SECRETORY MECHANISM : A REVIEW ARTICLE

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To the cytologist, secretion is the process whereby a cell elaborates, segregates and concentrates within its protoplasm, the characteristic substances emitted from the gland in which it occurs. The physiologist is concerned principally with extrusion of secretory products from the duct systems of glands, while the biochemist is concerned principally with the enzymatic chemistry and energetics involved in the synthesis of the secretory product. But there is a considerable overlap, for the cytologist is interested in the mechanism by which the secretory product leaves its cell of origin as well as the mechanism by which the cellular constituents transform the raw materials reaching them from the blood stream into the final product. Special emphasis of the cytologist lies in his insistence that secretion is essentially a cellular process and that an adequate account of it must include the architecture of the protoplasmic framework in which it proceeds.(13)

The fact that secretion is essentially an activity of the individual cell has developed slowly out of the efforts of 19th century anatomists and physiologists to understand the secretory process. Heidenhain, in 1875, first proposed a relationship between intracellular secretory granules and the specific products of the glands. At the close of the 18th century, the secretory products of glands were believed to be circulating preformed in the blood stream, but when it became evident by the middle of the 19th century that glands were composed of cells, this theory was replaced by the view that the secretory product was constructed in the cells of glands and mixed with fluids derived from the blood or lymph. In 1898, the Golgi apparatus was discovered by Golgi in the neurons of brain and spinal ganglia. As originally described, this "internal reticular apparatus" consisted of irregular, tortuous, anastomosing threads forming a circumnuclear network. Similar networks were soon discovered in all cell types and this structure rapidly became the center of a controversy which has continued to this day. An early and persistent concept was that secretion was essentially a protoplasmic degeneration leading to disintegration of the whole cell. Only late in the 19th century did it become clear that production of secretory substances involved synthesis and segregation of new material, and that discharge from the cell was a secondary phenomenon involving in some instances destruction of the whole cell, and in others mainly a cyclic release of the contained product.

In the early 1950's, improvements in techniques made possible the full-scale application of electron microscopy to the study of cells and tissues. Cells turned out to be much more elaborately organized, the most conspicuous finding being the remarkable amount of compartmentation of animal cells accomplished by extensive use of intracellular membranes. One of the first significant discoveries in cell

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morphology using the electron microscope was made by G.E. Palade in 1953, who worked out the fine structure of the mitochondria.[6]

Porter, Claude and Fullman[15], by means of a tissue culture technique, procured cells from chick embryos and using the electron microscope noticed the presence of a "lace-like reticulum" in the cytoplasm. Porter[14] called it the endoplasmic reticulum because of its general morphology and intracellular location, and identified it with the basophilic component (ergastoplasm) of the cytoplasm. With improvements in techniques of fixation, embedding and microtomy; Palade and Porter[10] were able to study the endoplasmic reticulum in cells **in situ.** Their observations showed that the endoplasmic reticulum was a network of membrane-bound, interconnected tubules, vesicles and flattened vesicles or cisternae.

Palade[7] found small particles in the ground substance of the cytoplasm. Originally called "the small particulate components of the cytoplasm" and later renamed ribosomes, they occurred either free in the cytoplasmic matrix or attached to the membrane of certain elements of the endoplasmic reticulum, called for this reason,rough-surfaced. The distribution of this component among various cell types paralleled to the distribution of cytoplasmic basophilia, i.e., of cytoplasmic ribonucleic acid (RNA) in general, which at that time was already associated with protein synthesis.

By coincidence, electron microscopic studies of cells and tissues were developing concomitantly with cell fractionation, i.e., the isolation of various subcellular components from tissue homogenate by differential centrifugation. Using these techniques conjointly, structural, chemical and functional characterizations of subcellular components were made possible.

Following the introduction of the technique for the separation of cell components, Claude succeeded in isolating from homogenated of liver and other tissues a fraction of particles referred to as "small granules" and later as microsomes. A number of studies showed that the incorporation of labeled amino acids proceeded at a high rate in microsomal proteins than that in the proteins of any other cell fraction both **in vivo** and **in vitro,** suggesting that microsomes were active in protein synthesis. Palade and Siekevitz[12] demonstrated that microsomes prepared from rat liver were fragments of the endoplasmic reticulum, most of them rough-surfaced, recognized as such on account of the ribosomes still attached to the outer surface of their membranes. All fragments appeared in closed vesicles and additional evidence from studies on pancreatic exocrine cells suggested that the breaking down of the reticulum was not due to mechanical tearing but rather to a generalized pinching off process that affected the endoplasmic reticulum when cell membranes were damaged or ruptured during cell homogenization.

There remained, however, numerous and obvious gaps in the secretory process. The relationship between free and attached ribosomes was still obscure, the functional meaning of the endoplasmic reticulum was still unsettled, and even the final conclusion that ribosomes synthesized proteins rested at the time on the incorporation of radioactive amino acids into mixtures of proteins rather than into a well-defined proteins known to be produced by the cells under investigation.

To clear up some of these problems, Siekevitz and Palade did numerous studies on the pancreas, an organ with a number of convenient features. The exocrine cells produce a large number of digestive enzymes, most of which had been extensively studied, so that their production could be followed quantitatively by measuring enzymatic activity. Moreover, these enzymes could be isolated from cell fractions by well established procedures. Also, the pancreatic exocrine cells are conveniently organized for the purpose of study and the subcellular compartments are present in unusually large amounts neatly packed in different parts of the cells.

The general features of the pancreatic exocrine cells are fairly constant for all mammalian species so far studied. The basal region of the cell is occupied by the nucleus, mitochondria and a large number of rough-surfaced cisternae of the endoplasmic reticulum. The apical region contains, especially in starved animals, numerous zymogen granules interspersed with a few rough-surfaced elements of the endoplasmic reticulum. In between these two regions, on the apical side of the nucleus, there is a well-developed Golgi zone occupied by numerous smooth surfaced vesicles, cisternae and vacuoles, that is,the typical components of a Golgi complex.[1]

Siekevitz and Palade[17] attempted a complete fractionation of guinea pig pancreatic exocrine cells and were only partially successful. However. they found that the digestive enzymes were in high concentration in a fraction consisting mainly of zymogen granules. They stated that the segregation of these enzymes into compact. membrane-bound zymogen granules could represent a protective device since these enzymes could autolyze the cell if free to move throughout it. Also. they found that one hour after feeding. the pancreatic microsomes of the guinea pig contained digestive enzymes in higher concentrations and in relatively larger amounts than before feeding. The increase which followed food intake was so substantial that, at the time mentioned, the microsome fraction approached in total and specific activity the crude zymogen fraction known to consist primarily of zymogen granules.[18] Thus, the partial discharge of zymogen granules due to feeding, increased the rate of synthesis of digestive enzymes. The change in microsomal activity coincided in time with notable modifications in morphology of the microsomes and of the endoplasmic reticulum of the acinar cells, the most important of these modifications being the appearance of intracisternal granules inside the cisternal elements of the rough-surfaced endoplasmic reticulum.[8] They seemed to be a promising finding. but further work suggested they represent an accident rather than a regular event.[11]

Siekevitz and Palade[19] studied chymotrypsin synthesis in the exocrine cell of the guinea pig pancreas. Fasting animals were fed and one hour later, received radioactive leucine intravenously. At various time intervals (1–45 minutes) after injection, the glands were removed and fractionated and from each cell fraction. chymotrypsin was isolated and measured. Chymotrypsin isolated from the ribosomes of the microsomal fraction had the highest specific radioactivity at the early time points (1–3 minutes). After the longer time intervals, the specific radioactivity of the enzyme increased in the microsomal contents and finally in the zymogen granule fraction. The results clearly showed that the site of protein synthesis was the

attached ribosomes. Later on, the radioactive enzyme seemed to move to the endoplasmic reticulum and later appeared in the zymogen granules. They also hypothesized from these experiments that attached ribosomes synthesized proteins for export, whereas free ribosomes synthesized protein for intracellular use.

Because electron microscopy and cell fractionation provided essentially static information, a procedure was needed that capable of tracing in time and space by an appropriate label, the secretory proteins in their movement from cell compartment to cell compartment; it possible, all the way from attached ribosomes to zymogen granules. Such a technique was autoradiography. It was based on the incorporation into proteins of amino acids labeled with a radioisotope Cells or tissues containing the labeled amino acids were placed against a photographic emulsion, and the beta particles from the isotope would later produced an image of the radioactive area of the object.

Caro and Palade[1] injected radioactve leucine intravenously into guinea pigs and the pancreases were then examined by autoradiography at various times afer injection. Using the time sequence established by light microscopic autoradiography, they selected a small number of time points, judged as most significant, for a more detailed study at the electron microscopic level. Three to five minutes after the injection of the labeled leucine, the large majority of grains appeared over cell regions occupied mainly by rough - surfaced elements of the endoplasmic reticulum, marking the newly synthesized proteins. A lesser number of grains were associated with the Golgi complex, specifically over the clusters of smooth vesicles at its periphery. After 20 minutes, the rough - surfaced endoplasmic reticulum was practically free of label, while almost all the grains were over structures of the Golgi complex, especially in large vacuoles filled with a dense content and located centrally in the Golgi region. They called them "condensing vacuoles" since their strong labeling indicated that intensive concentration of newly synthesized protein was being carried on these structures. After 4 hours, the label was almost entirely confined to the zymogen granules, while the Golgi was free of grains. Occasionally, it was possible to see the discharge of a labeled granule by a process which will be described later. Thus, it was shown that the secretory proteins passed through the Golgi complex where they were progressively concentrated in condensing vacuoles. When completely filled, the latter became zymogen granules, the content of which most probably came from the rough-surfaced endoplasmic reticulum.

Based on past studies, Siekevitz and Palade proposed a general hypothesis concerning the synthesis and and subsequent intracellular transporage of digestive enzymes. According to this hypothesis, the secretory proteins are synthesized on ribosomes attached to the membrane of the rough surfaced cisternae of the endoplasmic reticulum. They are subsequently transferred into the intracisternal space through which they travel to the Golgi region where they are concentrated in membrane - bound vacuoles which thereby become zymogen granules. These move away from the Golgi zone and accumulate progressively in the apical region of the cell.

Intracellular storage of digestive enzymes in the form of zymogen granules, first postulated by Heidenhain, was recently confirmed by Greene, Hirs and Palade.[2] The latter isolated a reasonably pure zymogen granule fraction fro m

bovine pancreatic homogentes and showed that it contained the same zymogens and enzymes as the bovine pancreatic juice.

A new point was added to the general hypothesis by Palade.[9] Upon discharge of zymogen granules, the membrane of the zymogen granule becomes continuous with that of the cell membrane at the apical pole of the exocrine cell. Through the exit thus created, the content of the granule is poured into the acinar lumen. By this method, the granules can be discharged without any break in the continuity of the cell membrane and without the risks involved by any membrane discontinuity.

Two points of the hypothesis had not really been proved by definite findings : the transfer of newly synthesized secretory proteins from the attached ribosomes to the cisternal space, and their further transfer from this space to the condensing vacuoles of the Golgi complex.

Redman and Sabatini,[16] using guinea pigs injected by radioactive leucine obtained liver microsomes by differential centrifugation. Puromycin was used to inhibit protein synthesis causing release of the unfinished proteins from the ribosomal fraction to the microsomal fraction. The results suggested that from the onset of protein synthesis the growing peptide chain is directed toward the cisternal space into which it diffuses upon its release from the attached ribosome.

Caro and Palade implied that the small vesicular elements at the periphery of the Golgi complex are involved in the transfer of proteins from the rough endoplasmic reticulum to the condensing vacuoles of the Golgi complex. Jamieson and Palade[3] further described these small, smooth-surfaced vesicles at the periphery of the Golgi complex and described in the vicinity a series of special elements of the endoplasmic reticulum which they designated as "transitional elements" because they are partly rough and partly smooth. Jamieson and Palade,[4] also using guinea pigs injected with radioactive leucine, isolated two fractions from incubated slices of exocrine pancreas. One fraction was of rough microsomes and the other one of smooth microsomes. Labeled proteins appeared initially in the rough microsomes and were subsequently transferred to the smooth microsomes. Later labeled proteins left the smooth microsomes to appear in the zymogen granule fraction. These data provide direct evidence that the small vesicles of the Golgi complex mediate the transport of secretory proteins from the rough endoplasmic reticulum to condensing vacuoles.

The transport of secretory proteins via the small vesicles of the Golgi complex implies transport in bulk or in mass, since each vesicle must contain and carry a large number of protein molecules. The transport must also be discontinuous, otherwise concentration of the solution of secretory proteins at the next step would not be possible. The previously described transitional elements of the rough-surfaced endoplasmic reticulum are frequently in contact with smooth-surfaced vesicles or protrude as smooth-surfaced blebs toward the Golgi complex. These blebs are comparable in size to the smooth-surfaced vesicles of the region. Likewise the smooth-surfaced membrane bounding the condensing vacuoles is often in contact with small vesicles or is thrown up into small surface blebs. It can be assumed then, that secretory proteins are transported from the transitional elements of the rough-surfaced endoplasmic reticulum to condensing vacuoles through intermittent connections; or alternatively

that transport is effected by discrete vesicles that shuttle between the two compartments. Both possibilities are compatible, but the latter is more consistent with the fine structural details of the Golgi complex.(5)

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