The formation of zymogen granules in the pancreas of the mouse

By S. K. MALHOTRA

(From the Cytological Laboratory, Department of Zoology, Oxford)

With 3 plates (figs. 1 to 3)

Summary

Electron-dense, granular (rarely vesicular) bodies, some 30 μm or more in diameter, are seen lying in the ground cytoplasm in the vicinity of the smooth-surfaced membranous complex. They often appear to be embedded in a mass of amorphous material, which is of about the same electron-density as the granules themselves. Granules and vesicles, similar in appearance to those mentioned above, have also been seen in contact with large vacuoles that appear to be developing into zymogen granules. The membrane that delimits such vacuoles sometimes appears to be disrupted, particularly where the granules seem to establish contact with the vacuoles. These vacuoles give the impression of having accumulated granular or vesicular material within them. They may perhaps be connected with the process of formation of zymogen granules.

In the vicinity of the smooth-surfaced membranous complex, consisting of γ-cytomembranes (Sjöstrand, 1956, 1959) and vacuoles, there appear, in quite considerable numbers, small electron-dense granules (or rarely vesicles). These, are about 30 μm or more in diameter and correspond to the description of ‘X-bodies’ of Hirsch (1961 a, b). Such granules have been seen lying in or near a fairly large, diffused mass of amorphous material in the ground cytoplasm (fig. 2, A). This amorphous material and the granules lodged in it are of about the same electron density. Sometimes a stream of such granules is also seen in close proximity to the large vacuoles associated with the smooth-surfaced membranous complex. It is not uncommon to find in micrographs situations, where granules or vesicles (resembling the X-bodies) are apparently in close contact with inclusions that are either empty-looking vacuoles or appear to be vacuoles that have accumulated some material in their interior and are stages in the formation of zymogen granules (figs. 2, 3, c; also see fig. 2, B in Malhotra, 1962b). It has also been noticed that the limiting membrane of these inclusions is not always complete, but gives the impression of ruptures at places (see arrows labelled d in figs. 2; 3, c). Small granules or vesicles may sometimes be seen at such places where the limiting membrane seems to be discontinuous (fig. 3, c). Though the various cytoplasmic inclusions seem to be fairly well preserved by the techniques employed in this investigation, it is difficult to rule out the possibility that these discontinuities in the limiting membrane of the vacuoles are artifacts. The contents of some of the vacuoles in the apical region studied in micrographs magnified about 100,000 times seem to be somewhat granular or vesicular (fig. 3, d). These vacuoles appear to be in the course of transformation into zymogen granules.

It is generally accepted that in the exocrine cells of the pancreas the zymogen granules are formed by progressive accumulation of proteins in the vacuoles associated with the smooth-surfaced membranous complex in the apical region of the cell (Farquhar and Wellings, 1957; Palade, 1956, 1961; Palay, 1958; Siekevitz, 1959; Siekevitz and Palade, 1960; Caro, 1961; Hirsch, 1961a, 1962; Sjöstrand and Hanzon, 1954a, b, 1961; Malhotra, 1962a). In suitable electron micrographs a sequence of stages can be constructed to show a gradual increase in the density of the contents of the vacuoles. There is a gradation from vacuoles that appear almost empty to those that resemble ripe zymogen granules (fig. 1, A, B; see also fig. 3 in Malhotra, 1962a). The largest of these prozymogen granules encountered in the micrographs are considerably bigger than the definitive zymogen granules (figs. 1, A, B; 3, D). It would therefore appear that after the process of filling up of the vacuoles has been completed, the entire inclusion undergoes shrinkage to the size of the definitive zymogen granule. This shrinkage is probably brought about by withdrawal of water and close packing of the contents. The possibility that while the smooth-surfaced membranous complex is concerned with the elaboration of the secretory products it is also functioning as a control of cellular water balance, has already been considered (see Oberling, 1959; De Robertis and others, 1960; Dalton, 1961). It is probable that the vacuoles seen in association with the smooth-surfaced membranous complex are in fact dilated chambers bounded by γ-cytomembranes; and these are produced for segregating the secretory products synthesized in other parts of the cell.

An integrated biochemical and electron microscopical study of the exocrine cell of the pancreas carried out conjointly by Palade and Siekevitz (see Siekevitz, 1959; Palade, 1961) has produced plausible evidence that the enzymatic proteins found in the definitive zymogen granules are synthesized in intimate relationship with the ribosomes attached to the membranous endoplasmic reticulum at the base of the cell (fig. 3, B). These proteins are believed to be transported through the cisternae of the endoplasmic reticulum (fig. 3, A) to the apical region, as intracisternal granules (visible by light and electron microscopy) or in solution (see Palade, 1961; Kurosumi, 1961; Hirsch, 1961a). It is not clearly understood how these proteins from within the cisternae of the endoplasmic reticulum get into the vacuoles that are associated with the smooth-surfaced membranous complex. These vacuoles are gener-

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**Fig. 1** (plate). Parts of the apical region of the pancreas of the mouse, showing the appearance that has been interpreted as gradual accumulation of secretory material in the vacuoles (z1 to z5), leading to the formation of definitive zymogen granules (z). z4 in B and z5 in A appear to be vacuoles filled with secretory material before shrinkage to the size of ripe zymogen granules has taken place. The lumen (l) of the acinus, bounded by the limiting membranes (cm) of the adjacent cells, is seen to contain electron-dense material, that has presumably been discharged into it. The projections of the cell membrane into the lumen of the acinus are seen at mv, g, γ-cytomembranes in association with vacuoles; m, mitochondria.

These electron micrographs were produced from tissue that had been fixed in a 4% solution of osmium tetroxide in distilled water (Malhotra, 1962a) and embedded in epikote 812 (Luft, 1961).
FIG. 1
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FIG. 2
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ally seen in micrographs as isolated inclusions; and there does not seem to be structural continuity with the elements of the endoplasmic reticulum. Palade (1961; also see Hagnenau and Hollmann, 1961; Porter, 1961; Robertson, 1962) believes that temporary continuity between the endoplasmic reticulum and the smooth-surfaced membranous complex is established to provide channels for transport. Hirsch (1961a) has considered another possibility. He conjectured that the membranes of the endoplasmic reticulum in the region of the smooth-surfaced membranous complex might rupture to liberate proteins in the ground cytoplasm. The liberated proteins might form the small, rounded objects which he named X-bodies (1961a, b). Hirsch considered that these X-bodies were probably 'taken up' by the vacuoles and 'packed up to large zymogen granules' (1961a).

The possibility of membranous continuity between the endoplasmic reticulum and smooth-surfaced membranous complex considered by Palade (1961) cannot be ruled out, but the appearance in micrographs of a diffused, amorphous material and of small, dense granules in the ground cytoplasm, and the existence of discontinuities in the membrane delimiting the vacuoles, may perhaps be suggestive of a process somewhat similar to that suggested by Hirsch (1961a). The granular or vesicular contents of some of the vacuoles (which appear to be early stages in the formation of the zymogen granules) may be an indication of the nature of the material that gradually accumulates in the vacuoles. Small granules and vesicles (resembling the X-bodies of Hirsch) seen in micrographs in contact with the vacuoles and developing zymogen granules probably constitute the material that is taken up by the vacuoles. The process of incorporation of the granules into the vacuoles is perhaps facilitated by temporarily established discontinuities in the membrane bounding the vacuoles described above (p. 117).

If these granules are or contain the enzymatic proteins synthesized in association with the ribosomes that are attached to the membranes of the endoplasmic reticulum, it would then appear that the secretory material is set free in the ground cytoplasm before it appears in the vacuoles. Perhaps the amorphous material seen in the micrographs is a mass of enzymatic proteins that are brought through the cavities of the endoplasmic reticulum to the apical region (Palade, 1961; also see Weiss, 1953). In the vicinity of the

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**FIG. 2 (plate).** The apical region of the exocrine cells of the pancreas of the mouse, showing some of the appearances which have been considered as probable stages in the formation of the zymogen granules. Arrows indicate small dense granules or vesicles which may be or may contain protein probably synthesized by the ribosomes attached to the endoplasmic reticulum at the base of the cell. Arrows labelled d indicate such granules in close contact with the vacuoles, which appear to be early stages in the formation of the zymogen granules and to be bounded by incomplete membranes. dm is a dense mass of amorphous material lying in the ground cytoplasm, which may have been set free by disintegration of the membranes of the endoplasmic reticulum. γ, γ-cytomembranes; m, mitochondria; r, mass of ribosomes.

A was produced from tissue fixed in a 2% solution of osmium tetroxide in sodium veronal buffer at pH 7.3 to 7.5 (Michaelis, 1930) and embedded in epikote (Luft, 1961).

B was produced from tissue fixed in a 1% solution of osmium tetroxide in distilled water and embedded in partially prepolymerized n-butyl methacrylate (Malhotra 1962a).
smooth-surfaced membranous complex, these enzymatic proteins are liberated, presumably by disintegration of the membranes of the endoplasmic reticulum. The granules, which appear to be taken up by the vacuoles, probably take their origin in this amorphous material. However, it also seems possible that the small granules lying in the amorphous material constitute the enzymatic proteins liberated from the endoplasmic reticulum, and that the diffuse mass has been produced by the dissolution of the granules during preparation of the tissue for electron microscopy.

The various appearances discussed above could be interpreted in ways other than that described here, but the interpretations made in this paper seem to make a logical sequence of stages in the formation of zymogen granules.

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References


FIG. 3 (plate). All the micrographs in this figure are from the pancreas of the mouse.

A and B show small parts of the endoplasmic reticulum (er) from the base of the exocrine cells. The cisternae are at places somewhat distended and their contents consist of slightly electron-dense, amorphous material, which may be protein synthesized by the ribosomes attached to the surface of the endoplasmic reticulum (see B). Intracisternal granules of the type seen in the guinea-pig (Palade, 1956) have not so far been observed in the pancreas of the mouse.

cm, cell membrane; m, mitochondria; n, nucleus showing pores (arrows) in its limiting membrane.

A was produced from tissue that had been fixed in a 1% solution of potassium permanganate in sodium veronal buffer and embedded in partially prepolymerized n-butyl methacrylate. Note the absence of ribosomes after fixation in KMnO₄ (Luft, 1956; Bradbury and Meek, 1960).

B was prepared by fixing the tissue in a 2% solution of osmium tetroxide in distilled water and embedding in partially prepolymerized n-butyl methacrylate (Malhotra, 1962a).

c, apical region of the exocrine cell, showing what are thought to be stages in the formation of zymogen granules. There are two bodies (a), which appear to be prozymogen granules; and the arrows (labelled d) indicate places where the limiting membrane of these seems to be discontinuous. Small granules are probably being absorbed into the contents of these prozymogen granules. The tissue was fixed in a 1% solution of potassium permanganate in sodium veronal buffer and embedded in partially prepolymerized n-butyl methacrylate.

D, from apical region of the exocrine cell. a is a part of a large vacuole, showing the accumulation of electron-dense material, apparently of granular or vesicular form (arrows); g, γ-cytomembranes associated with large vacuoles; z, zymogen granules. Arrow labelled d indicates a vacuole whose limiting membrane appears to be disrupted.

The tissue was fixed in a 1% solution of osmium tetroxide in distilled water and embedded in partially prepolymerized n-butyl methacrylate according to the technique described in Malhotra (1962a).
FIG. 3
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