SOME CHEMOCYTOLOGICAL OBSERVATIONS ON THE PARATHYROID GLAND OF THE FORMOSAN ROCK-MONKEY (*MACACA CYCLOPIS*)

JUN HARA AND KAZUYORI YAMADA

2nd Department of Anatomy, Nagoya University School of Medicine
(Director: Prof. Jun Hara)

Since chemocytological techniques have been introduced into the morphology of the parathyroid gland, several enzymorphological studies have been made on the gland of vertebrates (Dempsey, Greep and Deane, 1949; Yamanouchi, 1953; Pearse and Tremblay, 1958; Fujii, 1960; Balogh and Cohen, 1961; Tremblay and Cartier, 1961). While the endocrine organ is known to elaborate and release a polypeptide hormone, little is known about the chemical cytology of the glandular cells associated with their secretory activity. Our previous studies (Hara and Yamada, 1962; Hara, Yamada and Hotta, 1963; Yamada, 1963) have indicated that some chemocytological methods for the demonstration of proteins and amino acids prove to be useful for such cytology in the parathyroid gland of the rat and toad. In these animal species, however, the glandular cells are uniform in their properties. Thus, it has been desired to apply the chemocytological methods to the parathyroid gland of higher mammals in whom the glandular cells can be grouped into two cell types. Therefore, it is the purpose of the present paper to report some observations on the parathyroid gland of the Formosan Rock-monkey (*Macaca cyclopis*) employing the chemocytological methods named and those for the demonstration of glycogen and ribonucleic acid (RNA).

MATERIALS AND METHODS

Two male Formosan Rock-monkeys (*Macaca cyclopis*) aged 6 and 7 years respectively were employed in the present study. Bilateral parathyroid glands with attached neighboring thyroid tissues were removed from the animals which were sacrificed by ether anesthesia. The glands were fixed in 1 per cent trichlor acetic acid ethanol (1 g trichlor acetic acid in 100 ml 80 per cent ethanol) and Carnoy's fluid for a variety of periods ranging from 1 to 24 hours at room temperature. They were embedded in paraffin, sectioned at a thickness of 8 μ and subjected to the following several staining methods; Hansen's hematoxylin eosin and Heidenhain's azan for general morphology, coupled tetrazonium (Pearse, 1960), 2, 2′-dihydroxy-6, 6′-dinaphthyl disulfide (DDD) diazo blue B with prior thioglycolate treatment (Barrnett and Seligman,

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1952 and 1954) and 2-hydroxy-3-naphthoic acid hydrazide (HNAH) diazo blue B (Barrnett and Seligman, 1958) for proteins and amino acids, periodic acid-Schiff (PAS) (McManus, 1946) with prior β-amylase digestion for glycogen and methylgreen pyronin (Brachet, 1940) with ribonuclease control for ribonucleic acid (RNA).

RESULTS

General morphology:

Hematoxylin eosin and azan stained preparations reveal that the parathyroid gland of these monkeys is composed of a compact parenchyma encapsulated by a connective tissue layer which invades as trabeculae into the parenchyma and contains an abundance of blood vessels (Fig. 1). The parenchymal cells are grouped into two types, chief and oxyphil cells (Fig. 2). The chief cells are far more numerous in number than oxyphil cells and show usually cord-like or irregular arrangements (Fig. 2). They are mostly polygonal in shape, have a faintly eosinophilic granular cytoplasm and contain a relatively large nucleus at the center of the cytoplasm. The nucleus is round or oval in shape and provided with moderately staining chromatin granules and one or two round nucleoli. The oxyphil cells are small in number and occur singly or in groups between groups of chief cells (Fig. 2). They are larger in size than chief cells and primarily round or oval shaped, but occasionally of an irregular shape. Their cytoplasm exhibits intense eosinophilia which makes one distinguish them from chief cells. The nucleus of oxyphil cells is relatively small, round or oval in shape and situated at the center or periphery of the cytoplasm. Similar to the nucleus of chief cells it contains one or two nucleoli, but its chromatin granules stain rather deeply.

The interstitial connective tissues of the gland consist of fibers, cells and numerous vascular constituents as usually observed in the corresponding organ of rodents.

Chemocytology:

1. Coupled tetrazonium reactive proteins and amino acids:

When the parathyroid gland is stained by the coupled tetrazonium method, the cytoplasm of chief cells is found to contain moderately or weakly reactive fine granules (Fig. 3). These granules are varied in amount according to individual cells and scattered throughout the cytoplasm. Figures suggestive of their passage through the plasma membrane are hardly to be seen. The nucleus of chief cells is stained distinctly, and above all the nucleoli and nuclear membrane are differentiated clearly from other nuclear structures. The cytoplasm of oxyphil cells is characterized by an abundance of fine granules which react more intensely than those in chief cells and are not necessarily diffuse throughout the cytoplasm but frequently concentrated in the peripheral cytoplasm (Fig. 3). The nuclear tetrazonium reaction of oxyphil cells tends to be more intense than that of chief cells and here the nucleoli and nuclear

membrane are likewise easy to distinguish from other nuclear structures by their marked stainability.

The interstitial connective tissue cells and fibers present mostly a faint reaction for the tetrazonium stain. However, the vascular endothelial cells are distinctly tetrazonium reactive, especially in their cytoplasm.

2. 2, 2'-dihydroxy-6, 6'-dinaphthyl disulfide (DDD) diazo blue B reactive proteins and amino acids:

The cytoplasm of chief cells is found to contain granules of different sizes which stain bluish red with the DDD diazo blue B technique (Fig. 4). These granules are variable in amount and distribution depending upon individual cells. In some cells they are abundant, whereas being less in others. They tend to be concentrated at a juxtanuclear or peripheral area of the cytoplasm, but reveal not infrequently a diffuse distribution throughout the cytoplasm. The nucleus of chief cells is weakly DDD diazo blue B reactive. In addition to the intracellular localization of the DDD diazo blue B reactive granules, they are often seen to align themselves upon the outer surface of the plasma membrane and also to scatter in the interstitial connective tissue space. In the cytoplasm of most oxyphil cells DDD diazo blue B reactive granules tend to be rather less in amount as compared with those in the cytoplasm of chief cells (Fig. 4). Moreover, the outer surface of the plasma membrane is found to be studded by only a few number of them. The nucleus of oxyphil cells is similar in DDD diazo blue B stainability to that of chief cells.

Most of the interstitial connective tissue constituents color faintly or moderately with this stain. But, there occurs an exception which is the intense reaction of the cytoplasm of the vascular endothelial cells.

3. 2-hydroxy-3-naphthoic acid hydrazide (HNAH) diazo blue B reactive proteins and amino acids:

The HNAH diazo blue B method discloses varying amounts of bluish red granules of a nearly equal size within the cytoplasm of chief cells (Fig. 5). The granules are commonly disseminated dispersedly in the cytoplasm, but exhibit infrequently a concentration at either juxtanuclear or peripheral region of the cytoplasm. The nuclear HNAH diazo blue B reaction is generally faint in chief cells, except for the nuclear membrane which is visualized distinctly in a reddish blue shade. In contrast to DDD diazo blue B reactive granules HNAH diazo blue B reactive granules are scantily located outside the cytoplasm of chief cells. In the cytoplasm of oxyphil cells an appreciable abundance of intensely HNAH diazo blue B reactive granules of a nearly equal size is observed as compared with that of chief cells (Fig. 5). These granules tend to be condensed to a variable extent at a peripheral area of the cytoplasm beneath the plasma membrane. On the outer surface of the plasma membrane of oxyphil cells, however, only a few number of HNAH diazo blue B reactive granules are found to rest. Oxyphil cells are similar in nuclear HNAH diazo blue B staining intensity to chief cells.

All the interstitial connective tissue components react feebly for the HNAH

diazo blue B, excepting the intense stainability of the cytoplasm of the vascular endothelial cells.

4. Glycogen:

According to the PAS staining method employed in combination with prior β-amylase digestion procedure the cytoplasm of many chief cells is found to include a variable amount of glycogen granules of different sizes (Fig. 6). The distribution pattern of these granules within the cytoplasm is capricious depending upon each cell. In some cells they are scattered within the cytoplasm, but in others being aggregated at one locus of the cytoplasm which is juxtanuclear or peripheral in position. In occasional chief cells the cytoplasm is devoid of glycogen granules. The cytoplasm of oxyphil cells exhibits a marked clarity which is due chiefly to either absence of glycogen granules or presence of a negligible amount of them (Fig. 6). The cytoplasm is often so clear that one can easily distinguish it from that of chief cells.

In all the connective tissue constituents the polysaccharide granules are hardly demonstrated in any appreciable amount.

5. Ribonucleic acid (RNA):

The cytoplasm of chief cells exhibits RN-ase digestible diffuse moderate pyroninophilia, although the staining intensity varies to some extent with individual cells (Fig. 7). Within the cytoplasm exhibiting such pyroninophilia accumulations of intensely pyroninophilic granules of different sizes are visualized here and there. These granules are removed thoroughly by prior RN-ase treatment, consisting therefore of RNA. Their accumulations are situated at variable loci of the cytoplasm, thus either at juxtanuclear or peripheral regions. The diffuse pyronin reaction of the cytoplasm of oxyphil cells appears to be minimal in intensity and occasionally absent (Fig. 7). In addition, the cytoplasm does not contain any type of intensely reactive inclusions at all. The nuclear elements of both chief and oxyphil cells do not stain with pyronin, but the nucleoli show a rather strong pyroninophilia labile to prior RN-ase digestion.

No appreciable pyroninophilia indicative of the presence of RNA is discerned throughout the connective tissue components.

DISCUSSION

General morphology:

The parathyroid gland of the present monkeys is essentially similar in gross histological structure to that of the rat previously examined (Hara and Yamada, 1962). As widely known, the parenchymal cells of the monkey parathyroid gland can be classified into two types, chief and oxyphil cells. According to a series of rather classic investigations (Petersen, 1903; Verebely, 1907; Hartwich, 1922; Morgan, 1936), there occur, in the human parathyroid gland, a variety of morphological transitions between chief and oxyphil cells. This fact in the human parathyroid gland has tended to suggest that oxyphil

cells are regarded as a degenerative form of chief cells (Bargmann, 1939). In the parathyroid gland of the present monkey, however, oxyphil cells are not conceived to be a degenerative cell type, because their general cytology reveals nothing indicative of their degeneration.

Chemocytology:

1. Coupled tetrazonium reactive proteins and amino acids:

In the present study coupled tetrazonium reactive fine granules are detected in the cytoplasm of chief cells. Since figures suggestive of their passage through the plasma membrane are hardly to be seen, there is no convincing ground for regarding these protein granules as being associated with the cellular activity of secretion. Oxyphil cells are found here to be characterized by an abundance of intensely tetrazonium reactive fine granules which tend to be concentrated in the peripheral cytoplasm. Recent electron microscopic studies on the human and monkey parathyroid glands (Trier, 1958; Lange, 1961; Holzmann and Lange, 1963) have shown that the cytoplasm of oxyphil cells is significantly abundant in mitochondrial content as compared with that of chief cells. Accordingly, the abundance of tetrazonium reactive fine granules in the cytoplasm of oxyphil cells must be associated with the mitochondrial abundance.

2. 2, 2'-dihydroxy-6, 6'-dinaphthyl disulfide (DDD) diazo blue B reactive proteins and amino acids:

We (Hara and Yamada, 1962; Hara, Yamada and Hotta, 1963; Yamada, 1963) are the first to demonstrate the presence of DDD diazo blue B reactive granules in the cytoplasm of the rat and toad parathyroid cells and to put forward a novel theory that the sulfhydryl and disulfide groups containing protein granules are a reflection of the secretory activity of the cells. Previously, we (Hara, Yamada and Hotta 1963; Yamada, 1963) have considered the DDD diazo blue B reactive granules to be a carrier of parathormone, because this active principle is found on analysis to contain only an exceedingly small amount of cystine (Rasmussen, 1961). In the present study, chief cells of the monkey parathyroid gland are found to involve DDD diazo blue B reactive granules, the distribution patterns of which are suggestive of their passage through the plasma membrane and therefore of their participation in the secretory activity of the cells. Thus, our concept is taken to hold true in the chief cells of the parathyroid gland of the monkey which is phylogenetically higher than rodents and toads. In oxyphil cells of the present parathyroid gland DDD diazo blue B reactive granules tend to be rather less in amount than those in chief cells and the outer surface of the plasma membrane of this cell type is studded by only a few number of the granules. Therefore, according to our theory the oxyphil cells are concluded to be low in their secretory activity.

3. 2-hydroxy-3 naphthoic acid hydrazide (HNAH) diazo blue B reactive proteins and amino acids:

In a previous study on the parathyroid gland of bilaterally nephrectomized

rats (Hara, Yamada and Hotta, 1963) HNAH diazo blue B reactive granules within the cytoplasm of the parenchymal cells were conceived to represent mitochondrial proteins. In the parathyroid gland of the examined monkeys varying amounts of HNAH diazo blue B reactive granules tend to be disseminated diffusely throughout the cytoplasm of chief cells and extracellular HNAH diazo blue B reactive granules are scantily demonstrated. Such morphology of the granules is approximately identical with that seen in the parenchymal cells of the rat parathyroid gland. Therefore, our notion that the HNAH diazo blue B reactive granules represent mitochondrial figures, seems applicable to the chief cells studied here. This view is reliable, as intensely HNAH diazo blue B reactive granules are found here to be appreciably abundant in the cytoplasm of oxyphil cells in which mitochondria are shown to be condensed by recent electron microscopic studies (Trier, 1958; Lange, 1961; Holzmann and Lange, 1963). Accoding to the above discussion, the oxyphil cells should be characterized by an abundance of mitochondria and, in line with recent histochemical data (Balogh and Cohen, 1961; Tremblay and Cartier, 1961), be concluded to be a metabolically active cell type, although they have traditionally been believed to be degenerative (Bargmann, 1939).

4. Glycogen:

The cytophysiological significance of glycogen in the parathyroid cells has long been a subject of discussion among many authors (Petersen, 1903; Sundberg, 1924; Hara, Furuta, Murata and Yang, 1959; Isono, Isono and Komura, 1959; Fujii, 1960; Hara and Yamada, 1962). Our previous experimental studies (Hara, Yamada and Hotta, 1963) have certainly provided useful data for the recognition of the cytophysiological significance of the polysaccharide in the cells. According to our experiments the rat parathyroid cells show a noticeable decline in glycogen content when they are stimulated by bilateral nephrectomy. From this fact we have concluded that glycogen is utilized, possibly as an energy source, for the functional activity of the cells. In the present study chief cells of the monkey parathyroid gland are found to contain a variable amount of glycogen granules. In view of our previous results, therefore, this would imply that the chief cells are in varying states of functional activity. The oxyphil cells studied are of a poor glycogen content, which indicates that the polysaccharide is also utilized for the cellular metabolic activity, as they are concluded to be a metabolically active cell type.

5. Ribonucleic acid (RNA):

The general acceptance that RNA in combination with protein is concerned closely with protein synthesis in the cytoplasm, has been found to apply to the parathyroid cells, following Weymouth's (1957) and our (Hara, Yamada and Hotta, 1963) observations that they increase in RNA content, when the rate of protein synthesis in them is elevated by bilateral nephrectomy. It appears, thus, possible to interpret RNA as an indicator of protein synthetic activity in the parathyroid cells. Since the cytoplasm of chief cells of the monkey parathyroid gland exhibits varying degrees of RN-ase labile pyroninophilia, their protein synthetic activity seems to differ to some extent from

one another. The cytoplasm of oxphil cells of the examined parathyroid glands appears either minimal in RNA content or nearly devoid of the nucleic acid. This characteristic feature of the cells is supposed to represent a rather low protein synthetic activity in them and is consistent with our above notion that they are low in their secretory activity.

SUMMARY

The parathyroid glands of the Formosan Rock-monkey (*Macaca cyclopis*) have been microscopically examined, with particular interests in the chemocytological features of chief and oxyphil cells of the glandular parenchyma. From their stainability with a series of methods for the chemocytological demonstration of proteins and amino acids, glycogen and ribonucleic acid (RNA) the chief cells are concluded to be in a variety of states of secretory activity. The reactions of the oxyphil cells to the chemocytological stains named, indicate that this cell type is never degenerative as has traditionally been conceived, but is metabolically active.

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EXPLANATION OF PLATE FIGURES

FIG. 1. Parathyroid gland of a monkey. A compact parathyroid parenchyma is encapsulated by a highly vascular connective tissue layer. Carnoy's fixation, Hansen's hematoxylin eosin. ×120.

FIG. 2. Parathyroid gland of a monkey. Two oxyphil cells are seen between groups of chief cells. Carnoy's fixation, Heidenhain's azan. ×1300.

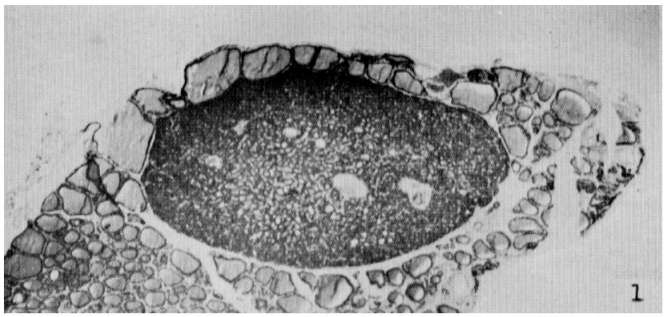
FIG. 3. Parathyroid gland of a monkey. An oxyphil cell is localized among groups of chief cells. Carnoy's fixation, Coupled tetrazonium. ×1300.

FIG. 4. Parathyroid gland of a monkey. An oxyphil cell is detected between chief cells. Trichlor acetic acid ethanol fixation, DDD diazo blue B. ×1300.

FIG. 5. Parathyroid gland of a monkey. An oxyphil cell is found to be surrounded by chief cells. Carnoy's fixation, HNAH diazo blue B. ×1300.

FIG. 6. Parathyroid gland of a monkey. Two oxyphil cells are visualized adjacent to chief cells. Carnoy's fixation, PAS and hematoxylin. ×1300.

FIG. 7. Parathyroid gland of a monkey. An oxyphil cell is demonstrated which is surrounded by chief cells. Carnoy's fixation, Methylgreen pyronin. ×1300.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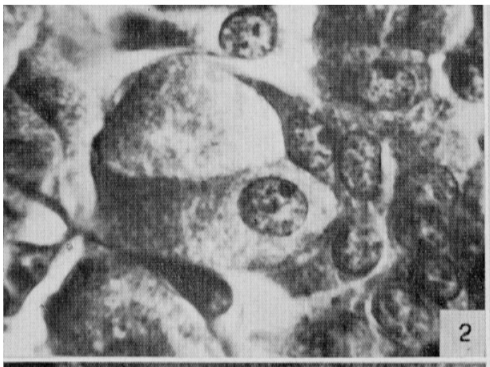
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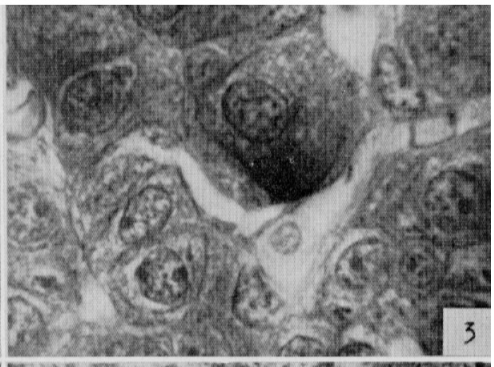
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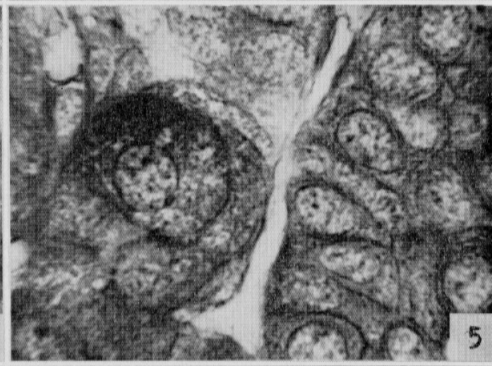
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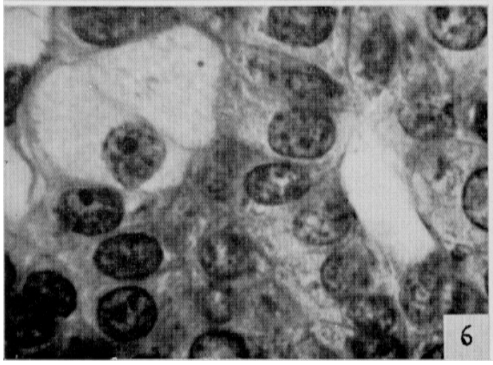
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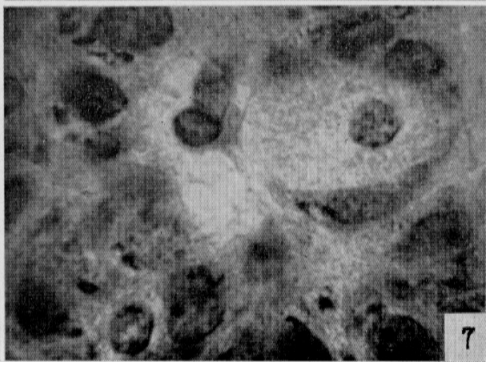
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