Observations on Two Types of Protein Granules in Primate and Avian Parathyroid Cells

My previous attempt to discover intracellular structures associated with the physiological functions of the parathyroid cells has been rewarded by the demonstration of a type of protein granule which is presumed to be correlated with the secretory activity of the cells[1]. A further approach in this line has recently led me to visualize two types of protein granules exhibiting positive 2,2'-dihydroxy-6,6'-dinaphthyl disulphide (DDD) diazo blue B and 2-hydroxy-3-naphthoic acid hydrazide (HNAH) diazo blue B reactions respectively in the cytoplasm of both the monkey (*Macaca cyclopis*) and quail (*Coturnix coturnix japonica*) parathyroid cells. The need for morphological examination of these granules in different cell types and under varied conditions for precise characterization of the granular reactions and for consequent elucidation of the cytophysiological significance of the granules has prompted the work recorded in the present report. Parathyroid glands were obtained from anaesthetized monkeys (*Macaca cyclopis*) and from live laying and non-laying quails (*Coturnix coturnix japonica*). The tissues were incubated in fixatives such as 1 or 2% trichlor acetic acid ethanol and Carnoy's fluid at room temperature for 1 to 24 h. Paraffin sections at a thickness of 8 μ were cut and stained with DDD diazo blue B and HNAH diazo blue B for the respective demonstrations of protein bound sulfhydryl and carboxyl groups, as prescribed originally by BARRNETT and SELIGMAN[2,3]. The former staining was, at times, done in combination with previous treatment with thioglycolic acid or N-ethyl maleimide potassium cyanide sequence for detecting protein bound disulfide groups[4,5]. The latter was occasionally accompanied by sodium hydroxide or ethanol treatment in order to distinguish protein bound side chain from α-terminal carboxyl groups[6,7].

In the parathyroid gland of the monkey, DDD diazo blue B reactive granules occur in the cytoplasm of both chief and oxyphil cells, being relatively smaller in amount in the latter cell type than in the former (Figure 1). The morphological distribution of the granules is worthy of attention, particularly in chief cells; the granules are frequently concentrated in peripheral loci of the cytoplasm, rest on the outer surface of the plasma membrane and are seen within the extracellular space. Such a distribution pattern appears suggestive of the passage of the granules through the plasma membrane. In the parathyroid cells of the quail, DDD diazo blue B reactive granules are almost identical in distribution pattern with those visualized in chief cells of the monkey parathyroid gland, thus occurring both in the cytoplasm and within the extracellular space (Figure 2). A larger amount of more intensely DDD diazo blue B reactive granules is discernible in the parathyroid parenchyma taken from laying quails than in that from non-laying birds (Figure 3). Since the parathyroid cells of laying birds are conceived to be more active in secretory function than those of non-laying individuals, this would imply that the amount and staining intensity of DDD diazo blue B reactive granules are closely correlated with the extent to which the parathyroid cells are elaborating their products of secretion.

In the monkey parathyroid gland HNAH diazo blue B reactive granules are thickly condensed in the cytoplasm of oxyphil cells (Figure 4), which is electronmicroscopically confirmed to contain an exclusive abundance of mitochondria[8]. So it appears that the majority of this type of protein granules represents a mitochondrial figure. In line with such interpretation, HNAH diazo blue B

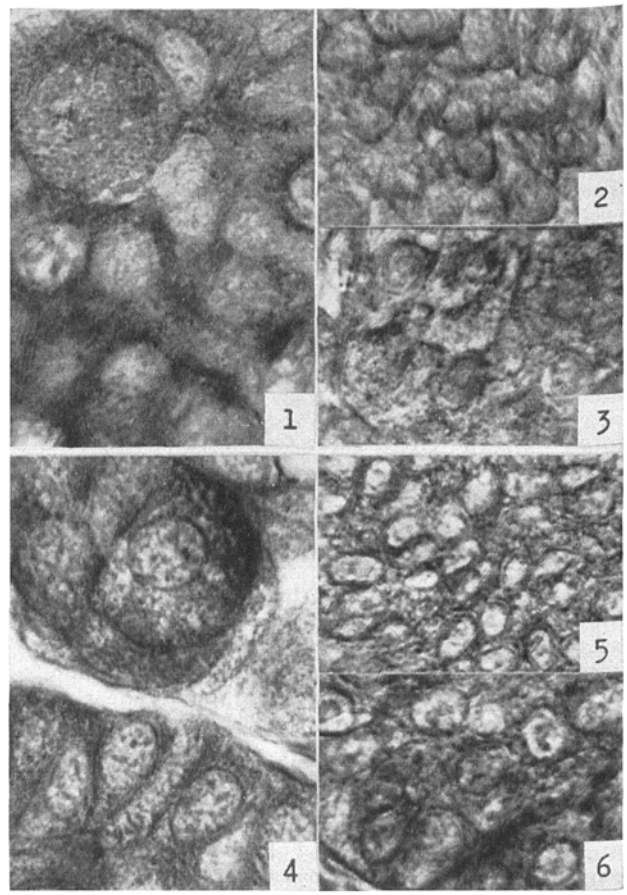

Fig. 1. Parathyroid gland of a monkey (*Macaca cyclopis*). In the cytoplasm of an oxyphil cell DDD diazo blue B reactive granules are relatively smaller in amount than in that of chief cells. ×1300.

Fig. 2. Parathyroid gland of a non-laying quail (*Coturnix coturnix japonica*). DDD diazo blue B reactive granules are demonstrated in the glandular parenchyma. ×1300.

Fig. 3. Parathyroid gland of a laying quail (*Coturnix coturnix japonica*). DDD diazo blue B reactive granules are more pronounced in amount and stainability than those in Figure 2. ×1300.

Fig. 4. Parathyroid gland of a monkey (*Macaca cyclopis*). HNAH diazo blue B reactive granules are thickly condensed in the cytoplasm of an oxyphil cell, while being moderate in amount in that of chief cells. ×1300.

Fig. 5. Parathyroid gland of a non-laying quail (*Conturnix coturnix japonica*). HNAH diazo blue B reactive granules are seen preponderantly in the cytoplasm of the glandular cells. ×1300.

Fig. 6. Parathyroid gland of a laying quail (*Coturnix coturnix japonica*). HNAH diazo blue B reactive granules are larger in amount and stain more intensely compared with those in Figure 5. ×1300.

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reactive granules tend to be distributed preponderantly in the cytoplasm of chief cells (Figure 4). However, it deserves attention that a small amount of HNAH diazo blue B reactive granules is localized outside the monkey parathyroid cells. With regard to the morphology of HNAH diazo blue B reactive granules, the quail parathyroid cells appear identical with chief cells of the monkey parathyroid gland, thus containing a preponderant distribution of the granules, while denoting the extracellular location of their small amount (Figure 5). Further, HNAH diazo blue B reactive granules are more prominent in amount and stainability in laying quails than in non-laying birds (Figure 6), pointing, therefore, to a parallelism of both properties of the granules to the secretory function of the parathyroid cells.

Previous thioglycolate reduction cannot decidedly increase the staining intensity of DDD diazo blue B reactive granules, and prior N-ethyl maleimide potassium cyanide sequence fails to yield a significantly intense reaction of the granules. Accordingly, protein bound sulfhydryl groups are primarily responsible for the reaction of the granules. HNAH diazo blue B reactive granules nearly fail to colour when the staining technique is accompanied by sodium hydroxide or ethanol treatment. Therefore, their stainability is considered to be due mainly to protein bound side chain carboxyl groups.

The present morphological examination of the two types of protein granules in different cell types and under varied conditions and the characterization of the granular reactions have provided an important clue to the accurate recognition of the cytophysiological significance of the granules. From their morphological features, DDD diazo blue B reactive granules can be conceived to reflect to an appreciable extent the cellular activity of secretion. This concept is particularly plausible in view of the recent electronmicroscopic evidence that in the human and deer parathyroid glands secretory granules can morphologically be followed from their formation in the glandular cells almost to their extrusion into the blood[9]. Moreover, in the light of the biologically activating effect of a sulfhydryl group containing reducing reagents like cysteine upon parathyroid hormone preparations[10,11], the chemical nature of the groups responsible for the granular reaction gives support to the theory that the granules exist in association with the hormone. The possibility that the granules display mitochondrial figures should be denied, because despite their extreme mitochondrial abundance[8] oxyphil cells have been shown to contain a relatively small amount of the granules in the monkey parathyroid gland. The morphology of HNAH diazo blue B reactive granules may indicate that their majority represents a mitochondrial pattern, whereas the extracellular presence of their small number is suggestive of their possible participation in the secretory function of the cells. In connection with the latter idea, the biochemical data may deserve attention in that parathyroid hormone preparations involve a significant number of glutamic and aspartic acid residues[11] and, therefore, of side chain carboxyls, which are found here to be the very groups responsible for the stainability of HNAH diazo blue B reactive granules.

Zusammenfassung. In Parenchymzellen der Epithelkörperchen von Affen (*Macaca cyclopis*) und Wachteln (*Coturnix coturnix japonica*)lassen sich zwei Typen von Proteingranula histochemisch unterscheiden. Die cytophysiologische Wertung dieser Granula wurde diskutiert.

K. YAMADA

Department of Anatomy, School of Medicine, University of Nagoya (Japan), April 14, 1964.

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Einbau von H^3-D-Glucose während der Oogenese bei *Apis mellifica* L.

In die wachsenden Oocyten der Insekten werden erhebliche Mengen verschiedenartiger Reservestoffe eingelagert, die insgesamt als Dotter bezeichnet werden können. Die Eizellen erreichen dadurch – meist in wenigen Tagen – in cytologischen Dimensionen betrachtet riesenhafte Ausmasse. Wie neuere, vor allem mit autoradiographischer[1] und serologischer[2] Methodik durchgeführte Untersuchungen über die Bildung der Proteinkomponenten des Dottersystems gezeigt haben, werden die hierbei erforderlichen Syntheseleistungen nicht von der Oocyte allein vollbracht: Die bei ihrem euplasmatischen Eiweissaufbau verbrauchte RNS stammt aus den polyploiden Nährzellen, und die Proteine der Dotterschollen werden bereits als Makromoleküle aus der Haemolymphe aufgenommen[3], wodurch das ausserordentlich schnelle Wachstum der Oocyten verständlich wird[4]. Über Kohlenhydrate liegen noch keinerlei entsprechende Befunde vor. Die vorhandenen Angaben sind vorwiegend deskriptiv und beziehen sich auf das weitverbreitete Vorkommen von Glykogen meist im Eizellplasma später Oogenesestadien[5,6], von PAS-positiven Dotterschollen[5,7–10] oder auch beidem nebeneinander[5,11–13]. Unter Verwendung von Glucose als Tracer wurde mit autoradiographischer Technik erstmals versucht, den genauen Zeitpunkt und

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