INTRACISTERNAL GRANULES IN THE EXOCRINE CELLS OF THE PANCREAS

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Plates 107 and 108

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In the course of an investigation concerned with the pancreas of the guinea pig and the microsomal fraction derived therefrom (1), it was found that the exocrine cells of the gland contain, during certain stages of their activity, a variable number of granules located in the cavities of the endoplasmic reticulum. The present paper describes this finding and discusses its implications in relation to the structure and function of the endoplasmic reticulum.

Materials and Methods

Young guinea pigs from the albino colony of these laboratories were used for this study. Small blocks of pancreas were removed under ether anesthesia and fixed for 2 hours at 0°C. in 1 per cent OsO_4 in acetate-veronal buffer (pH 7.4–7.6) (2). In many cases the osmolar concentration of the fixative mixture was increased to ~0.3 M by the addition of sucrose.[1] After fixation the specimens were washed and rapidly dehydrated in a series of graded alcohols. They were subsequently impregnated with *n*-butyl methacrylate and finally embedded in the same resin, by polymerization catalyzed with 2 per cent luperco CDB[2] at 47°C. (3). The embedded blocks were sectioned with a Porter-Blum microtome (4). The sections were mounted on carbon films (5) and examined in an RCA microscope, model EMU-2b, without removing the embedding plastic. The electron micrographs were taken at original magnifications of 8,000 to 16,000 and enlarged further photographically.

OBSERVATIONS

In the exocrine cell of the pancreas, the cisternal, tubular and vesicular elements of the endoplasmic reticulum are usually filled with a material of homogeneous appearance and low density. In certain cells, however, round bodies of high density and relatively large size are found embedded in this light material. In sections the bodies concerned appear, as a rule, as circular profiles (Fig. 1), an indication that in three dimensions they are of spherical shape. They consist of a dense material which appears homogeneous or finely granular at the present level of resolution. They are relatively sharply out-

[1] The addition of sucrose to the buffered OsO_4 solution results in a better preservation of the tissue as shown by J. B. Caulfield in work done in this laboratory.

[2] A Novadel-Agen Corporation product (Lucidol Division, Buffalo) containing in equal amounts a catalyst, *i.e.*, 2,4-dichlorobenzoyl peroxide, and a plasticizer; *i.e.*, dibutyl phthalate.

lined, although a limiting membrane has not been detected at their periphery. In keeping with the features mentioned, they can be adequately described as *intracisternal granules*. In sections, the profiles of the granules vary in size from 100 to 400 mμ. Since the smaller profiles are less dense and less sharply outlined than the larger ones (Fig. 2), it is assumed that the former represent lateral sections, and the latter, medial sections of the granules. It is concluded that most intracisternal granules measure between 250 and 350 mμ in diameter. Only occasionally larger elements (400 to 500 mμ) are encountered.

As already mentioned, the granules are located within the cavities of the endoplasmic reticulum, surrounded by a more or less thin layer of light material which usually separates them from the limiting membrane of this cavitary system (Figs. 1 and 2). In the case of the exocrine cell, this membrane bears small dense particles attached to the surface which faces the cytoplasmic matrix (1, 6). As other membranous systems of the cytoplasm are free of attached particles (1, 7), this structural detail indicates clearly that the vesicular elements in which the intracisternal granules are contained belong to the system described as the endoplasmic reticulum. Frequently a single granule is found in a circular profile of the system, but in elongated profiles rows of as many as six granules are encountered. Large, irregular elements, corresponding to distended cisternae, may contain from two to five granules (Figs. 1 and 2).

The number of intracisternal granules varies considerably from one gland to another and even from one cell to the next. In certain glands for instance they are absent or present in very small numbers, *i.e.*, one or two granules, per cell section. In other glands, most of the acinar cells contain an appreciable number of granules, sometimes as many as 100 to 150 per cell section (Fig. 1). These variations in number might be related to the secretory cycle of the cell, a problem which is now under investigation. When few, the granules are usually restricted to the endoplasmic reticulum of the basal region of the cell; when numerous, most of them still occur in the same location, but many are found within the cavities of the reticulum in the immediate neighborhood of the centrosphere region and in the apical zone of the cell (Fig. 1).

The endoplasmic reticulum of the pancreatic exocrine cell is distinguished by the regularity of its disposition, a feature which has been repeatedly stressed by various authors (1, 6, 8, 9, 15). In the extreme forms of this orderly disposition, the cisternal elements of the system are disposed parallel to one another at more or less regular intervals thus forming arrays of cisternae (1). Moreover in such cases the cisternal elements themselves are frequently of small and constant depth (about 30 mμ), a feature which enhances the orderly arrangement of the entire system. In such cases, intracisternal granules are usually absent. When they are present, the regular disposition of the endoplasmic reticulum is to a large extent disturbed, while the cavities

of its composing cisternae, tubules, and vesicles (Fig. 1) are considerably enlarged, and reach diameters of 400 mμ or more (Figs. 1 and 2).

The intracisternal granules are different in morphology and location from all the other granular components of the cell. They resemble only in part, namely in their high density and fine texture, the zymogen granules, but they differ from the latter in size, intracellular location, and relation to the endoplasmic reticulum. The zymogen granules measure about 600 mμ in diameter, are restricted to the apical zone of the cell, and do not have any direct relation to the rough surfaced part of the reticulum. They are apparently directly embedded in the cytoplasmic matrix, but, at least during certain stages of their formation and discharge, a membrane becomes visible at their periphery. This membrane bears no attached particles on its outer surface. It may be assumed that the zymogen granules are bound by a smooth surfaced membrane throughout their entire existence, and that the membrane of stored granules is not visible because it is tightly applied on a content of equal density.

Lipide droplets are sometimes encountered in the basal region of the cell; they generally are of larger size and appear to be directly embedded in the cytoplasmic matrix.

In light microscope literature, the possibility of a transformation of the acinar cells of the pancreas into islet cells has been repeatedly suggested and discussed. For this reason it was considered desirable to compare the morphology of the granules found in the endocrine cells of the pancreas with the intracisternal granules here described. The islet cells contain a variable number of granular inclusions similar in size and density to the intracisternal granules. Each of these inclusions, however, has a thin limiting membrane, free of attached particles, which is not directly applied on the inclusion but remains separated from it by a narrow ($\sim$10 mμ) space. The granules themselves have a dense homogeneous appearance in the α cells of the islets and a coarse, granular texture in the β cells. In the latter the inclusions show a considerable variety of appearances which could be explained by assuming that the granules are the result of the progressive filling of smooth surfaced vesicles by a dense, grainy material.

Intracisternal granules as here described have been encountered thus far in the pancreatic exocrine cells of the guinea pig. They are absent or extremely rare in the corresponding cells of the rat.

DISCUSSION

The presence of small granules in the basal cytoplasm of the acinar cells has been mentioned in studies of the pancreatic cells of the guinea pig by light microscopy. These granules which can be stained by safranine (10) and crystal violet (11) were considered by Mankowski as similar to the small granules

of the islet cells and their presence in acinar cells was used as an argument for the hypothesis that the two types of pancreatic cells are interchangeable (10). This view was thoroughly criticized by Bensley (11), who considered the two types of cells as permanently differentiated and assumed that the small granules found by Mankowski "result from a degeneration of the basophile material of the acinus cells."

In all probability the granules observed by Mankowski and described by Bensley correspond to the intracisternal granules of the present study. Their size, as measured in electron micrographs, is just above the limit of resolution of the light microscope. It can be assumed that their diameter can be further increased by heavy staining to render them clearly visible under light optics.

The information concerning the location of these granules is entirely new. Their presence inside the cavities of the endoplasmic reticulum gives a number of interesting indications concerning the structure and the function of this system. As it is known, the endoplasmic reticulum has been interpreted as a network of membrane bound cavities which permeates the entire cytoplasm (12–14), and various observations have pointed out both the polymorphism of the system and the frequency of its orderly disposition in pancreatic exocrine cells (1, 15). In the same cell, the layered cisternae of the endoplasmic reticulum have been interpreted as a system of imbricated lamellae (8), "cytoplasmic double membranes" (16), or paired "intracellular cytoplasmic membranes" (6). In these last interpretations, the cavitary nature of the system was denied or ignored; moreover it was assumed that under normal conditions, the paired membranes, *i.e.*, the membranes outlining the elongated profile of a cisterna, maintain a close parallelism at a small spacing and that a larger spacing or a lack of parallelism is the result of unsatisfactory fixation (6, 16). The present observations indicate that the elements of the endoplasmic reticulum have a cavity which can enlarge to accommodate one or more granules of $\sim$300 mμ in diameter. It is assumed that the granules and the accompanying modifications of the endoplasmic reticulum are not fixation artifacts and this assumption is based on (*a*) their occurrence in satisfactorily fixed regions of the specimen, (*b*) the highly regular structure of the granules, and (*c*) their demonstration by other preparatory methods in light microscopy (11). The observations also indicate that the general disposition and the size of the elements of the endoplasmic reticulum vary noticeably from one gland to another, and, to a lesser extent, from one cell to the next, and suggest that these variations may be of functional nature. In the wide spectrum of appearances taken by the endoplasmic reticulum in the exocrine cell of the pancreas, the one accompanying the presence of intracisternal granules may represent an extreme situation, opposite to the highly organized arrangement of relatively large cisternal elements which has been thus far presented as the "normal" condition.

Finally the presence of granules in the cavities of the endoplasmic reticulum

suggests that the system may be directly involved in the production or segregation of the material composing the granules. This suggestion derives additional support from the finding that the small dense particles attached to the limiting membrane of the system are ribonucleoprotein particles (1). As it is known, ribonucleic acid has been repeatedly associated with protein synthesis in general (17) and secretion processes in the pancreas in particular (18, 19). Future work will decide whether any biochemical connection underlies the morphological similarity between the intracisternal granules and the mature zymogen granules of the cell. Information thus far available does not exclude such a connection but it does not favor a direct transformation of one type of granule into the other.

SUMMARY

Dense, homogeneous granules of 250 to 350 mμ in diameter have been found inside the cavities of the endoplasmic reticulum in the acinar cells of the pancreas of the guinea pig. They apparently correspond to the fine granules described in the same material in light microscopy by Mankowski and Bensley. The location of these granules indicates that the endoplasmic reticulum is a cavitary system and suggests its participation in secretory processes.

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EXPLANATION OF PLATES

PLATE 107

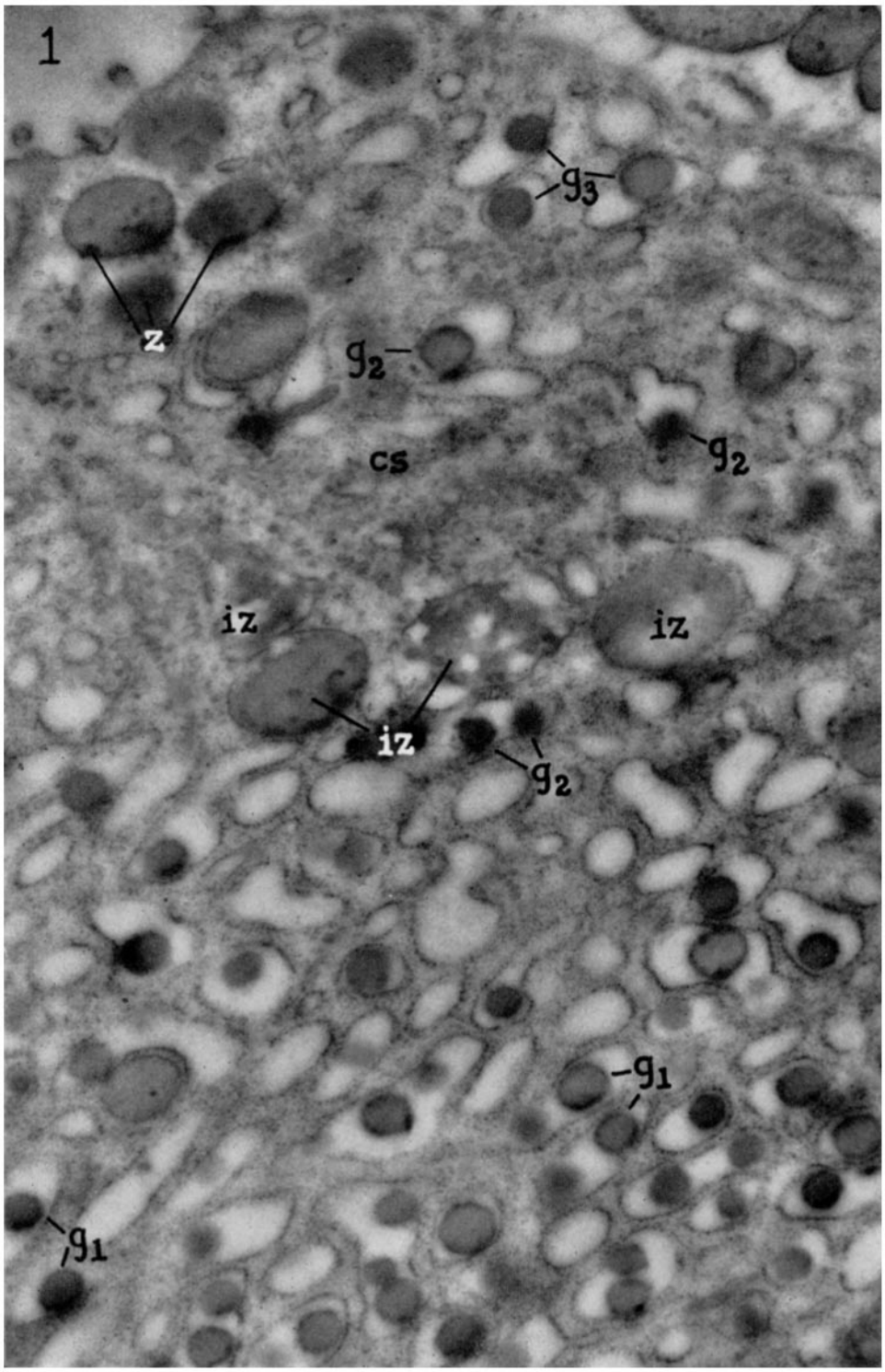
FIG. 1. Large field in the cytoplasm of an acinar cell of the pancreas (guinea pig).

The section cuts in the apical-basal direction through the cytoplasm and misses the nucleus. The lumen of the acinus appears in the upper left corner and part of a centro-acinar cell can be seen along the rest of the upper margin of the micrograph.

The lower half of the figure corresponds to the basal region of the cell. It is occupied by numerous rough surfaced profiles of the endoplasmic reticulum which appear distended and randomly disposed. Dense granules (g_1) are embedded in the light material that fills the cavities of the reticulum.

The centrosphere region of the cell (Golgi zone) can be seen at *cs* with four immature zymogen granules (*iz*) at its periphery. The apical region of the cell contains a few mature zymogen granules (*z*).

Note that most of the granules found in the cavities of the endoplasmic reticulum are located in the basal cytoplasm (g_1); a few, however, are found at the periphery of the centrosphere region (g_2) and in the apical zone of the cell (g_3). Magnification 26,000.



(Palade: Intracisternal granules in exocrine cells)

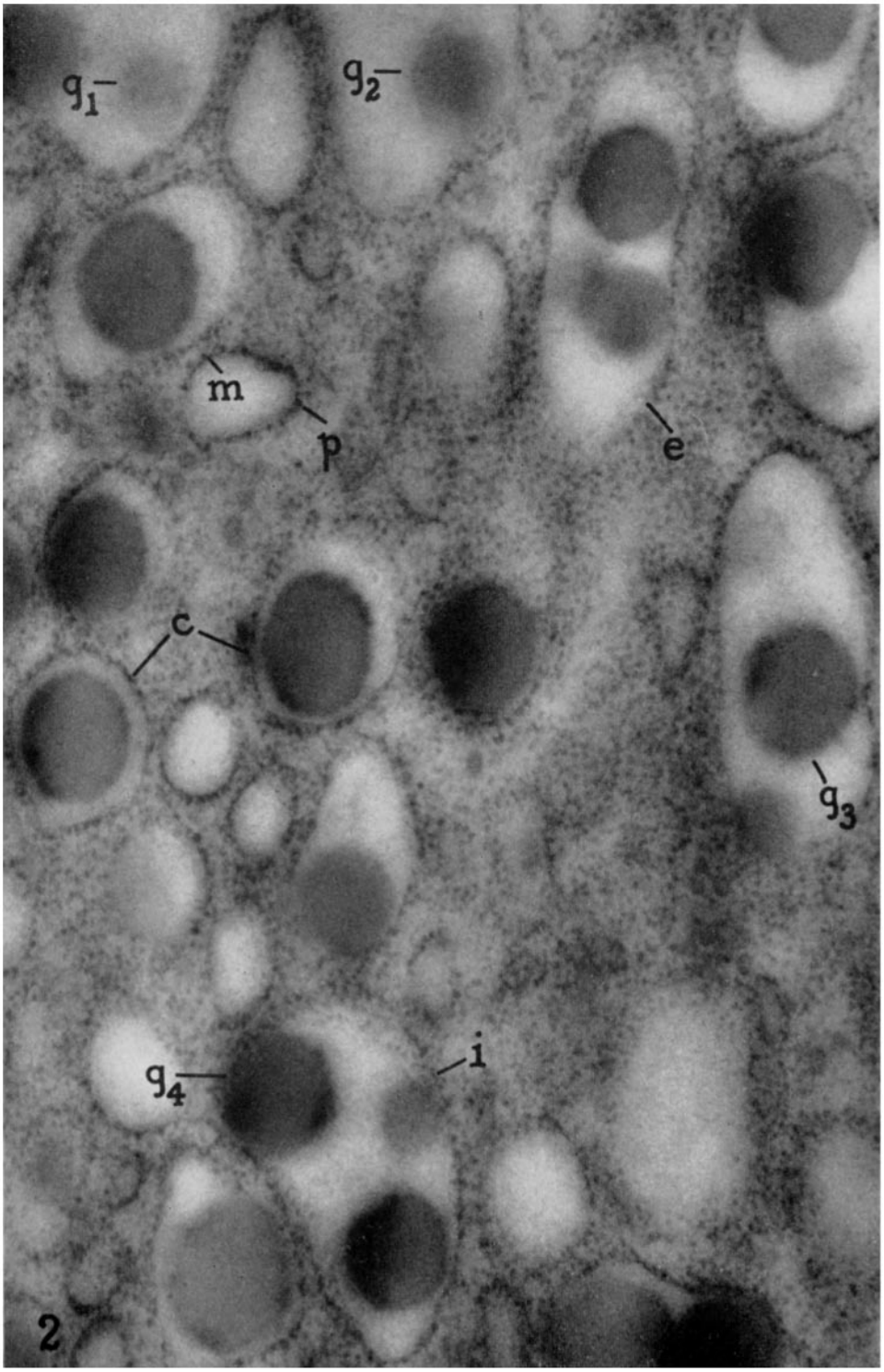
PLATE 108

FIG. 2. Small field in the cytoplasm of an acinar cell of the pancreas (guinea pig).

The field is occupied by circular (*c*), elongated (*e*), and irregular (*i*) profiles of the endoplasmic reticulum. They are limited by a thin membrane (*m*) visible only when normally sectioned, which bears small dense particles (*p*) on the surface that faces the cytoplasmic matrix. Numerous particles of similar size and density appear freely scattered in the matrix.

Dense granules (g_1 to g_4) are present in variable numbers in the cavities of the endoplasmic reticulum. There are three such granules in the profile marked *i*, two in that marked *e*, and one in each of those marked *c*.

The variation in size and density shown by the granules is, to a large extent, due to sectioning: g_1 and g_2 represent lateral sections, while g_3 and g_4 correspond to medial sections of granules. Magnification 58,000.



(Palade: Intracisternal granules in exocrine cells)