Ca^{++}-DEPENDENT DISASSEMBLY AND REASSEMBLY OF OCCLUDING JUNCTIONS IN GUINEA PIG PANCREATIC ACINAR CELLS

Effect of Drugs

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ABSTRACT

Incubation of guinea pig pancreatic lobules in Ca^{++}-free Krebs-Ringer bicarbonate solution (KRB) containing 0.5 mM ethylene glycol-bis(β-aminoethyl ether)N,N,N',N'-tetraacetate (EGTA) results in the progressive fragmentation of the occluding zonulae (ZO) with formation of multiple discrete junctions (fasciae occludentes) localized in the lateral and lumenal plasmalemma. After 1-2 h of such incubation, most ZO appear completely disassembled. This results in the disappearance of the heterogeneity in density of intramembrane particles on the P-fracture faces of the basolateral and lumenal plasmalemma. If Ca^{++} ions are reintroduced into the incubation fluid at this point, continous zonulae reform around the apices of the cells; in contrast, the density of intramembrane particles (imp) at the lumenal plasmalemma remains the same as in the basolateral region, at least for 3 h after Ca^{++} reintroduction. When added to the incubation fluid, cycloheximide (at a dose known to inhibit protein synthesis >95%) and cytochalasin B (at doses which disrupt microfilaments and modify the cell shape) had no effect on the organization of ZO, on their disassembly in Ca^{++}-free, EGTA medium, or on their Ca^{++}-dependent reformation. Likewise, the organization and disassembly of ZO were unaffected by colchicine; however, after treatment with the latter drug the reassembly was defective, with formation of strand networks on the lateral surface and incomplete segregation of the lumenal region. Antimycin A, on the other hand, when added to the Ca^{++}-EGTA medium, induced a large proliferation of long, infrequently anastomosed junctional strands, usually arranged to form ribbons, festoons, and other bizarre arrays. The possible relationship of these in vitro findings to the in vivo biogenesis and turnover of occluding junctions is discussed. It is suggested that the impairment of reassembly of zonulae by colchicine might be correlated with the disorder induced by the

J. Cell Biology © The Rockefeller University Press · 0021-9525/78/1001-0156$1.00
Volume 79 October 1978 156-172

drug on the general organization of pancreatic exocrine cells. Moreover, antimycin A could act by promoting the aggregation of a pool of free junctional strand components (or precursors) that might exist normally in pancreatic exocrine cells.

KEY WORDS exocrine pancreas · freeze-fracture · zonulae and fasciae occludentes · cell surface topology · colchicine · antimycin A

Since the introduction of freeze-fracture in biological studies, considerable information has accumulated on occluding (or tight) junctions. These structures, originally described in thin sections by Farquhar and Palade (19), are now known to be composed of elaborated arrays of interconnected strands, which appear as complementary ridges and furrows, respectively, in P- and E-fracture faces (see references 34 and 57 for reviews). Both the geometry of the arrays and the organization of individual strands can vary widely in different cell systems. Thus, in epithelium lining the lumen of cavity organs, occluding junctions are arranged mostly as continuous beltlike structures, localized at the apical margins of cells, that are designated as zonulae occludentes (ZO),[1] whereas in other epithelia (for instance, in most endocrine glands as well as in other tissues) they form discontinuous arrays of smaller size, called maculae or fasciae occludentes (34, 57). On the other hand, individual junctional strands appear mostly continuous in some systems (11, 21, 57); in others they are composed of discrete, closely adjacent bars, aligned in rows (11, 13, 21, 39, 55), while in others they consist of rows of smaller bars and particles (23, 47, 54, 64).

Studies carried out on embryonic and adult tissues, as well as on cultured tissues, have yielded information on the dynamics of occluding junctions. It has been shown that these structures are not always stable but can assemble, grow, and even disassemble depending on cell differentiation as well as on a variety of other physiological and experimental conditions. Assembly seems to involve, first, the aggregation of linear chains of intramembrane particles (imp), which then fuse to yield discontinuous junctional strands. Ultimately, the strands merge into continuous zonulae or fasciae occludentes (13, 14, 18, 39, 48, 49, 59). It has been suggested that disassembly of occluding junctions might take place by processes analogous to those mentioned above for junction assembly, but occurring in the reverse sequence (13, 48, 49). However, other studies indicate that, at least in some systems, the elimination of the arrays originated by the fragmentation of ZO might take place by endocytosis and lysosomal digestion of the plasmalemma patches bearing junctional strands (1, 14, 46, 57).

In most previous studies on occluding junction biogenesis and turnover, the experimental conditions used were insufficiently controlled to permit a detailed characterization of the processes (for instance, in terms of timetable, metabolic requirements, etc.) or an investigation of the underlying cellular mechanisms. Thus, in many embryonic and regenerating systems, emergence, displacement, and decay of occluding junctions occur concomitantly, making difficult the identification of whether a specific image represents assembly or degeneration (13, 14, 24, 25, 39, 48, 49, 59). Moreover, some of the changes in junctional size and complexity, which can be induced experimentally, are slow processes that take days to develop (18, 40, 45, 60); others are elicited by rough, unspecific treatments, such as exposure to proteolytic enzymes (14, 36, 43, 53).

In the present report, we will describe a convenient experimental model for studying in vitro the disassembly and reassembly of occluding junctions under controlled experimental conditions. We found that, in guinea pig pancreatic tissue lobules incubated without Ca^{++}, the ZO are progressively disarranged and finally disrupted to yield discrete fasciae occludentes; the process is rapidly reversed on reintroduction of Ca^{++} into the incubation fluid. Information on the role of ZO in maintaining the surface topology of pancreatic exocrine cells was also obtained. Moreover, since this Ca^{++}-dependent disassembly and reassembly of ZO is a well-reproducible process, it was possible to investigate whether it is interfered with by drugs, such as cycloheximide, cytochalasin B, colchicine, and antimycin A, which have known effects on individual cell structure and function. The initial part of this work has already been reported elsewhere in preliminary form (22).

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[1] *Abbreviations used in this paper:* EGTA, ethyleneglycol-bis(β-aminoethyl ether)N,N,N',N'-tetraacetate; ER, endoplasmic reticulum; imp, intramembrane particles; KRB, Krebs-Ringer bicarbonate solution; ZO, occluding zonula.

MATERIALS AND METHODS

Male albino guinea pigs were starved overnight, then stunned by a blow over the head. Pancreas tissue lobules were prepared as described by Scheele and Palade (51) and incubated in vitro at 37°C under 95% O_2–5% CO_2 in 100-ml Erlenmeyer flasks oscillating at 60 cycles/min in a water bath. Incubation fluids (10 ml) were the following: Krebs-Ringer bicarbonate solution (51) supplemented with an equilibrated mixture of aminoacids, glucose (14 mM), and soybean trypsin inhibitor (0.1 mg/ml) (KRB); Ca^{++}-free KRB containing 0.5 mM ethylene glycol-bis(β-aminoethyl ether)*N, N, N′, N′*-tetraacetate (EGTA). Drugs were added to the incubation fluids at times specified in the text and figure legends and at the following concentrations: colchicine: 10^{-6}, 10^{-5}, 2×10^{-5} and 10^{-4} M; cytochalasin B: 10^{-5}, $2{,}5 \times 10^{-5}$, and 5×10^{-5} M; cycloheximide: 10^{-4} M; antimycin A: 5×10^{-5} M. In some experiments the following secretagogues were used: caerulein (10^{-8} M); urecholine, (4×10^{-5} M); A23187 (2 μg/ml). The in vitro release of secretory proteins was estimated by the radiochemical procedure of Jamieson and Palade (29).

At the end of the incubations, the lobules were fixed with 1.5% glutaraldehyde + 1% paraformaldehyde in 0.12 M phosphate buffer, pH 7.3, then infiltrated with glycerol (at concentrations increasing from 10 to 30%) in the same buffer. Samples were frozen in Freon 22, cooled at −150°C in liquid nitrogen, then freeze-fractured according to Moor and Mühlethaler (41) in a Balzers freeze-etching device (Balzers AG, Balzers, Liechtenstein). The fracturing temperature was −100°C. Platinum-carbon replicas were washed in Na hypochlorite solution to remove organic material, then in distilled water, and finally recovered on 200-mesh copper grids.

Other lobules were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4, postfixed in 1% OsO_4 in the same buffer, stained in block with uranyl acetate, and embedded in Epon 812. Thin sections, cut with C. Reichert (Buffalo, N.Y.) and LKB ultramicrotomes (LKB Produkter, Bromma, Sweden), were doubly stained with uranyl acetate and lead citrate. Freeze-fracture replicas and thin sections were examined in Philips EM 200, EM 300, and EM 400 electron microscopes.

To measure the density of imp, small cardboard sheets bearing square or rectangular holes (sides between 2 and 5 cm, areas between 8 and 14 cm^2) were placed on top of well-resolved replicas printed at a final magnification of 100,000 and moved around until the image appearing through the hole was entirely accounted for by a flat portion (P-fracture face) of either the lumenal or the lateral plasmalemma of well-identified pancreatic acinar cells. Particles were then counted and the density was calculated by dividing their number by the area of the analyzed membrane portion (0.08–1.14 μm^2). The use of holes of different size and shape enabled us to carry out the counting on sufficiently large, flat areas of both the lateral and lumenal plasmalemma, even if the latter is quite irregular due to the presence of microvilli.

MATERIALS: The materials used were obtained from the sources specified below: soybean trypsin inhibitor, colchicine and cycloheximide: Sigma Chemical Co., St. Louis, Mo.; cytochalasin B: Ega Chemie, Steinheim bei Heidenheim/Brenz, Germany; antimycin A: Nutritional Biochemicals, Cleveland, Ohio; urecholine: Merck, Sharp & Dohme Canada Ltd., Montreal, Quebec, Canada. Caerulein and A23187 were the kind gifts of Farmitalia Labs for Basic Research, Milan, Italy and Eli Lilly and Co., Indianapolis, Ind., respectively. All other chemicals were reagent grade.

RESULTS

Control Cells

The appearance of normal exocrine cells of the guinea pig pancreas, both in thin sections and in freeze-fracture electron microscopy, has been described in detail previously (1, 9, 35). Here, only two points will be emphasized, since they are essential for the understanding of the rest of our work: (*a*) In both acinar and duct cells almost all junctional strands are integrated into well-organized ZO, which delineate circumferentially the lumenal portion of the plasmalemma (12, 35). Fasciae occludentes, localized on the lateral surface, were very rarely seen. The organization of the ZO is quite elaborate (Fig. 1) and usually includes continuous parallel strands (two to five in acinar cells), running close to one another perpendicular to the major axis of the cell, surrounded by a looser network of variable orientation. The ablumenal strand, which is also perpendicular to the cell axis, is interrupted from time to time by open-ended spurs. Communicating junctions (gap junctions [54]) of variable size are common on the lateral plasmalemma. (*b*) The P-fracture faces of flat surfaces of the basolateral and lumenal plasmalemma are characterized by a different density of imp. In particular, in acinar cells this difference is quite remarkable (12, 35): 2,768 ± 156 and 768 ± 52 imp/μm^2, respectively[2] (Fig. 1).

Both the ZO organization and the imp density of the various plasmalemma regions are not significantly modified in tissue lobules incubated in complete KRB for periods of time up to 5 h.

Ca-Dependent Disassembly and Reassembly of ZO

The changes in internal structure that we ob-

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[2] Number of analyzed cells: 19 and 18; total counted areas: 2.2 and 1.7 μm^2, respectively.

served in pancreatic acinar cells incubated in KRB-EGTA medium are analogous to those described previously by Amsterdam and Jamieson (1) after treatment with EDTA. The distribution of the chromatin in nuclei, the parallel arrangement of endoplasmic reticulum (ER) cisternae, and the structure of mitochondria and zymogen granules were essentially unchanged. In contrast, the Golgi complex was clearly altered; it was enlarged and showed many swollen condensing vacuoles containing loosely packed, segregated material (see Fig. 13). Alterations of the junctional complex (such as disarrangement of desmosomes, appearance of punctate occluding junctions between the lateral plasmalemmas of adjacent cells and, in a few cases, also opening of ZO were detected also in thin sections (not shown). However, a full appreciation of the changes induced in occluding junctions was revealed only by freeze-fracture (Figs. 2-6).

During the first 30 min of incubation in KRB-EGTA, the changes in the ZO were usually confined to the ablumenal seals. Their meshwork was progressively loosened and disarranged; after 10-15 min, long strands began to detach from the ZO body and to move ablumenally (Fig. 2). Eventually (15-60 min), these strands were progressively fragmented into pieces, which were dispersed in the lateral region of the plasmalemma and were often seen wound around communicating junctions (Fig. 3). The strand fragments retained their junctional function, as demonstrated by the continuity of ridges and furrows in adjacent P- and E-fracture faces (Fig. 3, see also Figs. 4-6).

In the majority of cells the arrangement of the lumenal parallel strands of the ZO remained unaffected for at least 40 min of KRB-EGTA incubation. However, with longer times these strands also were disarranged and fragmented, so that after 2 h most ZO were transformed into multiple, discrete fasciae occludentes. Of the latter, some remained around the cell apex in the plasmalemma regions originally occupied by the ZO, while others were displaced to the lateral and also to the lumenal region, where they appeared intermingled with the microvilli (figs. 4, 5, and 6). As a consequence, some acinar lumena become multichambered, partially obliterated, and distorted. At this stage many fasciae occludentes, especially those that had moved far away, were small in size. Some were composed of only one or a few short strands, either continuous or interrupted by short rows of imp. Continuity with rows of imp was seen sometimes also at the tips of the strands.

In strict correlation with the interruption of the ZO continuity, we observed a disappearance of the surface heterogeneity in imp density of plasmalemma P-fracture faces. Thus, in cells that, even after prolonged incubaton in KRB-EGTA, still maintained at least one continuous ZO junctional strand, the density of imp at the lumenal P-fracture face remained as low as in the controls. In contrast, in all cells with interrupted ZO, approximately the same high density of imp that in control cells occurs only at the basolateral plasmalemma, was found both at the basolateral and at the lumenal plasmalemma regions.

In lobules first incubated for various time intervals in KRB-EGTA, major changes in the occluding junctions were provoked by transfer to the complete KRB medium. In brief, the lateral and lumenal fasciae occludentes were drastically reduced and the ZO reassembled. These processes developed rapidly: reassembled ZO were observed in some cells already after 5 min and nearly in all cells by 30 min on (figs. 7-10). By that time, only a few small fasciae occludentes, formed by short strands and rows of particles, remained in the lateral surface. On the other hand, the increase in imp density on the P face of the lumenal plasmalemma occurring after ZO fragmentation was not reversed, at least within 3 h. Thus, most acinar cells of lobules first incubated for 2 h or more in KRB-EGTA and then transferred to plain KRB were characterized by a lumenal plasmalemma P face rich in particles (3,103 ± 226 imp/μm^2)[3] surrounded by continuous ZO (Figs. 7-10). The latter were not identical to those described in control cells. In particular, the ordered and peculiar geometry (parallel strands towards the lumen surrounded by a looser meshwork) was no longer evident and was replaced by an even, tighter network. The thickness of this network was often variable, with alternation of deep (10-12 strands) and shallow (two to three strands) portions (Figs. 7-10).

Effect of Drugs

The aim of these experiments was to obtain some indirect information about the cellular mechanisms which might be involved in the regu-

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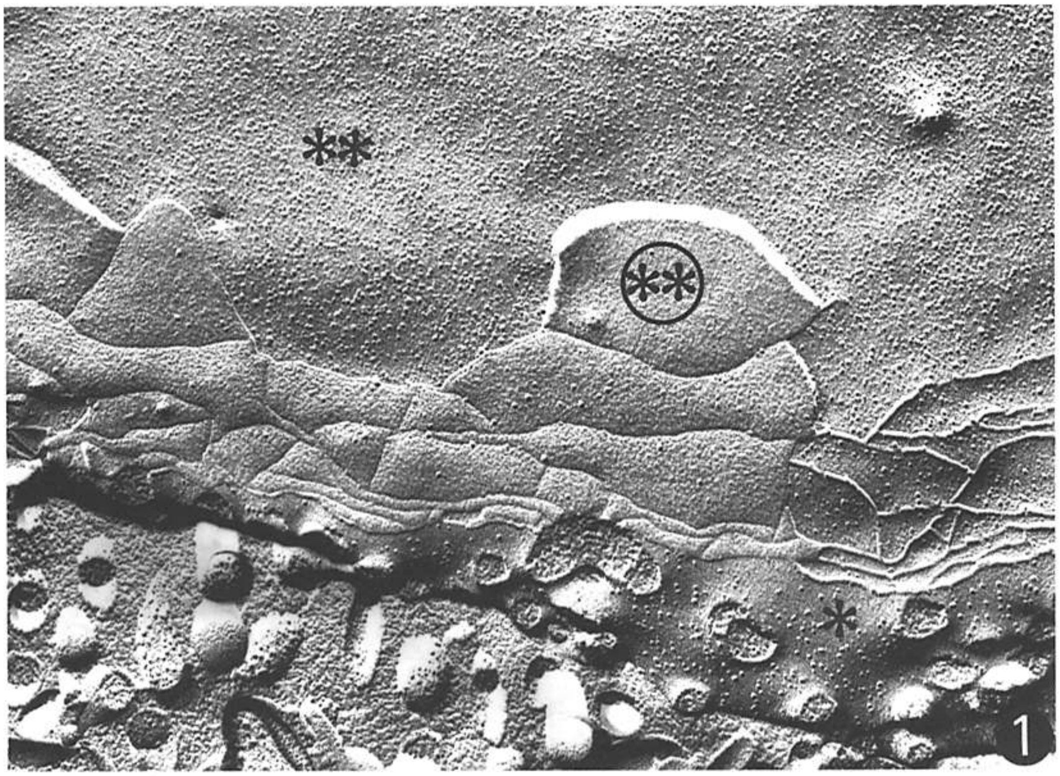
[3] Number of analyzed cells: 8; total counted area: 0.71 μm^2.



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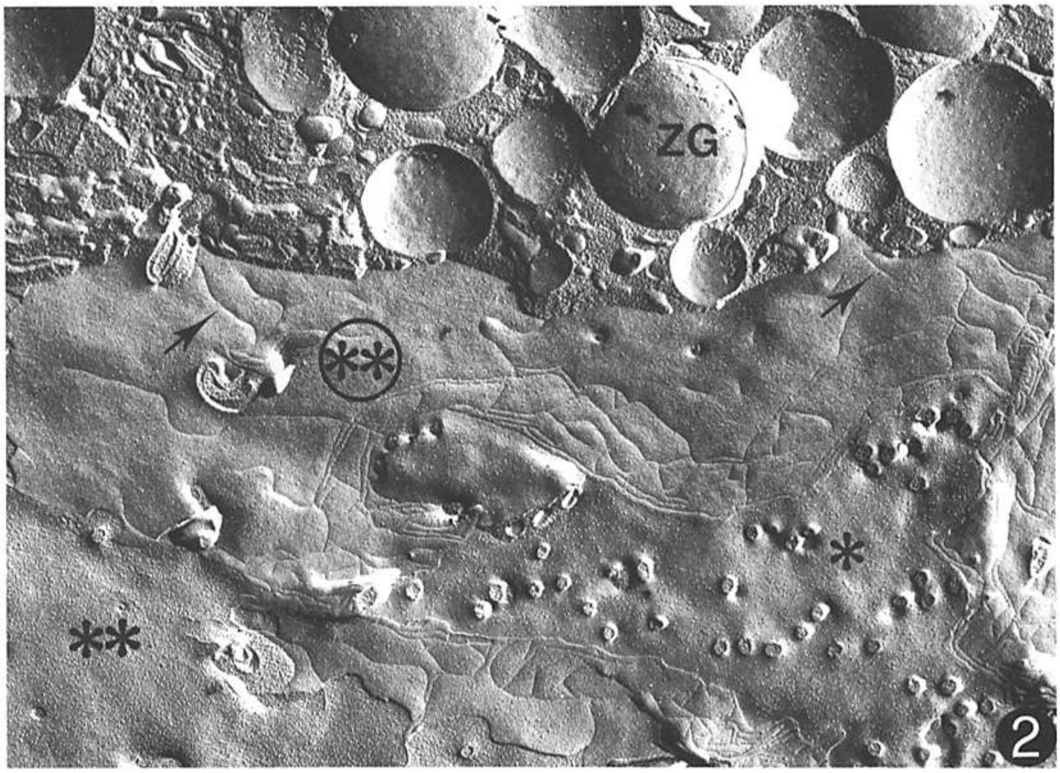

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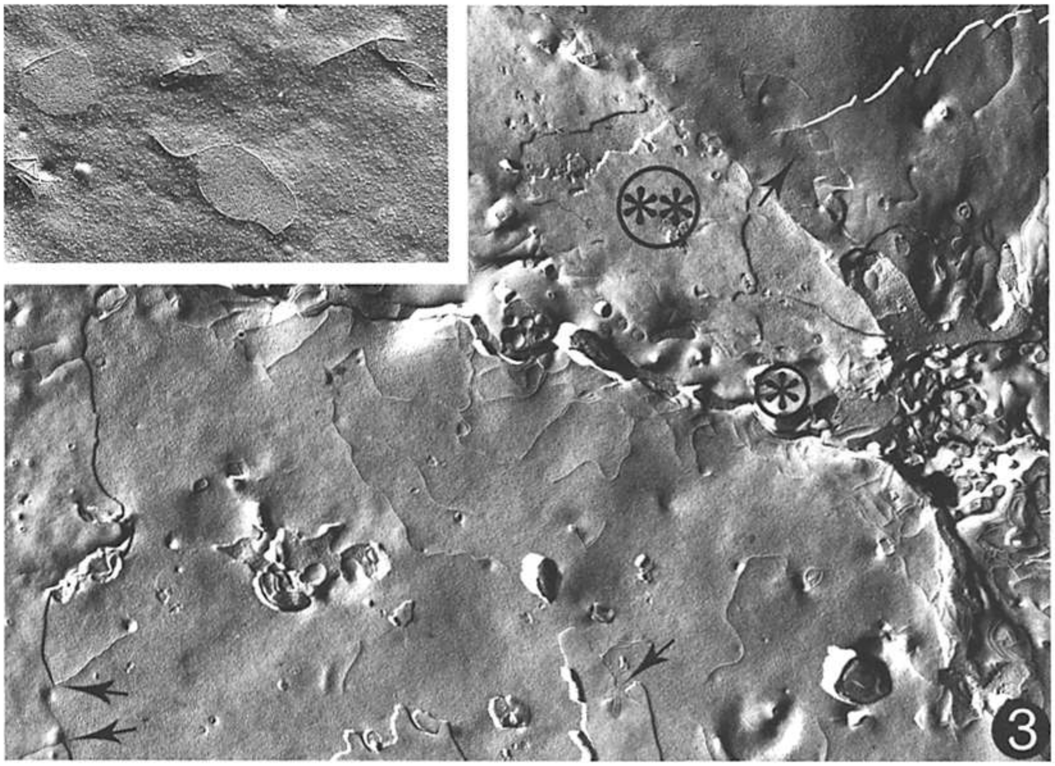
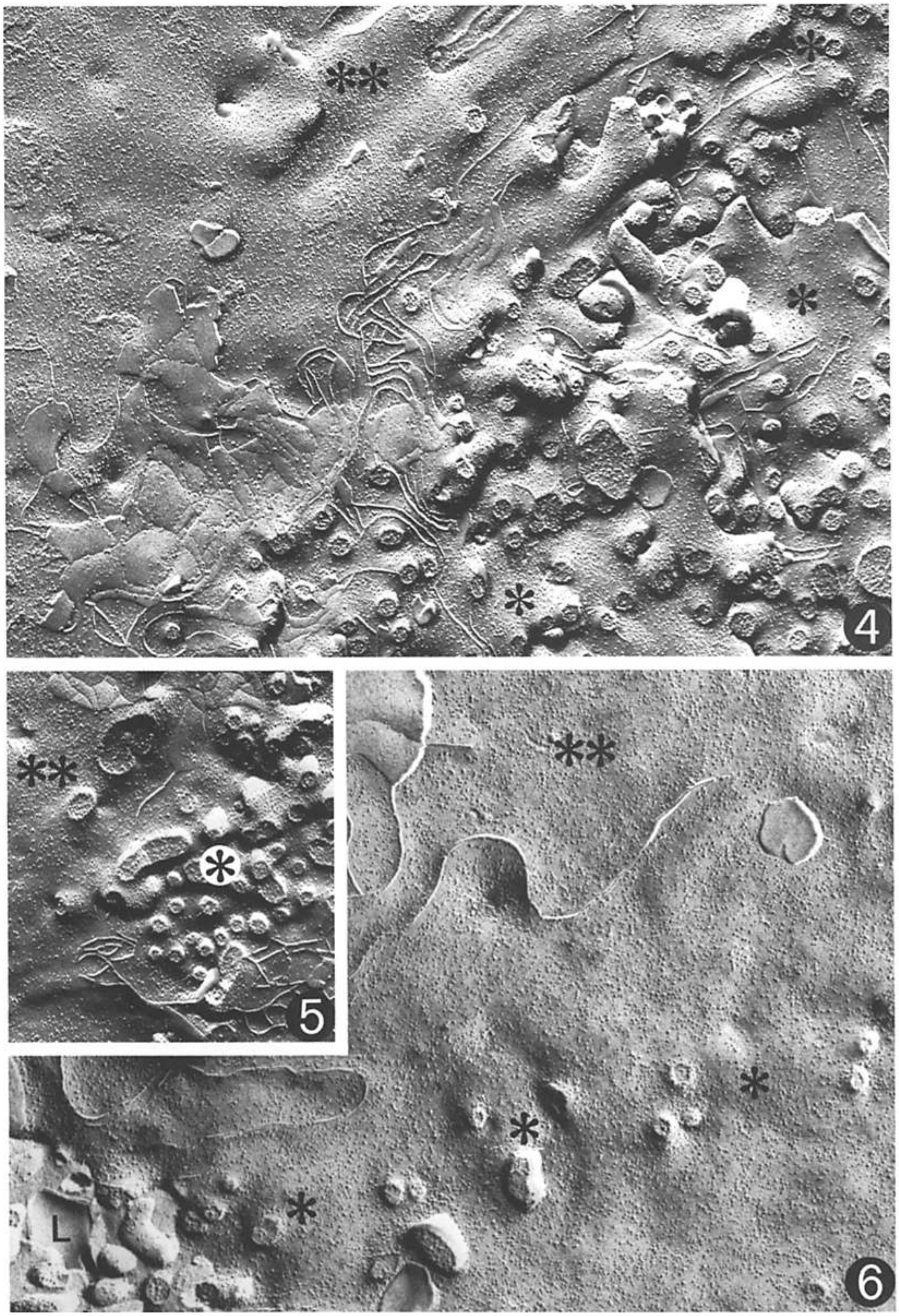


FIGURE 3 Pancreatic lobules incubated for 40 min in KRB-EGTA. A more advanced stage of ZO disorganization is shown. Note the numerous individual strands and small arrays present in the lateral plasmalemma. These structures still maintain a junctional function as indicated by the continuity of grooves and furrows in adjacent P- and E-fracture faces (arrows). Junctional strands wound around five small communicating junctions located in the lateral plasmalemma of a pancreatic acinar cell are shown in the *inset*. Circled double asterisk and circled single asterisk label the lateral and lumenal plasmalemma regions, E-fracture faces. × 14,000; *inset*: ×25,200.

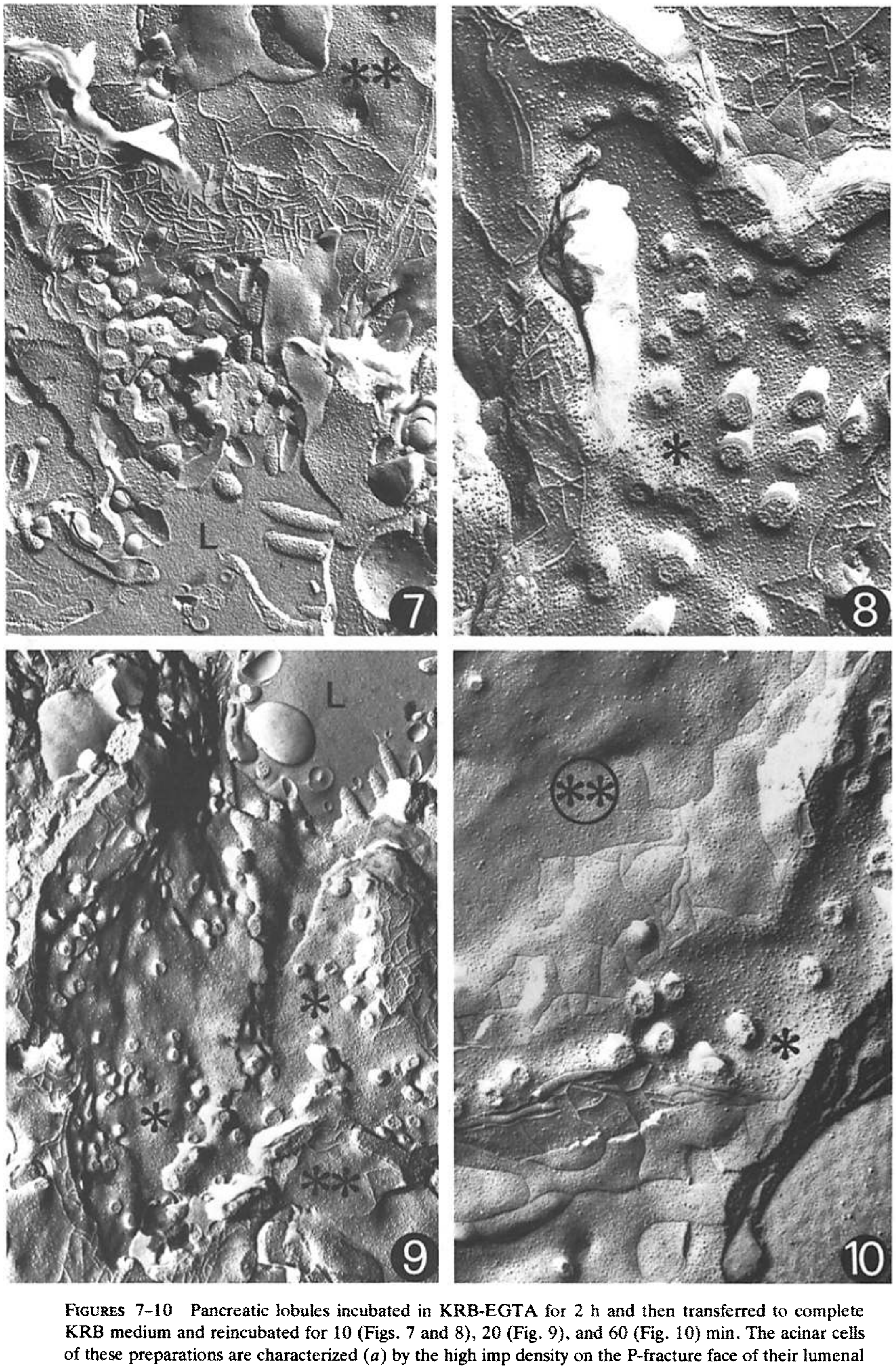
lation of the ZO dynamics in pancreatic tissue. In each series of experiments the possibility was first investigated that the drug used, when added to plain KRB medium, had an effect on the structure of pancreatic exocrine cells (in particular on the organization of the ZO) within 4 h of in vitro incubation. In further experiments the drugs were added to the KRB-EGTA medium in order to test

FIGURE 1 The field illustrates the typical appearance of the apical surface of normal guinea pig pancreatic acinar cells. Note the complex organization of the zonula occludens, formed by three strands running in parallel surrounded by a looser network located ablumenally. The density of imp on the P-fracture face is much higher in the lateral (double asterisk) than in the lumenal (single asterisk) region of the plasmalemma. The latter is also characterized by the presence of microvilli, which appear either cross-fractured or tangentially exposed. Circled double asterisk = lateral plasmalemma, E-fracture face. × 60,000.

FIGURE 2 Pancreatic lobules incubated in KRB-EGTA for 10 min. An early stage of ZO disorganization is shown. Note that the lumenal, roughly parallel strands of the junctions are unaffected. In contrast, the ablumenal meshwork is loosened and disarranged; individual strands and small strand arrays detached from the ZO are free in the lateral plasmalemma (arrows). The heterogeneity of imp density between the P-fracture faces of the lateral (double asterisk) and lumenal (single asterisk) plasmalemma regions (which is typical of normal acinar cells, Fig. 1) is still maintained. *ZG*, = zymogen granules; circled double asterisk = lateral plasmalemma, E-fracture face. × 23,500.



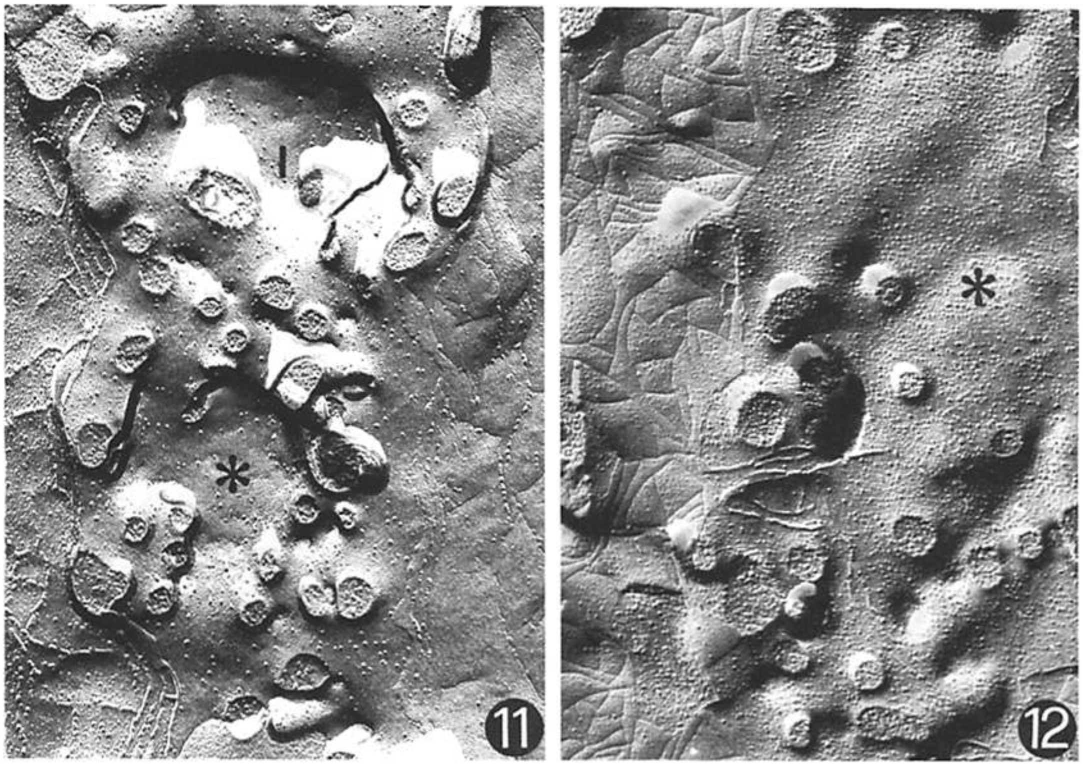
FIGURES 4–6 Pancreatic lobules incubated in KRB-EGTA for 2 h. At this time point of the experiment, the ZO of most acinar cells appear disassembled to yield discrete strands and small strand arrays. As a consequence, the lateral (double asterisk) and the lumenal (single asterisk) regions of the plasmalemma (the latter identified by the presence of microvilli) become directly continuous. Note that the density of imp on the P-fracture face is approximately the same in the two plasmalemma regions. *L* = acinar lumen. Fig. 4: × 38,500; Fig. 5: × 34,500; Fig. 6: × 50,000.



FIGURES 7–10 Pancreatic lobules incubated in KRB-EGTA for 2 h and then transferred to complete KRB medium and reincubated for 10 (Figs. 7 and 8), 20 (Fig. 9), and 60 (Fig. 10) min. The acinar cells of these preparations are characterized (*a*) by the high imp density on the P-fracture face of their lumenal plasmalemma (single asterisk) (as in the lobules incubated for 2 h in KRB-EGTA, see Figs. 4–6); and (*b*) by continuous ZO, which, however, do not exhibit the ordered, elaborate pattern visible in control cells (see Fig. 1), but are arranged more disorderly. *L* = acinar lumen; double asterisk and circled double asterisk = lateral plasmalemma, P and E faces. Fig. 7: × 32,000; Fig. 8: × 60,000; Fig. 9: × 24,500; Fig. 10: × 63,000.

their effect on the ZO disassembly induced by Ca^{++} withdrawal. Finally, the effect on ZO reformation was investigated in other lobules, incubated first in KRB-EGTA (usually for 120 min) and then in KRB (usually for 60 min). In many experiments the drugs were added to the second incubation fluid. In some cases, however, they were added to the first incubation or were even present throughout the whole incubation period. Here we will report the results obtained with four different drugs: cycloheximide (an inhibitor of polypeptide elongation); cytochalasin B (a mold metabolite that disrupts cytoplasmic microfilaments and also exerts a variety of other effects in the cells, especially at the level of the plasmalemma [2, 31, 65]); colchicine (which binds to tubulin-containing structures and thus causes microtubule depolymerization); and antimycin A (an irreversible inhibitor of the mitochondrial electron-transport chain). With each of these drugs, at least three complete experiments of ZO disassembly and reassembly were carried out.

The results obtained can be summarized as follows: Cycloheximide, at doses known to inhibit pancreatic protein synthesis by >95% (27), was without clear effect on the general ultrastructure of the cells (as already reported by Jamieson and Palade [27]) or on the organization of ZO. Moreover, no interference with the disassembly and reassembly of ZO was detected (not shown). Analogously, no effect on the ZO structure and dynamics was observed with cytochalasin B (Figs. 11 and 12), which, however, induced profound alterations in the organization of acinar cells, especially at their secretory pole (for details, see references 3 and 62).



FIGURES 11 and 12 Effects of cytochalasin B (2.5×10^{-5} M). Fig. 11 refers to a lobule incubated for 60 min in plain KRB containing the drug. Note that cytochalasin B had no significant effect on the organization of the ZO. In contrast, the lumenal surface of the cell (P-fracture face, asterisk) was clearly altered, with formation of large infoldings (*I*) and bulges. Fig. 12 is from a lobule first incubated in KRB-EGTA for 2 h and then in plain KRB for 60 min. Both incubation fluids contained cytochalasin B. The picture is analogous to those shown in Figs. 7-10. The high imp density on the P-fracture face of the lumenal plasmalemma (asterisk) and the continuity of the ZO indicate that the drug did not interfere with the disassembly of the junctions occurring in Ca^{++}-free medium or with the reassembly elicited by Ca^{++} reintroduction. Fig. 11: × 60,000; Fig. 12: × 68,500.

The results of the colchicine studies were more complex. At doses between 10^{-6} and 10^{-4} M, this drug induced the appearance of some pseudocrystalline structures and of images of autophagocytosis (63), while at least some microtubules were still evident. Moreover, the organization of the Golgi complex was clearly affected: clusters of vesicles and condensing vacuoles were scattered apparently at random in the whole cytoplasm (Fig. 13). These changes in the internal structure of acinar cells developed slowly (in about 60 min) and were not reversed (at least within 3 h) on drug withdrawal. By itself, colchicine did not induce detectable alterations in the ZO or interfere with the disassembly of this junction occurring in KRB-EGTA. In contrast, the reassembly of the ZO provoked by Ca^{++} reintroduction was clearly impaired. The degree of the impairment was variable. In a few cells, no sign of redistribution or fusion of the discrete fasciae occludentes could be detected even after 2 h of reincubation in complete KRB. In others, the reintroduction of Ca^{++} did result in a clustering of the fasciae and in the consequent formation of tightly packed junctional arrays both at the apical margins of the cells (Fig. 14) and in the lateral plasmalemma (Fig. 15); however, the coalescence of these arrays was only partial and therefore continuous ZO were not reestablished (Fig. 14). Finally, in some cells the junctional strands aligned themselves around the cell apex, but the resulting meshwork was very disordered and focal discontinuities were often observed (Fig. 16).

The effect of antimycin A was even more surprising. When added to plain KRB, this drug induced in acinar cells a vesiculation of the rough-

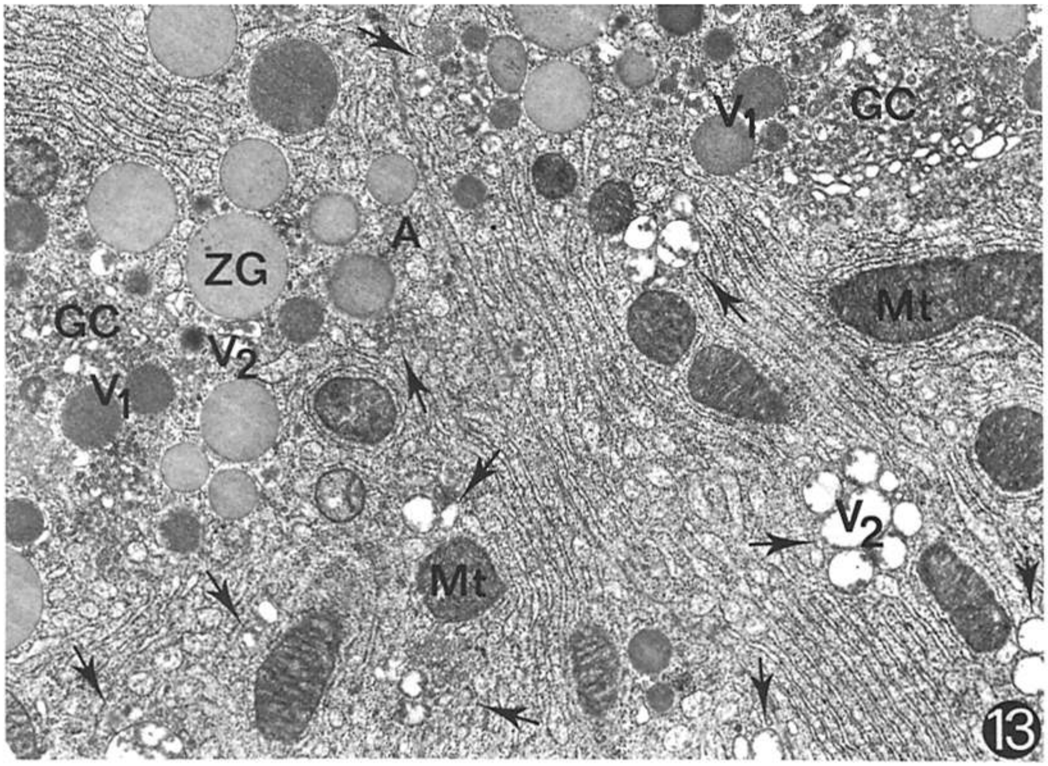
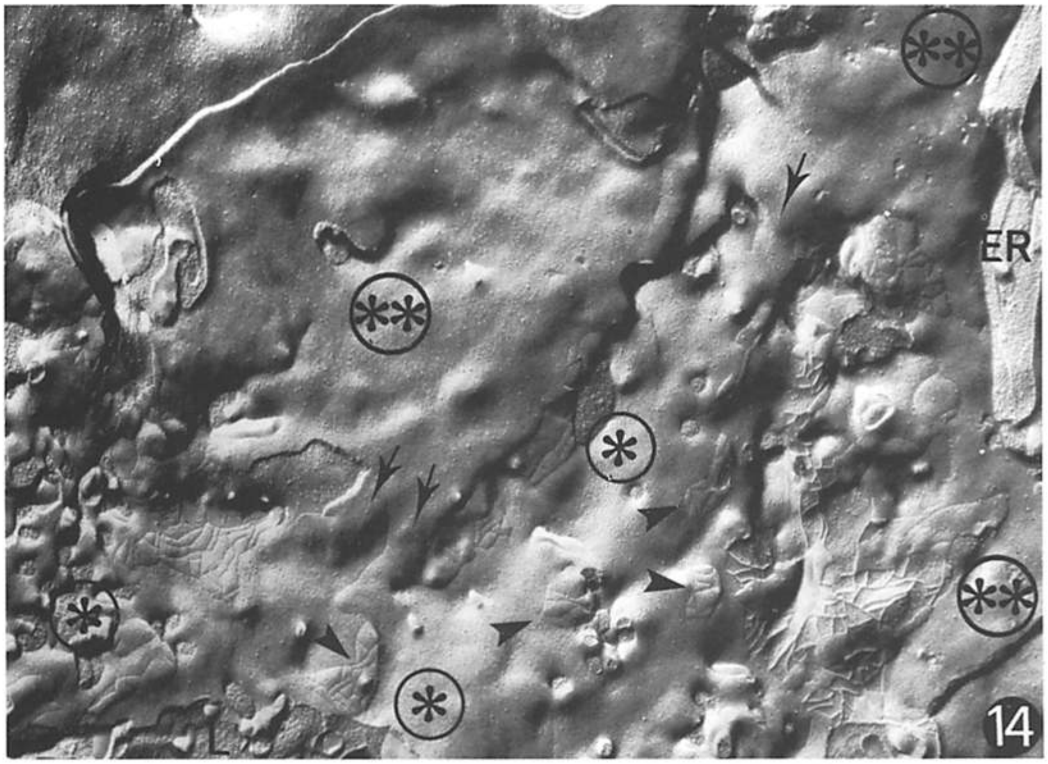


Figure 13 Effect of colchicine (10^{-5} M) on the Golgi complex. This picture is from a lobule incubated for 120 min in KRB-EGTA containing the drug. In two adjacent acinar cells the Golgi complex (*GC*) is quite prominent and includes short, parallel cisternae, numerous vesicles and condensing vacuoles. Of the latter organelles, some are uniformly occupied by the segregated zymogens (V_1); others, in contrast, are occupied only in part (V_2). The presence of these loose condensing vacuoles is not due to colchicine but to the Ca^{++} chelator. Numerous condensing vacuoles (mostly of the loose type), either isolated or in small groups, and clusters of vesicles are spread at random in the cytoplasm, away from the Golgi area (arrows). *ZG* = zymogen granule; *Mt* = mitochondrion; *A* = small autophagosome. × 15,500.



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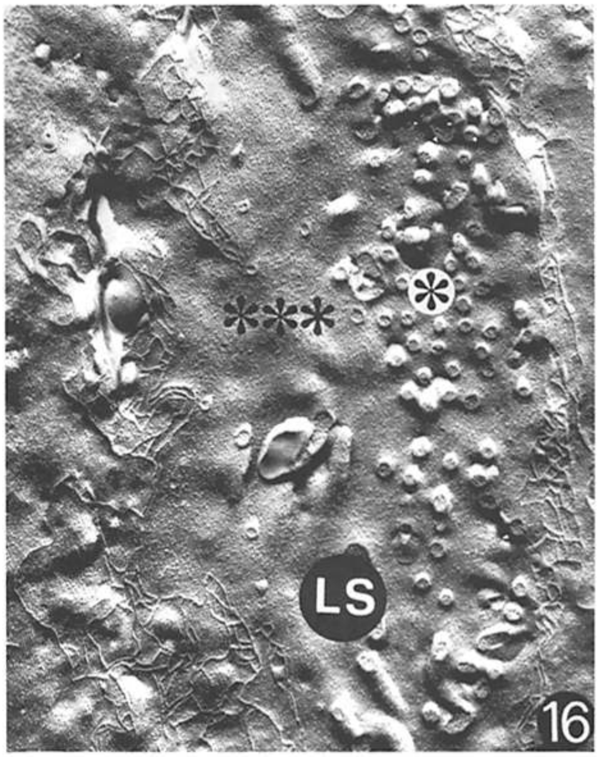

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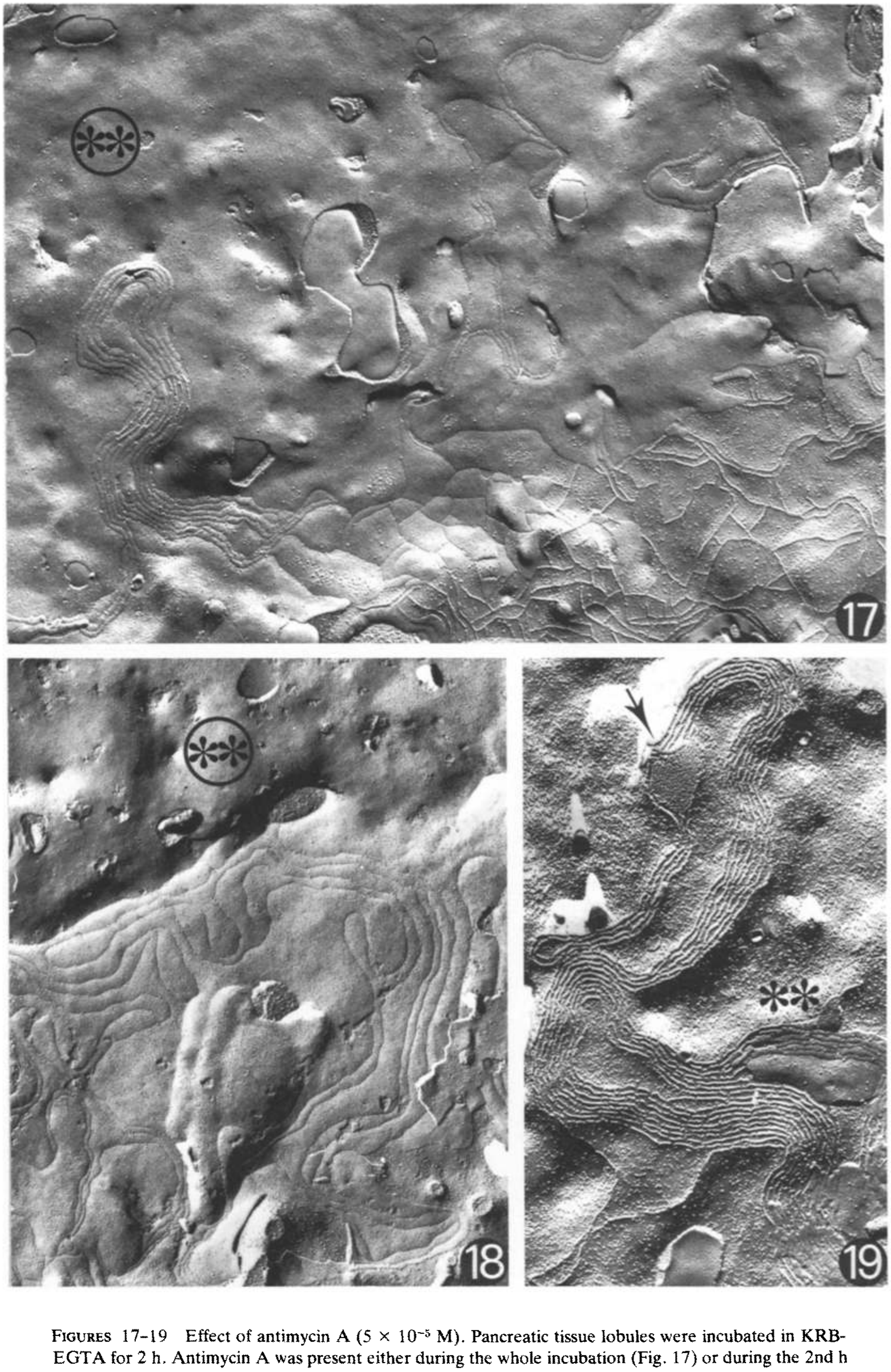
surfaced ER and swelling of some mitochondria (8, 28). The Golgi complex was also modified, with an increase in stacked cisternae, a decrease in the vesicles, and accumulation of fibrillar material. In most cells, the organization of the occluding junctions was unchanged. In others, the drug induced the appearance of small, bizarre arrays composed of junctional strands, which were localized in the lateral plasmalemma and were often continuous with the ZO (not shown). When added to the KRB-EGTA incubation fluid, antimycin A induced the formation of similar arrays which, however, were much more numerous, large, and elaborate (Fig. 17). In these acinar cells, the lateral surface was often covered by long, infrequently anastomosed junctional strands. Often, two to ten of these strands were arranged in parallel to form ribbons, festoons, whorls, and other bizarre figures, sometimes enclosing entrapped communicating junctions (Fig. 19). Less tightly packed arrays were formed as well (Fig. 18). Also, in lobules first incubated in KRB-EGTA for 2 h, and then in complete KRB containing antimycin A, we observed an apparent increase in junctional strands. Usually, however, the latter were organized to form elaborated, reformed ZO (not shown) rather than the peculiar arrays that developed when the drug was applied under Ca^{++}-free conditions.

DISCUSSION

Previous studies carried out in a variety of cellular systems, especially in embryonic tissues, support the idea that occluding junctions are dynamic structures that can adjust their organization in response to specific and unspecific stimuli. So far, however, the information concerning these junctions is still limited and fragmentary. This is due, on the one hand, to the fact that, in contrast to the situation which exists today for other junctions, such as the communicating junctions (15, 16, 30), little is known about the chemical composition of occluding junctions and about their relationships to the other components of the plasmalemma. Subcellular fractions enriched specifically in junctional strands have not yet been isolated, while the peculiar localization of the strands has prevented detailed cytochemical studies. On the other hand, in many morphological investigations the changes in occluding junctions have been insufficiently characterized in terms of timetable, metabolic requirements, and functional significance, and their correlation with underlying cellular events has not been taken into consideration or sufficiently investigated.

In these respects, the experimental model used in this work (i.e., guinea pig pancreatic tissue lobules incubated in vitro in salt solutions with or without Ca^{++} and containing different drugs) offers some distinct advantages. This depends in part on the surface morphology of pancreatic acinar cells. In these cells, in contrast to the situation existing in many other epithelia lining a lumen (49, 57), almost all the junctional strands are integrated in continuous, well-organized ZO, while discrete fasciae occludentes are very rare. This structural feature permitted us to establish that at least most of the fasciae occludentes that appear on the lateral and lumenal surfaces on incubation in KRB-EGTA do not exist before the treatment, but arise as a consequence of ZO fragmentation. Moreover, with respect to the ba-

FIGURES 14–16 Effect of colchicine (10^{-5} M) on the Ca^{++}-dependent reassembly of ZO. Pancreatic lobules were first incubated in KRB-EGTA for 2 h and then in plain KRB for 1 more h. Colchicine was present in both incubation fluids. In the cell shown in Fig. 14 the junctional strands have reassembled to form large meshworks located at the apical margins; however, a continuous, beltlike ZO has not reformed. Clear continuities between the lateral and lumenal plasmalemma (E-fracture faces, circled double asterisk and circled single asterisk, respectively) are indicated by arrows. The lumenal plasmalemma, identified by the characteristic craters corresponding to the bases of microvilli, is partially occupied by discrete arrays of junctional strands (arrowheads). *L* = acinar lumen; *ER* = cross fracture of parallel cisternae of the endoplasmic reticulum. Fig. 15 shows a large array of tightly packed strands localized in the lateral plasmalemma of an acinar cell. Fig. 16 shows a cell in which the Ca^{++}-dependent reassembly has yielded a continuous ZO. The latter, however, is abnormal because (*a*) it is very thin and disordered and (*b*) it encircles a very large lumenal surface (*LS*), part of which bears microvilli (and therefore probably corresponds to the original lumenal plasmalemma [single asterisk]), while the rest is smooth and is probably accounted for by plasma membrane originally belonging to the lateral region [triple asterisk]. The density of imp at the lumenal P-fracture face is as high as in the other cells with reassembled ZO (Figs. 7–10 and 12). Fig. 14: × 32,000; Fig. 15: × 35,000; Fig. 16: × 27,500.



FIGURES 17–19 Effect of antimycin A (5×10^{-5} M). Pancreatic tissue lobules were incubated in KRB-EGTA for 2 h. Antimycin A was present either during the whole incubation (Fig. 17) or during the 2nd h only (Figs. 18 and 19). Long, infrequently anastomosed junctional strands have proliferated in the lateral plasmalemma, yielding large, elaborate arrays of various shapes and configurations which sometimes enclose communicating junctions (Fig. 19, Arrow). Circled double asterisk and double asterisk: lateral plasmalemma, E- and P-fracture faces. Fig. 17: × 56,500; Fig. 18: × 25,000; Fig. 19: × 40,000.

solateral region of the plasmalemma, the P-fracture face of the lumenal region of acinar cells is characterized by a much lower density of imp (12) and this density was markedly increased after the interruption of ZO continuity by prolonged incubation in KRB-EGTA. This provided us with a valuable criterion for distinguishing the cells in which the ZO opening had really occurred from those which had resisted the KRB-EGTA treatment. This identification was necessary in order to investigate the dynamics of ZO reassembly which takes place after readmission of Ca^{++} into the incubation fluid. Finally, the reproducibility of the extracellular Ca^{++}-dependent changes of occluding junctions was quite good, especially in terms of evolution with time. This was a necessary prerequisite to the epxeriments in which the effect of drugs on the disassembly and reassembly of the ZO was investigated.

On the other hand, it should be acknowledged that our model also suffers distinct limitations. For instance, in our experiments it was impossible to investigate the correlation between ZO organization and paracellular ion permeability, measured by biophysical and physiological techniques, as done by others in different systems (4, 10, 11, 32, 38). Moreover, our system proved inadequate to investigate the effect of ZO disassembly (and of the consequent loss of heterogeneity of P-face imp density in the plasmalemma) on the localization of secretion granule discharge by exocytosis. In fact, in parallel biochemical studies we found that prolonged incubation in KRB-EGTA inhibits the stimulated enzyme release in pancreatic lobules (by 60–80%), even after reincubation in complete KRB for times up to 2 h (not shown). Analogous results were obtained recently in another laboratory.[4] As a consequence, even if we exposed these lobules to a variety of secretagogue drugs (caerulein, urecholine, and the Ca^{++} ionophore A23187), we were unable to observe convincing images of exocytosis, which, on the contrary, were quite common at the lumenal surface of stimulated control preparations.

Effect of ZO Disassembly and Reassembly on Cellular Surface Topology

The strict correlation between interruption of ZO continuity and loss of heterogeneity of P-face imp density at the surface of pancreatic exocrine cells confirms the hypothesis (which is also supported by results in another system [44]) that ZO are not only responsible for the segregation of the extracellular space, but also act as mechanical barriers which prevent the intermixing of membrane components of the lumenal and lateral regions of the plasmalemma. When the ZO is dismantled, the intermixing of these components is probably very rapid, as indicated by the fact that intermediate stages (such as gradients of imp across the continuities of the two plasmalemma regions) were never observed. It is possible that the function of the ZO would be in maintaining and not in establishing in the two regions the heterogeneous distribution of particles, since the latter did not reappear (at least within 3 h) after the continuity of the ZO was reestablished by reintroduction of Ca^{++} into the incubation fluid. The rapid intermixing of the plasmalemma components might explain why, in pancreatic acinar cells dissociated from the tissue, the distribution of various sugar residues (revealed by the binding of specific lectins) was found to be uniform over the entire cell surface (33).

The observation that after opening of the ZO the imp density of both the basolateral and lumenal plasmalemma was not significantly different from that found in the basolateral region of control cells strongly suggests that the total complement of plasmalemma imp is conserved throughout our experiments. In fact, in the acinar cells of the guinea pig pancreas, the lumenal plasmalemma accounts for only 5.1% of the total surface area (9). Therefore, the net relocation of basolateral imp to the lumenal plasmalemma which is needed in order to make the density homogeneous in the two compartments is small (<4% of the total basolateral imp) and is not expected to modify significantly the density of the basolateral region.

On the other hand, there is no reason to believe that the intermixing of the molecular components of the two plasmalemma regions was necessarily complete. On the contrary, some degree of surface heterogeneity, not detectable by freeze-fracture, might have remained since recent evidence demonstrates that, in membranes, while the bulk of the lipids and many proteins are free to diffuse laterally, other proteins move little or are immobile (20, 52).

Biogenesis and Turnover of Occluding Junctions

Two phenomena that we have demonstrated

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[4] G. A. Scheele, personal communication.

might be relevant in relation to the biogenesis and turnover of occluding junctions: the progressive fragmentation of the ZO into smaller arrays on treatment with KRB-EGTA and its reformation when Ca^{++} is reintroduced into the incubation fluid. The first process is slow (1–2 h), whereas the second occurs rapidly. The time needed for ZO disassembly might be prolonged by the fact that the central strands are probably protected by those located ablumenally, as suggested by the observation that all strands were never affected concomitantly but always in sequence, from the periphery to the lumen.

The occluding strands of the lateral plasmalemma were often seen wound around communicating junctions. Similar images were observed previously in a series of different tissues, (13, 14, 18, 34, 36, 48, 49, 55). In particular, in embryonic tissues it was suggested that interaction with strands might favor the association of communicating junction monomers. We believe that in our cells the process occurs in the opposite direction: junctional strands, while moving in the plane of the membrane, might remain caught by the communicating junctions, probably because the latter are sites of low fluidity with respect to the rest of the plasmalemma. The association of the two junctions is not stable since it is rapidly dismantled when Ca^{++} is reintroduced into the incubation fluid.

Due to the rapidity of the phenomenon, we were unable to identify clearly the intermediate stages in ZO reassembly. However, the concomitant disappearance of most lateral fasciae occludentes strongly suggests that at least part of the reformed ZO results from the clustering and coalescence of the former structures rather than through complete *de novo* assembly of junctional strands. Reassembly always occurs around the lumenal surface, i.e., at the place occupied by ZO in intact cells.

Our images suggest that the disassembly of ZO might be accompanied by a net decrease in the amount of junctional strands in the cells and that the opposite phenomenon occurs during reassembly. However, the attempts we made to check this point in quantitative terms yielded inconclusive results, as a consequence of the geometrical problems imposed by the displacement of the junctional strands over the cell surface. The suggestion that a partial disassembly and reassembly of junctional strands occurred in our experiments is also supported by the observation that the arrays located in the lateral plasmalemma are often constituted by short bars alternating or continuous with short rows of imp. In other systems, clear evidence indicates that junctional strands arise by alignment of imp followed by fusion of the resulting rows (13, 14, 18, 26, 39, 48, 49, 59). The reverse process has been suggested to take place when strands are disassembled (13, 48, 49). In contrast, we were unable to confirm another process that has been proposed to account for the disposition of occluding junctions, i.e., endocytosis and lysosomal digestion of plasmalemma fragments bearing junctional strands (11, 14, 46, 57). In this respect, it should be emphasized that endocytosis of junctional strands has been demonstrated clearly only in cells detached from their neighbors for various reasons (for instance, in experimental tissue dissociation and during cell differentiation). In all these cases, fragments of adjacent cells remain attached to the fasciae occludentes. Thus, the subsequent endocytosis might be related to the disposition of surface interactions no longer useful rather than to the physiological turnover of occluding junctions.

Taken together, the results discussed so far support the idea that zonulae and fasciae occludentes are not separate entities. Rather, all junctional strands might constitute a common pool. In pancreatic exocrine cells, the integration of these strands into continuous ZO appears greatly favored over ZO disassembly. This might explain why fasciae occludentes appear only when Ca^{++} is withdrawn from the extracellular fluid. Possibly, a difference in the balance between assembly and disassembly could explain why, in many epithelia lining a lumen, zonulae and maculae occludentes coexist also at physiological Ca^{++} concentrations and even why, in other systems, continuous zonulae are never assembled. The importance of Ca^{++} in the assembly of occluding junctions has been demonstrated also in other systems (10, 46) and might be of general occurrence, although it is not yet clear whether Ca^{++} acts directly (on junctional components) or indirectly (on the organization of the plasmalemma [42, 50] or even on the intracellular environment).

Effects of Drugs

We will now consider the effects of drugs on the disassembly and reassembly of occluding junctions. In this respect it should be emphasized that, due to the poor specificity of the drugs used, the interpretation is far from straightforward. However, some interesting data have emerged. First of all, we found that cytochalasin B, at doses which disrupt microfilaments and produce clear changes in cell shape, especially at the lumenal surface, has no effect on the organization and dynamic changes of the ZO. This result is surprising in view of the previous proposal that the development and function of occluding junctions might be controlled by cellular microfilaments (4, 40).

The effects of colchicine were more complex. Neither the organization nor the disassembly of ZO was influenced by this drug. Moreover, after reintroduction of Ca^{++} the junctional strands did reassemble to yield large arrays. However, in most cases the arrays either reformed away from the right place or failed to coalesce entirely to yield complete ZO. Thus, in colchicine-treated cells, the defect seems to involve not the ZO reassembly per se, but its ordered localization at the cell surface. This effect of the drug correlates nicely with that elicited on the Golgi complex, which is fragmented and dispersed throughout the cytoplasm. The importance of the Golgi complex in inducing the site of assembly of ZO has been suggested recently by other authors (61). The effect of colchicine on the Golgi complex is not limited to the exocrine pancreas but has been observed also in a number of other systems (17, 37, 58) and attributed to the disruption of the microtubules localized in the controsphere region of the cell (58). Alternatively, colchicine might act directly on the plasmalemma, since this membrane is known to contain large amounts of tubulin and to be profoundly affected by the drug, both in its physicochemical features and in its function (5–7, 56).

Finally, the results with cycloheximide demonstrate that the disassembly and reassembly of ZO does not depend on *de novo* protein synthesis. Moreover, antimycin A, especially when added to the KRB-EGTA medium, induced a remarkable proliferation of junctional strands. Such a proliferation is probably not an unspecific sign of degeneration because (*a*) in grossly damaged cells (both treated and nontreated with antimycin A) we observed characteristic alterations in the plasmalemma ultrastructure (such as generalized clustering of imp in a network organization); however, proliferation of junctional strands was never seen; and (*b*) when the antibiotic was added to complete KRB, even after prolonged exposure of the lobules to KRB-EGTA, the changes in occluding junctions were relatively minor. It is interesting that proliferations of junctional strands, although less impressive than that elicited by antimycin A, were observed previously in several cell systems after treatment with proteolytic enzymes (36, 43, 53). Many cell types seem therefore to possess the potentiality to increase their complement of junctional strands in response to apparently unspecific stimuli. Since this phenomenon occurs in antimycin A-treated cells, in which all the ATP-dependent functions are inhibited (28), it cannot be dependent on *de novo* synthesis of the strand components. Rather, it could be due to the association of junctional components (or precursors) not integrated in assembled strands, which might exist normally in these cell types.

The photographic assistance of Mr. P. Tinelli and F. Crippa is gratefully acknowledged.

The initial experiments of this work were carried out in collaboration with Drs. P. Galli and A. Brenna.

Received for publication 17 January 1978, and in revised form 16 June 1978.

REFERENCES

1. Amsterdam, A., and J. D. Jamieson. 1974. Studies on dispersed pancreatic exocrine cells. I. Dissociation technique and morphologic characteristics of separated cells. *J. Cell Biol.* **63:**1037–1056.
2. Axline, S. G., and E. P. Reaven. 1974. Inhibition of phagocytosis and plasma membrane mobility of cultivated macrophages by cytochalasin B. Role of subplasmalemmal microfilaments. *J. Cell Biol.* **62:**647–659.
3. Bauduin, H., C. Stock, D. Vincent, and J. F. Grenier. 1975. Microfilamentous system and secretion of enzymes in exocrine pancreas. Effect of cytochalasin B. *J. Cell Biol.* **66:**165–182.
4. Bentzel, C. J., B. Hainau, A. Edelman, T. Anagnostopulos, and E. L. Benedetti. 1976. Effect of plant cytokinins on microfilaments and tight junction permeability. *Nature* (*Lond.*). **264:**666–668.
5. Berlin, R. D. Microtubules and fluidity of the cell surface. 1975. *Ann. N. Y. Acad. Sci.* **253:**445–454.
6. Berlin, R. D., and J. P. Fera. 1977. Changes in membrane microviscosity associated with phagocytosis. Effects of colchicine. *Proc. Natl. Acad. Sci. U. S. A.* **74:**1072–1076.
7. Bhattacharyya, B., and J. Wolff. 1976. Polymerisation of membrane tubulin, Nature (*Lond.*). **264:**576–577.
8. Bieger, W., J. Seybold, and H. F. Kern. 1975. Studies on intracellular transport of secretory proteins in the rat exocrine pancreas. III. Effect of cobalt: lanthanum and antimycin A. *Virchows Arch. Abt. A Pathol. Anat.* **368:**329–345.
9. Bolender, R. P. 1974. Stereological analysis of the guinea pig pancreas. I. Analytical model and quantitative description of nonstimulated pancreatic exocrine cells. *J. Cell Biol.* **61:**269–287.
10. Cereijido, M., E. S. Robbins, C. A. Rotunno, W. J. Dolan and D. D. Sabatini. 1978. Polarized monolayers formed by epithelial cells on a permeable and translucent support. *J. Cell Biol.* **77:**853–880.
11. Claude, P. and D. Goodenough. 1973. Fracture faces of zonulae occludentes from tight and leaky epithelia. *J. Cell Biol.* **58:**390–400.
12. De Camilli, P., D. Peluchetti, and J. Meldolesi. 1974. Structural difference between luminal and lateral plasmalemma in pancreatic acinar cells. *Nature* (*Lond.*). **248:**245–246.
13. Decker, R. S., and D. S. Friend. 1974. Assembly of gap junctions during amphibian neurulation. *J. Cell Biol.* **62:**32–47.
14. Dermietzel, R. 1977. In vivo and in vitro formation of the junctional complex in choroid epithelium. A freeze-fracture study. *Cell Tissue Res.* **181:**427–442.
15. Duguid, J. R., and J. P. Revel. 1976. The protein components of the gap junction. *Cold Spring Harbor Symp. Quant. Biol.* **40:**45–48.
16. Ehrhart, J. C., and J. Chauveau. 1977. The protein component of mouse hepatocyte gap junctions. *FEBS* (*Fed. Eur. Biochem. Soc.*) *Lett.* **78:**295–299.

17. Ehrlich, H. P., R. Ross, and P. Bornstein. 1974. Effects of antimicrotubular agents on the secretion of collagen. A biochemical and morphological study. *J. Cell biol.* **62:**390–406.
18. Elias, P. M., and D. S. Friend. 1976. Vitamin A-induced mucous metaplasia: an in vitro system for modulating tight and gap junction differentiation. *J. Cell Biol.* **68:**173–188.
19. Farquhar, M. G., and G. E. Palade. 1963. Junctional complexes in various epithelia. *J. Cell Biol.* **17:**375–412.
20. Fowler, V., and D. Branton. 1977. Lateral mobility of human erythrocyte membrane proteins. *Nature* (*Lond.*). **268:**23–26.
21. Friend, D., and N. B. Gilula. 1972. Variations in tight and gap junctions in mammalian tissues. *J. Cell Biol.* **53:**758–776.
22. Galli, P., A. Brenna, P. De Camilli, and J. Meldolesi. 1976. Extracellular calcium and the organization of tight junctions in pancreatic acinar cells. *Exp. Cell Res.* **99:**183–187.
23. Gilula, N. B., D. W. Fawcett, and A. Aoki. 1976. The Sertoli cell occluding junctions and gap junctions in mature and developing mammalian testis. *Dev. Biol.* **50:**142–168.
24. Hasty, D. L., and E. D. Hay. 1977. Freeze-fracture studies of the developing cell surface. I. The plasmalemma of corneal fibroblasts. *J. Cell Biol.* **72:**667–686.
25. Hudspeth, A. J. 1975. Establishment of tight junctions between epithelial cells. *Proc. Natl. Acad. Sci. U. S. A.* **72:**2711–2713.
26. Humbert, F., R. Montesano, A. Perrelet, and L. Orci. 1976. Junctions in developing human and rat kidney. A freeze-fracture study. *J. Ultrastruct. Res.* **56:**202–214.
27. Jamieson, J. D., and G. E. Palade. 1968. Intracellular transport of secretory proteins in the pancreatic exocrine cell. III. Dissociation of intracellular transport from protein synthesis. *J. Cell Biol.* **39:**580–588.
28. Jamison, J. D., and G. E. Palade. 1968. Intracellular transport of secretory proteins in the pancreatic exocrine cell. IV. Metabolic requirements. *J. Cell Biol.* **39:**589–601.
29. Jamieson, J. D., and G. E. Palade. 1971. Condensing vacuole conversion and zymogen granule discharge in pancreatic exocrine cells. Metabolic studies. *J. Cell Biol.* **48:**503–521.
30. Larsen, W. L. 1977. Structural diversity of gap junctions. A review. *Tissue Cell.* **9:**373–394.
31. Lin, S., and C. E. Snyder. 1977. High affinity cytochalasin B binding to red cell membrane proteins which are unrelated to sugar transport. *J. Biol. Chem.* **252:**5464–5471.
32. Martinez-Palomo, A., and D. Erlij. 1975. Structure of tight junctions in epithelia with different permeability. *Proc. Natl. Acad. Sci. U. S. A.* **72:**4487–4491.
33. Maylié-Pfenninger, M. F., G. E. Palade, and J. D. Jamieson. 1975. Interaction of lectins with the surface of dispersed pancreatic acinar cells. *J. Cell Biol.* **67**(2, Pt. 2)**:**333*a*. (Abstr.)
34. McNutt, N. S., and R. S. Weinstein. 1973. Membrane ultrastructure at mammalian intercellular junctions. *Prog. Biophys. Mol. Biol.* **26:**45–101.
35. Meldolesi, J., P. De Camilli, and A. Brenna. 1977. The topology of plasma membrane in pancreatic acinar cells. *In* Hormonal Receptors in Digestive Tract Physiology. S. Bonfils, P. Fromageot, and G. Rosselin, editors. North Holland Publishing Co., Amsterdam. 203–212.
36. Metz, J., W. G. Forssman, and S. Ito. 1977. Exocrine pancreas under experimental conditions. III. Membrane and cell junctions in isolated acinar cells. *Cell Tissue Res.* **177:**459–474.
37. Mokalewski, S., J. Thyberg, and U. Friberg. 1976. In vitro influence of colchicine on the Golgi complex in A and B cells of guinea pig pancreatic islets. *J. Ultrastruct. Res.* **54:**304–317.
38. Mollgard, K., D. H. Malinowska, and N. R. Saunders. 1976. Lack of correlation between tight junction morphology and permeability properties in developing choroid plexus. *Nature* (*Lond.*). **264:**293–294.
39. Montesano, R., D. S. Friend, A. Perrelet, and L. Orci. 1975. In vivo assembly of tight junctions in fetal rat liver. *J. Cell Biol.* **67:**310–319.
40. Montesano, R., G. Gabbiani, A. Perrelet, and L. Orci. 1976. In vivo induction of tight junction proliferation in rat liver. *J. Cell Biol.* **68:**793–798.
41. Moor, H., and K. Mühlethaler. 1973. Fine structure in frozen-etched yeast cells. *J. Cell Biol.* **17:**609–628.
42. Oliver, J. M., T. E. Ukena, and R. D. Berlin. 1974. Effect of phagocytosis and colchicine on the distribution of lectin binding sites on cell surfaces. *Proc. Natl. Acad. Sci. U.S . A.* **71:**394–398.
43. Orci, L., M. Amherdt, J. C. Henquin, A. C. Lambert, R. H. Unger, and A. E. Renold. 1973. Pronase effect in pancreatic beta cell secretion and morphology. *Science* (*Wash. D. C.*). **180:**647–649.
44. Pisam, M., and P. Ripoche. 1976. Redistribution of surface macromolecules in dissociated epithelial cells. *J. Cell Biol.* **71:**907–920.
45. Pitelka, D. R., S. T. Hamamoto, J. G. Duafala, and M. K. Nemanic. 1973. Cell contacts in the mouse mammary gland. I. Normal gland in postnatal development of the secretory cycle. *J. Cell Biol.* **56:**797–818.
46. Pitelka D. R., and S. T. Hamamoto. 1977. Calcium chelation-induced disruption of occluding junctions by cultured mammary epithelial cells. *J. Cell Biol.* **75**(2, Pt. 2)**:**69*a*. (Abstr.).
47. Reale, E., L. Luciano, K. Franke, E. Pannese, G. Wermbter, and S. Jurato. 1975. Intercellular junctions in the vascular stria on spiral ligament. *J. Ultrastruct. Res.* **53:**284–297.
48. Revel, J. P., and S. S. Brown. 1976. Cell Junctions in development with particular reference to the neural tube. *Cold Spring Harbor Symp. Quant. Biol.* **40:**443–455.
49. Revel, J. P., P. Yip, and L. L. Chang. 1973. Cell junctions in the early chick embryo. A freeze-etch study. *Dev. Biol.* **35:**302–317.
50. Sauerheber, R. D., and L. M. Gordon. 1975. Spin label studies on rat liver plasma membrane: calcium effects on membrane fluidity. *Proc. Soc. Exp. Biol. Med.* **150:**28–31.
51. Scheele, G. A., and G. E. Palade. 1975. Studies on the guinea pig pancreas. Parallel discharge of exocrine enzyme activities. *J. Biol. Chem.* **250:**2660–2670.
52. Schlessinger, J., L. S. Barak, G. G. Hammes, K. M. Yamada, I. Pastan, W. W. Webb, and E. L. Elson. 1977. Mobility and distribution of a cell surface glycoprotein and its interaction with other membrane components. *Proc. Natl. Acad. Sci. U. S. A.* **74:**2909–2913.
53. Shimono, M., and F. Clementi. 1977. Intercellular junctions of oral epithelium. II. Ultrastructural changes in rat buccal epithelium induced by trypsin digestion. *J. Ultrastruct. Res.* **59:**101–112.
54. Simionescu, M., N. Simionescu, and G. E. Palade. 1975. Segmental differentiation in the vascular endothelium. The microvasculature. *J. Cell Biol.* **67:**863–885.
55. Simionescu, M., and N. Simionescu. 1977. Organization of cell junctions in the peritoneal mesothelium. *J. Cell Biol.* **74:**98–110.
56. Stadler, J., and W. W. Franke. 1974. Characterization of the colchicine binding of membrane fractions from rat and mouse liver. *J. Cell Biol.* **60:**297–302.
57. Staehelin, L. A. 1974. Structure and function of intercellular junctions. *Int. Rev. Cytol.* **39:**191–283.
58. Thyberg, J., and A. Hinek. 1977. Electron microscopic studies on embryonic chick spinal ganglion cells. In vitro effects of antimicrotubular agents on Golgi complex. *J. Neurocytol.* **6:**27–38.
59. Tice, L. W., R. L. Carter, and M. C. Cahill. 1977. Tracer and freeze-fracture observations on developing tight junctions in fetal rat thyroid. *Tissue Cell* **9:**395–417.
60. Tice, L. W., S. H. Wollman, and R. C. Carter. 1975. Changes in tight junctions of thyroid epithelium with changes in thyroid activity. *J. Cell Biol.* **66:**657–662.
61. Wanson, J. C., P. Drochmans, R. Mosselmans, and M. F. Ronveaux. 1977. Adult rat hepatocytes in primary culture. Ultrastructural characteristics of intercellular contacts and cell membrane differentiations. *J. Cell Biol.* **74:**858–877.
62. Williams, J. A. 1977. Effects of cytochalasin B on pancreatic acinar cell structure and secretion. *Cell Tissue Res.* **179:**453–466.
63. Williams, J. A., and M. Lee. 1976. Microtubules in pancreatic amylase release by mouse pancreas in vitro. *J. Cell Biol.* **71:**795–806.
64. Yee, A. G., and J. P. Revel. 1975. Endothelial cell junctions. *J. Cell Biol.* **66:**200–204.
65. Zigmond, S., and J. G. Hirsch. 1972. Cytochalasin B inhibition of D-2-deoxyglucose transport into leukocytes and fibroblasts. *Science* (*Wash. D. C.*). **176:**1432–1433.