somewhat different and more complex character.) For the first time there is the real prospect of understanding the logic behind gene deployment in pattern formation. As we have seen, the speculative ideas about compartments in this section are not supported by hard evidence. The best we have so far is a series of hints. But it is exactly this possibility, that compartments may have a wider significance, which makes the study of them at the present time so important and so interesting.

References and Notes

1. A. Garcia-Bellido, in *Cell Patterning* (Associated Scientific Publishers, London, 1975), p. 161.
2. C. Auerbach, *Trans. R. Soc. Edinb.* **58**, 787 (1936).
3. M. J. Pearson, *J. Cell Sci.* **16**, 113 (1974).
4. P. J. Bryant, *Dev. Biol.* **22**, 389 (1970).
5. A. Garcia-Bellido and J. R. Merriam, *ibid.* **24**, 61 (1971).
6. P. Ripoll, *Wilhelm Roux' Arch. Entwicklungsmech. Org.* **169**, 200 (1972).
7. C. A. Poodry and H. A. Schneiderman, *ibid.* **168**, 1 (1971).
8. P. J. Bryant and H. A. Schneiderman, *Dev. Biol.* **20**, 263 (1969).
9. A. Garcia-Bellido, *Genetics* **60**, 181 (1968).
10. _____, P. Ripoll, G. Morata, *Nat. New Biol.* **245**, 251 (1973); *Dev. Biol.*, in press.
11. W. Gehring, *Dev. Biol.* **16**, 438 (1967).
12. D. L. Lindsley and E. H. Grell, *Genetic Variations of* Drosophila melanogaster (Carnegie Institution of Washington, Washington, D.C., 1968).
13. G. Morata and P. Ripoll, *Dev. Biol.* **42**, 211 (1975).
14. A. Ferrus, *Genetics*, in press.
15. H. J. Becker, *Z. Indukt. Abstammungs-Vererbungsl.* **88**, 333 (1957).
16. P. Lawrence, *Symp. Soc. Exp. Biol.* **25**, 379 (1971).
17. _____, *Nat. New Biol.* **242**, 31 (1973).
18. _____, *J. Embryol. Exp. Morphol.* **30**, 681 (1973).
19. T. S. Sahota and W. E. Beckel, *Can. J. Zool.* **45**, 407 (1967).
20. P. Santamaria and A. Garcia-Bellido, in preparation.
21. P. A. Lawrence, *J. Cell Sci.* **1**, 475 (1966).
22. W. Gehring and R. Nöthiger, in *Developmental Systems: Insects*, S. Counce and C. H. Waddington, Eds. (Academic Press, New York, 1973), vol. 2, p. 211.
23. G. Morata and A. Garcia-Bellido, in preparation.
24. E. B. Lewis, *Am. Zool.* **3**, 33 (1963); in *Role of Chromosomes in Development*, M. Locke, Ed. (Academic Press, New York, 1964), p. 231; in *Heritage from Mendel*, P. A. Brink, Ed. (Univ. of Wisconsin Press, Madison, 1967), p. 17.
25. A. Garcia-Bellido and P. Santamaria, *Genetics* **72**, 87 (1972).
26. G. Morata and P. A. Lawrence, *Nature (Lond.)* **255**, 614 (1975).
27. P. A. Lawrence, F. H. C. Crick, M. Munro, *J. Cell Sci.* **11**, 815 (1972).
28. R. Nöthiger, *Wilhelm Roux' Arch. Entwicklungsmech. Org.* **155**, 269 (1964).
29. A. Garcia-Bellido, *Dev. Biol.* **14**, 278 (1966).
30. P. A. Lawrence, unpublished data.
31. W. Janning. *Drosophila Inf. Serv.* **50**, 151 (1973).
32. I. Hasenfuss, *Verh. Dtsch. Zool. Ges.* **66**, 71 (1973); *Zool. Jahrb. Anat.* **90**, 1 (1973); *ibid.*, p. 175.
33. P. A. Lawrence, in *Cell Patterning* (Associated Scientific Publishers, London, 1975), p. 3.
34. A. E. Warner and P. A. Lawrence, *Nature (Lond.)* **245**, 47 (1973).
35. P. A. Lawrence and S. M. Green, *J. Cell Biol.* **65**, 373 (1975).
36. B. Ripley, personal communication.

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Intracellular Aspects of the Process of Protein Synthesis

George Palade

In the early 1950's, during the near avalanche of discoveries, rediscoveries, and redefinitions of subcellular components made possible by electron microscopy, those prospecting in this newly opened field were faced with the problem of what to do with their newly acquired wealth. It could be increased by extending the inquiry on the horizontal to many other cell types prepared by many other techniques; it could be extended in further depth, instrumental resolution permitting ("ultra" was the preferred prefix of the period); or it could be used as a guide to monitor cell fractionation procedures of the type previously developed by Claude (*1*). The last alternative seemed particularly attractive since the small dimensions of many of the newly discovered structures suggested that they were relatively simple macromolecular assemblies. At their level, structure—as traditionally envisaged by the microscopist—was bound to merge into biochemistry, and biochemistry of mass-isolated subcellular components appeared to be the best way to get at the function of some of the newly discovered structures. The example provided by the work on isolated mitochondria was recent and still shining (*2*).

At the time, the structures of interest were the "small particulate component of the cytoplasm" (*3*), soon to become in succession "ribonucleoprotein particles" (*4*) and "ribosomes" (*5*), and the endoplasmic reticulum originally discovered by Porter, Claude, and Fullam (*6*) and then studied by Porter (*7*) and by Porter and myself (*8*). Philip Siekevitz joined me in 1955 and together we started a long series of integrated morphological and biochemical studies on the pancreas of the guinea pig, using primarily a combination of electron microscopy and cell fractionation procedures.

The choice of the pancreatic exocrine cell, a very efficient protein producer, as the object for our studies reflected in part our training, and in part our environment. I was coming from a medical school where I had acquired an interest in "microscopical anatomy" and "physiological chemistry" and great respect for the work of Claude Bernard, Rudolf Heidenhain, and Charles Garnier. Philip Siekevitz was coming from a graduate school with a Ph.D. in biochemistry and had recently worked out one of the first in vitro systems for protein synthesis (*9*). Our environment was the Rockefeller Institute for Medical Research, where a substantial amount of work had been done on the isolation, crystallization, and characterization of pancreatic secretory proteins [for example, see (*10*)]. But perhaps the most important factor in this selection was the appeal of the amazing organization of the pancreatic acinar cell, whose cytoplasm is packed with stacked endoplasmic reticulum cisternae studded with ribosomes. Its pictures had for me the effect of the song of a mermaid: irresistable and half transparent. Its meaning seemed to be buried only under a few years of work, and reasonable working hypotheses were already suggested by the structural organization itself.

The general aim of the project was to define the role played by the ribosomes, endoplasmic reticulum, and other subcellular components in the synthesis and subsequent processing of the proteins produced for export by the exocrine cells of the gland. The approach worked rather well for awhile (*11*), but after a few years we ran into the common limitations of the cell fractionation procedures then in use: imperfect separation, incomplete recovery, and incomplete representation of subcellular components in the fractionation scheme. To resume the advance of the in-

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This article is the lecture he delivered in Stockholm, Sweden, on 12 December 1974 when he received the Nobel Prize for Physiology or Medicine, a prize he shared with Albert Claude and Christian de Duve. The article is published here with the permission of the Nobel Foundation and will also be included in the complete volume of *Les Prix Nobel en 1974* as well as in the series Nobel Lectures (in English) published by the Elsevier Publishing Company, Amsterdam and New York. The lecture by de Duve appeared in the 18 July issue and the lecture by Claude will appear in a later issue.

quiry, Lucien Caro and I shifted to radioautography adapted to electron microscopy and obtained, in experiments performed in vivo, a reasonable approximation of the route and timetable followed by newly synthesized, radioactive proteins from their site of synthesis to their site of discharge from the cell (*12*). Radioautography has, however, its own limitations connected primarily with its low resolution, so that in subsequent experiments, uncertain radioautographic findings had to be checked by going back to cell fractionation procedures—this time with an advised mind. The experimental protocols were also changed to obtain better time resolution of the events under study, the major changes being the use of an in vitro subcellular system (*13*) and the adaptation by James Jamieson of an in vitro slice system (*14*), which later evolved into a lobule system (*15, 16*).

Analysis of the Secretory Process in the Pancreatic Exocrine Cell

Out of this combination of complementary techniques came a coherent representation of the secretory process, a "model" that has stood well the test of time. The current trend is to move from the subcellular to the molecular level in the analysis of the model, which means that its subcellular stage has been widely enough accepted.

The analysis of the secretory process of the pancreatic exocrine cell has not been the only research line pursued in our laboratory; membrane biogenesis, intercellular junctions, and structural aspects of capillary permeability are other examples. But the corresponding bodies of information are either less fully developed or still under scrutiny by us and by others; besides, none of them has affected the general thinking in our field to the same extent as the story of the secretory process.

With these considerations in mind, I believe that this unique and solemn occasion would be put to good use if I were to depart from the apparent tradition, which favors a summary of past or current work, and assess instead the available evidence on the secretory process, pointing out its strengths as well as its weaknesses and trying to figure out what can be done in the future to advance our knowledge still further.

Our analysis recognizes in the secretory process (*17*) of the pancreatic exocrine cell six successive steps or operations of which the object is the secretory proteins. These steps are: (i) synthesis, (ii) segregation, (iii) intracellular transport, (iv) concentration, (v) intracellular storage, and (vi) discharge. Each of them will be considered in some detail in what follows.

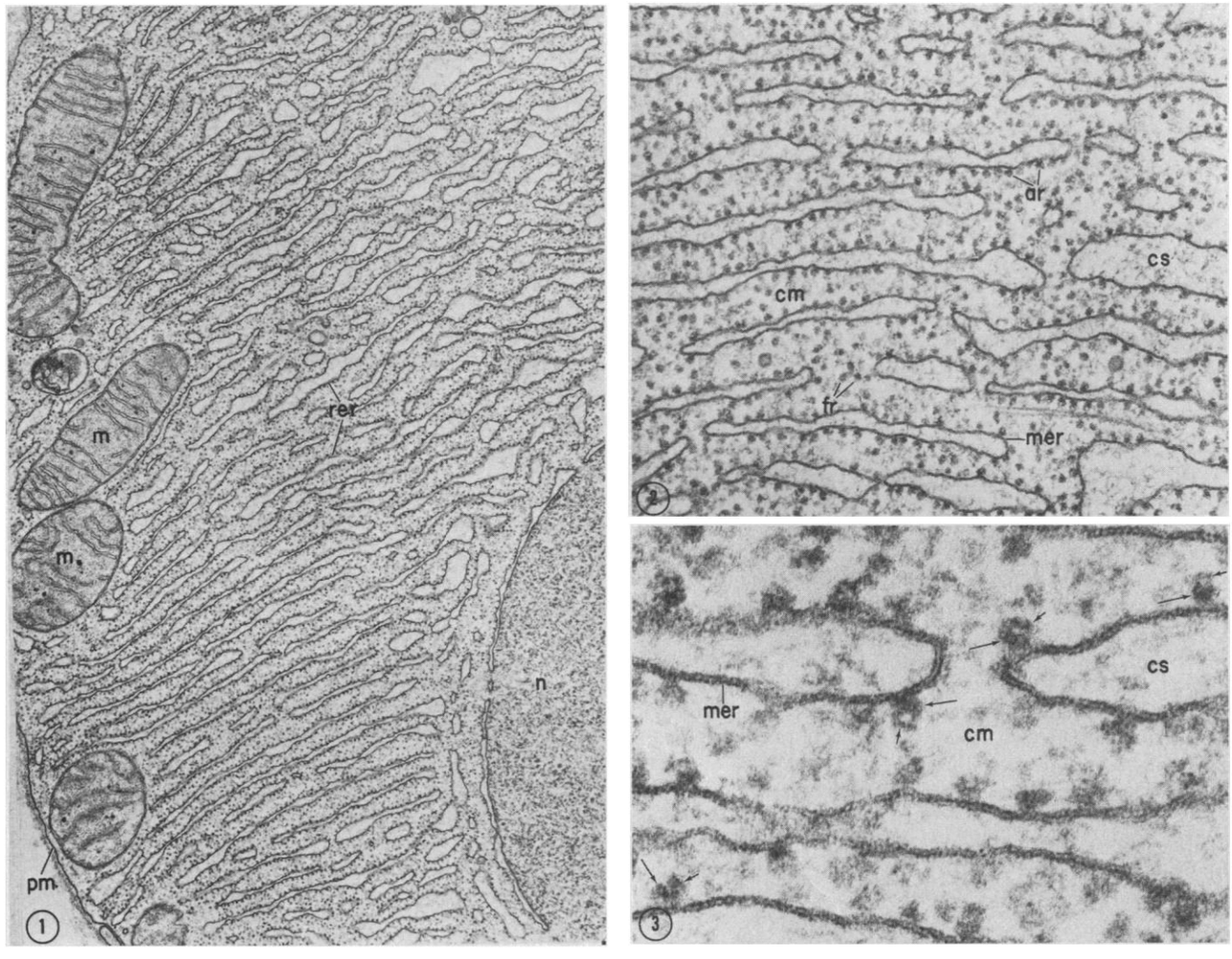


Fig. 1. Pancreatic exocrine cell. The basal region of the cell between the nucleus (*n*) and the plasmalemma (*pm*) is occupied by numerous cisternae of the rough endoplasmic reticulum (*rer*) and a few mitochondria (*m*) (× 9,000). Fig. 2. Pancreatic exocrine cell. Array of cisternae of the rough-surfaced endoplasmic reticulum is shown; *cs*, cisternal space; *cm*, cytoplasmic matrix (cell sol); *fr*, free ribosomes; *ar*, attached ribosomes; *mer*, membrane of the endoplasmic reticulum (× 37,000). Fig. 3. Pancreatic exocrine cell. A cytoplasmic region occupied by cisternal elements of the rough-surfaced endoplasmic reticulum is shown at high magnification; *mer*, membrane of the endoplasmic reticulum; *cs*, cisternal space; *cm*, cytoplasmic matrix (cell sol). The short arrows point to small subunits and the long arrows to large subunits of attached ribosomes (× 206,000).

1) Synthesis

Proteins for export are synthesized on polysomes attached to the membrane of the rough endoplasmic reticulum (Figs. 1 and 2). The first clear indication that this is the case came from early work with Siekevitz. After a short in vivo exposure to [^{14}C]leucine, radioactive chymotrypsinogen appeared preferentially associated with attached polysomes isolated from the guinea pig pancreas (*18*) (Table 1). The products of free polysomes were not investigated, but, by analogy with the situation studied by others in the liver (*19*), these polysomes probably synthesize proteins for intracellular use. Yet in all these cases, the results are to some extent ambiguous, since—as isolated—both polysome classes carry newly synthesized proteins irrespective of the latter's final destination. The differences are not qualitative, as would be expected for strict specialization; they are definitely large, but only quantitative.

This finding could have a trivial explanation: for example, leakage of newly synthesized proteins from cell compartments ruptured during tissue homogenization, followed by relocation by adsorption on the "wrong" class of polysomes. Available data indicate that artifactual relocation definitely occurs under these circumstances (*20*), but so far there is no reliable information concerning its extent. Alternatively, the dual location may have functional significance, since the position of the polysomes at the time of the initiation of translation is still unknown. Initiation in the free condition followed by enough elongation to expose either enzymic active sites or antigenic determinants before attachment seems unlikely but may occur, in principle. And the special sequence detected at the N-terminal of IgG light chains synthesized on detached polysomes (*21*) may function as a signal for attachment [for example, see (*22*)]. To understand the situation, we need more information than we have at present on the relationship between free and attached ribosomes, on the position of polysomes at the time of initiation, and on the duration of polysome attachment to the endoplasmic reticulum membrane.

Another aspect that should be considered at this point is the existence of two subclasses of attached polysomes: one synthesizing proteins for export and the other involved in the production of endoplasmic reticulum membrane proteins coupled with their insertion in this membrane (*23*). Much less is known about this second subclass, except that in its case the same uncertainties apply as to the location of the polysomes at the time of initiation. By analogy with a rather different system [chloroplast polysomes attached to thylakoid membranes during the synthesis of certain membrane proteins (*24*)], this type of attachment may be essentially transient, perhaps limited to a single round of translation for each site of attachment. It is generally assumed that all the soluble factors necessary for protein synthesis are present in molecular dispersion or in the form of soluble complexes in the cell sol or cytoplasmic matrix, but very few actual data are available in the case of the pancreatic exocrine cell—although this cell is potentially a rich source of aminoacyl-transfer RNA synthetases, transfer RNA's, and messenger RNA's. The presence of an active ribonuclease among the secretory proteins produced by the cell has discouraged work along such lines, but this whole field may be opened by using tissue taken from species known to have a very low pancreatic ribonuclease content. Pancreatic proteolytic zymogens do not appear to constitute a problem, since their activation is either nil or controllable during cell fractionation.

Table 1. Specific radioactivity of chymotrypsinogen isolated from attached and free polysomes in guinea pig pancreas after in vivo labeling with [^{14}C]leucine. Data are counts per minute per milligram of chymotrypsinogen (estimated from enzyme activity. [From (*18*)]

Fraction	Time after [^{14}C]leucine	
	1 minute	3 minutes
Attached polysomes	22,100	10,000
Free polysomes	2,800	3,000

2) Segregation

The newly synthesized secretory proteins are segregated in the cisternal space of the rough endoplasmic reticulum. The first evidence that this is the case came from work by Redman *et al.* (*13*) on pigeon pancreatic microsomes synthesizing [^{14}C]amylase in vitro. This radioactive secretory protein, initially associated with attached polysomes, preferentially appeared after about 3 minutes in the microsomal cavities. Experiments bearing on segregation were further refined in our laboratory by Redman and Sabatini (*25*) and Blobel and Sabatini (*26*). Their results indicate that the growing polypeptide chain is extruded through the microsomal membrane into the microsomal cavity, which is the in vitro equivalent of the cisternal space of the rough endoplasmic reticulum. Upon natural or experimentally induced termination, the newly synthesized chain separates with the microsomal vesicles and does not appear in the incubation medium, which topologically is the in vitro equivalent of the cell sol. Since it had already been established by Sabatini *et al.* (*27*) that the ribosomes are attached to the endoplasmic reticulum membrane by their large subunits (that is, the bearers of nascent chains) (Fig. 3), it was concluded that segregation is the result of a vectorial transport of the newly synthesized polypeptide from the large ribosomal subunit through the endoplasmic reticulum membrane to the cisternal space.

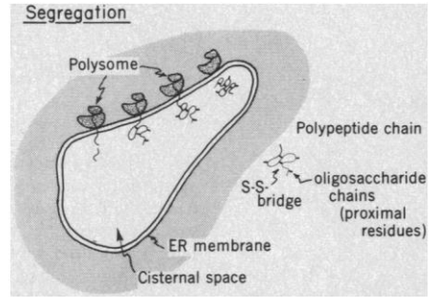


Fig. 4. Diagram of the segregation step; *ER*, endoplasmic reticulum.

This conclusion provides a satisfactory explanation for the basic structural features of the endoplasmic reticulum: a cavitary cell organ of complicated geometry that endows it with a large surface. All these features make sense if we assume that one of the main functions of the system is the trapping of proteins produced for export. With the exception of calcium ion accumulation in the sarcoplasmic reticulum (that is, the equivalent cell organ of muscle fibers) no other recognized function of the endoplasmic reticulum (such as phosphatide- and triacylglycerol synthesis, mixed-function oxygenation, or fatty acid desaturation) requires compellingly and directly a cavitary organ, at least according to our current knowledge. In detail, however, the forces and reactions involved in the trapping operation remain unknown. The interaction of the large ribosomal subunit with the endoplasmic reticulum membrane is understood only in very general terms (*26*), and precise information bearing on specific molecules involved in attachment is still lacking. Segregation appears to be an irreversible step: the nascent polypeptide is extruded in the cisternal space and, once inside, can no longer get out (Fig. 4).

The membrane of isolated microsomes was found to be highly permeable to molecules of about 10-angstrom diameter (*28*). If it is assumed that the same applies for the endoplasmic reticulum membrane in situ, it is reasonable to postulate that the

imprisonment of the polypeptide is the consequence of its conversion into a globular protein too large (> 20-Å diameter) to permeate the membrane. This postulate is in keeping with a series of findings which show that enzymes associated with the membrane, or present in the cisternal space, are responsible for disulfide bridge formation (*29*), hydroxylation of proline and lysine residues (*30*), proximal glycosylation of polypeptide chains (*31*), and perhaps partial proteolysis [for example, see (*21*)]. All these modifying operations are expected to affect directly or indirectly the tertiary structure of the secretory proteins which, once assumed, could render the proteins impermeant and their segregation irreversible (Fig. 4). Disulfide bridge formation aside, it would be of interest to know to what extent modifications of the type mentioned affect proteins produced for intracellular use. If the extent were nil or negligible, the differential modification of secretory proteins would provide an additional explanation for their segregation.

Available evidence either indicates or suggests that vectorial transport of secretory proteins to the cisternal space occurs in many other cell types [for example, plasma cells (*32*), fibroblasts (*33*), granulocytes (*34*), and parotid acinar cells (*35*)] in addition to hepatocytes and pancreatic exocrine cells. Vectorial transport and its corollary—segregation—are most probably obligate functional features for all protein-secreting cells, but further work is needed to check on the actual extent of their occurrence, as well as on possible exceptions (*36*).

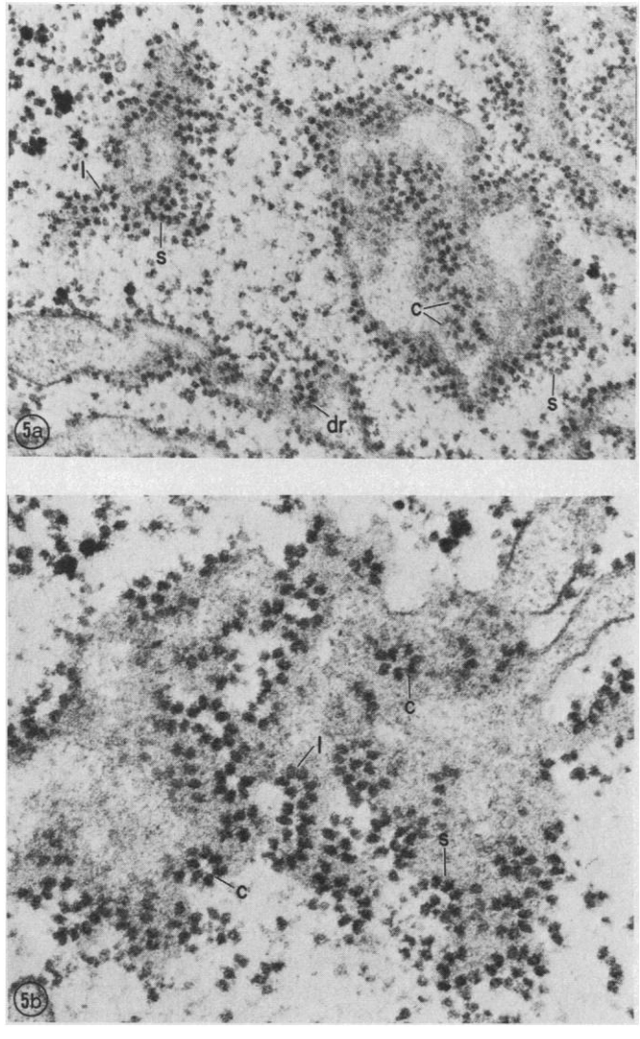
Although the endoplasmic reticulum membrane is characterized by high fluidity (*37*), the polysomes attached to its cytoplasmic aspect maintain regular, characteristic patterns (Fig. 5) of rather constant geometry (*3*). One may wonder what prevents them from assuming a random coil conformation; in other words, how does the cell succeed in securing fixed attachment sites on a highly fluid membrane? This riddle must have an interesting answer.

3) Intracellular Transport

From the cisternal space of the rough endoplasmic reticulum, the secretory proteins are transported to the Golgi complex. In the case we have studied, the pancreatic exocrine cell of the guinea pig, the terminus of the transport operations is a set of large vacuoles on the trans side of the complex (*12, 14*) which, because of their function (to be discussed later), are called condensing vacuoles.

Intracellular transport was first recognized in radioautographic experiments performed with Lucien Caro (*12*), but the details and requirements of this operation became evident only after James Jamieson and I shifted from intact animals to in vitro systems based on tissue slices (*14*). In such systems, short tissue exposure to radioactive amino acids ("labeling pulse") followed by effective removal of unincorporated label ("chase") became possible and, as a result, time resolution in our experiments was considerably improved.

Results obtained in pulse-chase experi-



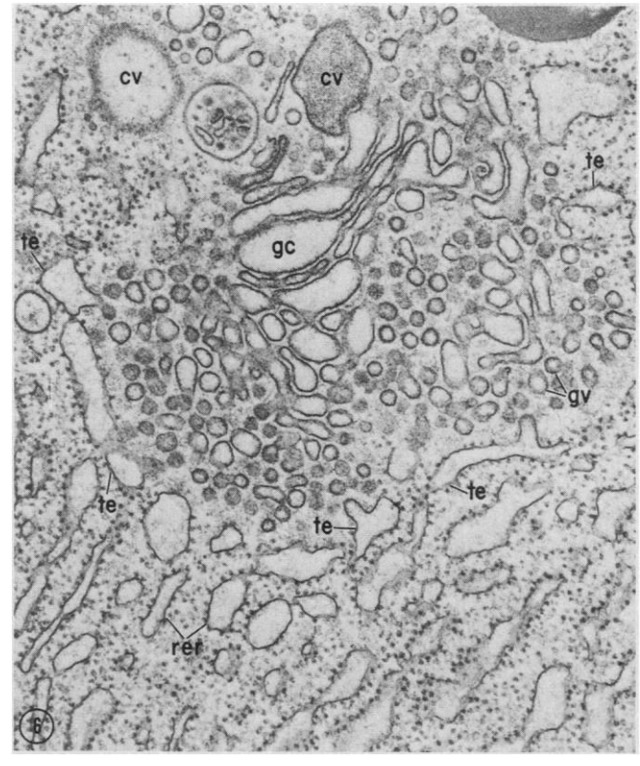


Fig. 5. Rat hepatocyte. The attached ribosomes (polysomes) form spirals (*s*), loops (*l*), circles (*c*), and double rows (*dr*) on the surface of the endoplasmic reticulum membrane (a: × 41,000; b: × 67,000). Fig. 6. Pancreatic exocrine cell, partial view of Golgi complex; *cv*, condensing vacuoles; *gc*, Golgi cisternae; *gv*, Golgi vesicles; *te*, transitional elements; *rer*, rough endoplasmic reticulum (× 19,000).

ments showed that the pathway followed by the secretory proteins leads from the rough endoplasmic reticulum to the transitional elements of this system (Fig. 6), then to the small peripheral vesicles on the cis side of the Golgi complex (*14*), and finally, in about 30 minutes, to condensing vacuoles (*38*) (Table 2 and Fig. 7). An unexpected and intriguing finding was that intracellular transport requires energy (*39*) supplied (in the system investigated) by oxidative phosphorylation. In the absence of adenosine triphosphate (ATP) synthesis, the secretory proteins remain in the rough endoplasmic reticulum, transport to condensing vacuoles being resumed upon resumption of ATP production. From these and other data, we concluded that the functional equivalent of a lock (or lock-gate) exists along the channels used for intracellular transport, that the lock is located at the level of the transitional elements of the endoplasmic reticulum, and that secretory proteins seem to flow vectorially to the Golgi complex when the lock is opened.

The general pathway followed in intracellular transport appears to be the same in a variety of cell types (*15, 40–43*), but direct evidence on the pre-Golgi lock has been obtained only in the case of the exocrine pancreatic cell. Extension to other systems of the inquiry dealing primarily with the lock-gate is clearly needed. In addition, many aspects of the transport operation remain either unknown or unsettled. The geometry of the connections between the endoplasmic reticulum and the Golgi complex is still a matter of debate: according to some investigators (*44*), the two compartments are permanently connected by continuous tubules; according to us (*14*), the connection is intermittent and is probably established by shuttling vesicles. The energy-requiring reactions are unknown, and equally unknown are the forces involved in transport and the means by which macromolecules are moved from the endoplasmic reticulum to the condensing vacuoles against an apparent concentration gradient.

We have uncovered an interesting process, but we are only at the very beginning of its analysis. Every one of the points mentioned above remains to be elucidated by further work.

4) Concentration

The secretory proteins reach the condensing vacuoles in a dilute solution that is progressively concentrated at these sites to a level comparable to that eventually found in mature secretion granules. The exact concentration in each of the compartments involved in intracellular transport is unknown, but the increase in the density of the content in condensing vacuoles (as seen in electron micrographs) and the increase in number of radioautographic grains associated with the same vacuoles (*38*) (Fig. 8) suggest that the incoming solution is concentrated by a large factor. The final result of the concentration step is the conversion of the condensing vacuoles into mature secretion granules (*12, 38*), usually called zymogen granules in the case of the pancreatic exocrine cell.

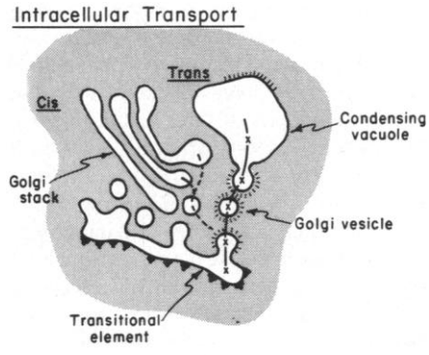


Fig. 7. Diagram of intracellular transport; ×—×, pathway followed in the pancreatic exocrine cell of the guinea pig; - - -, pathway followed in other glandular cells.

Concentration is not dependent on a continuous supply of energy. In situ, neither condensing vacuoles nor zymogen granules swell when ATP production is blocked; and in vitro, isolated secretion granules are rather insensitive to the osmolality of the suspension medium at, or below, neutrality (*45*). They are instead highly sensitive to variations in *p*H and lyse promptly above *p*H 7.2 (*46, 47*). These results rule out the hypothesis that concentration is achieved by ion pumps located in the membrane of the condensing vacuoles, and suggest that the cell uses for this step some other, energetically more economical mechanism. The synthesis of sulfate-containing macromolecules in Golgi elements and their presence in secretion granules in murine pancreatic acinar cells (*48*) as well as in other murine glandular cells (*49*) have been established by radioautography. Moreover, Tartakoff *et al.* (*50*) have recently detected a sulfated polyanion ($pI \simeq 3.4$), presumably a sulfated peptidoglycan, in the content of zymogen granules and in discharged secretion in the guinea pig pancreas. The formation of large aggregates by ionic interactions between this polyanion and secretory proteins, which are known to be predominantly cationic (*50*), could cause a reduction in osmotic activity within condensing vacuoles with concomitant outflow of water. In this case, energy would no longer be required past the synthesis of the polyanion, and concentration would depend primarily on the stability of the postulated aggregates.

This hypothesis remains to be validated by the isolation and characterization of the sulfated polyanion, and especially by the demonstration of relevant aggregate formation under conditions likely to prevail in vivo within condensing vacuoles. The hypothesis is particularly attractive because it could explain not only concentration per se, but also intracellular transport against an apparent chemical gradient. Such a gradient may not exist, or may be reversed, if the secretory proteins of every new batch were to be aggregated and thereby osmotically inactivated upon their entry into condensing vacuoles.

In the pancreatic exocrine cell of the guinea pig, concentration is effected in trans Golgi condensing vacuoles, but in the same cell of other species (rat, for instance), the step under discussion takes place in the last cisterna on the trans side of each Golgi stack. Finally, in many other glandular cells [for example, see (*51*)], the same operation is carried out in the dilated rims of the last two to three trans Golgi cisternae (Fig. 7). Moreover, in guinea pig

Table 2. Distribution of radioautographic grains after a pulse of labeled leucine in slices of guinea pig pancreas incubated in vitro. The pulse was L-[4,5-^{3}H]leucine (40 μM; 200 μc/ml); the chase was unlabeled leucine (2 mM). For each compartment of the secretory pathway, the maximal concentration figures are given in italics. [Simplified from (*38*)]

Cell compartment	Radioautographic grains (%)			
	Pulse (3 minutes)	Chase (minutes)		
		+7	+37	+117
Rough endoplasmic reticulum	*86.3*	43.7	24.3	20.0
Golgi complex				
Peripheral vesicles	2.7	*43.0*	14.9	3.6
Condensing vacuoles	1.0	3.8	*48.5*	7.5
Zymogen granules	3.0	4.6	11.3	*58.6*
Acinar lumen	—	—	—	7.1
Other compartments*	7.0	4.6	1.1	3.2

*Nuclei and mitochondria.

pancreatic lobules hyperstimulated in vitro, the usual condensing vacuoles are no longer present, and concentration of secretory proteins begins in the Golgi cisternae, preferentially in those located on the trans side of the stacks (*52*). There are, therefore, variations according to species, cell type, and physiological conditions in the location of concentration sites within the Golgi complex, and it would be of interest to find out whether these variations reflect changes in the distribution of the sulfated polyanion (or other functionally equivalent compounds) within the complex.

Radioautographic findings (*41, 42, 53*) and cell fractionation data (*54*) obtained on a variety of tissues indicate that terminal glycosylation of secretory proteins occurs in the Golgi complex. This operation is known to affect only a fraction, not the totality, of the proteins produced for export.

In addition, the Golgi complex appears to be the site of partial proteolysis of proinsulin (*55*) and perhaps other secretory proteins. It is also the site of synthesis of polysaccharides in plant cells [for example, see (*56*)]. The Golgi apparatus therefore has a multiplicity of functions in the processing of secretory products, but—with the exception of concentration—the location of the other activities among its elements is either uncertain or still unknown.

On the one hand, we have a rather extensive literature dealing with differences in cytochemical reactions within the same cisterna (*57*) or among the cisternae of the same stack (*58, 59*) without any obvious functional correlation. On the other hand, initial biochemical data on Golgi subfractions so far reveal no differences between Golgi cisternae and Golgi vacuoles (*60*).

Finally, at the level of the Golgi complex the secretory product is transferred from a high permeability membrane (that is, the membrane of the endoplasmic reticulum), to a membrane whose lipid composition approaches that of the plasmalemma in its high content of cholesterol and sphingomyelin and in the low degree of unsaturation of fatty acids in its phospholipids (*61, 62*). Such a membrane is expected to have low permeability, and therefore to be "exposable" without danger to the external medium at the time of discharge (see below).

In general, our knowledge of the functions of the complex is still rudimentary, primarily because the isolation of Golgi fractions from tissue homogenates was achieved only recently (*63*) and is still limited to a few sources [liver, pancreas (*61*), and kidney (*64*)]. The extent of compartmentation within the complex as well as the precise pathway followed by secretory products through it is still unknown. Finally, as a telling measure of our ignorance, it is worthwhile pointing out that we do not have any good idea about the functional meaning of the most prominent structural feature of the Golgi complex: the stacking of its cisternae.

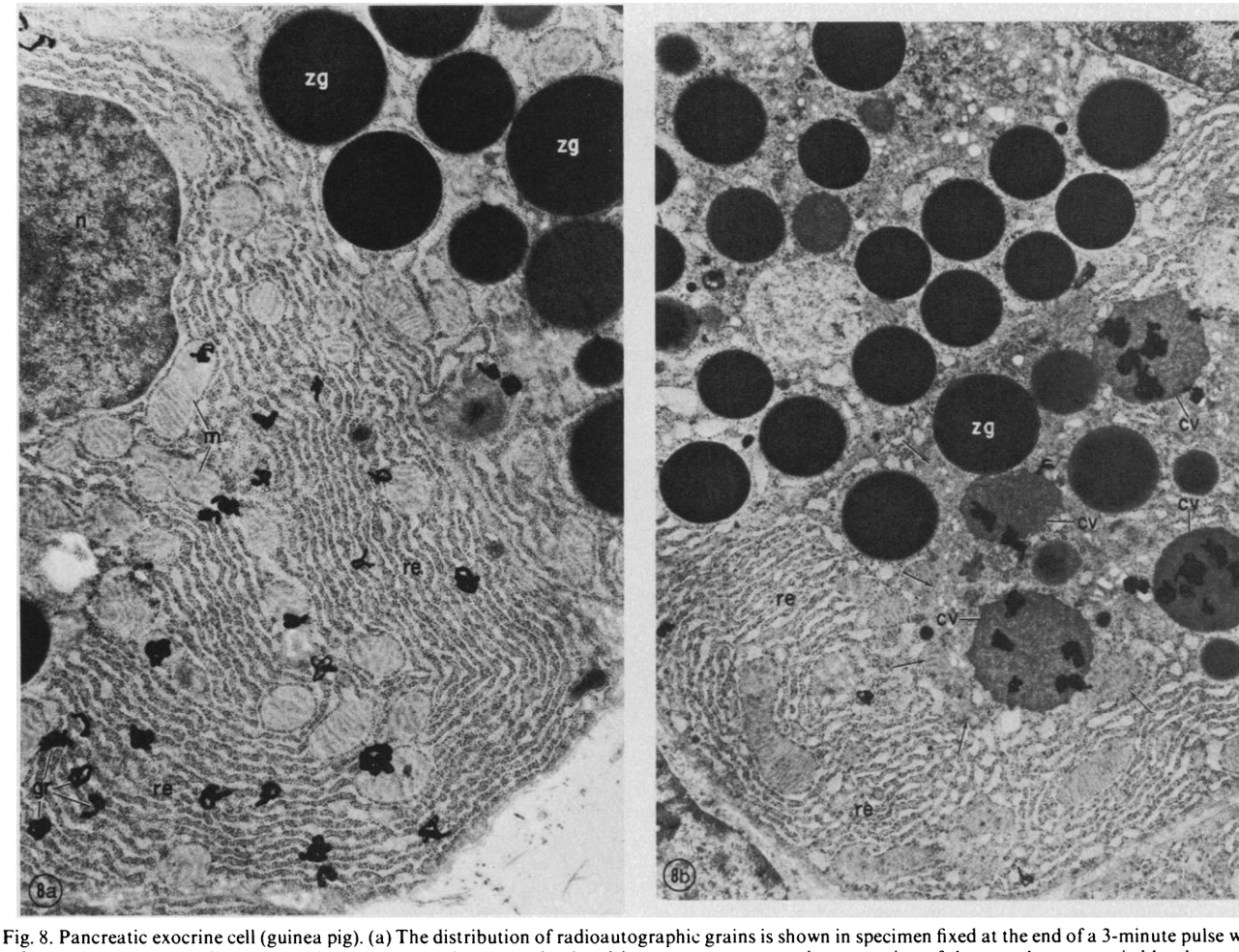


Fig. 8. Pancreatic exocrine cell (guinea pig). (a) The distribution of radioautographic grains is shown in specimen fixed at the end of a 3-minute pulse with L-[4,5-^{3}H]leucine; *gr*, radioautographic grains; *n*, nucleus; *m*, mitochondria; *zg*, zymogen granules; *re*, region of the cytoplasm occupied by the rough-surfaced endoplasmic reticulum. At this time, ~ 85 percent of the grains are found associated with such regions (× 9000). (b) The distribution of radioautographic grains is shown at the end of a 37-minute chase after a 3-minute pulse as in (a); *cv*, condensing vacuoles; *zg*, zymogen granules; *re*, region of the cytoplasm occupied by the rough-surfaced endoplasmic reticulum. The periphery of the Golgi complex is marked by arrows. At this time, ~ 50 percent of the radioautographic grains are associated with condensing vacuoles (× 9000). [Fig. 8, a and b, from (*38*)]

5) Intracellular Storage

Secretory proteins are temporarily stored within the cell in secretion granules that, as already mentioned, are condensing vacuoles that have reached the end of the concentration step. Their membrane comes, therefore, from the Golgi complex and their content is the product of attached polysomes, modified at subsequent steps as already described in previous sections.

In the cases so far investigated—that is, the exocrine pancreas of the cow (*47, 65*), rat (*66*), and guinea pig (*50*) and the parotid of the rabbit (*67*)—the content of the secretion granules (more precisely, the extract obtained from reasonably homogeneous secretion granule fractions) and the physiologically discharged secretion contain the same proteins in the same relative amounts (Fig. 9). Since no other intracellular source has been revealed or suggested by our evidence, we have concluded that the content of these granules is the sole precursor of the proteins found in the juice secreted by the gland.

In the case of glands that, like the exocrine pancreas, consist of an apparently homogeneous population of secretory cells that produce a complex mixture of secretory proteins, the question of specialization at the cellular or subcellular level was asked repeatedly and answered only in part. So far, all of the proteins looked for in the bovine pancreas [trypsinogen (*68*), chymotrypsinogen, deoxyribonuclease (*69*), and ribonuclease (*70*)] were detected by immunocytochemical procedures in all of the secretion granules of all cells examined. Each granule probably contains a sample of the mixture discharged by the gland, but it is hard to believe that all of these microsamples are quantitatively strictly identical. Specialization at the cellular level is well established in a number of endocrine glands that are characterized by a morphologically heterogeneous cell population [for example, see (*51*)]. Specialization at the subcellular level exists in polymorphonuclear neutrophil granulocytes (*34*). The formula used in the pancreas, that is, intracellular storage of a complex mixture in apparently equivalent quanta, probably explains the lack of short-term qualitative modulation of the secretory output [see (*16, 71*) for a more detailed discussion]. It can be assumed that this type of modulation is rendered unnecessary by the specialized nutritional habits of each species.

In the exocrine cells of the pancreas, secretion granules usually occupy the apical region between the Golgi complex and the acinar lumen. There are few microtubules in this region and few microfilaments, and there is no consistent pattern in their organization and distribution (except for the microfilaments associated with junctional elements and microvilli). In other cell types, it has been postulated that microtubules and microfilaments play a role in effecting secretory discharge (see below), as well as in directing or moving secretory granules to their sites of discharge. In pancreatic acinar cells, radioautographic findings show that newly formed (that is, labeled) granules are distributed at random within the preexisting granule population (*38*), and biochemical data indicate that newly synthesized and preexisting proteins are discharged at random from the total zymogen granule population (*16, 71*). With the evidence at hand, these results can be ascribed to slow diffusion leading to thorough mixing of old and new granules within the apical region. In other cell types, the situation may be different because of incomplete mixing within the granule population and uneven distribution of discharge sites (see below).

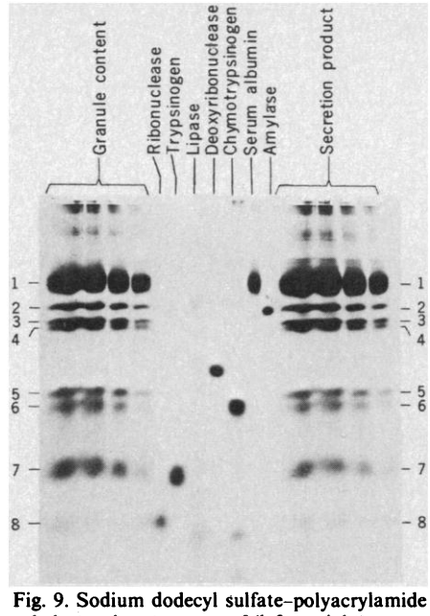


Fig. 9. Sodium dodecyl sulfate–polyacrylamide gel electrophoretograms of (left to right) zymogen granule content, standards, and secretion discharged by pancreatic lobules incubated and stimulated in vitro. Identification of bands: 1, unknown secretory protein and carrier bovine plasma albumin; 2, amylase; 3 and 4, procarboxypeptidases A and B and unknown secretory proteins; 5, unknown protein; 6, chymotrypsinogen; 7, trypsinogen; and 8, ribonuclease. [From (*50*)]

6) Discharge

Relatively early in the investigation of the secretory process, it was found that secretion granules discharge their content into glandular lumina (Fig. 10) by a process originally called "membrane fusion" (*72*) and later termed exocytosis (*73*). Morphological findings suggest that in preparation for discharge, the membrane of the secretion granule fuses with the plasmalemma and that subsequent reorganization [that is, progressive elimination of layers (Figs. 11 and 12)] leads to fission of the fused membranes within the area of fusion. The final result is continuity established between the granule compartment and the extracellular medium (lumen), concomitant with continuity of the granule membrane and the plasmalemma all around the orifice through which the granule content reaches the lumen (Fig. 13). This operation allows the discharge of the secretory product while ensuring the maintenance of a continuous diffusion barrier between the cell sol and the extracellular medium. At the beginning, a few alternatives were considered, but by now exocytosis is recognized as a widely occurring, probably general mechanism for the discharge of macromolecular secretory products.

The membrane fusion involved in secretory discharge has a high degree of specificity. The membrane of secretion granules fuses only with the plasmalemma, although there are at the time of this event and at comparable distances around the interacting pair many other types of cellular membranes. In the exocrine cells the specificity is even more stringent, since ability to fuse is limited to the apical or luminal domain of the plasmalemma. The only permanently operative alternative is preliminary fusion of granule membrane to granule membrane leading eventually to discharge of two or more secretion granules in tandem (*74*). This type of specificity suggests the existence of complementary recognition sites in each interacting membrane which may be involved in binding preliminary to fusion. In some respects the postulated situation is reminiscent of the interaction between a hormone and its membrane receptor (*75*), except that in this case the events are intracellular and receptors as well as agonists are assumed to be membrane-bound.

Exocytosis has been extensively studied in a variety of secretory cells and by now its basic requirements for calcium ion and energy are well established (*76, 77*). Our own data demonstrated a stringent energy requirement for secretory discharge in the exocrine pancreatic cell and, hence, the existence of a second energy-dependent lock that controls the flow of secretory products from secretion granules into the acinar lumina (*52*). Our data also showed that discharge can proceed in the absence of continuous protein synthesis (*52*).

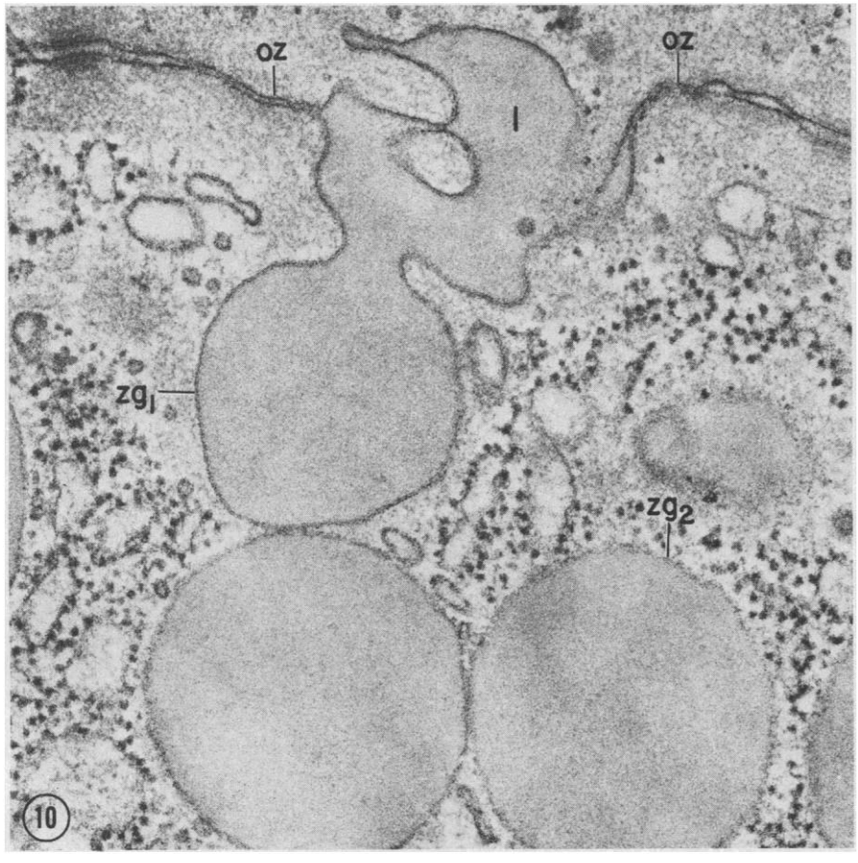


Fig. 10. Pancreatic exocrine cell, apical region; *l*, lumen; *oz*, occluding zonules; zg_1, discharging zymogen granule; zg_2, zymogen granules still in storage (× 110,000).

In certain glandular cells, pancreatic exocrine cells included, discharge is intermittent and well integrated with other activities of the organism. In such cases, the cell, which without stimulation discharges at a slow, liminal rate, responds to stimulation by either hormones or neurotransmitters by a dramatic step-up in the rate of exocytosis. The stimulus-secretion coupling (*77*) often involves a cyclic nucleotide generating system (adenylate cyclase in most cases) and one or more protein kinases (*78*). But this coupling also involves a depolarization of the plasmalemma. In the case of the pancreatic exocrine cell, stimulation definitely leads to membrane depolarization (*79*), while the activation of a cyclic nucleotide system is still uncertain [compare (*80*) and (*78*)]. The final target of the protein kinases is unknown in secretory cells. A hypothesis advanced a few years ago ascribes this role to tubulin (*81*), but the evidence in this case is open to question. Results obtained on other systems (*82*) suggest that the target might be a membrane protein.

In recent years, a number of agents activating or inhibiting exocytosis have been described. Among the latter, colchicine and the vinca alkaloids have received considerable attention (*83*), the general assumption being that their inhibitory effect implies the involvement of microtubules in exocytosis. At present the situation is rather confused, and a reasonable interpretation of the numerous and in part contradictory data is hardly possible. A distinction should be made between agents directly affecting fusion-fission and agents affecting the superimposed regulatory systems that activate and inactivate the coupling between stimulation and secretion. Colchicine appears to affect the basic mechanism rather than its controls, since it inhibits discharge in hepatocytes (*84*, *85*), that is, in cells that appear to lack a stimulus-secretion coupling. In these cells the effect has been localized at discharge, the last step in the secretory process, all previous steps being unaffected (*85*). But the involvement of microtubules remains open to question since, at least in hepatocytes, the inhibitory effect is prompt and reaches its maximum long before the depolymerization of the microtubules becomes morphologically detectable. Hence, alternative targets should be considered, especially because colchicine binds to membranes (*86*) and inhibits a number of transport mechanisms in the plasmalemma (*87*).

As already mentioned, there is no elaborate organization involving microtubules and microfilaments in the apical region of the pancreatic exocrine cells. A rather modest fibrillar feltwork (terminal web) is found under the luminal plasmalemma, but there is no fibrillar lining on the cytoplasmic aspect of the membrane of the

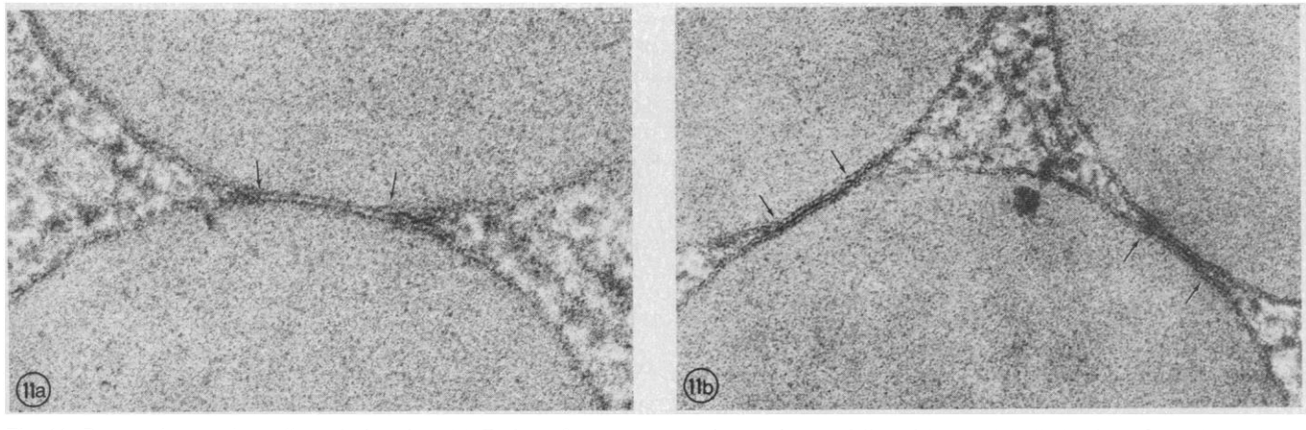


Fig. 11. Pancreatic exocrine cells, apical region. (a) Fusion of zymogen granule membranes followed by partial elimination of membrane layers (arrows) (×165,000). (b) Fusion of zymogen granule membranes (arrows) (×120,000).

zymogen granules while still in storage. However, a fibrillar shell (*88*) often appears around discharging zymogen granules when their membrane is already in continuity with the plasmalemma. It is continuous with the terminal web, it may consist of contractile proteins (actin? myosin?), and it may promote the expulsion of the secretion granule content.

Effects of Exocytosis and Intracellular Transport on Membrane Distribution

The end result of exocytosis is, on the one hand, discharge of a secretory product and, on the other hand, relocation of secretory granule membranes in the plasmalemma. Under normal steady-state conditions, excess membrane must be removed from the receiving compartment (lumen) and membrane must be added to the donor compartment (secretion granules or Golgi complex), since the distribution of membrane amounts among these compartments remains relatively constant with time.

The procedures used by the cell to recover and redistribute membrane after exocytosis are unknown. Morphological findings suggest coupled endocytosis; in a few cases, namely in nerve endings (*89*) and anterior pituitary cells (*90*), recovery of organized membrane in the form of endocytic vesicles has convincingly been demonstrated with the help of cytochemical tracers. Moreover, in the case of pituitary cells, the recovered membrane was eventually traced to trans Golgi vacuoles and cisternae (*90*). But the exact nature of this membrane and its ultimate fate remain a matter of speculation.

In the case of discharge, the membranes of the secretory granules can be viewed as a set of individual vesicular containers that move forward from the Golgi complex to the surface during exocytosis and presumably back to the Golgi complex during coupled endocytosis. In the pancreas (*91*) as well as in the parotid (*92*), the rate of synthesis of the proteins of the granule membranes is generally slower than the rate of synthesis of the secretory proteins contained in the granules. Hence, reutilization or recycling of the membrane containers is possible in principle, but so far has not been proved.

Assuming that a similar shuttling system of membrane containers operates between the rough endoplasmic reticulum and the Golgi complex, recent evidence indicates that there is no mixing among either the lipid (*61, 62*) or the protein (*60, 93*) components of the membranes of the two compartments in the pancreas (guinea pig) and in the liver (rat). These findings impose stringent limitations on membrane interactions since they suggest that lateral diffusion of components is prevented at the time the membranes of the two compartments establish continuity, and that incoming membrane is removed from the receiving compartment according to a nonrandom formula (*60*).

The situation may appear unexpectedly complicated, even confusing, but in fact it makes sense since the final result of the restrictions mentioned is the preservation of functional specificity for the membrane of

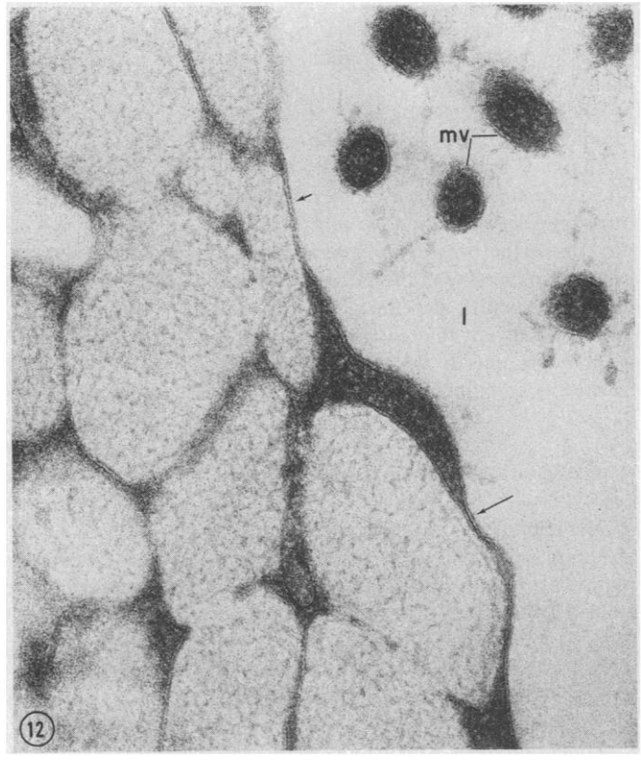


Fig. 12. Intestinal epithelium, goblet cell (rat). Fusion of secretion granule membranes with the plasmalemma; long arrow, simple fusion; short arrow, fusion with partial elimination of membrane layers; *l*, lumen; *mv*, microvilli (× 105,000).

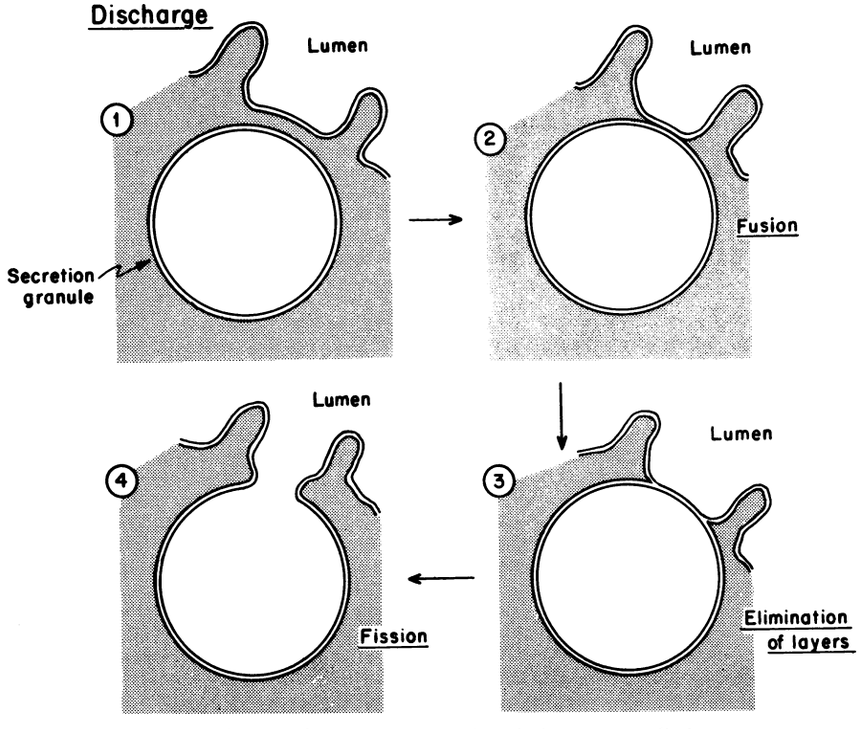


Fig. 13. Diagram of membrane interactions during secretory discharge.

each compartment. This specificity is implied in both the old concept of "marker enzyme" and in the newer ideas on sequential modification of secretory proteins as they move along the secretory pathway. The most convincing example is that of the successive glycosylation of glycoproteins (*41, 42, 54*). The main difficulty is that we do not have at present any clear idea about the means used by the cell to carry through the various steps of the secretory process while imposing and maintaining the restrictions mentioned.

These are intriguing and challenging problems that stress the need for extending the inquiry from the processed product to the processing apparatus, especially to the membranes that outline the compartments that form the processing apparatus. Further understanding of the secretory process is now becoming dependent on adequate information on the chemistry of these membranes and on the reactions involved in their interactions.

Variations on a Common Theme

The functional analysis of the pancreatic exocrine cell gave us a reasonably good representation of the steps generally involved in the secretory process. In addition, it helped us understand a series of special cases in other cell types which now appear to be recognizable variations on the theme already described (Fig. 14).

Endocrine cells producing peptide or protein hormones follow the same sequence of operations but apparently discharge their secretory product along the entire plasmalemma (*51*), instead of discharging within restricted plasmalemmal domains as exocrine cells do. In many secretory cells (such as fibroblasts, chondrocytes, and plasma cells), the concentration step is omitted, secretion granules of usual appearance are absent, intracellular storage is reduced in duration or eliminated, and discharge seems to take place continuously. In such cells, the applicability of the last three steps of the general scheme was in doubt and the possibility of direct discharge from the cisternal space of the endoplasmic reticulum was considered (*94*). But recently, equivalents of secretion granules were recognized in special fibroblasts, that is, odontoblasts (*95*), as well as in ordinary fibroblasts after treatment with colchicine (*96*). Their secretory process now appears as a variation on the common theme with the variant step resulting from lack of extensive concentration in the Golgi complex. In plasma cells the equivalent of the secretion granule is still not yet identified (*42*).

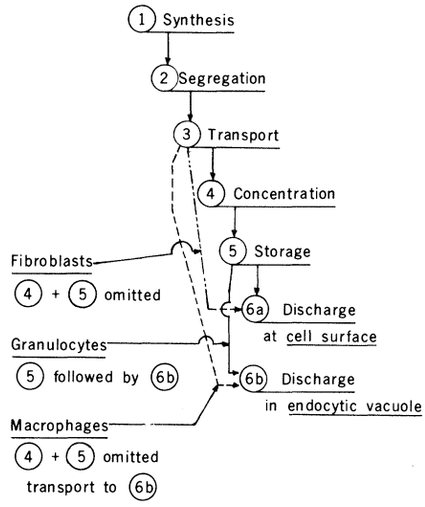


Fig. 14. Secretory process: variations on a common theme.

In polymorphonuclear neutrophil and eosinophil granulocytes, secretion granules are preferentially discharged into endocytic vacuoles (*97*), discharge at the cell surface occurring only under special conditions (*98*). In eosinophils, the entire population of secretion granules consists of primary lysosomes, while in neutrophils the population includes "specific granules" in addition to primary lysosomes. In both cell types, all secretory proteins—irrespective of their nature—appear to be produced and processed according to the general scheme worked out for the pancreatic exocrine cell, except for the variant already mentioned at the discharge step (*99*).

In macrophages, discharge of secretory proteins is also preferentially effected into endocytic vacuoles, but in addition the concentration step is apparently omitted. A dilute solution of acid hydrolases is carried, probably by small vesicles (the local equivalent of primary lysosomes), from the Golgi complex to endocytic vacuoles. The latter are also able to fuse with secondary lysosomes, which provide a secondhand source of hydrolases (*100*). The variation on the common theme used by macrophages seems to be applied in all cells capable of autophagy and low-efficiency heterophagy—including cells specialized in protein production for export, such as the hepatocytes, exocrine cells of the pancreas, and cells of the anterior pituitary. A special problem arises in this case in connection with the separation of regular exportable proteins from lysosomal hydrolases. The separation seems to be reasonably efficient, although not perfect, since acid phosphatase activity has been repeatedly detected by histochemical procedures within regular secretion granules—mature and immature—and within trans Golgi cisternae (*58, 101*). In addition, it has been postulated that in a number of cell types lysosome formation takes place in a special compartment, called GERL (Golgi–endoplasmic reticulum–lysosomes) (*102*), intercalated between the endoplasmic reticulum and trans Golgi elements. It is evident that all these cells are capable of handling concomitantly, and probably in the same production apparatus, two "incompatible" lines of secretory proteins, but the means by which the products are separated or their inactivation prevented (in case of mixing) remain unknown. This riddle must also have an interesting answer.

Finally, another variation on the common theme has been found in glandular cells, which produce protein or glycoprotein hormones and are faced with an excess of stored product (*51, 101*). In this case the secretion granules are discharged directly into secondary lysosomes by simple membrane fusion. The process, called crinophagy, was originally discovered in pituitary mammotrophs (*101*), but further work has shown that it probably occurs in all the cells of the anterior pituitary (*51*) and probably in those of many other glands. The use of lysosomes for degrading excess secretory proteins stresses once more the need for understanding protective means against lysosomal hydrolases, which must be at work along the entire secretory pathway beginning with the endoplasmic reticulum.

On the Generality of the Secretory Process

The evidence already discussed stresses the role played by the endoplasmic reticulum and the Golgi complex in the production and processing of secretory proteins. The stress put on secretion leads, however, to an apparent impasse. Since every eukaryotic cell possesses both an endoplasmic reticulum and a Golgi complex, it follows that all eukaryotic cells secrete proteins or that the organs of the secretory pathway have additional, perhaps more general and more important functions than secretion, which have been ignored or are still unknown.

This problem actually concerns fewer cell types than generally assumed, since secretion of macromolecules has been recognized in recent years as an important activity in a wide variety of cells. Interestingly enough, all plant eukaryotes are secretory cells, since they produce and discharge the polysaccharides and proteins of their cell walls (*103*). Among animal eukaryotes, male (*104*) and female (*105*) gametes pro-

duce protein for extracellular use (*106*), and so do secretory nerve cells (*107*), including adrenergic (*76*) and presumably cholinergic (*108*) neurons. Smooth muscle cells have been recently recognized as producers of collagen, elastin, and other proteins of the intracellular matrices (*109*), and the same probably applies for a variety of epithelia (including the vascular endothelium) in relation to the production of the corresponding basement membranes (*110*).

For those animal cells for which a protein product for extracellular use has not been identified, an acceptable answer is provided by the production of lysosomal enzymes. As already mentioned, the production of these enzymes involves the same secretory apparatus (that is, the endoplasmic reticulum and the Golgi complex) and the same sequence of steps (except for extracellular discharge) as in bona fide glandular or secretory cells. It appears, therefore, that—for the moment and with the evidence at hand—the problem can be solved in favor of the first alternative; that is, all eukaryotic cells produce secretory proteins, the basic general secretory functions being the production of cell wall components in plant cells and the production of lysosomal enzymes in animal cells. To some extent, each type of basic production must be represented in the other kingdom. On top of these lowly but ubiquitous secretory activities appears to be superimposed the production of highly specialized proteins exported by a variety of differentiated cell types. Our attention has been focused on the latter long enough to lose proper perspective and to assume (as we did until recently) that the secretion of proteins is a specialized function restricted to a few, highly differentiated, glandular cells.

Notwithstanding the conclusion just stated, the second alternative—that is, the involvement of the secretory pathway in another general but still unrecognized function—is not excluded. Among the nonsecretory functions postulated for the endoplasmic reticulum and the Golgi complex is the production of cellular membranes, plasmalemma included [for example, see (*56*)]. At present this postulate rests only on suggestive evidence, most of it morphological. This situation brings us back to the necessity of obtaining detailed and—if possible—comprehensive data on the chemistry and function of the different membranes of the secretory pathway and on their interactions. With this type of information, the second alternative could be put to test, and in the same time our understanding of the secretory process and of the basic organization of eukaryotic cells could be further advanced.

References and Notes

1. A. Claude, *J. Exp. Med.* **84**, 51 (1946); *ibid.*, p. 61.
2. G. H. Hogeboom, W. C. Schneider, G. E. Palade, *J. Biol. Chem.* **172**, 619 (1948); E. P. Kennedy and A. L. Lehninger, *ibid.* **179**, 957 (1949).

G. E. Palade, *J. Biophys. Biochem. Cytol.* **1**, 59 (1955).

4. ———, in *Microsomal Particles and Protein Synthesis*, R. B. Roberts, Ed. (Pergamon, New York, 1958), pp. 36–49.
5. R. B. Roberts, in *ibid.*, pp. vii–viii.
6. K. R. Porter, A. Claude, E. Fullam, *J. Exp. Med.* **81**, 233 (1945).
7. K. R. Porter, *ibid.* **97**, 727 (1953).
8. G. E. Palade and K. R. Porter, *ibid.* **100**, 641 (19 K. R. Porter and G. E. Palade, *J. Biophys. Biochem. Cytol.* **3**, 269 (19 G. E. Palade, *ibid.* **2** (Suppl.), 85 (1956).
9. P. Siekevitz, *J. Biol. Chem.* **195**, 549 (1952).
10. J. H. Northrop, M. Kunitz, R. M. Herriott, *Crystalline Enzymes* (Columbia Univ. Press, New York, 1948).

G. E. Palade and P. Siekevitz, *J. Biophys. Biochem. Cytol.* **2**, 171 (1956); *ibid.*, p. P. Siekevitz and G. E. Palade, *ibid.* **4**, 203 (1958); *ibid.*, p. 309; *ibid.*, p. *ibid.* **5**, 1 (1959).

L. G. Caro and G. E. Palade, *J. Cell Biol.* **20**, 473 (1964).

13. C. M. Redman, P. Siekevitz, G. E. Palade, *J. Biol. Chem.* **241**, 1150 (1966).

J. D. Jamieson and G. E. Palade, *J. Cell Biol.* **34**, 577 (1967).

J. D. Castle, J. D. Jamieson, G. E. Palade, *ibid.* **53**, 290 (1972).

16. G. A. Scheele and G. E. Palade, *J. Biol. Chem.* **250**, 2660 (1975).
17. For convenience, the term "secretory process" will be used in the rest of the text as a shorthand for "the process of protein secretion."

P. Siekevitz and G. E. Palade, *J. Biophys. Biochem. Cytol.* **7**, 619 (1960); *ibid.*, p. 631.

19. C. M. Redman, *J. Biol. Chem.* **244**, 4308 (1969); S. J. Hicks, J. W. Drisdale, H. N. Munro, *Science* **164**, 584 (1969).
20. A. Tartakoff and G. E. Palade, unpublished observations.
21. C. Milstein, G. G. Brownlee, T. M. Harrison, M. B. Mathews, *Nature (Lond.)* **239**, 117 (1972).
22. G. Blobel and D. D. Sabatini, *Biomembranes* **2**, 193 (1971).
23. G. Dallner, P. Siekevitz, G. E. Palade, *J. Cell Biol.* **30**, 73 (1966); *ibid.*, p. 97.
24. N. H. Chua, G. Blobel, P. Siekevitz, G. E. Palade, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 1554 (1973).

C. M. Redman and D. D. Sabatini, *ibid.* **56**, 608 (1966).

26. G. Blobel and D. D. Sabatini, *J. Cell Biol.* **45**, 146 (1970).
27. D. D. Sabatini, Y. Tashiro, G. E. Palade, *J. Mol. Biol.* **19**, 503 (1966).
28. H. Tedeschi, J. M. James, W. Anthony, *J. Cell Biol.* **18**, 503 (1963).
29. C. B. Anfinson, *Harvey Lect.* **61**, 95 (1966).
30. B. R. Olsen, R. A. Berg, Y. Kishida, D. J. Prockop, *Science* **182**, 825 (1973).
31. J. Molnar, G. B. Robinson, R. J. Winzler, *J. Biol. Chem.* **240**, 1882 (1965).
32. B. Mach, H. Koblet, D. Gros, *Proc. Natl. Acad. Sci. U.S.A.* **59**, 445 (1968).
33. M. G. Grant and D. J. Prockop, *N. Engl. J. Med.* **286**, 194 (1972).

D. F. Bainton and M. G. Farquhar, *J. Cell Biol.* **39**, 299 (19 *ibid.* **45**, 54 (1970).

35. V. Herzog and F. Miller, *Z. Zellforsch. Mikrosk. Anat.* **107** 403 (1970).
36. B. Lisowska-Berstein, M. E. Lamm, P. Vassali, *Proc. Natl. Acad. Sci. U.S.A.* **66**, 425 (1970).
37. M. J. Rogers and P. Strittmatter, *J. Biol. Chem.* **249**, 895 (1974); *ibid.*, p. 5565.

J. D. Jamieson and G. E. Palade, *J. Cell Biol.* **34**, 597 (1967).

———, *ibid.* **39**, 589 (1968).

40. R. M. Swenson and M. Kern, *Proc. Natl. Acad. Sci. U.S.A.* **57**, 417 (1967).
41. P. Wuhr, A. Herscovics, C. P. Leblond, *J. Cell Biol.* **43**, 289 (19 A. Haddad, M. D. Smith, A. Herscovics, N. J. Nadler, C. P. Leblond, *ibid.* **49**, 856 (1971).

D. Zagury, J. W. Uhr, J. D. Jamieson, G. E. Palade, *ibid.* **46**, 52 (1970).

C. R. Hopkins and M. G. Farquhar, *ibid.* **59**, 276 (1973).

44. D. J. Morré, T. W. Keenan, H. H. Mollenhauer, in *Advances in Cytopharmacology*, F. Clementi and B. Ceccarelli, Eds. (Raven, New York, 1971);

A. Claude, *J. Cell Biol.* **47**, 745 (1970).

J. D. Jamieson and G. E. Palade, *J. Cell Biol.* **48**, 503 (1971).

46. L. E. Hokin, *Biochim. Biophys. Acta* **18**, 379 (1955).
47. L. J. Greene, C. H. W. Hirs, G. E. Palade, *J. Biol. Chem.* **238**, 2054 (1963).

N. B. Berg and R. W. Young, *J. Cell Biol.* **50**, 469 (1971).

R. W. Young, *ibid.* **57**, 175 (1973).

50. A. M. Tartakoff, L. J. Greene, G. E. Palade, *J. Biol. Chem.* **249**, 7420 (1974).
51. M. G. Farquhar, *Mem. Soc. Endocrinol.* **19**, 79 (1971).

J. D. Jamieson and G. E. Palade, *J. Cell Biol.* **50**, 135 (1971).

M. Neutra and C. P. Leblond, *ibid.* **30**, 137 (1966).

54. H. Schachter, I. Jabbal, R. L. Hudgin, L. Pinteric, J. McGuire, S. Roseman, *J. Biol. Chem.* **245**, 1090 (1970).
55. D. F. Steiner, J. L. Clark, C. Nolan, A. H. Rubenstein, E. Margoliash, F. Melani, P. E. Oyer, in *13th Nobel Symposium, Stockholm* (Interscience, New York, 1970), p. 123.
56. M. Dauwalder, W. G. Whaley, J. E. Kephart, *Subcell. Biochem.* **1**, 225 (1972).

M. G. Farquhar, J. J. M. Bergeron, G. E. Palade, *J. Cell Biol.* **60**, 8 (1974); L. Ovtracht and J. P. Thiéry, *J. Microsc. (Paris)* **15**, 135 (1972).

58. A. B. Novikoff, E. Essner, S. Goldfischer, in *The Interpretation of Ultrastructure*, R. J. C. Harris, Ed. (Academic Press, New York, 1962), pp. 149–192.

D. S. Friend, *J. Cell Biol.* **41**, 269 (1969).

J. J. M. Bergeron, J. H. Ehrenreich, P. Siekevitz, G. E. Palade, *ibid.* **59**, 73 (1973).

J. Meldolesi, J. D. Jamieson, G. E. Palade, *ibid.* **49**, 109 (1971); *ibid.*, p. 130.

62. T. W. Keenan and D. J. Morré, *Biochemistry* **9**, 19 (1970).

B. Fleischer, S. Fleischer, H. Ozawa, *J. Cell Biol.* **43**, 59 (1969); B. Fleischer and S. Fleischer, *Biochim. Biophys. Acta* **219**, 301 (1970); D. J. Morré, R. L. Hamilton, H. H. Mollenhauer, R. W. Mahley, W. P. Cunningham, R. D. Cheetham, V. S. LeQuire, *J. Cell Biol.* **44**, 484 (19 J. H. Ehrenreich, J. J. M. Bergeron, P. Siekevitz, G. E. Palade, *ibid.* **59**, 45 (1973).

64. B. Fleischer and F. Zambrano, *J. Biol. Chem.* **249**, 5995 (1974).
65. P. J. Keller and E. Cohen, *ibid.* **236**, 1407 (1961).
66. J. C. Palla, thesis, Université d'Aix-Marseille (1970).

J. D. Castle, J. D. Jamieson, G. E. Palade, *J. Cell Biol.* **64**, 182 (1975).

68. J. P. Kraehenbuhl and J. D. Jamieson, *Proc. Natl. Acad. Sci U.S.A.* **69**, 1771 (1972).
69. ———, unpublished observations.
70. R. G. Painter, K. T. Tokuyashu, S. J. Singer, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 1649 (1973).
71. A. M. Tartakoff, J. D. Jamieson, G. A. Scheele, G. E. Palade, *J. Biol. Chem.* **250**, 2671 (1974).
72. G. E. Palade, in *Subcellular Particles*, T. Hayashi, Ed. (Ronald, New York, 1959), pp. 64–83.
73. C. de Duve, in *Lysosomes, Ciba Foundation Symposium* (Little, Brown, Boston, 1963), p. 126.
74. A. Ishikawa, *J. Cell Biol.* **24**, 369 (1965).
75. P. Cuatrecasas, *Proc. Natl. Acad. Sci. U.S.A.* **68**, 1264 (1971).
76. W. W. Douglas and R. P. Rubin, *J. Physiol. (Lond.)* **159**, 40 (1961); *ibid.* **167**, 288 (1963); M. Schramm, *Annu. Rev. Biochem.* **36**, 307 (1967).
77. W. W. Douglas, *Br. J. Pharmacol.* **34**, 451 (1968).
78. H. Rasmussen, *Science* **170**, 404 (1970).
79. E. K. Mathews and O. H. Petersen, *J. Physiol. (Lond.)* **231**, 283 (1973).
80. R. G. Kulka and E. Sternlicht, *Proc. Natl. Acad. Sci. U.S.A.* **61**, 1123 (1968).
81. D. P. B. Goodman, H. Rasmussen, F. DiBella, C. E. Guthrow, Jr., *ibid.* **67**, 652 (1970).
82. R. J. Dilorenzo, K. G. Walton, P. F. Curran, P. Greengard, *ibid.* **70**, 880 (1973); E. M. Johnson, T. Ueda, H. Moeno, P. Greengard, *J. Biol. Chem.* **247**, 5650 (1972).
83. P. E. Lacy, S. L. Howell, D. A. Young, C. J. Fink, *Nature (Lond.)* **219**, 1177 (1968); J. A. Williams and J. Wolff, *J. Cell Biol.* **54**, 158 (1972).
84. Y. LeMarchand *et al.*, *Biol. Chem.* **248**, 6862 (1973); O. Stein and Y. Stein, *Biochim. Biophys. Acta* **306**, 142 (1973).
85. C. M. Redman, D. Banerjee, K. Howell, G. E. Palade, *J. Cell Biol.*, in press.

J. Stadler and W. W. Franke, *ibid.* **60**, 297 (1974).

87. L. Wilson, J. R. Bamberg, S. B. Mizel, L. M. Grisham, K. M. Creswell, *Fed. Proc.* **33**, 158 (1973).
88. A fibrillar feltwork exists also at the periphery of the Golgi complex in association with the transitional elements of the endoplasmic reticulum. Its function, and the function of fibrillar coats or layers occasionally found around Golgi vesicles and vacuoles, are unknown.

J. E. Heuser and T. S. Reese, *J. Cell Biol.* **57**, 315 (1973); B. Ceccarelli, W. P. Hurlbut, A. Mauro, *ibid.*, p. 449.

G. Pelletier, *J. Ultrastruct. Res.* **43**, 445 (1973); M. G. Farquhar, E. Skutelsky, C. R. Hopkins, in *The Anterior Pituitary*, A. Tixier-Vidal and M. G. Farquhar, Eds. (Academic Press, New York, 1975), pp. 83–135.
J. Meldolesi, *J. Cell Biol.* **61**, 1 (1974).
92. J. D. Castle, thesis, Rockefeller University (1974).
93. L. M. G. van Golde, B. Fleischer, S. Fleischer, *Biochim. Biophys. Acta* **249**, 318 (1971).
R. Ross and E. Benditt, *J. Cell Biol.* **27**, 83 (1965).
M. Weinstock and C. P. Leblond, *ibid.* **60**, 92 (1974).
96. B. R. Olsen and D. J. Prockop, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 2033 (1974).
97. D. Zucker-Franklin and J. G. Hirsch, *J. Exp. Med.* **120**, 569 (1964); D. F. Bainton, in *Phagocytic Mechanisms in Health and Disease*, R. C. Williams and H. H. Fudenberg, Eds. (Straton Intercontinental Medical, New York, 1972), pp. 123–136.
98. P. M. Henson, *J. Immunol.* **107**, 1547 (1971).
99. Another exception is the fact that specific granules and primary lysosomes are formed on opposite sides of the Golgi complex of the neutrophil granulocytes (*34*).
100. Z. A. Cohn, M. E. Fedorko, J. G. Hirsch, *J. Exp. Med.* **123**, 157 (1966).
101. R. E. Smith and M. G. Farquhar, *J. Cell Biol.* **31**, 319 (1966).
P. M. Novikoff, A. B. Novikoff, N. Quintana, J. Hauw, *ibid.* **50**, 859 (1971).
103. P. Albersheim, W. D. Bauer, K. Keestra, K. W. Talmadge, in *Biogenesis of Plant Cell Wall Polysaccharides*, F. Loewus, Ed. (Academic Press, New York, 1973).
104. D. W. Fawcett, *Biol. Reprod.* **2**, 90 (1970).
E. Anderson, *J. Cell Biol.* **37**, 514 (1968); D. Szollosi, *Anat. Rec.* **159**, 431 (1967).
106. In many species, female gametes produce vitellus proteins by using the secretory pathway in part or in toto [R. G. Kessel, *Z. Zellforsch. Mikrosk. Anat.* **89**, 17 (1968)].
107. W. W. Douglas and A. M. Poisner, *J. Physiol. (Lond.)* **172**, 1 (1964).
108. V. P. Whittacker, *Adv. Cytopharmacol.* **2**, 311 (1973).
R. Ross, *J. Cell Biol.* **50**, 172 (1971).
110. E. D. Hay, in *The Epidermis*, W. Montagna and W. C. Lobitz, Eds. (Academic Press, New York, 1964), p. 97; A. M. Cohen and E. D. Hay, *Dev. Biol.* **26**, 578 (1971).
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NEWS AND COMMENT

Discovery of Pulsars: A Graduate Student's Story

Holmbury St. Mary, Surrey. Perhaps the most dramatic scientific event of the last decade was the discovery in 1967 of the celestial objects known as pulsating radio stars or pulsars. The radioastronomy group at Cambridge, England, announced their detection of a wholly novel class of stars which beamed out pulses of radio waves with extraordinary rapidity and precision. The pulsars were soon determined to be neutron stars, the long-postulated embers of stellar evolution that astronomers had assumed were too faint ever to be detectable from Earth. And the public who followed these events enjoyed the frisson of learning that the Cambridge astronomers had for a time considered the signals might originate from another civilization, in token of which they had nicknamed the pulsars LGM stars, for "little green men."

Just how pulsars came to be discovered is a historically important case study in serendipity. The manner of the discovery is also of topical interest because of a dispute that has blown up over the award of last year's Nobel prize for physics. For the first time, the prize went to astronomers, and the Nobel committee honored Martin Ryle, leader of the Cambridge radioastronomy team, and his colleague Anthony Hewish, under whose supervision pulsars were discovered (see *Science*, 15 November 1974).

Hewish's citation was "for his decisive role in the discovery of pulsars," the qualification being there because, as Hewish has always acknowledged, the first finder of pulsars was his graduate student, Jocelyn Bell, now Burnell. Nobel prize committees have a reputation for doing their homework thoroughly, and if anyone thought the credit had been wrongly distributed, they kept their doubts to themselves, at least until recently. This March, after a lecture at McGill University, Montreal, English theoretical astronomer Fred Hoyle was reported to have described the award to Hewish as a scandal. According to *The Times* of London, Hoyle declared that Burnell's finding had been kept secret for 6 months while her directors " 'were busily pinching the discovery from the girl, or that was what it amounted to.' " But the victim of the alleged conspiracy disagreed. " 'It's a bit preposterous and he has overstated the case so as to be incorrect,' " she told *The Times*'s reporter.

There the matter might have rested, but a few days later Hoyle wrote from Rice University, Houston, where he was teaching at the time, to say that his views had been "only crudely represented." While not in fact repudiating any of the remarks attributed to him in the initial article, Hoyle proceeded to lay out his case more precisely. The actual discovery of pulsars, he said in his letter to *The Times* (published 8 April), seemed to have taken place in the 2-month period up to September 1967, but the finding was not published until February 1968, by which time it had accrued 5 authors including Hewish and Bell, together with four others who were cited for help in the discussions that led to the report. The publication consisted of two parts: the detection of the first pulsar, and a follow-up investigation. The second part of the process was guided by Hewish, but could have been done equally well by other observatories, Hoyle opined. The discovery of the signals by Burnell, and her finding that the source of the signals changed position with the stars, was what constituted the crucial step: "Once this step had been taken, nothing that happened from there on could have made any difference to the eventual outcome." Hoyle continued:

> There has been a tendency to misunderstand the magnitude of Miss Bell's achievement, because it sounds so simple—just to search and search through a great mass of records. The achievement came from a willingness to contemplate as a serious possibility a phenomenon that all past experience suggested was impossible. I have to go back in my mind to the discovery of radioactivity by Henri Becquerel for a comparable example of a scientific bolt from the blue.
>
> I would add that my criticism of the Nobel award was directed against the awards committee itself, not against Professor Hewish. It seems clear that the committee did not bother itself to understand what happened in this case.

It says nothing as to the merits or demerits of Hoyle's argument to note that his letter is part of a long series of dissensions which have made British astronomy a game quite unfit for children. The dispute has led, in one way or another, to Hoyle's resignation from his Cambridge Institute of Theoretical Astronomy (see *Science*, 2 June 1972) and to the resignation of Margaret Burbidge, now at the University of California, San Diego, from the directorship of the Royal Greenwich Observatory (see *Science*, 30 November 1973).

Hoyle's letter presented Hewish with the virtually no-win choice between saying nothing, thus seeming to have nothing to say, and defending his role in the discovery at Burnell's expense, with the risk of appearing somewhat ungallant. His decision was to reply with restraint (11 April), saying in effect that Burnell had been using his telescope, under his instructions, to make a sky survey which he had initiated. There were several problematical features of the pulsed source—a slight variability in its time of appearance, the possibility that