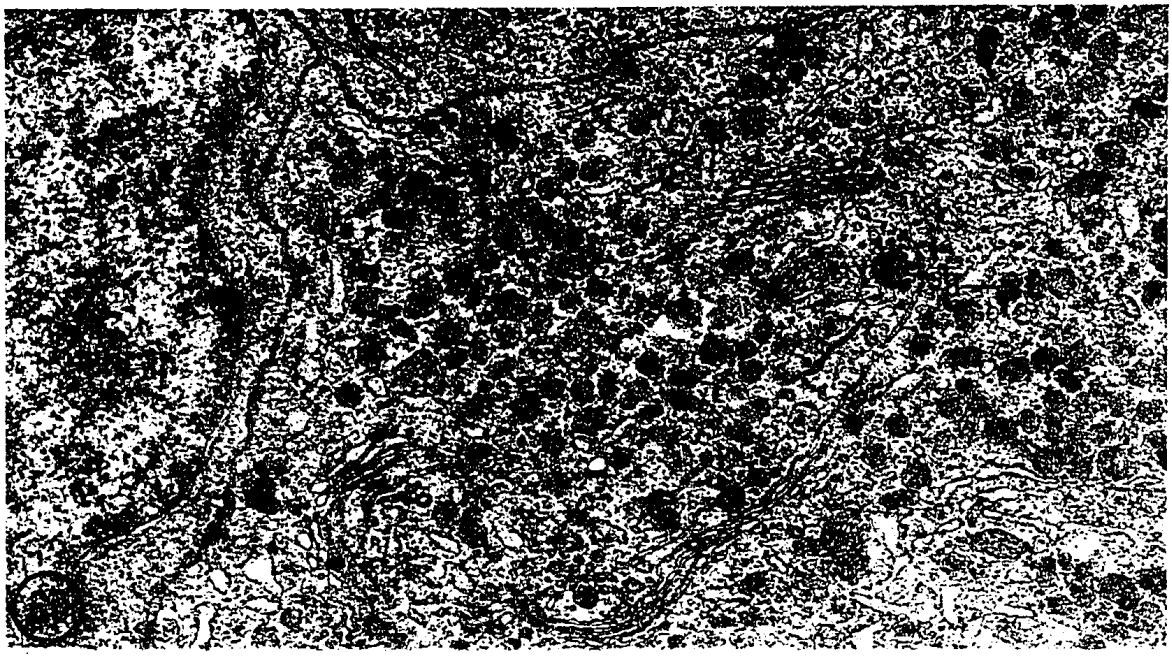
Light Cell in the Ultimobranchial Gland of the Trout

The light cells in the ultimobranchial gland of the trout were generally oval or elongate in shape. The cytoplasm contained variable number of secretory granules. The secretory granules were round or oval in shape and measured from 150 to 250 mμ in diameter.

Fig. 59 An area of the light cell in the ultimobranchial gland of the trout showing the presence of secretory granules (sg) and the development of the endoplasmic reticulum (Rer). Uranyl and lead. X 13,650.

Fig. 60 Golgi region of the light cell showing the presence of saccules and smooth-surfaced vesicles. Variable number of secretory granules (sg) and some fibrils (f) are observed. Uranyl and lead. X 13,650.


59



The secretory granules were lined by a smooth limiting membrane. The contents of the secretory granules consisted of finely granular material which varied in density (Figs. 59 and 60).

The endoplasmic reticulum consisted of the rough-surfaced variety. The membranous cisternae were distributed randomly throughout the cytoplasm (Fig. 59). The Golgi complex was invariably developed in the light cells. In the well developed Golgi area, the saccules were arranged in concentric layers. Clusters of smooth-surfaced vesicles and variable number of secretory granules were observed in the Golgi region (Fig. 60).

In addition, the cytoplasm of the light cell also contained variable number of mitochondria and some fibrils.

3. Discussion

The Ultimobranchial Gland in the Frog

In the present study, on the basis of the light and electron microscopic observations, two distinct cell types can be recognized in the parenchyma of the ultimobranchial gland of the frog.

The light cell seems to be homologous to the light cell in the chick and the mammalian thyroid

light cell. The cytoplasm of the light cell contains numerous secretory granules which appear morphologically similar to those in the other species. The contents of the secretory granules also stain differently in either osmium tetroxide or glutaraldehyde fixation. The presence of the majority of the secretory granules in the basal region of the cell together with images of the fusion of the limiting membrane of the secretory granule with the cell membrane seem to suggest the secretion of the product into the surrounding connective tissue. Further evidence of the functional role of the secretory granules is the depletion of these granules in the hypercalcemic frogs induced by vitamin D_2 (Robertson, 1968b).

The epithelial cell present in the parenchyma is morphologically different from the light cell. The cytoplasm of the epithelial cell is characterized by the presence of filaments which tend to give the cell a dense and compact appearance. Dense granular material in the lumen of the follicle is presumably secreted by the epithelial cell. Images of similar material which is being extruded into the lumen from the epithelial cells provide further evidence of the difference between the light and the epithelial cells.

The material in the lumen and those found in the cytoplasm of the epithelial cells are P.A.S.-positive and this could presumably be a mucous-like substance.

The general organization of the ultimobranchial gland of the frog is similar to the cyst-like structure in the ultimobranchial gland in the chick. However, Robertson (1968b) classified the different cell types as storage cells and degenerating cells. He considered them to be similar, but in different stages of maturation.

The physiological significance of the ultimobranchial gland in the frog seems to be similar to those in other species. It has been shown that osteocytic osteolysis occur in the frog and parathyroid hormone seem to have a stimulating effect (Bélanger and Drouin, 1966). Ultimobranchialectomy impairs the ability of the frog to control hypercalcemia (Robertson, 1969). Therefore, the evidence seems convincing that the ultimobranchial gland may be involved in the regulation of the calcium homeostasis in the frog.

The Ultimobranchial Gland in the Fish

Ultrastructurally, light cells in the trout show several common characteristics with the light cells of the ultimobranchial gland in the chick. The cytoplasm is characterized by the presence of variable number of secretory granules. Morphologically, the

secretory granules appear similar to those in the light cells of the ultimobranchial gland in the chick (Chan et al., 1969). Furthermore, the presence of calcitonin in the ultimobranchial glands of dogfish and salmon has been confirmed by Copp et al. (1967b, 1968).

The physiological role of the ultimobranchial gland in the fishes is not known. However, several studies seem to suggest a possible relation of the gland to calcium homeostasis and bone metabolism. Rasquin and Rosenbloom (1954) observed numerous abnormalities including skeletal malfunction, renal pathology and the hypertrophy of the ultimobranchial gland in the teleost, Astyanax mexicanus, raised in total darkness for several months. They postulated a parathyroid-like function for the gland. The hypertrophy of the ultimobranchial gland was considered by them to produce a condition comparable to hyperparathyroidism, the kidney and skeletal defects being then attributable to it. However, the possibility exists that the hypertrophy of the ultimobranchial gland is the effect rather than the cause of the increased level of serum calcium.

Similarly, the ultimobranchial gland of the trout is hypertrophied when subjected to toxic levels of fluorides (Neuhold and Sigler, 1960). They also consider the ultimobranchial gland to be homologous

to the parathyroid gland in the mammals. However, chronic fluoride administration in rats has no stimulating effect on the parathyroid function (Raisz and Taves, 1967).

Studies on the effect of porcine calcitonin in fishes have produced inconsistent results. Catfish, Ictalurus melas, with cellular bone gives a positive response to procine calcitonin (Louw et al., 1967). Similarly, Chan et al. (1968) found that similar extracts administered to the European eel, Anguilla anguilla L, resulted in a progressive hypocalcemia. However, in killifish, Fundulus heteroclitus, which has acellular bone, there is a lack of response (Pang and Pickford, 1967). It is not known whether the difference in response can be attributed to the difference in bone types.

The functional significance of the ultimobranchial gland in the fish is not known. However, fishes may be subjected to surges of calcium absorption due to dietary conditions which could result in elevated levels of plasma calcium (Urist, 1966). In this regard, calcitonin may play an important role in the calcium homeostasis.

General Conclusion

Electron microscopy of the light cell in the rat thyroid gland shows an ultrastructural pattern of organization similar to that of other secretory cells. Light cells have an abundance of secretory granules, measuring 150 to 300 mμ in diameter. Other cytological features include the presence of rough-surfaced endoplasmic reticulum, Golgi complex and mitochondria.

The ultrastructure of the light cell in the chick ultimobranchial gland is similar to that in the rat thyroid. The cytoplasm is characterized by the presence of variable number of secretory granules measuring 100 to 250 mμ in diameter. The presence of rough-surfaced endoplasmic reticulum, Golgi complex and light granules are probably related to the secretory nature of the cell.

The light cell in the ultimobranchial gland of the frog resembles that in the chick. Similarly, the light cell in the fish ultimobranchial gland retains many cytological features in the light cells of the various species.

There is a marked effect of the fixative on the appearance of the secretory granules in the light cells of the various vertebrate species. Following osmium tetroxide fixation, the secretory

granules appear as light vesicles which contain some finely granular material. In glutaraldehyde fixation, followed by postfixation with osmium tetroxide, the contents of the secretory granules are retained as dense granules.

The common cytological features of the light cells in the various vertebrate species seem to indicate that they develop from a common ancestral cell type. Therefore, the light cell in the rat thyroid gland is homologous to the light cell in the ultimobranchial glands of the non-mammalian vertebrates.

The light cells in the rat thyroid gland and the chick ultimobranchial gland are sensitive to changes in the serum calcium level. In the parathyroid hormone treated rats, the light cells respond to the hypercalcemia by discharging the secretory granules. In addition, the hyperplasia of the light cells, with increased development of the endoplasmic reticulum and the Golgi complex are morphological evidence of hyperactivity. In the rats fed a low calcium diet, the secretory granules in the light cells seem to increase in number. Presumably, these secretory granules must have accumulated in the absence of the stimulus for discharge in hypocalcemia. The light cells in the chick ultimobranchial gland react in a

similar manner to the hyper- and hypocalcemia as induced by the different dietary calcium level. The results suggest that the light cells in the various species are responsible for the secretion of calcitonin and that calcitonin is associated with the membrane-limited secretory granules.

The hyperplasia of the light cells after hypophysectomy demonstrates the absence of pituitary control of the light cell. However, the presence of numerous secretory granules in the light cells may be due to the lack of discharge in the hypothyroid state. Furthermore, it has been demonstrated that the light cells in the hypophysectomized rats are sensitive to the elevation in serum calcium level as induced by a phosphate deficient diet.

The present study has provided conclusive evidence that the light cell in the rat thyroid gland is the site of production of calcitonin and that calcitonin is contained in the membrane-limited secretory granules. The light cells respond to hypercalcemia by discharging the secretory granules whereas in hypocalcemia the secretory granules tend to accumulate. The presently reported maintenance of function of light cells in hypophysectomized rats and enchancement

of function in hypophysectomized rats fed a phosphate deficient diet is new knowledge. Furthermore, it has been demonstrated that the light cells in the rat is homologous to the light cells in the ultimobranchial glands of the various vertebrate species. The ultrastructural changes in the light cells of the chick ultimobranchial gland in hyper- and hypocalcemia have provided further evidence of the involvement of the gland in the regulation calcium homeostasis.

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