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REVIEW ARTICLE

A Portrait of the Pancreatic B-Cell

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„Yes, I have a pair of eyes" replied Sam „and that's just it. If they wos a pair o'patent double million magnifin'gas microscopes of hextra power, p'raps I might be able to see through a flight o'stairs or a deal door; but bein' only eyes, you see, my wisdom's limited".
(Sam Weller, In „Pickwick Papers", Charles Dickens)

Key words: Insulin-producing cell, insulin biosynthesis, insulin release, emiocytosis, exocytosis, endocytosis, membrane recycling, islet junctional complexes, tight junctions, gap junctions, cell coat, electron microscopy, freeze-etching, radioautography, cytochemistry.

Before 1950, correlation between the structure of the insulin-producing B-cells and their functional activity was essentially limited to the determination, by light microscopy, of the degree of cytoplasmic granularity (Fig. 1) using specific stains such as that described by Gomori [14]. The advent of electron microscopy opened a whole new stratum of biological organization, stretching from about 2.000 Å — the limit of resolution of the light microscope — down to 50 Å, and later 20 Å, for most biological specimens. The first explorers of these new territories drastically extended our knowledge of the organization of cells, and created an atmosphere of great excitement for morphological sciences. The result was a growing appreciation of the fact that there is a physiological, as well as a biochemical meaning in the minute-scale organization of cells. This organization turned out to be much more elaborate than previously assumed, and many intracellular membrane-bounded compartments could be described.

On the basis of the relationship between the number of storage secretory granules (beta granules) and the amount or degree of development of other organelles, two major structural patterns of B-cells can be recognized. In one pattern (Fig. 2), typical of resting B-cells, the predominant cytoplasmic component is represented by numerous storage secretory granules, each bounded by a smooth-surfaced membrane and containing a material, whose shape and electron density vary between different species of animals. In the second pattern (Fig. 3), typical of B-cells after prolonged secretory stimulation, the cytoplasm contains only a few beta granules, but numerous mitochondria, abundant rough endoplasmic reticulum cisternae (RER), and a large Golgi complex. Between these two extreme situations, all gradations are also recognized. Thus, it becomes necessary to account for the production and disappearance of the beta granules in order to explain the secretory process.

In this review, we will discuss the following problems, essentially from a morphological standpoint: a) the biosynthesis of (pro)insulin; b) the formation of the secretory granules; c) the release of the secretory granules; d) the recycling of membrane constituents associated with secretion, and e) the intercellular contacts within the islet.

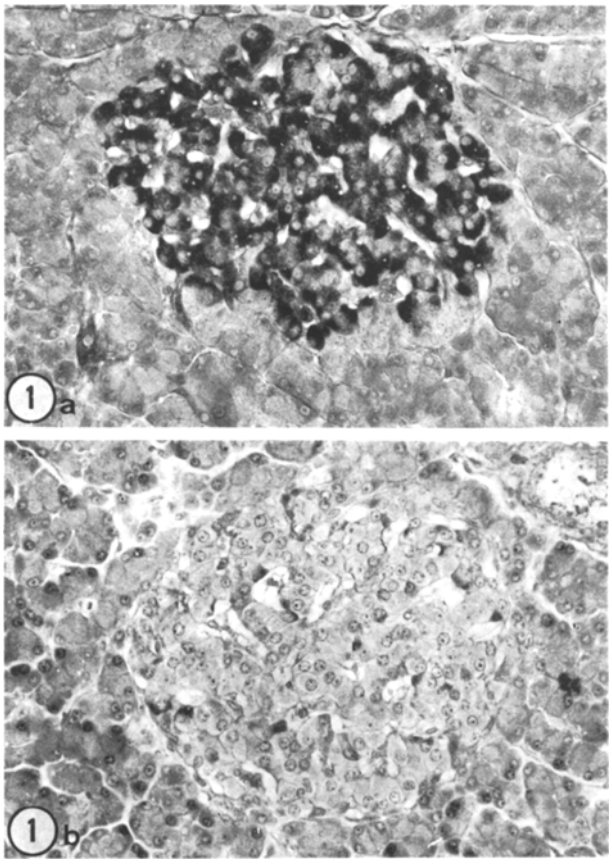
a) (Pro)insulin Biosynthesis

In a series of ultrastructural studies comparing the sequential changes in the organelles of the B-cells under different experimental conditions, Lacy and coworkers proposed a general hypothesis concerning the formation of secretory granules [25, 26, 27, 28, 64]. According to this classical scheme, the secretory substance was elaborated in the rough endoplasmic reticulum, condensed into a visible core within the cisternae, and became a mature secretory granule upon detachment of the ribosomes from the endoplasmic reticulum membrane. The starting point and the end of the proposed sequence were satisfactorily established, since Bauer and coworkers [1] were able to give evidence for insulin synthesis in microsomes (the cell fraction formed mainly by endoplasmic reticulum membranes) and for its subsequent transfer into the secretory granules. However in these early electronmicroscopic studies, the involvement of the

Golgi complex in the processes of cellular secretion was only occasionally acknowledged [11], despite the frequent hypertrophy of the Golgi complex in actively secreting B-cells.

Later, improvements in preparative techniques concerning mainly fixation and embedding allowed the Golgi complex to be clearly implicated in the channelling movement of secretory product within the B-cell [22, 32, 33, 51] (Figs. 4, 8b). At that time, the passage of secretory product through the Golgi complex was assumed on the basis of indirect evidence, and as such was speculative; attempts to obtain an experimental basis for the sequential involvement of the RER, the Golgi complex and the secretory granules along the intracellular pathway in the B-cell secretory process were therefore carried out by electron microscopic radioautography. When we started to study the endocrine pancreas by radioautography, the pattern of the secretory process in the exocrine pancreas had already been established with this technique due to the pioneering work carried out in several laboratories [5, 6, 19, 20, 62, 63]. Therefore, in our work, we had on our side the benefit of the experience of these predecessors.

After a series of failures when the labelling experiments were carried out with whole pancreatic glands (either in vivo or in vitro), satisfactory results were obtained with isolated islets or pancreatic cultures [18, 41, 45]. In particular, an integrated biochemical and radioautographic study was made possible with thinly spread pancreatic endocrine cells in culture (monolayer cultures) [46]. With this system, it is possible to obtain a clear-cut pulse-labelling of secretory proteins in B-cells. The design of the experiment is quite simple: islet cells are exposed to a short pulse of tritiated leucine, which is incorporated into protein, and the radioactivity is then chased with non-radioactive leucine for varying periods of time. The results of these studies (Table 1; Figs. 5, 6) show, after isolation of IRI peptides on G-50 sephadex column, that a substantial amount of radioactivity appears in the proinsulin fraction at the end of the 5 min pulse (0 chase) whereas no radioactivity is detectable in the insulin fraction. Radioautographs prepared from B-cells at the same time point (Table 1; Fig. 6a) show that up to 78% of the radioautographic grains are over elements of the rough endoplasmic reticulum,



Unless otherwise stated, all micrographs refer to rat islets. Freeze-etching micrographs were taken in the central part of islets, which consists mostly of B-cells in the rat [10].

Key to abbreviations

ac = acinar cells
cc = cell coat
cm = cell membrane or plasma membrane
cv = coated vesicles
cw = cell web
d = desmosome
ev = endocytotic vesicle
G = Golgi complex
gj = gap junction
ics = intercellular space
IGC = inner cisternae of the Golgi complex
m = mitochondrion
mg = maturing granule
mp = microvillous process
mt = microtubule
mv = microvesicle
n = nucleus
np = nuclear pore
OGC = outer cisternae of the Golgi complex
RER = rough endoplasmic reticulum
sg = secretory granule
Te = transitional elements of the rough endoplasmic reticulum
tj = tight junction

Fig. 1. Islets from control (a) and sulfonylurea-treated (b) rats. Aldehyde fuchsin stain [15] a) The deep staining of the cytoplasm (black in the figure) characterizes well-granulated B-cells. b) B-cell degranulation is striking. $\times 230$

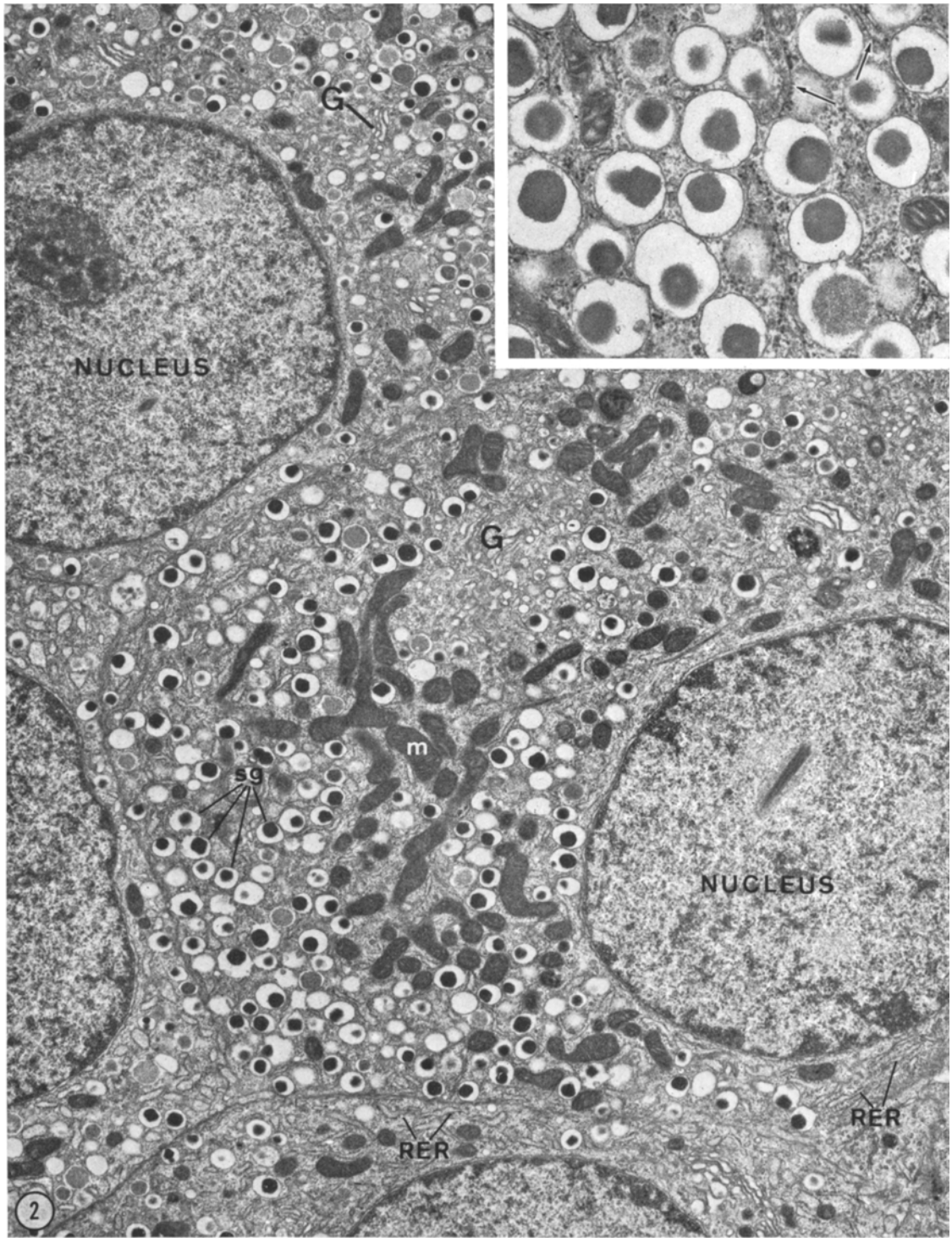


Fig. 2. Low magnification electron micrograph of granulated B-cells. The cytoplasm contains numerous storage secretory granules, whereas the rough endoplasmic reticulum is present in small amounts and the Golgi complexes are reduced in size and complexity. The inset shows a small field in the cytoplasm of a well-granulated B-cell. Elements of rough surfaced endoplasmic reticulum are scanty and interspersed between the granules (arrows). Such an image is characteristic of a cell in an inactive storage phase. Each storage granule is bounded by a smooth limiting membrane and contains a dense core of variable shape. $\times 9500$; Inset $\times 23000$

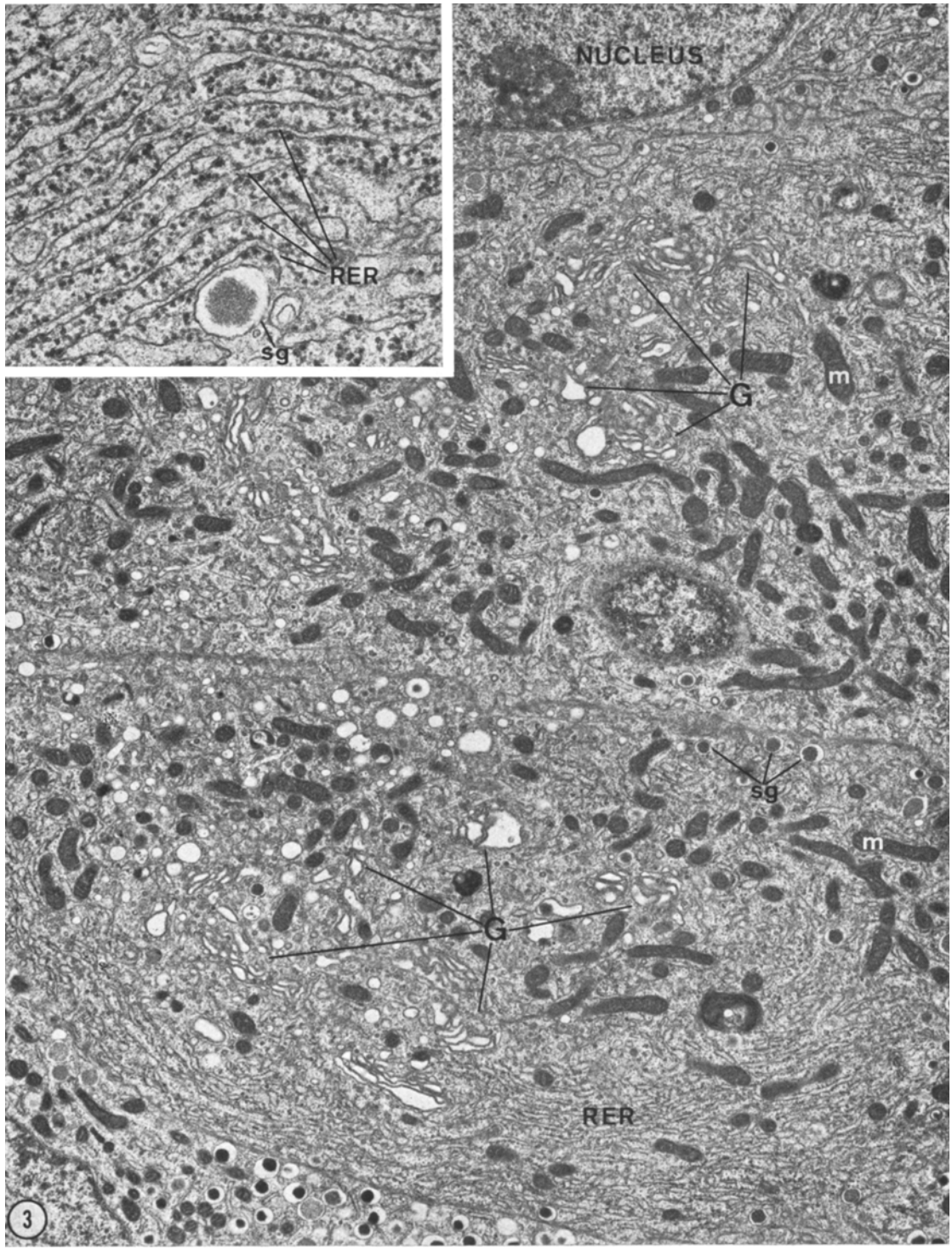


Fig. 3. Low magnification electron micrograph of B-cells containing only a few secretory granules. As is characteristic for cells that are specialized to synthesize exportable (secretory) proteins, the cytoplasm is occupied by abundant arrays of rough-surfaced endoplasmic reticulum (RER) and large Golgi complexes. The mitochondria (m) are also numerous and preferentially located in the Golgi area. The inset shows a small field in the cytoplasm of a degranulated B-cell. The numerous elements of rough surfaced endoplasmic reticulum appear as flattened cisternae which bear ribosomes attached to the outer surface of their limiting membrane. ×9500; Inset ×46000

where the labelled amino acid is presumably being incorporated into newly synthetized protein. At this time point, only a few grains appear over the elements of the Golgi region. Ten minutes after the end of the pulse, chromatography reveals that radioactivity is still present in the proinsulin fraction, although a drastic change is observed in radioautographs where most of the grains appear over structures of the Golgi complex (Table 1) while only a few grains appear over elements of the rough endoplasmic reticulum. The situation remains essentially the same thirty minutes after the pulse-labelling, (Fig. 6b) but there is a further change at sixty minutes, when part of the radioactivity has migrated from the "proinsulin" into the "insulin" peak and up to 50% of the radioautographic grains are seen over secretory granules which are localized in the Golgi region (Table 1; Fig. 6c; see also Fig. 7). Finally at ninety minutes, chromatography shows that radioactivity is predominantly present in the insulin fraction and at this time point, radioautography shows labelled secretory granules to be scattered throughout the B-cell cytoplasm (Fig. 6d).

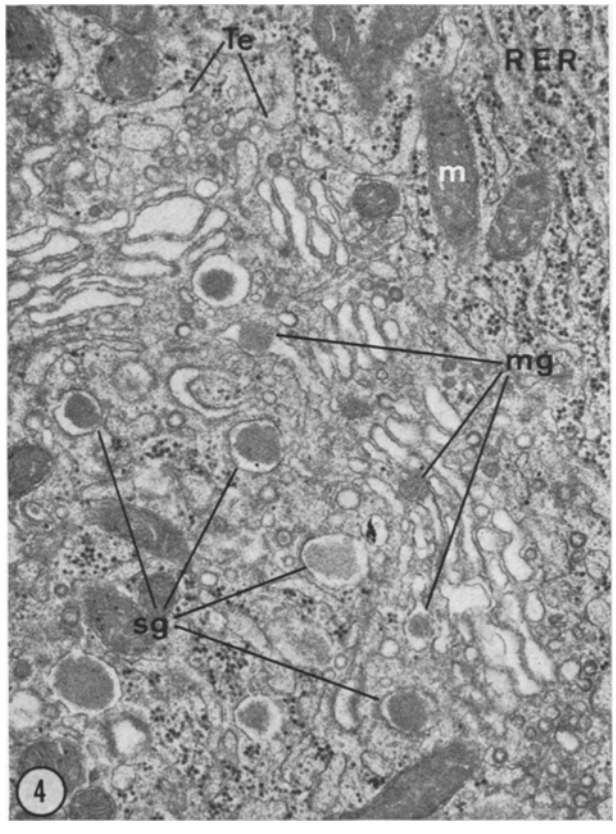


Fig. 4. The large Golgi complexes in actively synthesizing B-cells consist of stacks of curved cisternae arranged in concentric layers. Each stack has an outer or convex surface and an inner or concave surface. Three rounded masses (mg) consisting of a material similar in density and texture to the content of the beta granules (sg) are present within expanded regions of the innermost Golgi cisternae. Such images clearly implicate the Golgi complex in the intracellular pathway of secretory granules formation, and establish that the membrane limiting the secretory granule is acquired in the Golgi region (see also Fig. 8b). × 26000

b) Formation of the Secretory Granules

With the concept that granule formation occurs in the Golgi area, the need arises to account ultrastructurally for the process of transfer of the newly synthetized protein from the rough endoplasmic reticulum to the Golgi complex [51]. When one analyses carefully electronmicroscopic pictures of the Golgi region (Fig. 8), one is struck by the fact that Golgi stacks, which are usually bent so as to form crescent-like structures, are intimately associated with the RER cisternae. RER cisternae are close not only to the convex face of the crescent-shaped Golgi complex (Fig. 8a), but also close to its concave face (Fig. 8b). When approaching the surface of Golgi stacks, RER cisternae loose their ribosomes on that face oriented towards the Golgi stacks (transitional elements of the RER). Characteristically, at the convex face of the Golgi, the transitional elements undergo a process of vesicular budding (Fig. 8a), resulting in accumulations of smooth-surfaced microvesicles. It is generally considered that the main function of such microvesicles is to convey the secretory product from the RER to the Golgi. Indeed the budded vesicles are assumed to coalesce so as to give rise to the outer Golgi cisternae, both budding and fusion processes occurring through repetitive membrane fission and fusion. At the biochemical level, the processes of membrane fission and fusion probably represent the energy requiring event shown by Kemmler and coworkers [23] to be associated with the translocation of the proinsulin to the Golgi complex. As described above, whereas the transitional elements are located on both sides of the Golgi complex, the formation of the secretory granules seems to occur mostly at the extremities of the stacks and at the concave face of the complex (Figs. 4, 8b).

The Golgi complex thus appears to be asymmetric, and this apparent asymmetry is confirmed by histochemical techniques and by freeze-etching. When one performs osmic impregnation of the endocrine pancreas [58], one finds black deposits of osmium within the RER cisternae as well as within the outermost cisternae of the Golgi apparatus (Fig. 9). Conversely, the histochemical demonstration of the enzyme acid phosphatase [52] is possible mainly within the innermost cisternae of the Golgi as well as in a number of secretory granules (Fig. 10). Freeze-etching is now a well-established technique for studying the macromolecular organization of membranes [3, 38, 39, 61] (Figs. 11, 12). When examined

by this technique, all biological membranes share a common basic appearance in freeze-etching but differ one from another mainly by the number of membrane-associated particles [4] (see legend Fig. 12). These particles are assumed to be, at least in part, proteins, and their number has been used to characterize different membrane types. It has been shown for cisternae have only a few (up to 800/μ^2) (Fig. 13). So far we have not been able to examine sufficient material to determine whether the drop in the number of membrane particles within different regions of the Golgi apparatus occurs gradually from the convex to the concave side of the complex or whether it occurs in different areas of the same cisterna. Never-

Table 1*. *Distribution of Autoradiographic Grains over Cell Components*[a]

	Percent of autoradiographic grains				
		Chase incubation			
Cell component	5 Min (pulse)	+15 Min	+30 Min	+60 Min	+90 Min
RER and cytoplasm	78.3	27.3	19.3	22.4	28.6
Gogi complex	4.8	54.6	58.3	12.6	10.2
Secretory β granules	8.4	4.6	11.8	51.2	49.5
Mitochondria	4.8	5.8	5.2	5.3	6.9
Nuclei	3.6	7.5	5.2	8.2	4.6
No. of grains counted	383	372	328	316	430

* From Orci et al., 1973 [46]; courtesy of Journal of Ultrastructure Research.
[a] Examples shown in Fig. 6

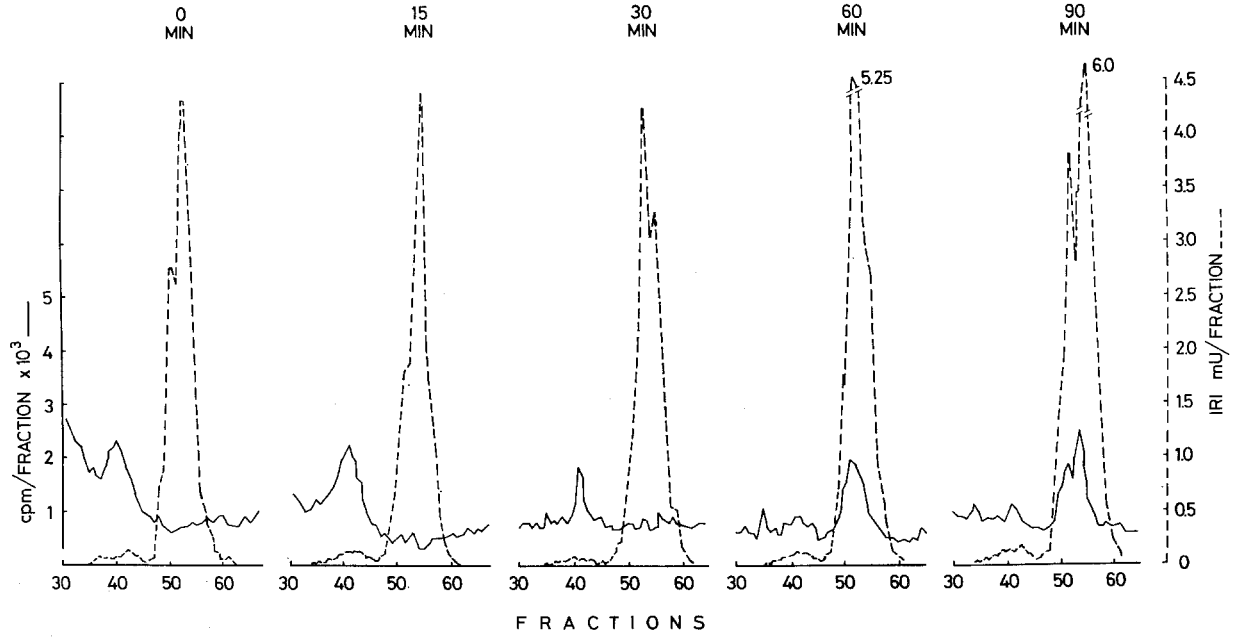


Fig. 5. Incorporation of leucine-H^3 into IRI (immunoreactive insulin) in pancreatic cell monolayer cultures. (from Orci *et al.*, 1973, ref. 46; courtesy of Journal of Ultrastructure Research).

example that metabolically active membranes have a large number of particles, while relatively inert ones have only few particles. When one measures the number of membrane-associated particles in the membranes of the Golgi region, one obtains a surprising result. Certain Golgi cisternae exhibit a large number of particles (up to 4000/μ^2), whereas other theless, we take the drop of the number of particles from the Golgi membranes to the secretory granule membrane (see Fig. 12) as an indication that membranes of the Golgi complex undergo extensive rearrangement before they are used to form the limiting membrane of the secretory granule. All the asymmetries noted with freeze-etching and cyto-

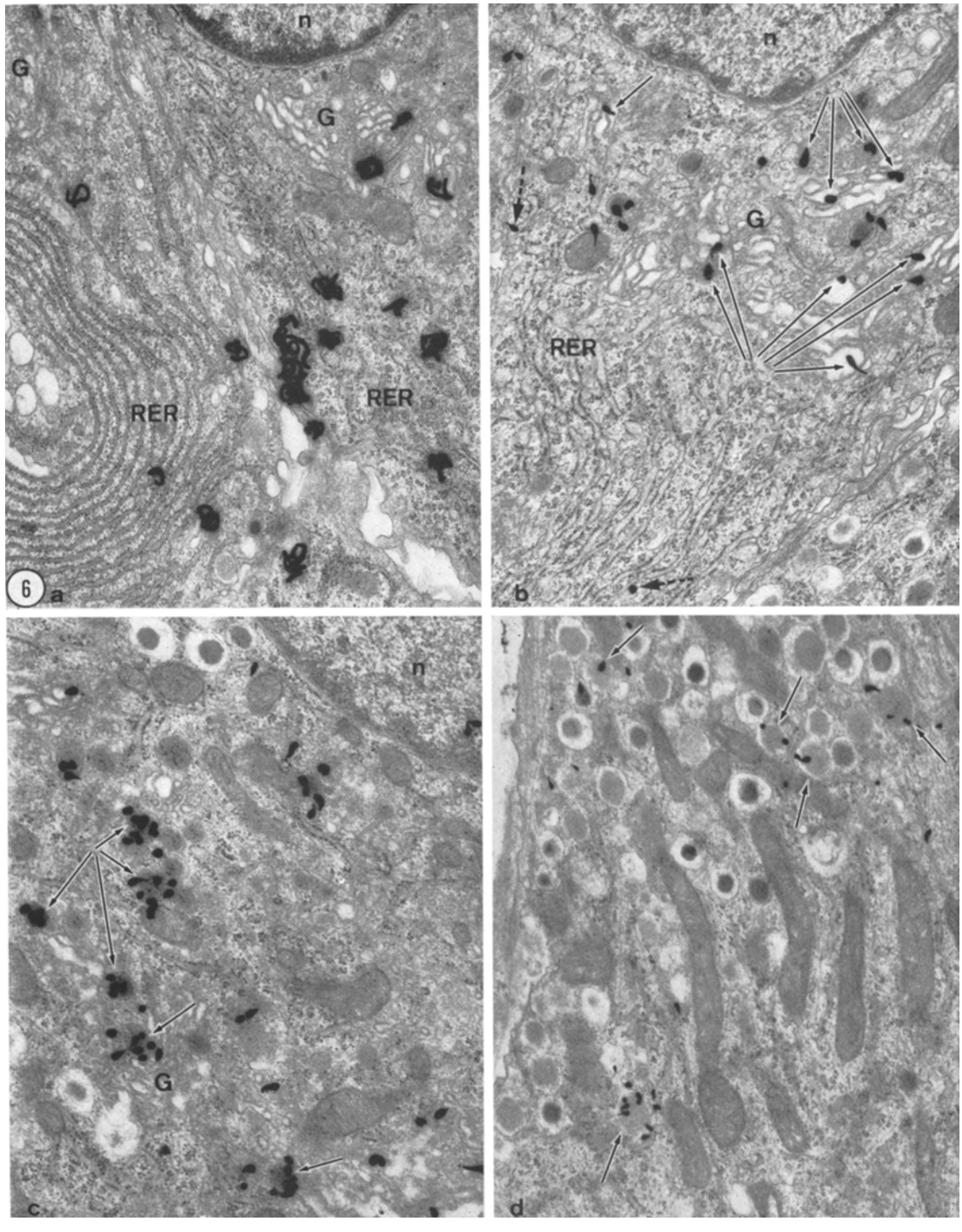
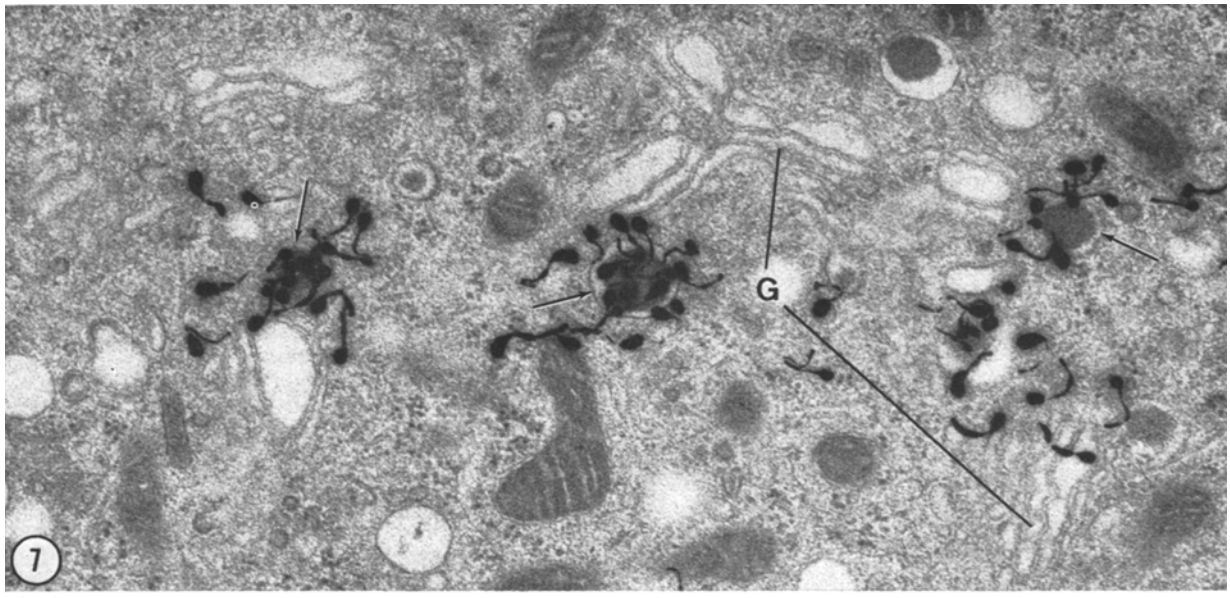


Fig. 6. Pancreatic endocrine cells after a 3-day monolayer culture. Electron microscopic radioautography of B-cells after pulse labelling with ^{3}H-leucine for 5 min (a), after an additional 30 min (b), 60 min (c) and 90 min (d) of incubation in a "chase" medium. Emulsion developed in Microdol — X (a) or in a fine grain "physical" developer (b—d). (*a*) Most silver grains are located over elements of the rough endoplasmic reticulum or the surrounding cytoplasmic matrix. A few grains are also found over the Golgi complex. (*b*) Most silver grains (solid arrows) overlie elements of the Golgi complex. A small number (dotted arrows) remain over elements of the rough endoplasmic reticulum or cytoplasmic matrix. (*c*) Silver grains are highly concentrated over secretory granules within the Golgi area (arrows). (*d*) Several labelled secretory granules (arrows) are scattered throughout the cytoplasm. a : ×15000; b : ×15500; c : ×17000; d : ×15000 (from Orci *et al.*, 1973, ref. 46; courtesy of Journal of Ultrastructure Research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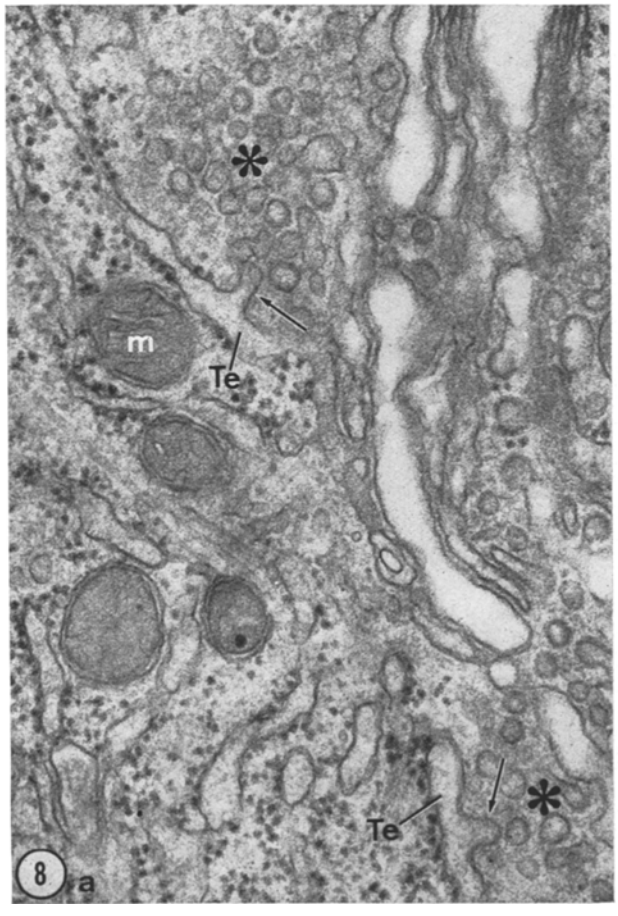
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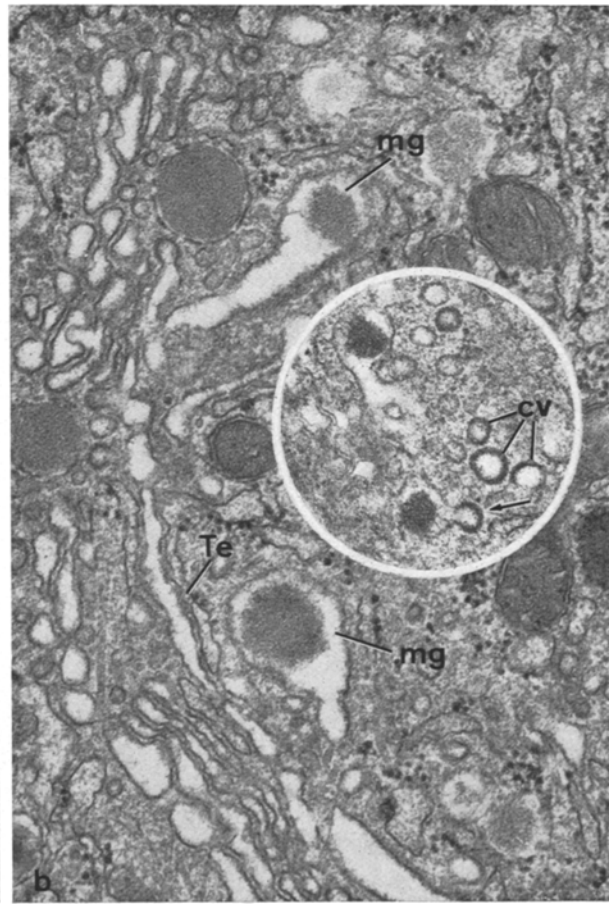
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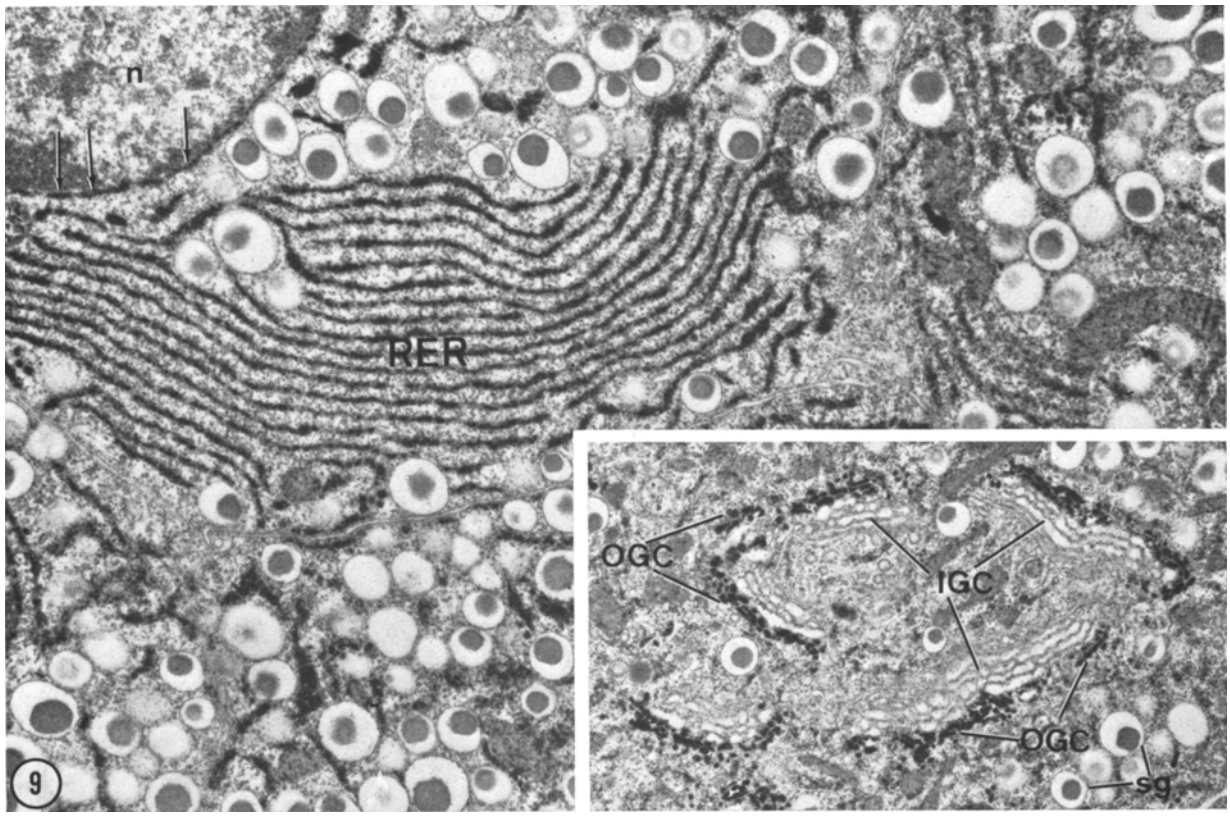

Fig. 7. Radioautograph of a B-cell from an isolated islet after a 60-min chase period following a 5-min pulse-labelling with leucine-H^3. Emulsion developed in a fine grain "physical" developer. Radioautographic grains are mainly associated with secretory granules in close proximity to the Golgi cisternae. ×29000

Fig. 8. Small fields in the Golgi region. (*a*) Profiles of endoplasmic reticulum adjacent to the outermost cisternae of the Golgi complex appear often partly smooth- and partly rough-surfaced. They represent the transitional elements of the rough endoplasmic reticulum (Te). The arrows point to vesicles budding from smooth-surfaced regions of the transitional elements. Two collections of similar vesicles are indicated by the asterisks. (*b*) Transitional elements of the rough endoplasmic reticulum at the concave face of the Golgi complex lie in close relationship with the innermost Golgi cisternae, but without interposed microvesicles. Although vesicular movement seems the most common mode of transport from the RER to the Golgi complex, B-cells may occasionally utilize such transitional elements as continuous pathways or channels from the RER to the Golgi complex. *Inset:* Collections of coated vesicles (cv) in the cytoplasmic region circumscribed by the inner Golgi cisternae. The arrow points to a coated vesicle opening into a Golgi cisterna containing a maturing secretory granule. It has been suggested that such coated microvesicles may function as carriers of substances involved in the conversion of proinsulin to insulin [51]. a : ×71000; b : ×67000; Inset : ×39000

Fig. 9. Small fields of B-cells in an isolated islet incubated in unbuffered OsO_4 for 40 h after glutaraldehyde fixation, according to Friend and Murray [13]. Black deposits are present within the cisternae of the endoplasmic reticulum and of the nuclear envelope (arrows), as well as in the outer cisternae of the Golgi complex (OGC) and their associated vesicles (Inset). Virtually no deposit is found within the inner cisternae of the Golgi complex (IGC) and their associated vesicles, nor in the secretory granules (sg). ×17000; Inset : ×11500

chemical techniques can therefore be interpreted as evidences for functional differentiations existing between the initial compartment of the secretory chain, the RER, and its terminal region, the mature secretory granules, and that these changes occur mostly in the Golgi region.

Another problem in integrating morphological and biochemical concepts is encountered in the identification of the site of conversion of proinsulin to insulin. From what we have discussed up to now, it seems clear that conversion of proinsulin to insulin begins somewhere between the RER and the mature secretory granules, that is at a time when the newly synthetized protein passes through the Golgi area [51]. This is in agreement with the data of Howell, Fink, and Lacy [17], who showed that labelled proinsulin was localized predominantly in the microsomal fraction, and labelled insulin in the secretory granules fractions. The containers of the converting enzymes are not known, but the best candidates at the moment are a distinct population of vesicles, the so-called coated vesicles [51], commonly found at the concave face of the Golgi complex (Inset Fig. 8b).

c) Release of the Secretory Granules

The next step of obvious interest in the natural history of the secretory process, is the mechanism by which the content of the secretory granules is released into the extracellular space. Electronmicroscopic observations of B-cells at definite intervals following acute stimulation of insulin secretion led Lacy to introduce the concept of emiocytosis (exocytosis) [26, 27, 64]. In this process, the granule membrane merges with the plasma membrane and the granule-core is extruded into the extracellular space where it undergoes dissolution. Since this original description, images of plasma membrane — granule membrane fusion have been obtained which strongly suggested

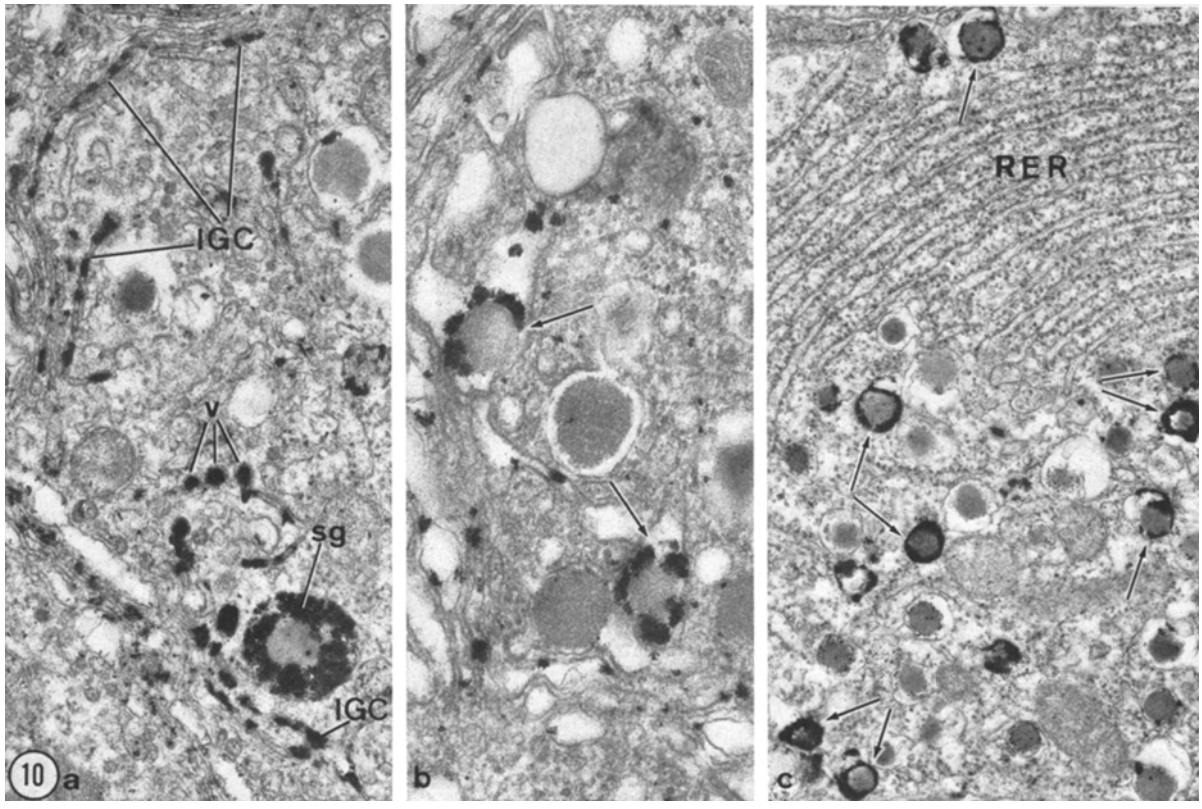

Fig. 10. Small fields of B-cells in an isolated islet incubated in a modified Gomori medium for revealing the sites of acid phosphatase activity (for details, see ref. 52). In *a*, the reaction product is localized mainly within the inner Golgi cisternae (IGC) and adjacent vesicles (v). In *b*, the reaction product is localized around two forming secretory granules within the Golgi cisternae (arrows). In *c*, acid-phosphatase reaction product labels secretory granules scattered throughout the cytoplasm (arrows). a : ×30000; b : ×42000; c : ×23000

that this suspected mechanism may be operative [44, 51, 56] (Fig. 14a). Studies with conventional thin-section electron microscopy do not allow, however, a quantitative appreciation of emiocytosis, probably owing to the infrequency with which the point of opening of the secretory granule at the plasma membrane coincides with the plane of a thin section. Thus, the view of Lacy was challenged by several authors, including myself, who advocated alternative routes of insulin release [7, 30, 31, 51]. Again we turned to freeze-etching, which by its exquisite capacity to expose membrane surfaces, allows us to quantify the event of emiocytosis (Fig. 14b—d) under different conditions of insulin release. We actually

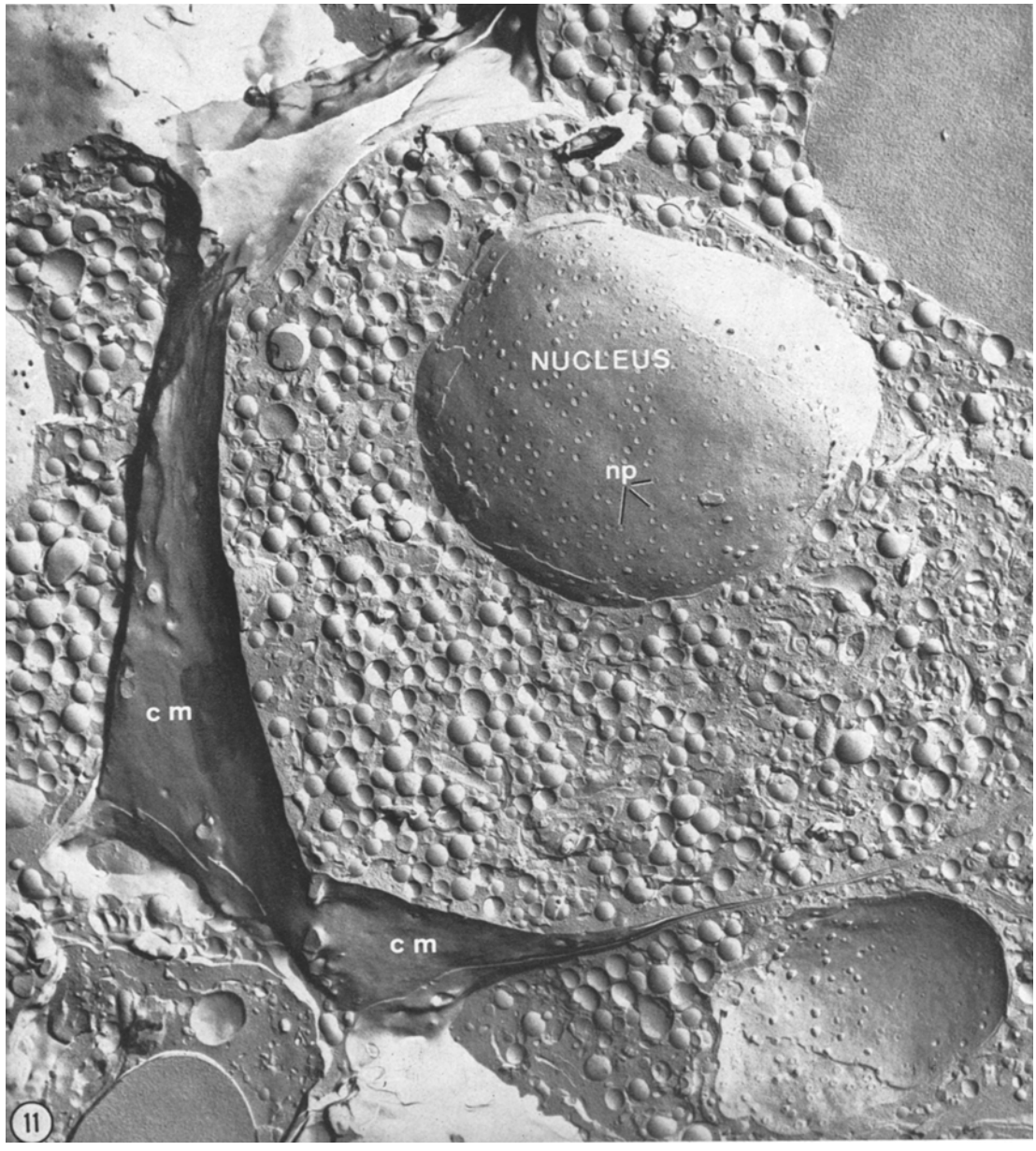

Fig. 11. Freeze-etch replica from an isolated islet. The freeze-etching technique characteristically reveals face views of membranes. The cells illustrated here have been cleaved in such a way that the fracturing process followed the cell membranes (cm) along their longitudinal plane for some distance before breaking through the interior of the cells, revealing the nucleus and numerous small globular profiles within the cytoplasm. Most of the globular profiles represent the membranous sacs of secretory granules. Freeze-fracturing splits membranes so as to expose their inner, hydrophobic matrix. For details, see the following figures. ×11000

found that the emiocytotic figures (in freeze-etching) were strikingly increased upon stimulation of insulin release (Fig. 15), thus reinforcing the original view that this process indeed represents an important modality for the secretion of insulin (42).

filamentous cell web are considered to play a critical role (for review see ref. 59). Experiments performed in order to test the contribution of the microtubular-microfilamentous system to the process of insulin release [29, 43] will however not be considered here,

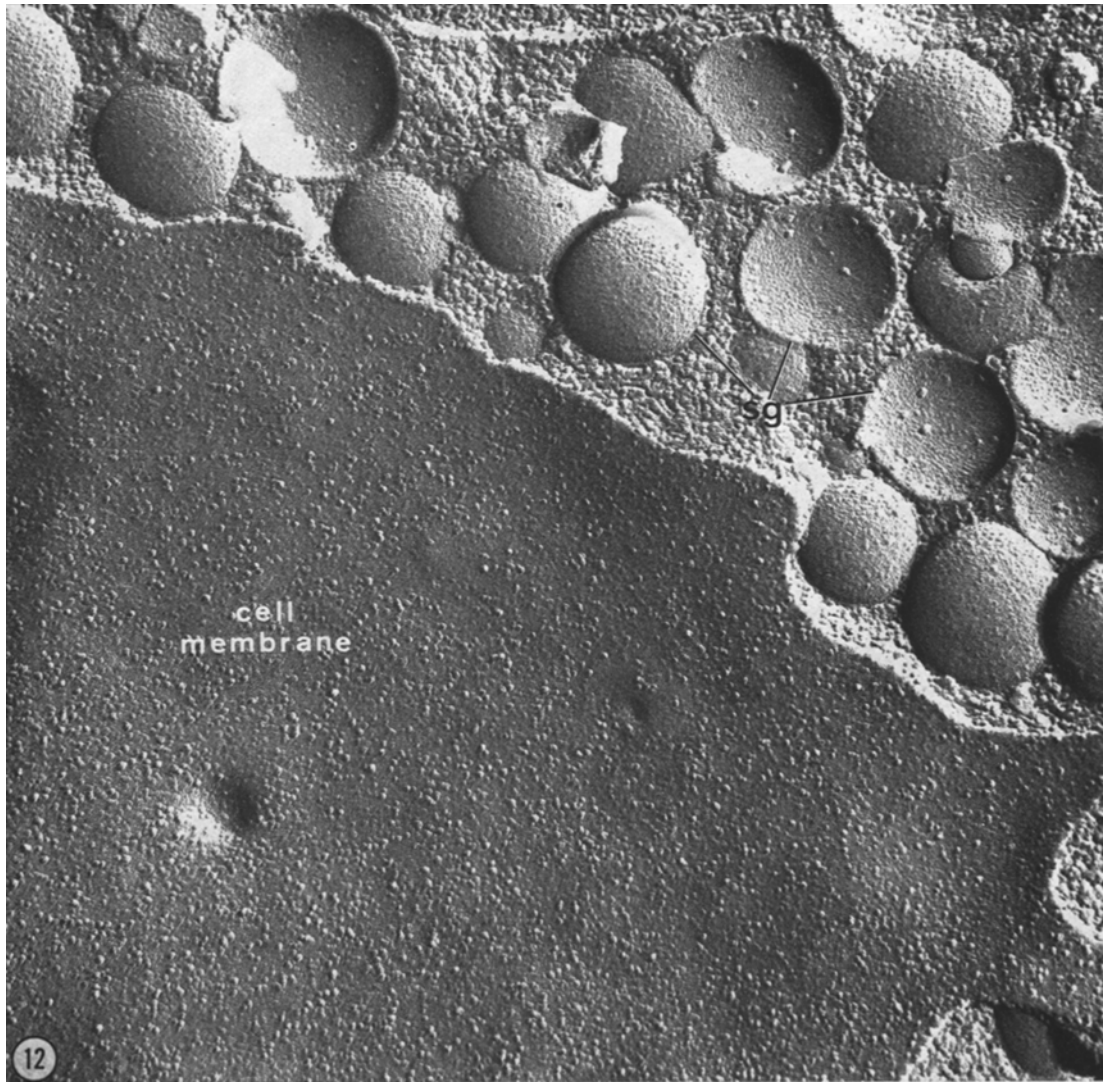

Fig. 12. At high magnification, a prominent feature of an exposed membrane matrix is a mosaic-like structure consisting of smooth areas (probably representing lamellar lipid regions) studded by particles (protein-containing structures). A striking difference is noticeable in the number of particles per unit membrane-area between the cell membrane ($\sim 2000/\mu^2$) and the granule limiting membrane ($\sim 200/\mu^2$). Moreover, the average diameter of membrane-associated particles varies from about 90 Å (plasma membrane) to about 135 Å (granule membrane). $\times 69\,500$

It is obvious that before the formation of the emiocytotic figure the granule must move to the cell membrane. In all cell processes which involve directional movement, such as intracellular translocation of secretory granules, microtubules and the micro-

since they have been reviewed in Dr. Malaisse's 1972 Minkowski Lecture [34]. We wish now to turn our attention to another active event taking place at the level of the cell membrane, one also associated with the emiocytotic process.

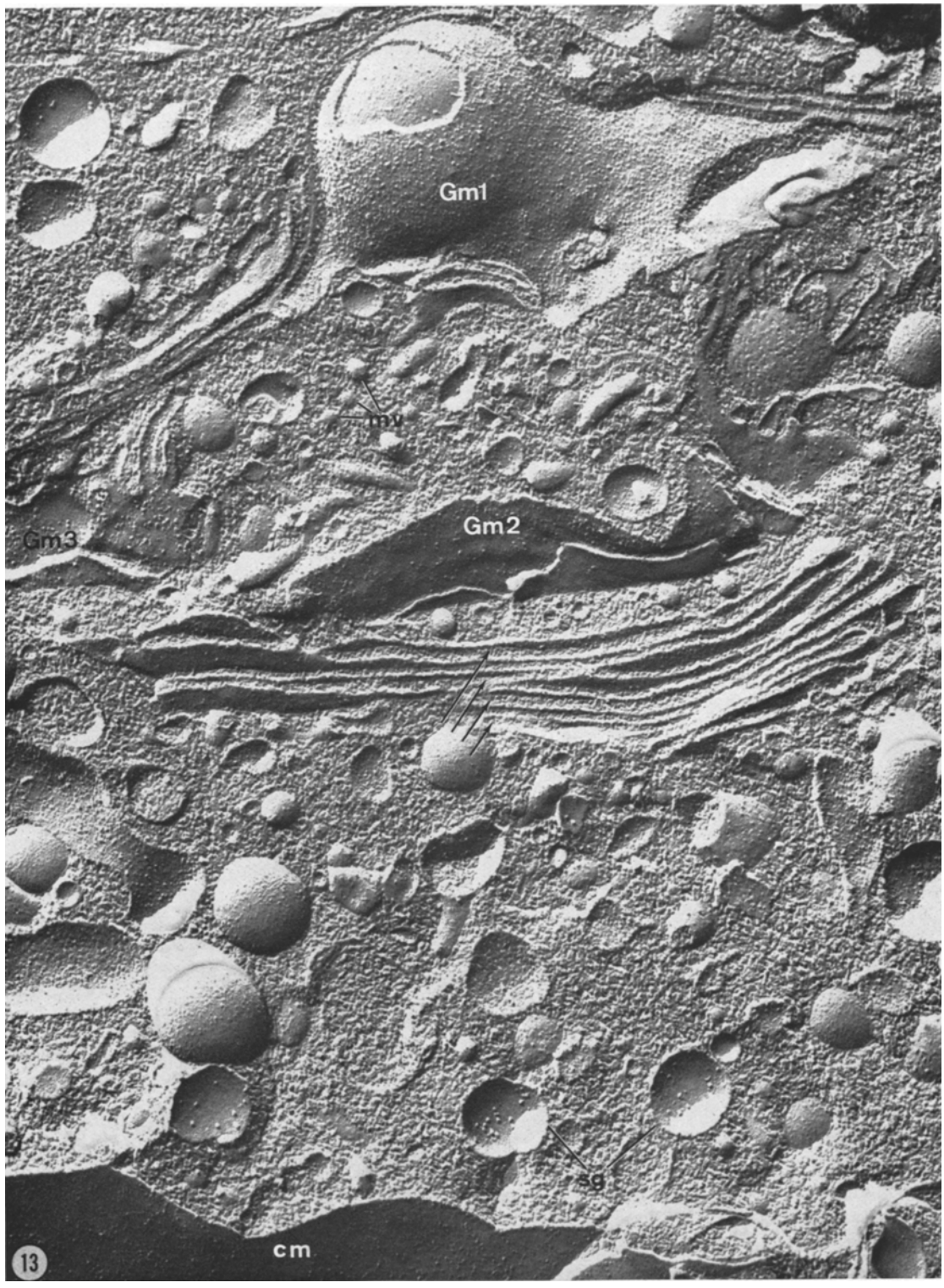

Fig. 13. Freeze fracture across a Golgi complex. The cisternal membranes are seen in cross-sectional (parallel arrows) as well as in face views (Gm 1, Gm 2, Gm 3). A striking difference in particle density is observed between Gm 1, Gm 2 and Gm 3. This change in the number of membrane-associated particles together with the observation that the Golgi-derived granule membranes (sg) carry only a few particles (see also Fig. 12) indicate that Golgi membranes undergo modifications before they are used for enclosing the secretory product. $\times 56000$

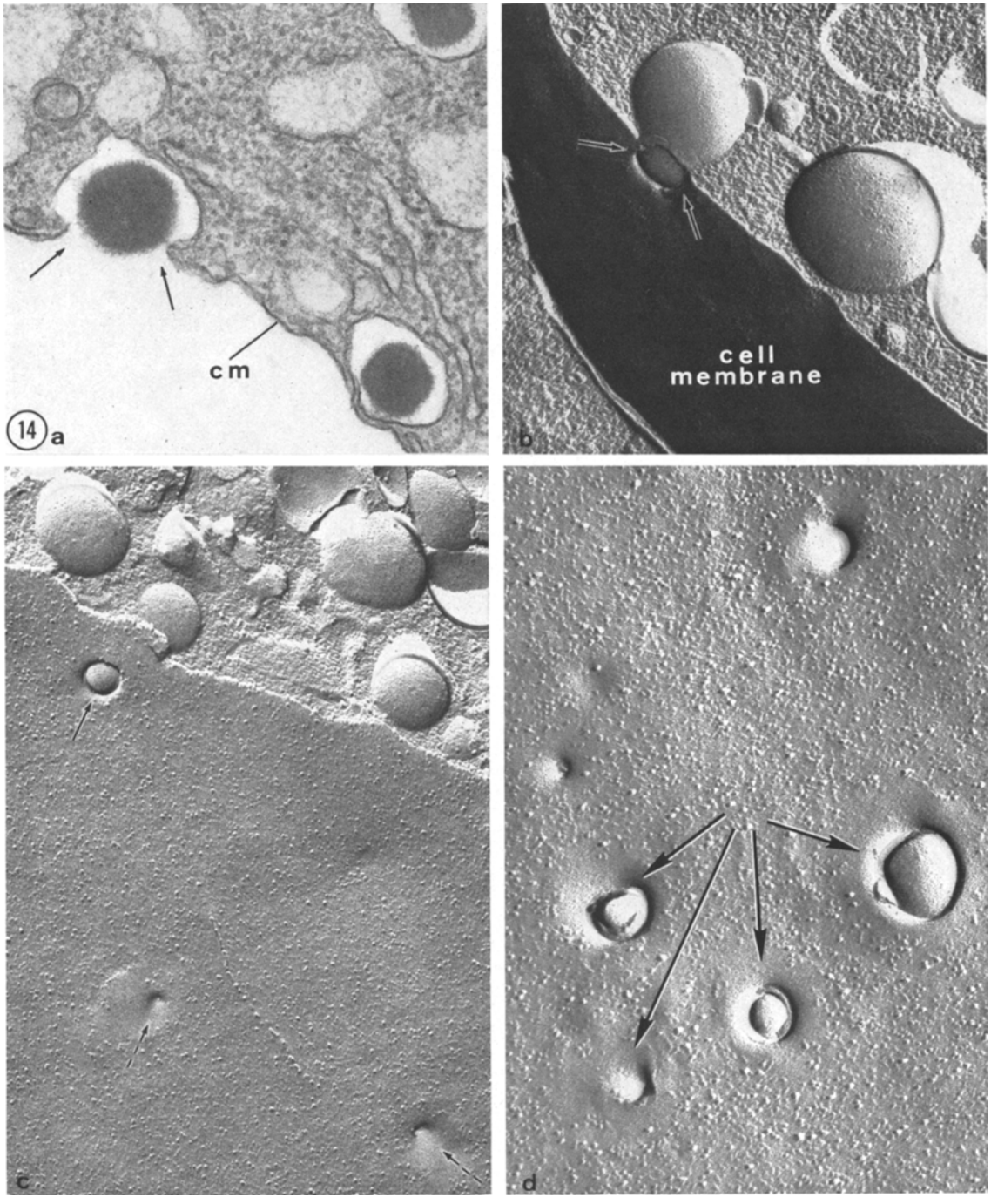

Fig. 14. *a*) Thin section of a B-cell. A granule core is being discharged into the extracellular space through the opening (arrows) created by the coalescence of the granule-limiting membrane with the plasma membrane (cm). *b*) Freeze-etch replica of a B-cell depicting a secretory granule connected to the extracellular space by a stoma (arrows) in the plasma membrane. (Orci *et al.*, 1973, ref. 42; courtesy of Science) *c*) The arrows point to sites representing probable stages of emiocytotic events on the plasma membrane. Note that, in the sites indicated by the dotted arrows, the membrane appears virtually free of particles. This aspect could be the result of the recent addition of the poorly particulated granule-limiting membranes to the richly particulated plasma membrane. Such a transient phenomenon indicates the capability for membrane-associated particles to perform translational movements along the plane of the membrane. *d*) Replica of a freeze-etched human islet. This micrograph clearly shows emiocytotic stomata occurring at the plasma membrane (arrows). Note that the portion of plasma membrane rimming each stoma is poorly particulated. a: × 44 000; b: × 45 000; c: × 46 000; d: × 69 500

Fig. 15. Freeze-etch replica of an isolated islet incubated for 3 h in the presence of 3.0 mg/ml glucose. Numerous emiocytotic stomata (arrows) appear on the exposed face of the plasma membrane. ×35000

d) Membrane Recycling Associated with Secretion

A direct consequence of emiocytosis would be that during periods of increased secretory activity, the surface area of the B-cell would increase considerably, as a result of the addition to it of many granule membranes. In order to keep the cell with a rather constant volume there must be a withdrawal of membrane, equivalent to the amount added by emiocytosis. This withdrawal could theoretically be performed by two mechanisms:

a) By endocytosis, that is the reversal of the process of exocytosis. In this process, plasma membrane is transferred back into the cytoplasm in the form of various membrane-bound vesicles and vacuoles.

b) By a gradual and morphologically undetectable breakdown of cell membrane.

Peroxidase tracing was employed to seek evidence for the first mechanism [47]. If endocytosis occurs, peroxidase will be taken up within vesicles derived from the plasma membrane and labelled vesicles could be followed in their intracellular route (Inset Fig. 16). Such an experiment has been performed under conditions of minimal and maximal insulin release (incubation of islets without glucose or with glucose at 3.0 mg/ml). While relatively few peroxidase-containing vesicles could be detected in the B-cells in the absence of glucose (Fig. 16), a marked accumulation of vesicles containing peroxidase was present within the cytoplasm of cells incubated in the

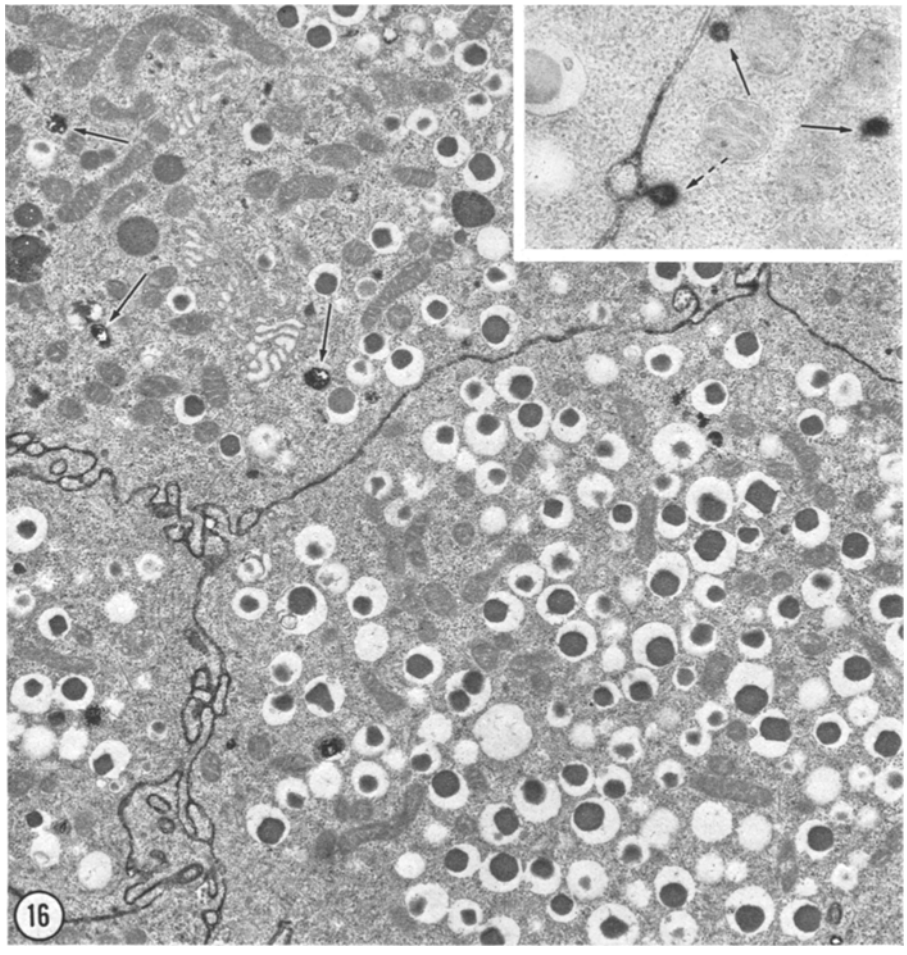

Fig. 16. Electron micrograph of B-cells in an islet incubated for 60 min in the presence of peroxidase and absence of glucose. The dense reaction product is present in the intercellular space and in a few vesicles (arrows) within the cytoplasm. The inset illustrates the route of entry of the marker. Endocytotic invaginations (dotted arrows) carrying the electron-dense marker pinch off from their surface connection so as to form peroxidase-containing, membrane-bound vesicles within the cytoplasm (solid arrows). ×11000; Inset ×30000

presence of a high glucose concentration (Fig. 17). However, since the intensity of this process varies somewhat from cell to cell, we have applied the stereological principles of morphometry for the measurement of the surface density of peroxidase-positive vesicles. As judged from such quantitative data, the endocytotic activity over 60 min of incubation was three times greater in the presence of glucose than in its absence (surface density = 0.429 $\pm$ 0,026 and 0.135 $\pm$ 0.020, respectively; n = 9 in each case; $p < 0.0005$).

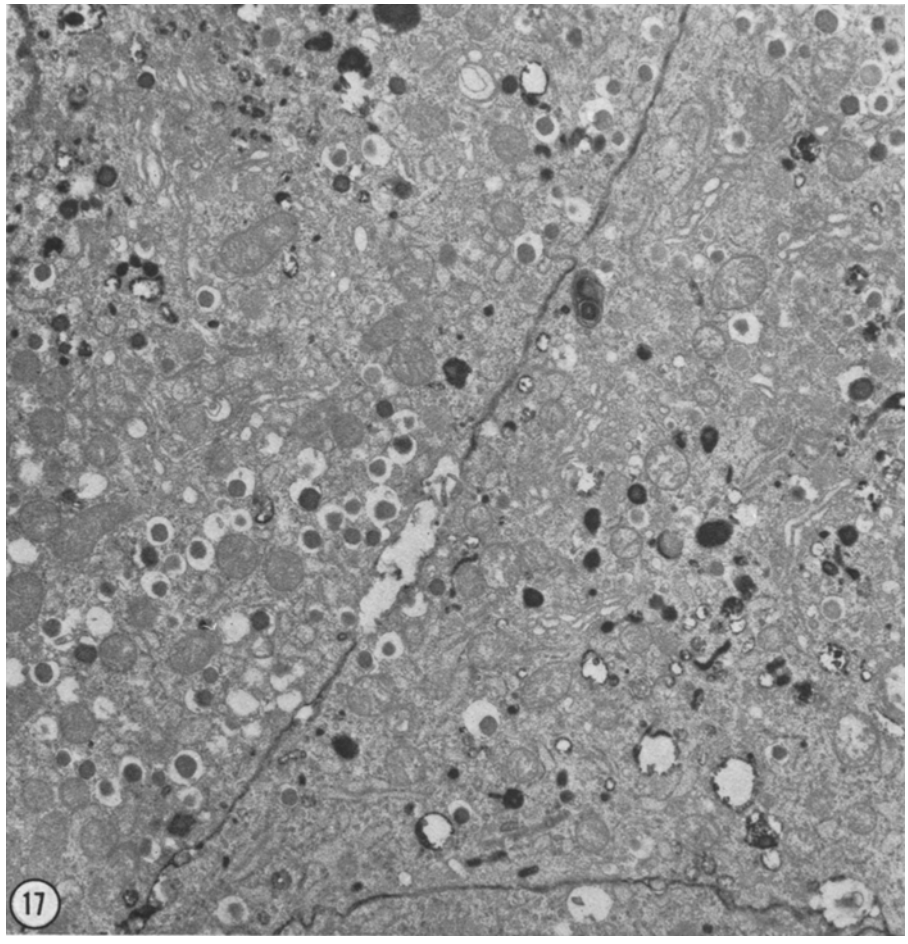

Fig. 17. Electron micrograph of B-cells in an islet incubated for 60 min in the presence of 3.0 mg/ml glucose and peroxidase. Numerous vesicles of various sizes containing reaction product are present within the cytoplasm. $\times$ 10 000

These findings thus indicate that the release of secretory granules by emiocytosis is not associated with the loss of intracellular membranous material, since a relocation of B-cell plasma membrane via endocytotic vesicles occurs concomittantly and consequently to insulin release [47].

e) Intercellular Contacts within the Islet

After having discussed the organization of individual B-cells, we might ask ourselves what are the mechanisms by which individual B-cells become linked one with another so as to form a functional entity within the islet of Langerhans, such as physiologists imply every time they measure the insulin output of an islet, *in vivo* or *in vitro*. Among these mechanisms, one has to take into consideration the autonomic innervation of the islet cells (Fig. 18) and the properties of cell surfaces.

It is well known that islet cells are provided with both cholinergic and adrenergic innervation (for review see ref. 9) and recently it has been shown that some nerve endings share with islet cells membrane differentiations ensuring electrical coupling [49].

There is growing evidence for a possible control of the hormonal output by the nervous system and in this context, it might be worthwhile to recall that the spiny mouse (*Acomys cahirinus*) shows an abnormal insulin secretory response after stimulation and a apparent lack of nerve endings among islet cells [44].

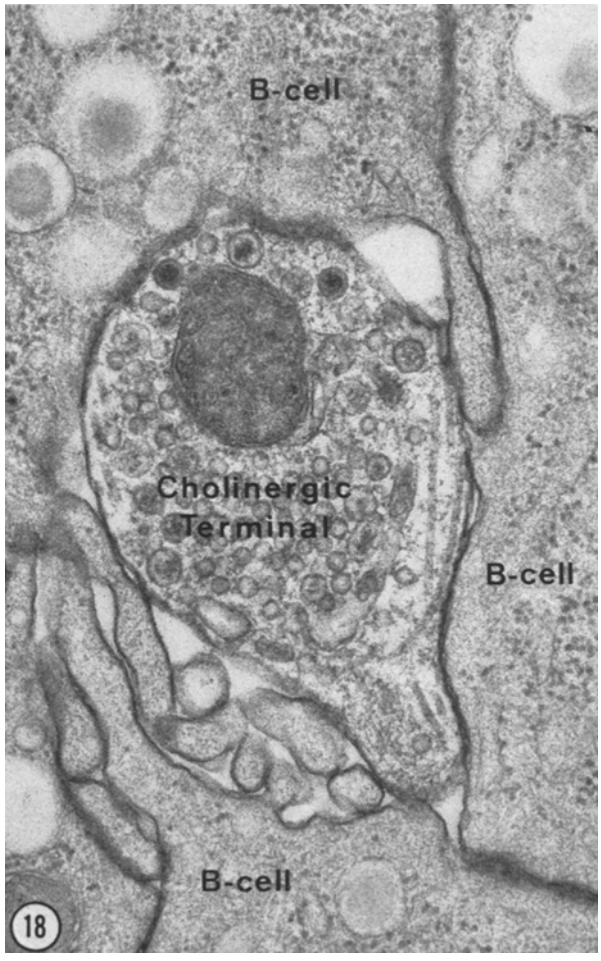

Fig. 18. Nerve ending of cholinergic type having several points of close contact with B-cells. Some endings have been shown to possess cytological characteristics for both electrical and chemical transmission [49]. ×40000

As far as the properties of cell surfaces are concerned, intensive investigations performed in the last ten years and directed primarily to the understanding of the basic behaviour of cancer cells have pointed out two components of the cell membrane which could be implicated in the maintenance of functional relationships between individual cells within a tissue. These two components are the cell coat and the intercellular junctions. The cell coat (for review see ref. 36, 57) is now understood as a thin layer of glycoproteins and glycolipids which are tightly bound to the outer surface of the plasma membrane. This layer can be demonstrated with special histochemical techniques and when such techniques were applied to isolated islets of Langerhans (Fig. 19), we were able to demonstrate the presence of a definite cell coat around all cell types of the islet [35, 50]. So far, we do not have evidence pointing to a specific role

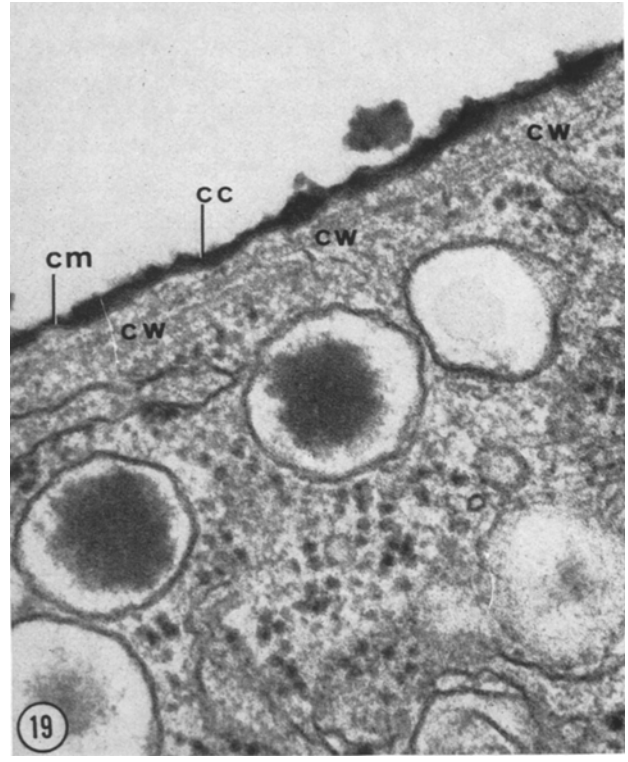

Fig. 19. Pancreatic monolayer cell culture fixed in glutaraldehyde-Alcian blue [2]. The free surface of a B-cell is heavily coated with stained material (cc = cell coat). It has been shown in a variety of cell types that the cell coat is composed of glycoproteins and glycolipids. ×60000

of the cell coat in B-cell secretion but experiments in which the cell coat is specifically removed by enzymatic digestion are under way and will hopefully throw some light on this point.

Turning our attention to the intercellular junctions, we know that one of these, the gap junction, may be of importance for the mediation of intercellular communication ,since in other tissues such structures have been shown to allow for electrical and metabolic coupling between individual cells. Indeed gap junctions, first recognized by conventional thin section electron microscopy [60] and then identified unambiguously on freeze-etch replicas [16, 24, 37], have been shown to be the site of a low resistance pathway between cells through which ions and small molecules (mw $<$ 500) can diffuse freely from one cell to another

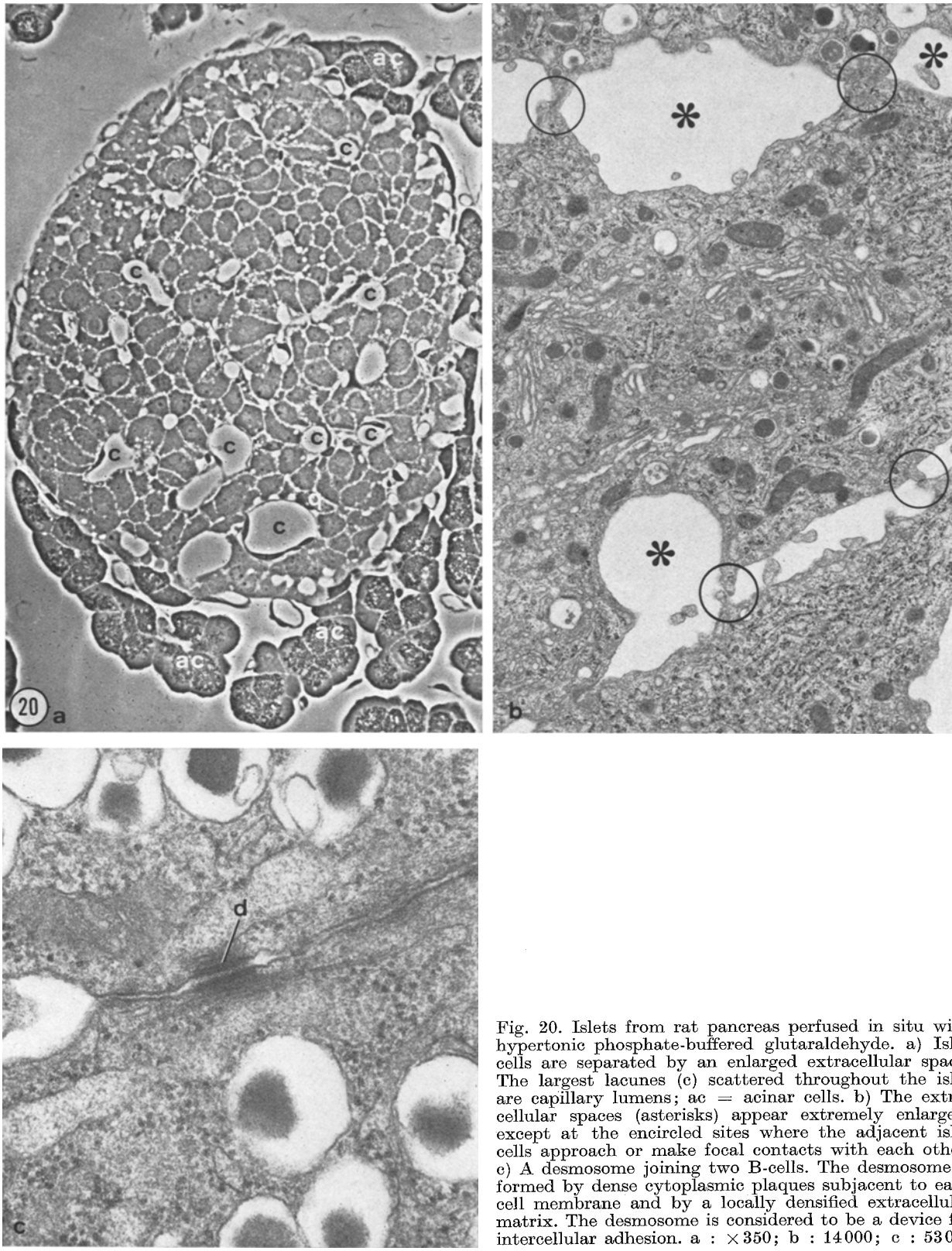

Fig. 20. Islets from rat pancreas perfused in situ with hypertonic phosphate-buffered glutaraldehyde. a) Islet cells are separated by an enlarged extracellular space. The largest lacunes (c) scattered throughout the islet are capillary lumens; ac = acinar cells. b) The extracellular spaces (asterisks) appear extremely enlarged, except at the encircled sites where the adjacent islet cells approach or make focal contacts with each other. c) A desmosome joining two B-cells. The desmosome is formed by dense cytoplasmic plaques subjacent to each cell membrane and by a locally densified extracellular matrix. The desmosome is considered to be a device for intercellular adhesion. a : ×350; b : 14000; c : 53000

[21, 55]. So far, islet cells have been described to make focal contacts with each other [51] (Figs. 20a, b) and to carry desmosomes (Fig. 20c), an intercellular junction which has no coupling capacity, but is mainly effective as an attachment device between cells against disruptive shearing forces.

However, when the technique of freeze-etching was applied to islets, its capacity for showing surface differentiation was again effective in revealing many aggregates of membrane associated particles characteristic of gap junctions [53] (Fig. 21). Later, using the more difficult technique of lanthanum staining of the extracellular space, these junctions were also demonstrated by the conventional thin section technique but is was immediately evident that most of them would always escape detection by this method, mainly because of their small size. Islet cells of all animals species so far examined seem to be equipped

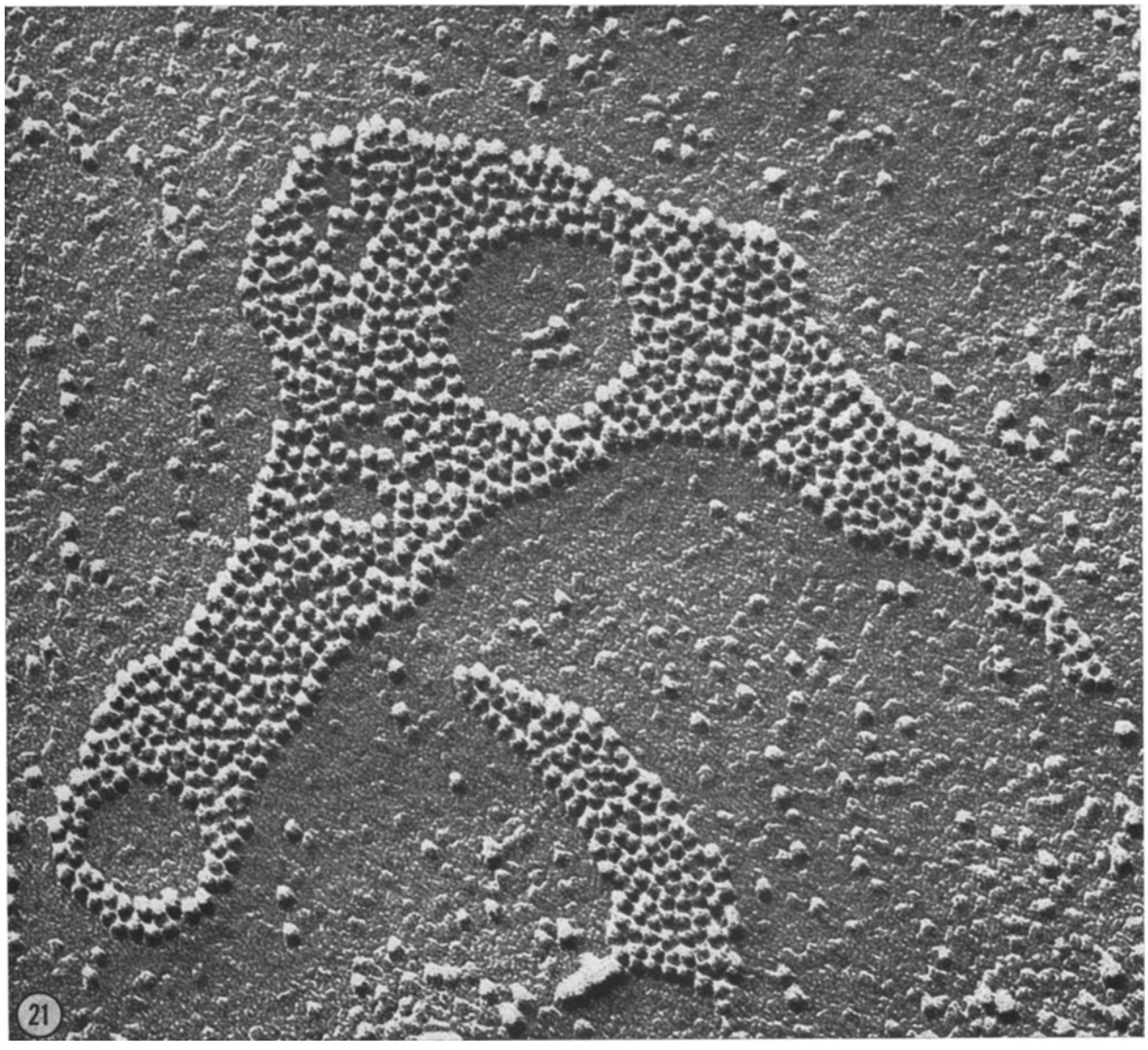

Fig. 21.Freeze-etch replica of islet cells. Fracture face of a cell membrane showing two macular gap junctions. These are composed of closely packed membrane-associated particles. The particles present in the gap junction are remarkably regular in both shape and dimension, contrasting with the heterogeneity frequently exhibited by the particles in the undifferentiated zones of the plasma membrane. The three-dimensional model of the gap junction that is most consistent with currently available morphological and physiological data proposes, in the center of each particle, a pore which could represent an intercellular diffusion channel isolated from the extracellular space and bridging 2 adjacent cells. $\times 203000$

for intercellular coupling by the means of gap junctions; recently we were fortunate to examine human islet cells and were able to observe the same membrane differentiation [54] (Fig. 23b).

Another junctional specialization revealed on islet cells by freeze-etching and frequently associated with gap junctions is the so-called tight junction [40, 53] (Fig. 22, 23). However, with the exception of tubular epithelia where a continous tight junction is present on the lateral surface of the cells and probably seals the lumen of the tube towards the extracellular fluid [8, 12, 16, 14, 48], the functional role of tight junction in parenchymal tissue is still a mystery. This holds true for the islet cells as well,

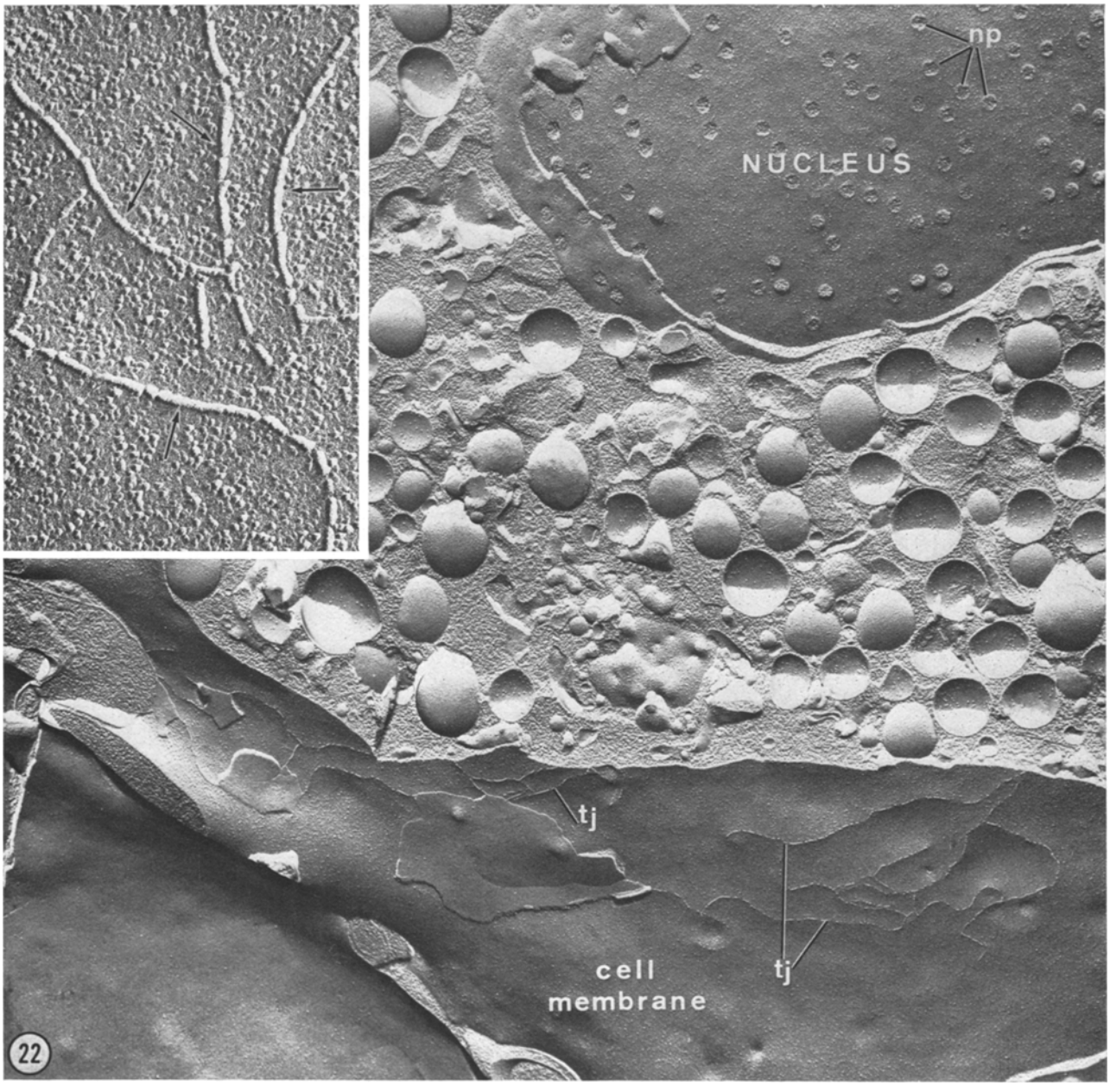

Fig. 22. Freeze-etch replica of islet cells. Focal tight junctions (tj) appear on the exposed face of the plasma membrane as membrane-associated linear elements. The inset shows at high magnification the branching and anastomosing ridges characteristic of the tight junctions. In suitable conventional thin sections, a tight junction can be seen as the merging of the outer leaflets of two contiguous cell membranes. No extracellular space is present in the fused area. ×21000; Inset ×81000

where under normal conditions the tight junctions are focal and rather short. By incubating the islets in pronase [40] or pancreatic proteases [50] we have been able to increase dramatically the number and the length of tight junctions (Fig. 24). However we feel that this experiment, rather than throwing light on the role and function of tight junctions, demonstrates the enormous plasticity of junctional differentiation. Again with the painstaking technique of lanthanum tracing, we could show that tight and gap junctions occur not only between cells of identical type (B or A for example) but also between cells of different types (B and A).

Taken together, these results, however fragmentary and incomplete, leave open the possibility of multiple approaches so as to test a hypothesis which considers an islet as a large functional syncytium working as a single multihormonal unit. For example, intercellular exchanges of a variety of molecules including the cyclic nucleotides through gap junctions is a fascinating possibility.

On the basis of what has been discussed up to now, one can present a schematic view (Fig. 25) summarizing the respective roles and sequence of participation of various cell constituents in the B-cell secretory cycle. Integrated with current notions on protein synthesis, this secretory cycle includes: 1. incorporation of amino acids into proteins at the ribosomes attached to the membranes of the endoplasmic reticulum and vectorial transfer of these nascent proteins through the membrane of the RER into the cisternal space; 2. transport of these proteins to the Golgi complex; 3. where the conversion of proinsulin to insulin may begin and where the secretory product is packed to form beta granules; 4. migration of the beta granules towards the plasma

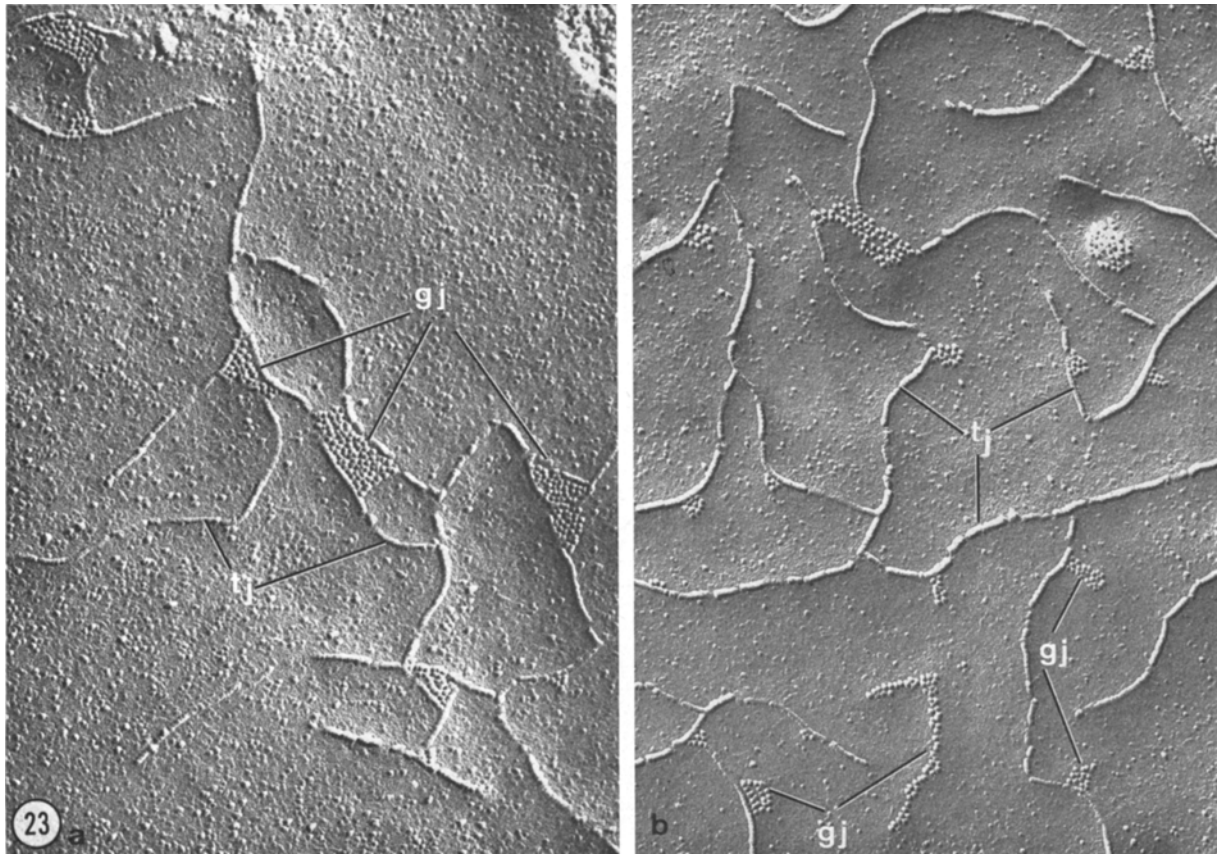

Fig. 23. In both rat islet (*a*) and human islet (*b*) a close association of small gap junctions with the elements of tight junctions is frequently observed. The small size of both types of junctions and the limited areas that they involve explain why they could easily escape detection in conventional thin sections. a : 48000; b: ×63000

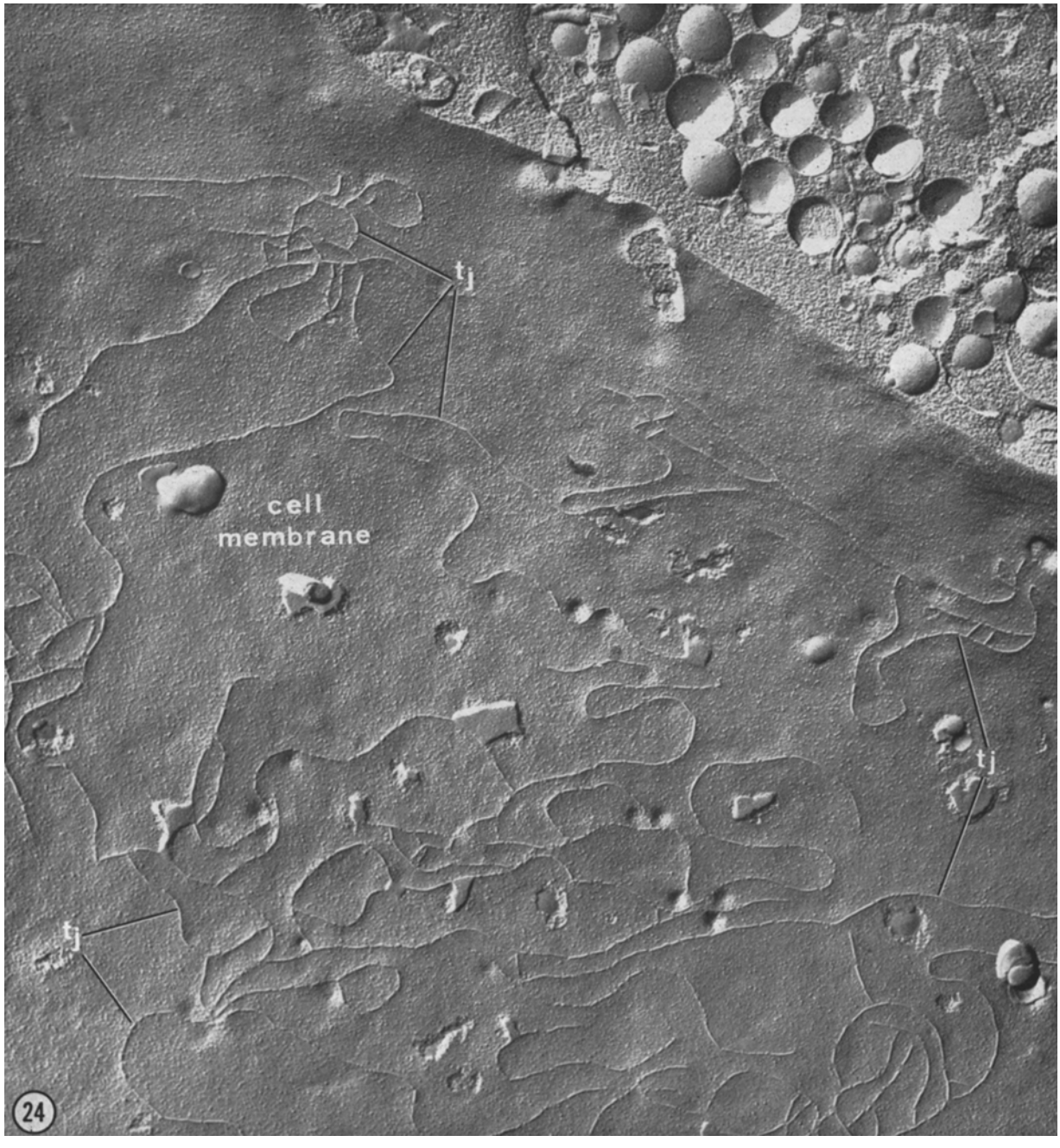

Fig. 24. Replica of freeze-etched islet cells treated with pancreatic protease (Sigma, type I, 10 μg/ml) for 90 min. The tight junctional elements are numerous and involve a large area of the cell membrane. ×27000

membrane via microtubular-microfilamentous system, discharge of the content of the granules into the extracellular space, and 5. recapturing of fragments of plasma membrane. These different events are probably influenced by the processes of intercellular communication occuring at the cell surface, and by the autonomic nervous system.

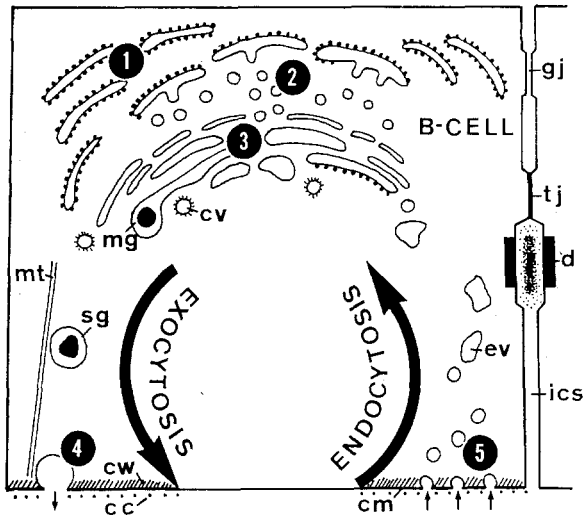

Fig. 25. Diagram summarizing the sequence of participation of various cell constituents in the B-cell secretory cycle. (See text).

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