# MEMBRANES OF THE HUMAN PEPSINOGEN GRANULE

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Robertson's comprehensive study of the ultrastructure of cell membranes and membranous cell organelles (Robertson, 1957) demonstrated that many cytoplasmic membranes have basically a similar appearance. The appearance of these membranes when cut in cross-section and seen in the electron microscope is two dark lines about 20 A thick, separated by a less dense space of about 35 A. Conceiving of these membranes as universal in cells, Robertson called them "unit membranes." Robertson reported unit membranes in the plasma membranes of many types of cells, in the membranes of the nuclear envelope, in the vesicles of the smooth-surfaced endoplasmic reticulum, and in mitochondria. Numerous other investigators have also reported density patterns similar to Robertson's unit membrane.

Recently, however, Suganuma (1961) has shown clearly with the electron microscope that the plasma membrane of *Staphylococcus aureus* displays in sections a density pattern characterized by a single layer about 50 A thick. Karrer (1960) has reported that the membranes of the cisternae of the rough-surfaced endoplasmic reticulum in the lung phagocytes are composed of a narrow, single-layered membrane unlike the unit membrane (Karrer, 1960). The present study reports another example of a variant from the unit membrane appearance. This variant is seen by electron microscopy in the envelope of certain pepsinogen granules from the "chief cells" in the human stomach.

Although the fine structure of gastric mucosa has been studied by several other investigators (Challice *et al.*, 1957; Dalton, 1951; Hally, 1959; Helander and Ekholm, 1959; Kurosumi *et al.*, 1958; Lawn, 1960; Sedar, 1955, 1959; and Vial and Orrego, 1960), the envelope of the pepsinogen granule has been clearly visualized only by Helander and Ekholm.

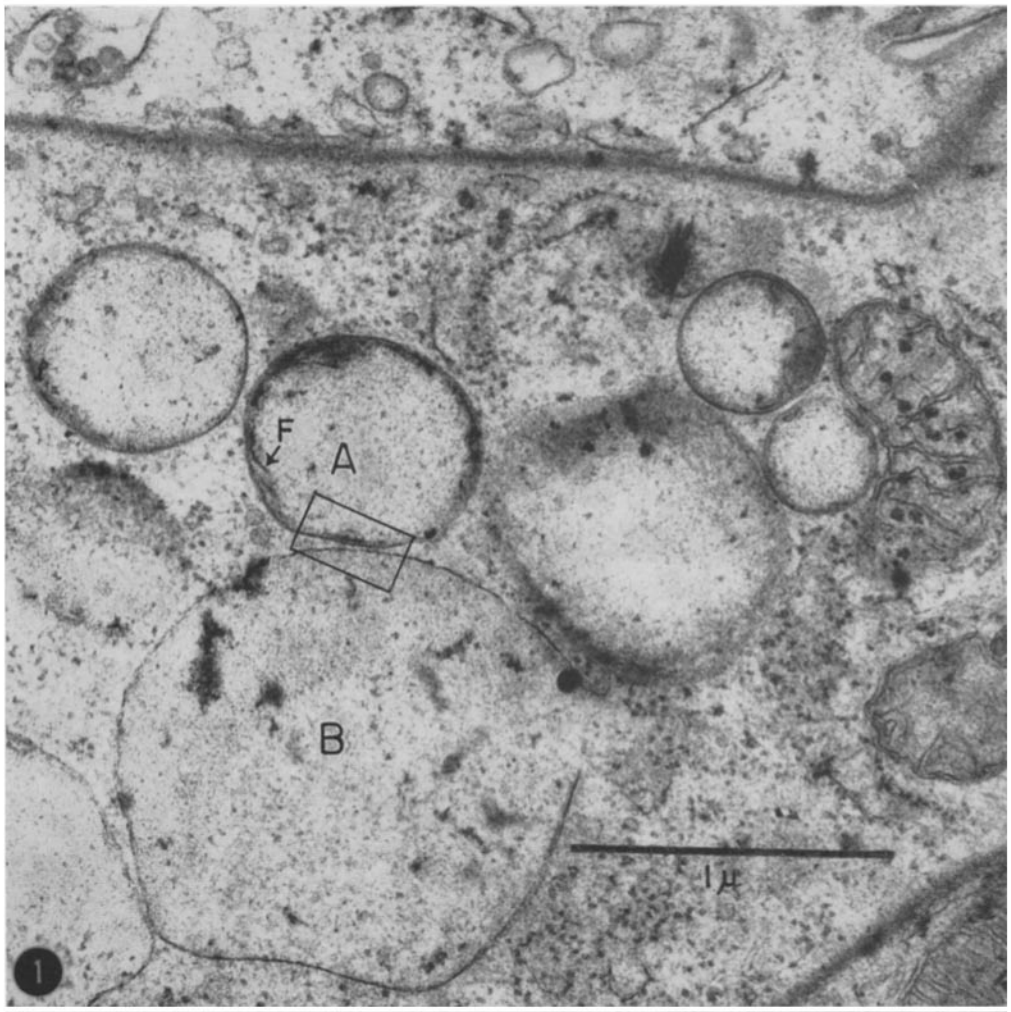
Tissue for the present study was obtained by peroral passage of a biopsy instrument (Brandborg *et al.*, 1955) into the stomach of human subjects without anesthesia. Biopsies of the full thickness of the mucosa were obtained. They were fixed in collidine-buffered $OsO_4$ (Bennett and Luft, 1959) and embedded in Epon resin (Luft, 1961). The ultrathin sections were stained with lead hydroxide (Watson, 1958) and examined with a modified RCA EMU-2A electron microscope. Cells were identified by criteria developed in a previous study (Lillibridge, 1960). Distribution of membrane densities were evaluated with a modified Kipp and Zonen densitometer (Bennett *et al.*, 1953).

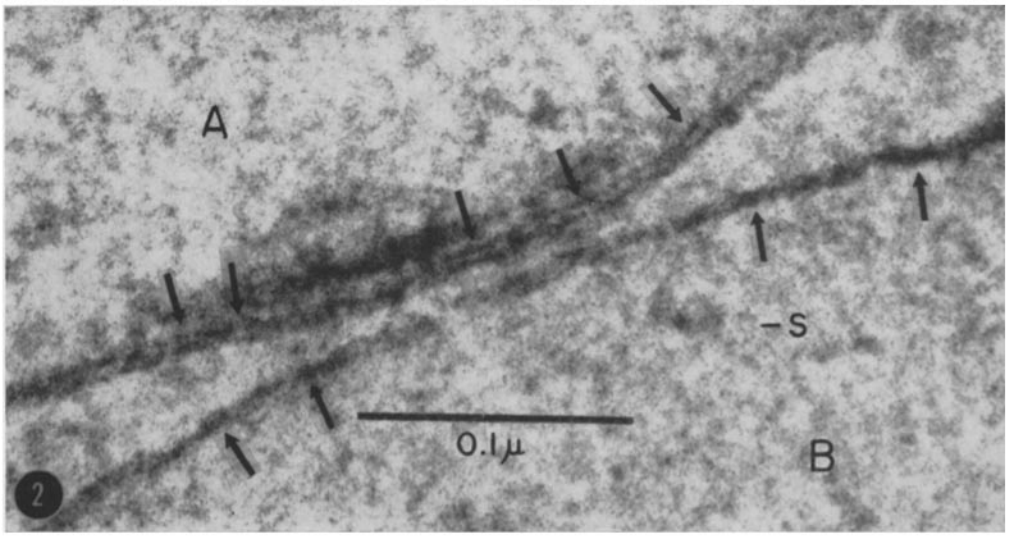
The secretion granules in human pepsiniferous (chief) cells appear in electron micrographs of thin sections as pale, homogeneous, oval structures enclosed by a membrane. These granules range in diameter from ½ to 3 microns. The smaller granules which are less than about 1 micron across usually are enveloped in a typical unit membrane. The larger granules have a thin, single-layered membrane which occasionally appears not to enclose the granules completely (Fig. 1).

Since measurements of the width of membranes with calipers give data which depend in considerable degree on photographic factors such as grain and contrast, the membranes in this study were measured by a densitometer. Fig. 2 illustrates two adjacent pepsinogen granules, one large, one small, each enclosed by differing membranes. Densitometric tracings from these membranes were taken at the sites indicated by the arrows. The tracings taken across each membrane were superimposed and are shown in Figs. 3 and 4.

The tracings across the membrane of the smaller granule (labeled *A* in Figs. 1 and 2), show two peaks of density with an average peak-to-peak distance on the tracings of about 50 A (Fig. 3). The over-all width is about 80 A. This is characteristic of tracings of typical unit membranes. In addition, tracings from membranes of the smaller granules show subsidiary peaks of density superimposed on the two main peaks and in the intervening trough.

In contrast, tracings across the membrane of the larger granule (labeled *B* in Figs. 1 and 2) display a single peak having an over-all width of about 50 A (Fig. 4). The single-layered appearance of the membranes enclosing the larger gran-



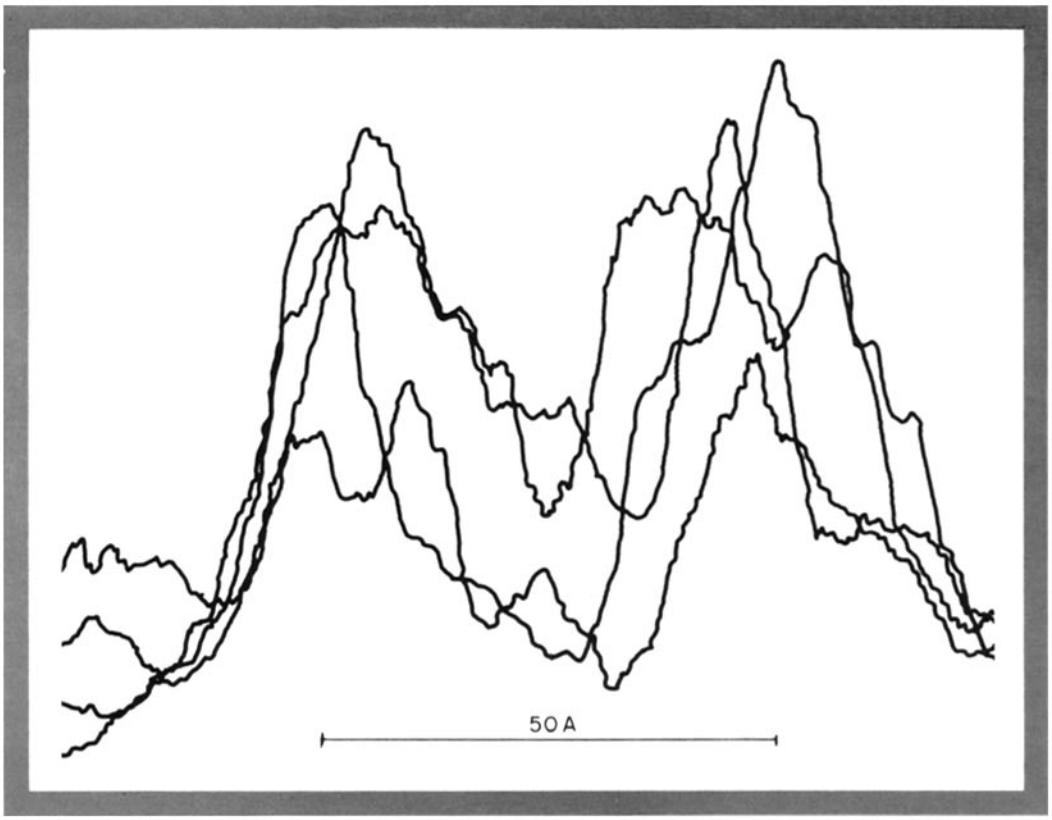



FIGURE 3

Superimposed densitometric tracings taken across the membrane enclosing granule *A* at the sites indicated by arrows in Fig. 2.

ules similar to granule *B* cannot be explained by poor resolution because the granule immediately adjacent does display the typical unit membrane appearance. Nor would oblique alignment of a typical unit membrane in a 500-A-thick section account for the appearance of a single dense line of the same width as the usual center-to-center spacing (50 A) of the perpendicularly oriented

FIGURE 1

An electron micrograph of the apex of a human pepsiniferous (chief) cell showing secretion granules of various sizes enclosed by different types of membranes. The smaller pepsinogen granules (*e.g. A*) are enclosed by typical unit membranes. Small fragments, *F*, of membrane may be found lying unattached within the smaller granule near the enclosing membrane. The larger granules (*e.g. B*) have thin, apparently single-layered membranes, which, in the case of granule *B*, appear not to enclose the granule completely. × 42,000.

FIGURE 2

An enlargement of the area of granules *A* and *B* enclosed within the rectangle in Fig. 1. Densitometric tracings (Figs. 3 and 4) were made across each membrane at the sites indicated by the arrows. The size of the slit (1.2 × 0.5 mm. actual size) relative to the micrograph is indicated by line *S*. × 360,000.

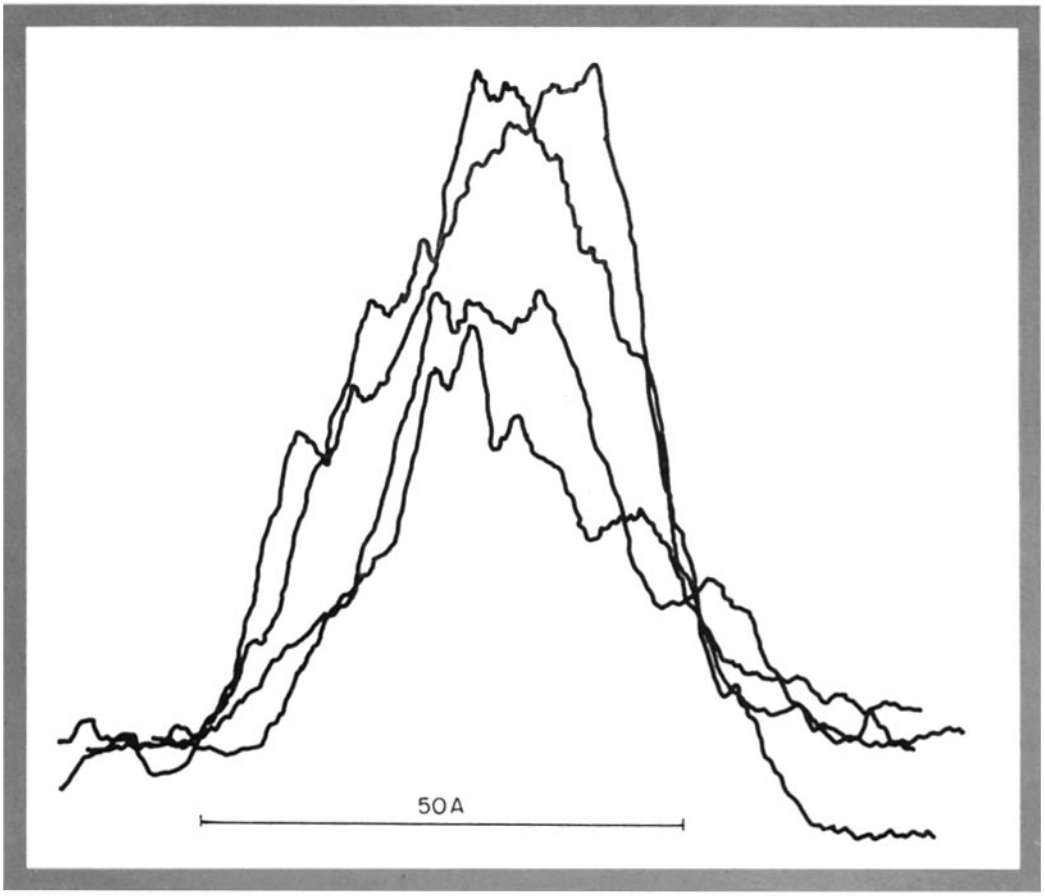



FIGURE 4

Superimposed densitometric tracings taken across the membrane enclosing granule *B* at the sites indicated by arrows in Fig. 2.

unit membrane. Since each picture was selected for focus from a series of five through-focus pictures, spurious images from diffraction effects are minimal.

In conclusion it appears that in the human pepsiniferous (chief) cell of the gastric mucosa the pepsinogen granules of less than 1 micron in diameter are enveloped in typical unit membranes, but the granules of more than 1 micron across have a different type of membrane which is thinner and has a single-layered appearance. These findings are supported by densitometry.

Whether this difference in appearance is due to alteration in basic molecular configuration or to some more subtle change reflected only in reactivity with $OsO_4$ remains to be answered.

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