Experiments on fixation for electron microscopy

I. Unbuffered osmium tetroxide

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With 5 plates (figs. 1 to 5)

Summary

The effect of fixation with a simple solution of osmium tetroxide in distilled water was studied by electron microscopy. Exocrine cells of the pancreas and cells of the proximal tubule of the kidney of the mouse were used as test-objects. Partially prepolymerized methacrylate was used for embedding. There did not appear to be any marked disorganization of the cell inclusions. The appearance of the inclusions in the electron micrographs was similar to what is generally seen after fixation with the buffered osmium tetroxide solution of Palade.

Introduction

Osmium tetroxide is more widely used than any other fixative for electron microscopy, because it is known to preserve the cell structure better than other mixed or unmixed fixatives; in fact the microscopical appearance of a cell after it has been placed in osmium tetroxide solution is very similar to that of the living cell (Strangeways and Canti, 1927; Palade, 1952; Porter and Kallman, 1953; Baker, 1960). Osmium also provides a desirable degree of contrast for the study of thin sections in electron microscopy.

In the early stages of the development of preparative techniques for studies of biological materials by electron microscopy, simple solutions of osmium tetroxide in distilled water were used for the fixation of tissue culture cells for whole mounts and for sectioning (see Pease, 1960, for references). Since 1952 it has been a routine procedure to use osmium tetroxide in buffered solutions. This change seems to have been due to the influence of experiments made by Palade (1952). He found that the penetration of osmium tetroxide into blocks of tissue was preceded by a wave of acidity which grossly altered the structure of the cells. Palade demonstrated that these alterations could be prevented by using osmium tetroxide in a solution buffered slightly on the alkaline side of neutrality (pH 7·3 to 7·5). He recommended the use of Michaelis’s (1931) acetate / veronal buffer (without sodium chloride) for this purpose. Palade’s fluid has probably been more used in electron microscopy than any other fixative.

Palade’s fluid is hypotonic to the body-fluids of mammals; and it appears that Palade regarded this as of little importance so far as preservation of cell structure was concerned (see Pease, 1960). Zetterqvist (1956) and Caulfield (1957) have employed variants of Palade’s buffered osmium

tetroxide solution, with the addition of indifferent salts to increase tonicity. Sjöstrand (1956) has used unbuffered osmium tetroxide solution made isotonic by adding indifferent salts.

I am studying the effect on fixation of the osmotic pressure caused by the non-fixative ions that are generally added to fixing fluids for electron microscopy. This project is intended to be completed in a series of papers. The present paper, the first of this series, is concerned with the results of control experiments, in which the fixative, osmium tetroxide, is used by itself in aqueous solution, without the addition of any salt.

**Material and methods**

It was decided to select the exocrine cell of the pancreas of the mouse as the main test-object for this series of experiments on fixation. Nearly all the cells of the exocrine gland are essentially similar in their structure, and they are therefore convenient for electron microscopy. Moreover, the various cytoplasmic inclusions are remarkably well organized for demonstrative purposes. The voluminous endoplasmic reticulum, with its ribonucleoprotein granules, is oriented into cisternae disposed parallel to one another and at more or less regular intervals in the basal region of the cell. The filamentous mitochondria show characteristic and well-developed cristae, regularly arranged. The more or less localized \( \gamma \)-cytomembranes (non-granular membranes), with their associated large vacuoles and small vesicles, represent a highly organized membranous complex ('Golgi apparatus') such as is generally seen in cells that actively secrete protein. The product of secretion is in the form of large zymogen granules; these constitute the most prominent cytoplasmic inclusion in the exocrine cell. All these cytoplasmic inclusions, as well as its nucleus, make the exocrine cell a suitable object for a comparative study of fixation. Further, a good deal of work has already been done on this cell, by both light and electron microscopy.

Cells of the first ('proximal') tubule of the kidney of the mouse were also used as test-objects, because these cells have large mitochondria, which are very sensitive indicators of the quality of fixation. The mitochondria are disposed in a characteristic way between the infoldings of the cell membrane at the base of the cell. The lumen-end of this cell is thrown into delicate microvilli, regularly arranged. It was considered that the appearance of these structures in electron micrographs might give an indication of the quality of fixation.

An unbuffered 1% aqueous solution of osmium tetroxide was used as fixative. Glass-distilled water was passed through an 'elgastat' deionizer and the effluent water was used for dissolving osmium tetroxide. This 'deionized' water had a pH of 5·9 to 6·3. The depression of the freezing-point by the osmium tetroxide, as determined by the use of a Beckman thermometer, was \( 0°-055°-0°06° \) C. The very slight variation in the depression of the freezing-point was probably due to slight differences in different samples of deionized...
water obtained from the deionizer. The depression by a so-called 'isotonic' sodium chloride solution (0.933\%) is 0.571° (see Heilbrunn, 1952).

The procedure for fixation, dehydration, and embedding was adapted from Pease (1960). The fixative was precooled to just above freezing-point. The period of fixation was always less than 1 h, since a short time is considered better for the preservation of the total constituents of the cell. A small piece of the tissue was minced with a razor blade in a drop or two of the precooled osmium tetroxide solution on a narrow strip of white cardboard, and within 20 sec the tissue was transferred to the fixative in a container kept among blocks of ice. Only very small pieces were selected for embedding. The embedding was done in \textit{n}-butyl methacrylate, partially prepolymerized to a thick syrup. The methacrylate was used as obtained from the manufacturer, without removal of the stabilizer (hydroquinone). The moist benzoyl peroxide provided by the manufacturer was dried over phosphorus pentoxide before use. After the benzoyl peroxide had dissolved in methacrylate monomer, the solution was dried with anhydrous sodium sulphate to ensure removal of any traces of moisture that might still remain. The technique employed for preparation of the tissue was standardized as follows:

1. Fix in ice-cold 1\% aqueous solution of osmium tetroxide for 30 min.
2. Allow the tissue to warm up to room-temperature while still in the fixative (20 min).
3. Pass directly to 70\% ethanol. First rinse the tissue with ethanol and leave in a fresh lot of 70\% ethanol for 10 min.
4. Transfer to 80\% ethanol; leave for 5 min.
5. Transfer to 95\% ethanol; leave for 5 min.
6. Transfer to absolute ethanol, previously dried with anhydrous copper sulphate. Give two changes during the first 10 min, and then leave for 15 min.
7. Transfer to a mixture of absolute ethanol (dried) and \textit{n}-butyl methacrylate monomer in equal volumes; leave for 10 min.
8. Soak the tissue in \textit{n}-butyl methacrylate monomer (without benzoyl peroxide) for 10 min.
9. Transfer to gelatin capsules containing partially prepolymerized methacrylate. Leave the capsules standing at room-temperature for a day to ensure thorough penetration of methacrylate.
10. Complete the polymerization at 50° C; this usually takes about 24 h.

Sections were cut on the Huxley ultramicrotome manufactured by the Cambridge Instrument Co. Ultrathin sections, mounted on grids coated with formvar, were examined in the Akashi transcope (model TRS 50 E1) operated at 50 kV, with anode aperture of 100 \(\mu\) and objective aperture of 50 \(\mu\).

Results

Criteria for judging electron micrographs are based on the results that have been achieved by careful fixation in Palade's buffered osmium tetroxide and embedding in suitable media. There are no general criteria for good fixation.
for all kinds of cells. But, in general, the appearance of the nucleus and mitochondria is often considered to be fairly sensitive indicator of 'good' or 'bad' fixation. For the purpose of this paper it is intended to record the appearance of the various cell inclusions produced by the experimental technique, and to compare this with what is already known about these inclusions.

**Exocrine cell of the pancreas**

The general appearance of this cell, when processed by the technique described above, is shown in fig. 1. The cell inclusions are fairly well preserved and their identity can easily be recognized. There is no gross distortion or vacuolization, suggestive of bad fixation.

The nucleoplasm is rather uniformly granular. Small aggregates of electron-dense granules appear scattered at random in the nucleus. The nuclear membrane is well preserved and sharply defined. Its double nature is quite obvious; the inner and outer membranes are closely apposed to each other. The outer nuclear membrane forms a bulge in some places and this bulging makes the space between the two membranes thicker than elsewhere. The total thickness of the nuclear membrane seems to vary between 30 and 60 mμ, but between 30 and 40 mμ seems to be the usual thickness. The outer nuclear membrane is wavy and looks like the membranes of the endoplasmic reticulum; it bears small dense particles, of the size of the ribonucleoprotein granules seen on the surface of the endoplasmic reticulum (fig. 4, B), and this makes the outer membrane indistinguishable from the membranes of the endoplasmic reticulum. This fact led Sjöstrand and Hanzon (1945a) to believe that the pancreatic exocrine cell has a single nuclear membrane. Moreover, the density of the contents of the space between the nuclear membranes is similar to that of the cisternae of the endoplasmic reticulum (fig. 4, c). These similarities lend support to the view that the apparent space between the nuclear membranes is a continuation of the cisternae of the endoplasmic reticulum (Watson, 1955; Porter, 1959; see Barer and others, 1960; Pease, 1960; Picken, 1960).

The two nuclear membranes are interrupted by nuclear pores, which do not seem to be abundant in the pancreatic exocrine cell (fig. 4, B, C). The average diameter of these pores in fixed material is about 40 mμ (Watson, 1955). Sometimes there are minute granules in the gap of the nuclear pores (fig. 4, C). These granules may, however, be artifacts; it is difficult to be certain.

The mitochondria do not show any marked alteration in their characteristic ultrastructure, which seems to be very well preserved (figs. 2, A, B; 3, B; 4, A, B). The outer membrane is well defined, and the cristae are clearly demonstrated. Some of the cristae appear to extend all the way across the mitochondrion.

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**Fig. 1 (plate).** Micrograph showing the general appearance of the pancreatic exocrine cell after fixation with unbuffered osmium tetroxide and embedding in partially prepolymerized methacrylate. *cm*, limiting cell membrane; *er*, endoplasmic reticulum; *n*, mitochondria; *n*, nucleus; *ngc*, complex associated with the non-granular membranes (γ-cytomembranes); *z*, zymogen granules. *zt* to *z4* are probably different stages in the formation of zymogen granules.
FIG. 2
S. K. MALHOTRA
others only partially so. The total thickness of the cristae does not seem to be more than 17 mμ. The mitochondrial matrix (fig. 2, B) is fairly well preserved and appears structureless in the micrographs. The diameter of the mitochondria seems to vary from about 0.35 to 0.6 μ.

The endoplasmic reticulum, with its small ribonucleoprotein particles, occupies the greater part of the cytoplasm (figs. 1; 2, A, B; 4, A–C). It does not appear to have undergone any marked disorganization. Its membranes are well defined. It is best demonstrated at the base of the cell, where the flattened cisternae are rather regularly placed, more or less parallel to one another (fig. 2, A). The cisternae are mostly about 30 mμ wide, but wider dilatations (40 to 80 mμ) also appear in the micrographs. Most of the cisternae are placed about 40 mμ apart from one another, but others are closer and others again farther away.

The ribonucleoprotein particles are quite clearly shown in the micrographs, especially where the elements of the endoplasmic reticulum are sectioned transversely (fig. 4, A, B,).

The aggregates of paired non-granular membranes (γ-cytomembranes of Sjöstrand, 1956), which are generally seen in intimate association with vacuoles and granules (or small vesicles) in electron micrographs of the exocrine cell of the pancreas, are illustrated in figs. 3, A, B and 4, B. It would appear that this membranous complex is well preserved and very clearly demonstrated. In each aggregate the paired membranes are closely packed and are placed more or less parallel to one another. The membranes of a pair appear to be joined at their ends. The vacuoles are bounded by a membrane, which appears as a single dense line in the micrographs. The third component of this membranous complex appears mostly in the form of small vesicles, delimited by a thin membrane. The contents of the smallest vesicles often appear almost as dark as the limiting membrane. The vesicles are often seen to be embedded in a substance that looks homogenous or slightly granular; the electron density of this substance is somewhat greater than that of the rest of the ground cytoplasm (fig. 3, A, B). This membranous complex, consisting of paired non-granular membranes, vacuoles, and granules, was first described by Dalton and Felix (1954) in the epithelial cells of the mammalian epididymis. It is commonly described as Golgi apparatus. The structure and appearance of this complex is very similar to that described by Sjöstrand and Hanzon (1954b), Palay (1958), and Hirsch (1961b) in the pancreatic exocrine cell.

The zymogen granules, which are scattered about in the apical region of the cell, constitute the most prominent cytoplasmic inclusion (figs. 1; 3, A, B; 4, A). They are more or less homogeneously dense bodies and often appear ovoid.

Fig. 2 (plate). Exocrine cell of pancreas fixed with unbuffered osmium tetroxide and embedded in partially prepolymerized methacrylate.

A, shows endoplasmic reticulum at the basal end of the cell. m, mitochondria.
B, shows a mitochondrion and endoplasmic reticulum in two adjacent cells. cm, limiting cell membrane.
in electron micrographs. The longer diameter is about 1 \( \mu \). The zymogen granules are delimited by a sharply outlined membrane. The thickness of this membrane does not seem to be more than 6 \( \mu \), which is the thickness of each non-granular membrane (\( \gamma \)-cytomembrane).

In addition to the cytoplasmic inclusions described above, the apical region of the exocrine cell contains nearly spherical or ovoid inclusions, mostly in the vicinity of the aggregates of non-granular membranes (figs. 1; 3, A, B). Some of these are of the same size as the vacuoles associated with the aggregates of non-granular membranes, but others are bigger and some are larger than the zymogen granules. They are bounded by a single membrane, which closely resembles in appearance the membranes delimiting the zymogen granules and the vacuoles associated with non-granular membranous aggregates. The contents of these inclusions vary greatly in electron density. Some of these bodies appear more or less empty, while others almost resemble ripe zymogen granules (fig. 3, A, B). It appears that these inclusions represent different stages in the formation of zymogen granules. It would thus appear that there is a gradual condensation of electron-dense material in the vacuoles, leading to the formation of definitive zymogen granules. The vacuoles seem to enlarge at first as the process of condensation progresses; after condensation has been completed, the entire inclusion appears to undergo shrinkage to the size of the ripe zymogen granule (fig. 3, A).

The limiting cell membrane is well preserved and quite sharply demarcated. A part of the limiting membranes shown in fig. 4, A was photographically enlarged to estimate the size of the intervening space between the two adjacent cells. The maximum width of this space was about 10 \( \mu \); this is very close to the measurement (11 \( \mu \)) given by Sjöstrand and Hanzon (1954a) for this space.

**Cells of the first ('proximal') convoluted tubules of the kidney**

Micrographs showing the various structures in the tubular cells of the kidney fixed in an unbuffered 1% solution of osmium tetroxide are shown in figs. 4, D–F and 5, A, B. The nucleoplasm is fairly uniformly granular. The double nuclear membrane is clearly seen (fig. 4, F). The outer and inner membranes are closely opposed to each other. At the base of the cell, the infoldings of the cell membrane into the cytoplasm are very clearly revealed (fig. 5, A, B). The membranes appear in the form of thin, dense lines. The two membranes of an infolding are closely apposed to each other. The distance between two membranes is fairly constant; the width of the space between the two membranes of an infolding is less than 20 \( \mu \) (compare Porter, 1959). Porter

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**Fig. 3 (plate).** Pancreatic exocrine cell from tissue fixed with unbuffered osmium tetroxide and embedded in partially prepolymerized methacrylate.

A and B, show non-granular membranes in association with vacuoles and small vesicles (ngc). x1 to x5 probably represent the gradual condensation of protein in the vacuoles, leading to the formation of definitive zymogen granules (z). m, mitochondria.
has given 35 to 40 mμ as the total thickness of an infolding (8 mμ being the thickness of each of the two membranes); Sjöstrand and Rhodin (1953) gave an average of 27 mμ. A careful examination of the micrographs reveals that there is a single, thin, dense line at the basal surface of the cell (fig. 5, A, B); this is the cell membrane. The double membranes of an infolding therefore represent two cell membranes with a small intervening space between them. Pease (1955b) has shown that the double membranes are in fact invaginations of the cell membrane.

The cytoplasm between each double infolding and the next is filled with long mitochondria (fig. 5, A). The arrangement of the mitochondria, more or less parallel to the major axis of the cell, is clearly shown. The characteristic structure seen by electron microscopy does not appear to have been visibly affected by fixation in strongly hypotonic, unbuffered osmium tetroxide solution. The outer and inner membranes are clearly seen; the cristae are regularly arranged. The cristae are much more densely packed than in the exocrine cell of the pancreas; most of them seem to extend throughout the diameter of the mitochondrion. The average thickness of the cristae is 16 mμ (compare Sjöstrand, 1956).

The microvilli constituting the brush border at the lumen end of cells appear as cylindrical processes, arranged perpendicularly to the cell surface (Pease, 1955a, b). They are of rather uniform diameter, about 60 mμ or a little more (compare Sjöstrand and Rhodin, 1953). In sections prepared by the technique described above, the microvilli are generally so closely packed (fig. 4, D) that the brush border resembles a honeycomb when examined in transverse section (fig. 4, E). Pease (1955b) considers that this appearance of the brush border is characteristic of material that has been removed from the animal in small pieces and subsequently put into the fixative. He is inclined to believe that the close packing of the microvilli is caused by swelling, as this region of the cell is particularly sensitive to osmotic changes.

Vacuoles of various sizes, each bounded by a membrane, and small dense particles are scattered throughout the cytoplasm (fig. 5, B). The dense particles are probably ribonucleoprotein granules, which are especially abundant in the cells of the proximal tubules (Pease, 1955b).

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**Fig. 4 (plate).** All the micrographs on this plate are from tissue fixed with unbuffered osmium tetroxide and embedded in partially prepolymerized methacrylate.

A, pancreatic exocrine cell, showing cell membrane (cm), a mitochondrion, and zymogen granules (z). Cisternae of the endoplasmic reticulum, with ribonucleoprotein particles scattered on their surface, are seen dispersed throughout the cytoplasm.

B, pancreatic exocrine cell, showing non-granular membranes (ngc) associated with vacuoles and small vesicles, a mitochondrion, and cisternae of the endoplasmic reticulum with ribonucleoprotein particles, in the greater part of the cytoplasm. Arrows indicate pores in the nuclear membrane (nm).

C, magnified view of a part of fig. 1, showing pores (arrows) in the nuclear membrane (nm). er, endoplasmic reticulum; z, zymogen granule.

D and E, microvilli constituting the brush border of the proximal tubule cells of the kidney. D, longitudinal section; E, transverse section.

F, a part of the first (proximal) tubule cell of the kidney, showing nuclear contents, nuclear membrane (nm), mitochondria, and infoldings (arrow) of the cell membrane.
The tubules are surrounded by a basement membrane. This is seen in the electron micrographs as a narrow, fairly dense band, at least 42 μ wide, quite homogeneous in appearance (fig. 5, A, B). Its homogeneous appearance has been considered as indication of good preservation, as poor preservation results in the production of granules (Pease, 1955a).

Discussion

The appearance of the various inclusions in the exocrine cell of the pancreas fixed with unbuffered osmium tetroxide appears to be essentially similar to what is seen in this cell after fixation with buffered osmium tetroxide (Palade, 1952, 1958, 1961; Sjöstrand, 1956, 1959; Sjöstrand and Hanzon, 1954 a, b; Palay, 1958; Bradbury and Meek, 1960). Palade (1952) experimented with an unbuffered solution of osmium tetroxide (without addition of any salts). He observed that in the exocrine cell of the pancreas of the rat, the nucleoplasm had the appearance of a reticular precipitate; the mitochondria and zymogen granules were swollen, and the zymogen granules appeared as circular spots, some of them surrounded by a diffused halo. Moreover, he found distinct vacuolization of the ground cytoplasm, causing disorganization of the endoplasmic reticulum. Palade attributed these defects to a wave of acidity, produced when osmium tetroxide reacts with the tissues; this acidity affects the tissues before they are fixed. Such defects in preservation of the cell inclusions are not obvious in my electron micrographs. The experiment was repeated several times, but the microscopical appearance of the pancreatic exocrine cell was always more or less the same. It appears that hydrogen ion concentration does not play a major role in the quality of preservation of the pancreatic exocrine cell. It was therefore thought desirable to use unbuffered osmium tetroxide solution for fixation of some other kind of cells; and the scope of this study was widened to the tubular cells of the kidney. The appearance of the various structures of these cells in my electron micrographs is similar to that described by Sjöstrand and Rhodin (1953) and Pease (1955 a, b). These authors have used buffered osmium tetroxide solution (pH 7.2 to 7.4) for fixation.

The results described in this paper make one doubt the value of buffering solutions of osmium tetroxide. This finding is in conformity with the results obtained by Sjöstrand and his associates (see Sjöstrand, 1956), who fixed a variety of tissues with fluids varying in pH from 4 (or even 2) to 8, and did not notice any marked difference in the results. They produced comparable micrographs of different kinds of animal cells by fixing in unbuffered osmium tetroxide solution, which had been made isotonic by the addition of indifferent salts.

Fig. 5 (plate). Cells of the convoluted tubules of the kidney fixed with unbuffered osmium tetroxide and embedded in partially prepolymerized methacrylate.

A and B, basal region of the cell showing infoldings of the cell membrane and large mitochondria lying between the infoldings. Small dense particles are seen throughout the cytoplasm in B. bm, basement membrane. Arrows indicate the single cell membrane at the base of the cell.
FIG. 5
S. K. MALHOTRA
Sjöstrand has stressed the importance of using isotonic fixatives, on the ground that hypotonic solutions are likely to cause swelling of cells. Rangan (1960), working on malignant tumours, observed that the cytoplasmic contents were better preserved when the fixative was isotonic than hypotonic; but hypotonic rather than isotonic fixative gave better preservation of the nucleoplasm. Baker (1958) has studied the effect of adding indifferent salts to the unmixed fixing fluids on the appearance of cells by light microscopy. He did not observe any marked change in the quality of preservation of cell structure when osmium tetroxide was used as fixative. He has discussed at length the use of indifferent salts in fixing fluids for light microscopy.

Embedding in methacrylate monomer is capable of causing serious damage to tissues during polymerization (Borsko, 1956; Birbeck and Mercer, 1956; Pease, 1960). The defects in preservation of cell inclusions observed by Palade (1952) in electron micrographs of tissues fixed in unbuffered osmium tetroxide may perhaps have been accentuated by polymerization damage. Tissues fixed in an unbuffered solution of osmium tetroxide (without addition of any salts) may perhaps be more susceptible to polymerization damage during embedding than tissue preserved by fixation in a solution whose tonicity has been raised by the addition of buffering or neutral salts. Pease (1960) believes that tissue fixed in Luft's (1956) potassium permanganate solution in acetate / veronal buffer (at the same concentration and pH as used by Palade for osmium tetroxide) is 'unduly sensitive' to damage during the polymerization of methacrylates. Yasuzumi and Ishida (1957) have recorded that washing of the tissue with water after fixation is often accompanied by a loss of cellular substances and drastic distortion. Rangan (1960) also considers that inferior preservation of material fixed in hypotonic solution of osmium tetroxide may be due to washing in water.

A few remarks on the role of complex associated with the γ-cytomembranes in the formation of zymogen granules is included here, though it does not directly concern the main subject of this paper. Palay (1958) has illustrated a process of gradual condensation of electron-dense material in the vacuoles that form a part of the complex in the pancreatic exocrine cell of the mouse. He states that 'a sequence of stages can be constructed from the smallest clear vacuoles through intermediate vacuoles containing dense material to the definitive secretory droplets'. Farquhar and Wellings (1957) also have observed material resembling zymogen granules in density within the 'Golgi vacuoles' of the exocrine cells; and they consider that the 'Golgi complex' may be the site of secretory granule formation in this cell. Caro (1961) injected labelled leucine-H₃ into the guinea-pig, and after 20 min the label was recorded in the Golgi complex, especially in large vacuoles partially filled with a dense material. These findings support the opinion of certain light microscopists that the zymogen granules are synthesized within the vacuoles of the Golgi apparatus (Junqueira and Hirsch, 1956; Hirsch, 1959, 1961 a, b; also see Palay, 1958).
Sjöstrand and Hanzon (1954b) have described the formation of zymogen granules in intimate association with the Golgi apparatus. They thought that the small granules fused to form bigger granules, which grew to give rise to the zymogen granules. Later Sjöstrand (1959) discussed the role played by the Golgi apparatus in the formation of zymogen granules. He considered that the membrane bounding the zymogen-precursor-granules synthesizes the secretory products, which gradually accumulate in the granules.

Siekevitz and Palade (1960) and Palade (1961) have made a biochemical study of the pancreatic exocrine cell. They conclude that the digestive enzymes produced by the cell are synthesized in or on the ribonucleoprotein particles attached to the membranous endoplasmic reticulum. These enzymes are segregated into the cisternae of the endoplasmic reticulum, sometimes in the form of intracisternal granules (visible by electron microscopy), which are passed on to the ‘centrosphere (Golgi) zone’; in this zone the digestive enzymes are condensed into definitive zymogen granules. There is no evidence in my micrographs that intracisternal granules exist in the pancreas of the mouse. This is in agreement with the findings of Palay (1958).

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