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Review of Cytology

EDITED BY

G. H. BOURNE J. F. DANIELLI

ASSISTANT EDITOR
K. W. JEON

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EDITED BY

G. H. BOURNE
Yerkes Regional Primate Research Center
Emory University
Atlanta, Georgia

J. F. DANIELLI
Worcester Polytechnic Institute
Worcester, Massachusetts

ASSISTANT EDITOR
K. W. JEON
Department of Zoology
University of Tennessee
Knoxville, Tennessee

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List of Contributors

Numbers in parentheses indicate the pages on which the authors' contributions begin.

A. C. ALLISON (119), *Division of Cell Pathology, Clinical Research Centre, Northwick Park, Harrow, Middlesex, England*

JAY C. BROWN (277), *Department of Microbiology, University of Virginia School of Medicine, Charlottesville, Virginia*

PHILIP DAVIES (119), *The Merck Institute for Therapeutic Research, Rahway, New Jersey*

MICHAEL D. GERSHON (1), *Department of Anatomy, Columbia University, College of Physicians and Surgeons, New York, New York*

KLAUS HAUSMANN (197), *Lehrstuhl für Zellenlehre, Universität Heidelberg, Heidelberg, West Germany*

RICHARD C. HUNT (277), *Department of Biochemistry, Oxford University, Oxford, England*

ELADIO A. NUNEZ (1), *Department of Anatomy, Columbia University, College of Physicians and Surgeons, New York, New York*

ROY C. PAGE (119), *Department of Pathology and Periodontics and the Center for Research in Oral Biology, University of Washington, Seattle, Washington*

ELIANE REGARD (81), *Université Paris-Sud, Laboratoire de Biologie-Vertébrés, Orsay, France*

TIMOTHY TREFFRY (159), *Department of Biochemistry, The University, Sheffield, United Kingdom*

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ELADIO A. NUNEZ AND MICHAEL D. GERSHON

Department of Anatomy, Columbia University, College of Physicians and Surgeons, New York, New York

I. Introduction

The thyroid gland of the mammal is a bilobed structure located at the base of the neck on either side of the trachea. The most important function of the thyroid gland is the synthesis, storage, and secretion into the blood of two iodinated amino acid hormones, L-thyroxine (tetraiodo-L-thyronine) and 3,5,3′-triiodo-L-thyronine. Thyroid hormones (thyroxine and triiodothyronine) are required for normal growth and development and for normal metabolic activity. Thyroid hormones act by accelerating general and specific metabolic processes of the body, leading to an increase in oxygen consumption and heat production (Tata, 1964). The importance of the thyroid gland was

known as early as 1895, when Murray demonstrated the therapeutic nature of extracts of the thyroid glands of animals in the treatment of hypothyroidism. The crystallization of thyroxine was achieved in 1919 (Kendall, 1919), and its chemical structure was determined in 1926 (Harington, 1926). The existence of triiodothyronine was first reported by Gross and Pitt-Rivers in 1952. Studies by Oppenheimer and his associates (1972) and others (Hervas *et al.*, 1976) strongly indicate that L-thyroxine may be a prohormone and that triiodothyronine is actually the only hormone of the thyroid gland that stimulates metabolism.

The mammalian thyroid gland is also responsible for the elaboration, storage, and secretion of a second type of hormone, calcitonin, which lowers the calcium concentration of blood. Calcitonin was discovered by Copp and his associates in 1962. By perfusing the thyroid-parathyroid complex of a dog with hypercalcemic blood, they induced the release of a factor, designated calcitonin, which lowered the concentration of calcium in the blood of a recipient animal. In 1963–1964, Hirsch *et al.* (1964) and Foster *et al.* (1964b) demonstrated that the thyroid gland, and not the parathyroid as first proposed by Copp *et al.* (1962), was the source of calcitonin. Materials extracted from thyroid tissue of the dog, rat, rabbit, ox, pig, goat, sheep, and human have all been effective in lowering the concentration of calcium in the blood when injected into a recipient rat (Hirsch and Munson, 1969). Isolation and purification of the hormone has been carried out in four mammalian species, human, pig, cow, and sheep (Brewer and Ronan, 1969; Neher *et al.*, 1968; Potts *et al.*, 1968; Raulais *et al.*, 1974). The hormone from all four species is a polypeptide with a molecular weight of about 3200, and it contains 32 amino acids in a single chain. Purified polypeptides are effective in lowering blood calcium in the rat. However, they differ in potency, and this difference has been attributed to the large variation in the amino acid sequence of the four molecules (Hirsch and Munson, 1969). Human calcitonin has been synthesized in the laboratory (Sieber *et al.*, 1968).

In lower vertebrates (birds, reptiles, amphibia, and fishes), calcitonin is found not in the thyroid gland but in the ultimobranchial body (Birov, 1971; Copp *et al.*, 1967; Cutler *et al.*, 1974; O'Dor *et al.*, 1969; Tauber, 1967). The ultimobranchial body is a separate and distinct gland. Avian and fish calcitonin has been purified and, like the hormone in mammals, consists of 32 amino acids (Potts *et al.*, 1970).

The major action of calcitonin is on bone. It prevents the removal of calcium from the skeleton (Aliapoulios *et al.*, 1966a; Hirsch, 1967; Wallach *et al.*, 1967). Calcitonin exerts this action by inhibiting all forms of bone resorption, both osteoclasia (Friedman and Raisz, 1965)

and osteocytic osteolysis (Whalen *et al.*, 1975). If excess calcitonin reaches the circulation during development, the resultant inhibition of bone resorption results in the retention of cartilage (Whalen *et al.*, 1975). Indications are that calcitonin does not enhance bone formation or alter the number of osteoblasts (Rasmussen and Bordier, 1974). However, it also acts on the kidney, where it decreases tubular resorption of calcium (Ogata and Kimura, 1973). These effects of calcitonin have been made use of. Synthetic human calcitonin has been demonstrated to have a very positive therapeutic effect in the treatment of adult Paget's disease (Doyle *et al.*, 1974; Haymovits *et al.*, 1975) and familial hyperphosphatasemia (Horwith *et al.*, 1976; Woodhouse *et al.*, 1972), diseases characterized by excessive resorption of bone. However, calcitonin may also play a role in the mediation of some diseases. There are indications that it is involved in the pathogenesis of certain metabolic diseases such as pseudohypoparathyroidism (Aliapoulios and Rose, 1970; Aliapoulios *et al.*, 1966b), hyperparathyroidism (Tashjian and Voekel, 1967), osteopetrosis in mice (Walker, 1966b), and parturient paresis in cattle (Young and Capen, 1968; Mayer *et al.*, 1975). But the physiological significance of calcitonin in the mammal is still not clear, since neither humans nor rats become hypercalcemic following total thyroidectomy (Editorial, 1973). Despite this, suggestions that it has a role in the adult have been put forward. It has been proposed that calcitonin is physiologically active in protecting animals against hypercalcemia during the period of calcium absorption following a meal (Munson *et al.*, 1974). Other suggestions are that calcitonin is required in liver regeneration (MacManus *et al.*, 1975) and that it is important in protecting the skeleton from excessive resorption throughout life (MacIntyre, 1971).

Microscopically, the mammalian thyroid gland is composed of a large number of closed, oval to spherical sacs referred to as follicles. Each follicle is lined by a single layer of cuboidal epithelial cells, called follicular cells, which surround a central lumen. The lumen is filled with a semifluid material which appears amorphous, the colloid. There are approximately 2 to 3 $\times$ 10^7 follicles in the human thyroid gland, and the individual follicles vary in size from 50 to 100 μm (Rhodin, 1974). A basement membrane is clearly evident around each follicle, and a rich capillary network is present in the interfollicular tissue. Every follicular cell borders a capillary, and many sympathetic nerve fibers accompany the blood vessels (Rhodin, 1974).

The lining follicular cells are responsible for the formation of thyroid hormone. They synthesize a complex glycoprotein, thyroglobulin, which they release into the follicular lumen by exocytosis (Nadler

et al., 1964). They also trap iodine and transport it from the blood to the follicular lumen, where thyroglobulin is iodinated. The iodinated thyroglobulin molecules are then stored in the lumen of the follicle in the form of colloid. Through the action of apical pseudopods (Ekholm and Smeds, 1966; Wetzel *et al.*, 1965), or invagination of the apical plasma membrane (Nunez *et al.*, 1972a; Seljelid *et al.*, 1971), the stored iodinated thyroglobulin is reabsorbed by the lining follicular cells in the form of colloid droplets. The intracellular colloid droplets eventually fuse with lysosomes, and the thyroglobulin undergoes degradation. This results in the liberation of thyroid hormone and secretion into the blood of thyroxine and triiodothyronine (Wetzel *et al.*, 1965).

The secretion of thyroid hormone is under the control of a negative feedback mechanism regulated by the hypothalamus. The hypothalamus secretes thyrotropin-releasing hormone (TRH) which stimulates the secretion of thyrotropin or thyroid-stimulating hormone (TSH) from the anterior pituitary. TSH then acts on the follicular cells, increasing thyroid hormone release. In turn, the output of TRH, and thus TSH, is governed by the circulating level of thyroid hormone, a decrease in the latter increasing the discharge of TRH and a rise diminishing it (Bowers *et al.*, 1970).

At the ultrastructural level, follicular cells are characterized by many evenly spaced microvilli projecting into the colloid from the apical surface. Beneath the surface plasma membrane, a heterogeneous population of tiny vesicles is found. They are considered as representing transport vesicles carrying thyroglobulin or thyroperoxidase to the follicular lumen (Nadler *et al.*, 1964; Novikoff *et al.*, 1974). The nucleus is typically near the base of the cell but has no distinctive features. The cytoplasm contains elaborate rough-surfaced endoplasmic reticulum which consists of many widely dilated profiles. A well-developed Golgi apparatus is usually found lying between the nucleus and the lumen of the follicle. Mitochondria are scattered throughout the cell. At least four types of rounded structures are present in follicular cells. They are: (1) colloid droplets which are particularly conspicuous after the administration of exogenous TSH, (2) membrane-limited dense granules which stain for acid phosphatase, (3) phagolysosomes which are bodies that arise from the fusion of colloid droplets and lysosomal dense granules, and (4) autophagic vacuoles. Apical pseudopods are not usually observed in normally active follicular cells. They are, however, especially numerous after TSH stimulation. A basement membrane borders the cell. For a more extensive discussion of the cytophysiology and ultrastructure of thyroid follicular cells, see the reviews by Fujita (1975) and Pantić (1974).

In addition to follicular cells, the mammalian thyroid gland contains a second type of epithelial cell. These cells have been given various names but are generally referred to as parafollicular cells. In contrast to follicular cells, parafollicular cells are relatively uncommon in the thyroid gland. As early as 1876, they were reported to be morphologically distinct from follicular cells. At that time, Baber (1876) reported that in the canine thyroid gland a second epithelial cell type, described as parenchymatous, differed markedly in size and shape from the follicular cells among which it lay. Since then, in addition to being described as parafollicular by Nonidez in 1931, they have been called protoplasm-rich cells (Hürtle, 1894), ovoid cells (Bensley, 1914), interfollicular cells (Takagi, 1922), clear cells (Zechel, 1933), light cells (Stux *et al.*, 1961), neurohormonal cells (Sunder-Plassmann, 1939), mitochondria-rich cells (Seecof, 1927), acidophilic cells (Hamperl, 1937), macrothyrocytes (Idelman, 1962; Kroon, 1958), argyrophilic cells (Sandritter and Klein, 1954), gray cells (Godwin, 1937), stem cells (Ponse, 1951), and most recently, C cells (Pearse, 1966a).

Nonidez, in his excellent histochemical studies of the dog thyroid gland in the early 1930s (1931, 1932, 1933), provided the first evidence that parafollicular cells differ functionally from follicular cells. From observations of tissue impregnated by the silver nitrate method of Cajál, he noted that parafollicular cells, but not follicular cells, contained brown to black cytoplasmic granules. In the early 1950s, Sandritter and Klein (1954), Sandritter *et al.* (1956), and others (Dejardin, 1955; Dumont, 1956a; Gabe, 1959) demonstrated the existence of still other cytochemical differences between parafollicular and follicular cells in such species as the dog, rabbit, and guinea pig. In 1960 it was further reported that parafollicular cells of the thyroid gland of the rat, unlike follicular cells, do not respond to TSH administration (Isler *et al.*, 1960). However, despite these findings and suggestions that parafollicular cells are secretory in nature (Altman, 1940; Arimitsu, 1937; Bensley, 1914; Ohkubo, 1934; Sato, 1959; Sugiyama, 1954), the general consensus during this period, 1876–1962, was that parafollicular cells were nonsecretory (Grossi and Servide, 1961; Tashiro, 1962; Waller, 1960) and represented a stage in the life cycle of follicular cells (Saito, 1956; Voitkevitch, 1963), that they might be nerve cells (Sunder-Plassmann, 1939), or that they might be ectopic parathyroid cells (Getzowa, 1907). Even as late as 1954 some workers denied the existence of parafollicular cells as a separate cell type within the thyroid gland, stating that they were artifacts of the procedures used in their demonstration (Ehrenbrand, 1954; Ludwig, 1953, 1954).

Following the discovery of calcitonin in 1962 by Copp *et al.*, there was immediate interest in the possibility that parafollicular cells

might be the cellular source of the polypeptide. Foster *et al.* (1964a) examined the canine thyroid gland after perfusion with a hypercalcemic solution, noted the apparent degranulation of a cell identified as a parafollicular cell, and suggested that parafollicular cells were responsible for the production of calcitonin. With the use of immunofluorescence methods, the specific localization of calcitonin in parafollicular cells was soon demonstrated by Bussolati and Pearse (1967) and Kracht and associates (1968a). This, then, was the beginning of a period of great interest in the morphology, function, and cytophysiology of the parafollicular cells of the mammalian thyroid gland, which has extended into the present. This interest has resulted in a large number of publications during the last dozen years. We have attempted to review these investigations and, on the basis of this review, to propose a role for the parafollicular cell in mammalian physiology. However, it is not the aim of this report to review the literature concerning the hormone calcitonin, since several excellent reviews have already been published on this subject (Haymovits and Rosen, 1972; Hirsch and Munson, 1969; Queener and Bell, 1975).

II. Histochemical Studies of Mammalian Thyroid Parafollicular Cells

A. Identification

Parafollicular cells of the thyroid gland have been distinguished from follicular cells and other thyroid elements by various histochemical procedures. The techniques described in the following discussion have most often been used during the past decade to identify parafollicular cells. A short discussion of each of these methods follows, and criticisms of the techniques are listed in Table I.

1. *Cajál Silver Nitrate*

As early as 1931, Nonidez demonstrated that the silver nitrate method of Cajál could distinguish parafollicular cells from follicular cells. More recently, further modifications of this method, used by Fitzgerald (1964), DeGrandi (1970), Sawicki and Bajko (1974), and Blähser and Kraus (1972), have simplified the procedure and demonstrated that silver nitrate impregnation is a reproducible and sensitive method for parafollicular cell identification in most, but not all, mammalian species. Silver nitrate has been used to demonstrate the presence of parafollicular cells in the thyroid glands of dogs, asses, sheep, rabbits, cats, rats, wolves, pigs, hamsters, and guinea pigs (Biddulph,

TABLE I
CRITICISM OF HISTOCHEMICAL PROCEDURES EMPLOYED TO IDENTIFY PARAFOLLICULAR CELLS

Technique	Source of criticism
Cajál silver nitrate	Kracht *et al.* (1970); Lietz (1971); Sawicki and Bajko (1974); Velický (1970)
Masked metachromasia	Blahošová *et al.* (1974); DeGrandi (1970); Ljungberg (1970a); Maunder and Rost (1972); Roszkiewicz (1974a)
Colloidal iron	Sawicki (1975); Velický (1970)
Nonspecific esterases	Mietkiewski *et al.* (1974); Mikhailov (1972); Sawicki (1975)
α-Glycerophosphate dehydrogenase	Birov (1971); Lietz (1971); Mietkiewski *et al.* (1973a); Roszkiewicz (1974a); Sawicki (1975); Stachura (1971b); Zabel (1973)
Acid phosphatase	Beskid and Rosciszewska (1968); Mietkiewski *et al.* (1974); Mikhailov (1972); Roszkiewicz (1974a); Sawicki (1975)
Amine precursor uptake	Lietz (1971); McMillan *et al.* (1974)
Immunohistochemistry	Kalina *et al.* (1970)

1968; DeGrandi, 1970; Nonidez, 1931; Pearse, 1968c; Sawicki and Bajko, 1974; Van Dyke, 1945; Young *et al.*, 1968). It is not known whether the argyrophilic properties of parafollicular cells are due to lipid interaction or to the interaction of the secretory granules with silver nitrate (Lietz, 1971; Roediger, 1973a).

2. *Masked Metachromasia*

Masked metachromasia can be demonstrated in parafollicular cells by staining with a metachromatic basic dye such as toluidine blue, azure A, or methylene blue after a Feulgen-type hydrolysis of suitably fixed material (Lietz and Zippel, 1969a; Sawicki, 1971; Solcia and Sampietro, 1965a). Similar results have been obtained with lead hematoxylin (Sawicki, 1975). Masked metachromasia of parafollicular cells is believed to be due to the presence in their cytoplasm of secretory granules containing a polypeptide with a high concentration of acidic groups and a random-coil conformation (Bussolati *et al.*, 1969c; Maunder and Rost, 1972; Solcia *et al.*, 1968a,b). The phenomenon of masked metachromasia was first noted in other polypeptide-containing cells by Manocchio in 1964. Solcia and Sampietro (1965a,b) were the first to develop this method for the demonstration of parafollicular cells. They stained the cells with toluidine blue or azure A after treatment with hot, dilute hydrochloric acid. According to them, the treat-

ment with hydrochloric acid eliminates diffuse metachromasia in the tissue and increases the metachromasia of the parafollicular cells. Recent modifications of the method include a fluorescence technique involving the acridine dye coriphosphine O (Bussolati *et al.*, 1969c). Other workers have employed thiazine dyes such as gentian violet B (Guglielmone, 1971) and 1,9-dimethylmethylene blue (Petkó, 1974c). Reinforcement of masked metachromasia in parafollicular cells has been claimed in the treatment of tissue with potassium ferricyanide and ammonium heptamolybdate, supposedly to decrease dehydration of the metachromasia (Petkó, 1974b).

The silver nitrate method has been reported to be intensely positive in parafollicular cells of the thyroid gland of the rat (Gittes *et al.*, 1968; Kameda, 1968, 1970). However, investigators using masked metachromasia have found that in the rat, in contrast to other species, this method shows cells only faintly (Roszkiewicz, 1974a). This may be due to the small number of secretory granules in the cytoplasm of rat parafollicular cells (Roszkiewicz, 1974a; Stachura, 1971a; Williams, 1966), but it may also indicate that in the rat masked metachromasia is simply less sensitive than other histochemical procedures. It is interesting to note that in certain mammals, such as hibernators (Olivereau, 1970; Olivereau and Fontaine, 1970), as well as some nonhibernators (Lietz and Donath, 1970a; Ljungberg, 1970a), parafollicular cells stain well with such dyes as toluidine blue, cresyl fast violet, and hematoxylin-eosin even without prior acid hydrolysis, whereas in most nonhibernating species the reaction of unhydrolyzed tissue is very weak (Lietz and Donath, 1970a; Hedhammer *et al.*, 1974).

3. *Colloidal Iron*

The reaction with colloidal iron has been reported to show a fairly high degree of selectivity in the demonstration of parafollicular cells in the thyroid glands of some mammals (Lietz and Zippel, 1969a; Roszkiewicz, 1974a). According to Lietz and Zippel (1969a), colloidal iron is similar to masked metachromasia and indicates the presence of acidic groups in the cytoplasm of parafollicular cells. However, it has been reported that in the guinea pig colloidal iron is much less selective than masked metachromasia for parafollicular cells, since a strong reaction with colloidal iron occurs in both parafollicular cells and follicular cells (Sawicki, 1975).

4. *Enzymic Reactions*

As early as 1955–1956, cholinesterase (acetyl-or butyrylcholinesterase) was reported (Dejardin, 1955; Dumont, 1956b) to be a particularly good indicator of parafollicular cells of the mammalian thyroid

gland (Pepler and Pearse, 1967). For example, parafollicular cells exhibit a strong cholinesterase reaction in pigs, dogs, rabbits, guinea pigs, and rats (Carvalheira and Pearse, 1967a,b; Gauguin *et al.*, 1973; Mietkiewski *et al.*, 1973a; Roediger, 1973b; Roszkiewicz, 1974a). The role of cholinesterase in parafollicular cell activity is not clear (Roszkiewicz, 1974a), however, its involvement in the synthesis of the membranes surrounding parafollicular cell granules and in the secretion of granules has been postulated (Pearse and Welsch, 1969; Stachura, 1971a).

Pearse (1966a) and others (Mietkiewski *et al.*, 1973a; Sawicki, 1975) used reactions for nonspecific esterases and acid phosphatase as selective markers for parafollicular cells. Unfortunately, it has also been reported that both esterase and acid phosphatase are much less selective in identifying parafollicular cells than other histochemical methods (Roszkiewicz, 1974a; Sawicki, 1975). The very strongly positive reaction with α-glycerophosphate dehydrogenase noted by Foster *et al.* (1964a) in parafollicular cells of the dog thyroid was subsequently regarded by Pearse (1966a) and others (Beskid and Rosciszewska, 1968; Roediger, 1973b; Sawicki, 1975) as a good parafollicular cell marker. However, studies on such rodents as rats (Birov, 1971; Stachura, 1971b; Roszkiewicz, 1974a), rabbits (Mietkiewski *et al.*, 1973a), and guinea pigs (Birov, 1971) have indicated that this reaction is useless for distinguishing parafollicular cells from follicular cells.

Reactions with other enzymes such as adenosine triphosphate (Ansari, 1967; Lietz and Zippel, 1969b; Mikhailov, 1972; Rother, 1970) and alkaline phosphatase (Mikhailov, 1972), as well as several other oxidoreductases (Beskid *et al.*, 1968; Mietkiewski *et al.*, 1974; Mikhailov, 1972; Pearse, 1969; Rother, 1970), have also been studied. However, their reliability and reproducibility as specific indicators of parafollicular cells have yet to be established. Parafollicular cells have been reported to contain high α-glycerophosphate menadione reductase activity (Pearse, 1969; Rojo-Ortega *et al.*, 1971), which is considered by some (Pearse, 1969) to be a manifestation of high lipid turnover. This implies a possible involvement of phospholipids in hormone secretion.

In summary, it seems clear that enzymic markers are not noncontroversial indicators of parafollicular cells. Of the available methods, cholinesterase seems clearly the most reliable, even if its significance to the economy of the parafollicular cell remains to be established.

5. *Amine Precursor Uptake*

The ability of mammalian parafollicular cells to take up and convert exogenous L-5-hydroxytryptophan (5-HTP) to serotonin (5-hydroxy-

tryptamine, 5-HT), or L-3,4-dihydroxyphenylalanine (L-dopa) to dopamine (DA), has been employed by many workers to identify parafollicular cells in the thyroid gland (Dahlström and Ericson, 1972; Englund *et al.*, 1972; Falck *et al.*, 1964; Gershon and Nunez, 1970; Larson *et al.*, 1966; Owman and Sundler, 1968; Pearse, 1966a,b; Ritzen *et al.*, 1965; Tjälve and Slanina, 1971). Both of these amino acids, 5-HTP and L-dopa, are decarboxylated in the cytoplasm of parafollicular cells to give the corresponding amines, 5-HT and DA, respectively (Ericson, 1972b; Håkanson *et al.*, 1971a; Owman and Sundler, 1968). The amines are then stored in the cytoplasm and can be converted into fluorescent compounds in tissue that has been freeze-dried and exposed to formaldehyde vapor of appropriate relative humidity. This permits detection of the amines in parafollicular cells by fluorescence microscopy (Gershon and Nunez, 1970; Owman and Sundler, 1968; Pearse, 1966a). Ritzen *et al.* (1965) and Gershon and Ross (1966a,b) documented this process by injecting tritium-labeled L-dopa or 5-HTP and localizing labeled amine in parafollicular cells by autoradiography. Thus, by using either the histochemical fluorescence method or light microscope autoradiography, the distribution of parafollicular cells in the mammalian thyroid gland has been shown in many species including the dog (Gershon *et al.*, 1971; Pearse, 1966b), mouse (Gershon and Ross, 1966b; Larson *et al.*, 1966; Tjälve and Slanina, 1971), rat (Dahlström and Ericson, 1972), and bat (Gershon and Nunez, 1970).

Pearse (1966c, 1968a) has classified cells that share the ability to produce a low-molecular-weight protein or polypeptide and the ability to take up and decarboxylate exogenous 5-HTP or L-dopa in a group referred to as the amine precursor uptake and decarboxylation (APUD) series of endocrine cells. Thyroid parafollicular cells, as well as the cells of the ultimobranchial body, are considered to belong to this APUD series. Other cell types in the series include pituitary corticotrophs and melanotrophs, pancreatic endocrine cells, and gastrointestinal enteroendocrine cells (Pearse, 1966a). All these, like parafollicular cells, are likely to have as at least one of their functions the synthesis and secretion of polypeptides.

6. *Immunohistochemical Methods*

Specific demonstration of calcitonin in parafollicular cells has been obtained by the use of fluorescein-labeled antibody to calcitonin (Bussolati and Pearse, 1967; Bussolati *et al.*, 1969a; Kalina *et al.*, 1970; Kracht *et al.*, 1968a,b) and unlabeled antibody-immunoperoxidase bridge techniques (LiVolsi *et al.*, 1973; McMillan *et al.*, 1974; Peng, 1975; Peng *et al.*, 1975; Tashjian *et al.*, 1974; Wolfe and Tashjian,

1974). Moreover, the use of these methods for the identification of calcitonin-containing parafollicular cells has clearly shown that parafollicular cells can easily be distinguished from follicular cells in the thyroid gland of the human and other species. A comparison of routine histochemical methods, such as those described above, with the immunoperoxidase technique shows that the immunohistochemical method is more sensitive and more specific in the demonstration of parafollicular cells in the normal thyroid gland (DeLellis *et al.*, 1974).

7. *Miscellaneous Methods*

The staining of parafollicular cells with strong dyes such as pseudoisocyanin and aldehyde fuchsin after oxidation of the tissue with performic acid (Dörrenhaus *et al.*, 1971; Lietz and Zippel, 1969b) has been claimed to be specific. However, these dyes have not been widely used, and thus it is difficult to judge their reliability and potential in the identification of parafollicular cells. Also, the use of other histochemical methods, such as histophotometry, to determine the calcitonin content and granulation of parafollicular cells has yet to be proved reproducible and reliable (Lietz *et al.*, 1969; Rouais *et al.*, 1973).

Foster *et al.* (1964a) and others (Harcourt-Webster and Stott, 1966; Seecof, 1927) referred to parafollicular cells as mitochondria-rich cells and hoped to distinguish them by their mitochondrial content. Electron microscope studies (see Section III,A,1) and histochemical studies (Mikhailov, 1972; Pearse, 1968a) of thyroid tissue have clearly shown that parafollicular cells are not particularly rich in mitochondria. In the human thyroid gland, however, there are cells, referred to as Ashkinay, oxyphilic, Hürtle, or oncocytic cells, which are in fact rich in mitochondria as revealed electron microscopically and histochemically (Hamperl, 1962; Raikhlin and Smirnova, 1970; Roth *et al.*, 1962; Tremblay and Pearse, 1960). These cells are clearly different from parafollicular cells (Roediger, 1975). Their function in the human thyroid gland is not known, but it has been thought by some workers that they are dystrophic persisting follicular cells (Friedman, 1949; Roediger, 1975). Others, however, have suggested, on the basis of enzyme histochemical studies, that they have an independent function (Kraevski *et al.*, 1974).

In summary, many methods have been applied for the demonstration of parafollicular cells. Of these, the best in terms of sensitivity and specificity appear to be amine precursor uptake and immunohistochemistry. The Cajál silver nitrate method, masked metachromasia, and cholinesterase histochemistry seem to be acceptable second