IN VITRO STIMULATION OF ENZYME SECRETION AND THE SYNTHESIS OF MICROSOMAL MEMBRANES IN THE PANCREAS OF THE GUINEA PIG

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ABSTRACT

Several mechanisms have been suggested to explain how secretory cells remove from the plasmalemma the excess membrane resulting from the insertion of granule membrane during exocytosis: intact patches of membrane may be internalized and then reutilized within the cell; alternatively these membranes may be either disassembled to subunits or degraded. In the latter case new membranes should be synthetized at other sites of the cell, probably in the rough-surfaced endoplasmic reticulum (RER) and the Golgi complex. In the present research, membrane subfractions were obtained from rough microsomes (derived from fragmented and resealed RER cisternae) and from smooth microsomes (primarily contributed by Golgi stacks and vesicles) of the guinea pig pancreas by incubation at 4°C for 4 hr in 0.0005 M puromycin at high ionic strength followed by mild (pH 7.8) alkaline extraction with 0.2 M $NaHCO_3$. Such treatments release the majority of nonmembrane components of both microsomal fractions (i.e., contained secretory enzymes, ribosomes, and absorbed proteins of the cell sap) and allow the membranes to be recovered by centrifugation. The effect of in vitro stimulation of enzyme secretion (brought about in pancreas slices by 0.0001 M carbamoyl choline) on the rate of synthesis of the phospholipid (PLP) and protein of these membranes was then investigated. In agreement with previous data, we observed that in stimulated slices the synthesis of microsomal PLP was greatly increased. In contrast, the synthesis of microsomal membrane proteins was unchanged. These results suggest that exocytosis is not coupled with an increased rate of synthesis of complete ER and Golgi membranes and are, therefore, consistent with the view that excess plasma membrane is preserved and reutilized, either as discrete membrane patches or as membrane macromolecules, throughout the secretory cycle.

It is now firmly established that, in the acinar cells of the pancreas, stimulation of secretion ultimately results in the discharge of digestive enzymes stored within zymogen granules by a process akin to exocytosis (1–5). Such a process consists of fusion of the limiting membrane of the granule with the apical portion of the plasmalemma and therefore results, at least temporarily, in the incorporation of the first membrane into the latter. The mechanism of removal of excess membrane from the acinar lumen is still unknown. As proposed by Palade (1), intact patches of membranes may be internalized from the cell surface and reutilized in the packaging of new zymogen granules. Alternatively these membranes may be degraded as suggested originally by Fawcett (6), new membranes being synthesized at other sites in the cell.

The results of a series of papers by the Hokins

(7–15), dealing with the turnover of pancreatic phospholipids (PLP),[1] seem to be consistent with the latter hypothesis, since, in slices of pancreas stimulated with either cholinergic drugs (7) or pancreozymin (8), incorporation of ^{32}Pi into PLP was found to be greatly enhanced. Such an increment is due to the synthesis of new lipid molecules (9, 10) and does not involve all PLP but is restricted to a few of them, primarily to phosphatidylinositol (PI) (9–11). As far as cellular localization is concerned, the newly synthesized PLP were recovered in the microsome fraction upon differential centrifugation of the homogenate (12); furthermore, radioautographic studies indicated that both the major components of such fraction, the rough-surfaced endoplasmic reticulum (RER) and the Golgi apparatus, are involved in the process (13).

Were the PI effect concerned with the formation of new intracellular membranes, one would expect the synthesis not only of PLP but also of membrane proteins to be increased upon stimulation. Data available indicate that incorporation of radioactive amino acids into microsome proteins is either unchanged or decreased after stimulation (16, 17).[2] Such observation, however, does not demonstrate conclusively that the synthesis of microsomal membrane proteins is not increased. In fact it is known that microsomal proteins are heterogeneous, being contributed by membranes, by (pro)enzymes segregated within the lumen of microsomal vesicles, by soluble proteins, absorbed onto the outer surface of membranes, and by ribosomes. Moreover, the rate of labeling of all these proteins is not the same. In particular, it is known that labeling of exportable proteins is much greater than that of sedentary proteins, such as those bound to the membranes (21, 22).

In the present work we have proceeded to the subfractionation of rough and smooth microsomes isolated from the guinea pig pancreas, by separating nonmembrane proteins from the membranes, and we have tested the effect of in vitro stimulation of enzyme secretion (brought about by carbamoyl choline, a cholinergic drug) on the incorporation of L-leucine-^{14}C into the proteins of the latter. Our results, showing that in stimulated cells incorporation is unchanged, clearly indicate that exocytosis is not coupled with a neosynthesis of complete microsomal membranes.

METHODS

Male albino guinea pigs (gift of Sigurtà Drug Co., Milan, Italy) weighing 450–500 g were fasted overnight. They were stunned by a blow to the head and bled by cardiosection. The pancreata were quickly removed and immersed in ice-cold incubation medium. Tissue slices were prepared and incubated under 95% O_2–5% CO_2 in Krebs-Ringer bicarbonate solution supplemented with L-amino acids and glucose as described by Jamieson and Palade (9).

Sets of slices obtained from the same animals were incubated either with or without carbamoyl choline chloride (carbachol) (0.0001 M). After 20 min of stimulation the slices were washed with warm incubation medium and then transferred to radioactive media. Incorporation was carried out at 37°C for 30 min. ^{32}PK monophosphate was used at the concentration of 80 μCi/ml (1.16 mM); L-leucine-^{14}C at the concentration of 0.75 μCi/ml (0.133 mM).

Cell Fractionation Procedures

At the end of the incubations the slices were homogenized in 5 ml of 0.3 M sucrose by means of 20 up-and-down strokes in a loose-fitting Dounce homogenizer (Kontes Glass Company, Vineland, N.J.). Isolation of rough and smooth microsome fractions was carried out as described previously (22, 23), except that a Spinco SW 41 rotor was used instead of a SW 39.

In order to detach bound ribosomes, isolated rough and smooth microsome fractions were resuspended in 1 ml of M KCl, 0.005 M $MgCl_2$, and 0.0005 M puromycin, pH 6, and transferred to Spinco SW 41 tubes. After 4 hr of incubation at 4°C these suspensions were mixed with 4 ml of 2.5 M sucrose containing 1 M KCl and 0.005 M $MgCl_2$, and overlaid with 1 ml of 1.8 M sucrose-1 M KCl-0.005 M $MgCl_2$. The tubes were filled with 0.3 M sucrose. Centrifugation at 40,000 rpm for 2 hr yielded a thick band at the interface between 1.8 M and 0.3 M sucrose and a small pellet. The pellet was resuspended and combined with the KCl-puromycin load. Such a preparation is referred to hereafter as KCl-puromycin-released subfraction.

[1] *Abbreviations used:* ATP, adenosine triphosphate; NADH and NADPH, nicotinamide adenine dinucleotide and dinucleotide phosphate, reduced forms; Pi, inorganic phosphate; PI, phosphatidylinositol; PLP, phospholipids; RER, rough-surfaced endoplasmic reticulum; RNA, ribonucleic acid; TCA, trichloroacetic acid.

[2] It has been reported that in the pigeon the in vivo stimulation of secretion brought about by injection of either cholinergic drugs or pancreozymin increases the rate of amino acid incorporation into total proteins of pancreatic slices incubated in vitro (18, 19). However, no such effect could be found in the guinea pig (20).

The band, which contained the bulk of microsomal membranes, was collected by means of a spatula, carefully resuspended in 3 ml of 0.17 M NaCl by means of a ground-glass homogenizer, diluted with ~ 9.5 ml of 0.2 M $NaHCO_3$, pH 7.8, and centrifuged at 50,000 rpm in a Spinco 50 Ti rotor for 90 min. The resulting supernatant and pellet were collected. They will be indicated hereafter as $NaHCO_3$-extracted and membrane subfractions, respectively.

Electron Microscopy

Pellets of rough and smooth microsomes and of microsomal subfractions obtained after KCl-puromycin treatment and after KCl-puromycin + $NaHCO_3$ treatments were processed as described previously (24). Thin sections encompassing the entire depth of the pellets were examined in a Philips EM 200 electron microscope.

Assays

Lipids were extracted and purified as described by Folch et al. (24). Lipid phosphorus was estimated according to Ames (25). Proteins were precipitated with cold 10% trichloroacetic acid (TCA) (final concentration), dissolved in 1 M NaOH and assayed according to Lowry et al. (26). RNA was determined on washed TCA precipitates with the orcinol reaction (27). α-amylase activity was determined as described by Bernfeld (28). All cell particulates and extracts for amylase assay were treated with 0.2% Triton X-100 buffered with 0.1 M K phosphate at pH 6.9 to allow full access of the substrate to the enzyme.

For radioactivity determinations, purified lipid extracts were dried and then dissolved in a toluene-base liquid scintillation fluid. Proteins were precipitated with ice-cold 10% TCA (final concentration) and washed twice with 5% TCA. Nucleic acids were removed by heating for 15 min at 90°C the precipitate resuspended in 5% TCA, lipids by an overnight extraction at room temperature with 3:1 ethanol-ether, followed by a second 2 hr extraction. The precipitate was dissolved in hyamine 10 × and mixed with a dioxane-toluene–base liquid scintillation fluid.

All counting rates given are corrected for background. ^{14}C counts were also corrected for quenching by means of an internal standard.

MATERIALS

All chemicals were reagent grade. Carbamoyl choline chloride was a gift of Farmitalia Labs. for Basic Research, Milan, Italy. Puromycin hydrochloride was obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio. L-leucine-^{14}C (uniformly labeled; SA:260 mCi/mmole) and K monophosphate-^{32}P (SA:250 mCi/mmole) were purchased from New England Nuclear, Langen, Germany. Enzyme grade sucrose was obtained from Mann Research Labs. Inc., New York. Solvents were from E. Merck AG, Darmstadt, Germany.

RESULTS

Isolation of Microsomal Fractions

The isolation of microsomal membranes has been carried out by removing from microsomes the nonmembrane components while leaving the structure and composition of membrane apparently unaffected.

In previous studies (28), we have observed that washing rough and smooth microsomes, isolated from guinea pig pancreas, with slightly alkaline buffers results in the quantitative extraction of the segregated digestive (pro)enzymes and in the release of a large proportion of the RNA, while PLP can be recovered, along with some proteins, in a fraction containing recognizable membranes. In the meantime, in a series of elegant experiments Blobel, Sabatini, Adelman, and Nonomura (29, 30, 31) have shown that, in the liver microsomes, up to 90% of the ribosomes can be detached from the membranes by treatment with puromycin at high ionic strength. We have now demonstrated that, with a few modifications,[3] such a procedure is effective also in the pancreas. Hence subfractions of membranes virtually free of ribosomes and segregated enzymes can be obtained from both rough and smooth microsomes by incubation with puromycin—1 M KCl, for 4 hr at 4 C followed by mild alkaline extraction with 0.2 M Na HCO_3, pH 7.8.

Table I shows the gross chemistry of the subfractions isolated from rough and smooth microsomes as well as the distribution of the segregated enzyme α-amylase. The morphology of rough microsomes and of the membrane subfractions isolated therefrom by the KCl-puromycin and the KCl-puromycin + $NaHCO_3$ treatments is shown in Fig. 1.

[3] Concerning the pH of the incubation fluid (pH 6 instead of 7.5) as well as the length and temperature of the incubation. In fact, with concentrations of KCl lower than 1 M (0.25 and 0.5) or incubation times shorter than 4 hr (1–2 hr), only a limited detachment of bound ribosomes was obtained. Conversely, when microsomes suspended in puromycin- 1 M KCl were incubated at 4°C for 12 hr or heated at 37°C for 15 min after 1 hr incubation at 4°C, the yield of recovered membranes was reduced and they appeared morphologically damaged.

TABLE I

Gross Chemistry and α-Amylase Activity of Rough and Smooth Microsome Fractions and Subfractions (Guinea Pig Pancreas)

	Proteins		PLP		RNA		α-Amylase	
	mg/g pancreas (wet weight)	%	μmoles of lipid P/mg protein	%	μg/mg protein	%	u*/mg protein	%
Rough microsomes	4.8	100.0	132.0	100.0	270.2	100.0	11.8	100.0
KCl-puromycin released	2.7	56.7	17.8	7.9	332.5	68.7	16.9	81.0
$NaHCO_3$ extracted	0.4	9.1	60.0	4.2	179.0	5.7	26.5	19.4
Membranes	1.4	29.8	324.0	70.8	41.0	4.6	0.5	1.3
Recovery		95.6		82.9		79.0		101.7
Smooth microsomes	4.2	100.0	277.8	100.0	43.5	100.0	12.2	100.0
KCl-puromycin-released	1.9	45.0	36.4	6.1	68.4	71.5	18.2	69.0
$NaHCO_3$ extracted	0.4	7.5	67.0	1.9	28.2	4.8	30.4	18.5
Membranes	1.4	34.6	572.5	71.0	4.3	3.4	0.2	0.6
Recovery		87.1		79.0		79.7		88.1

Values given are the averages of two highly consistent experiments.
* 1 amylase unit = 1 mg maltose formed/min at 30°C.

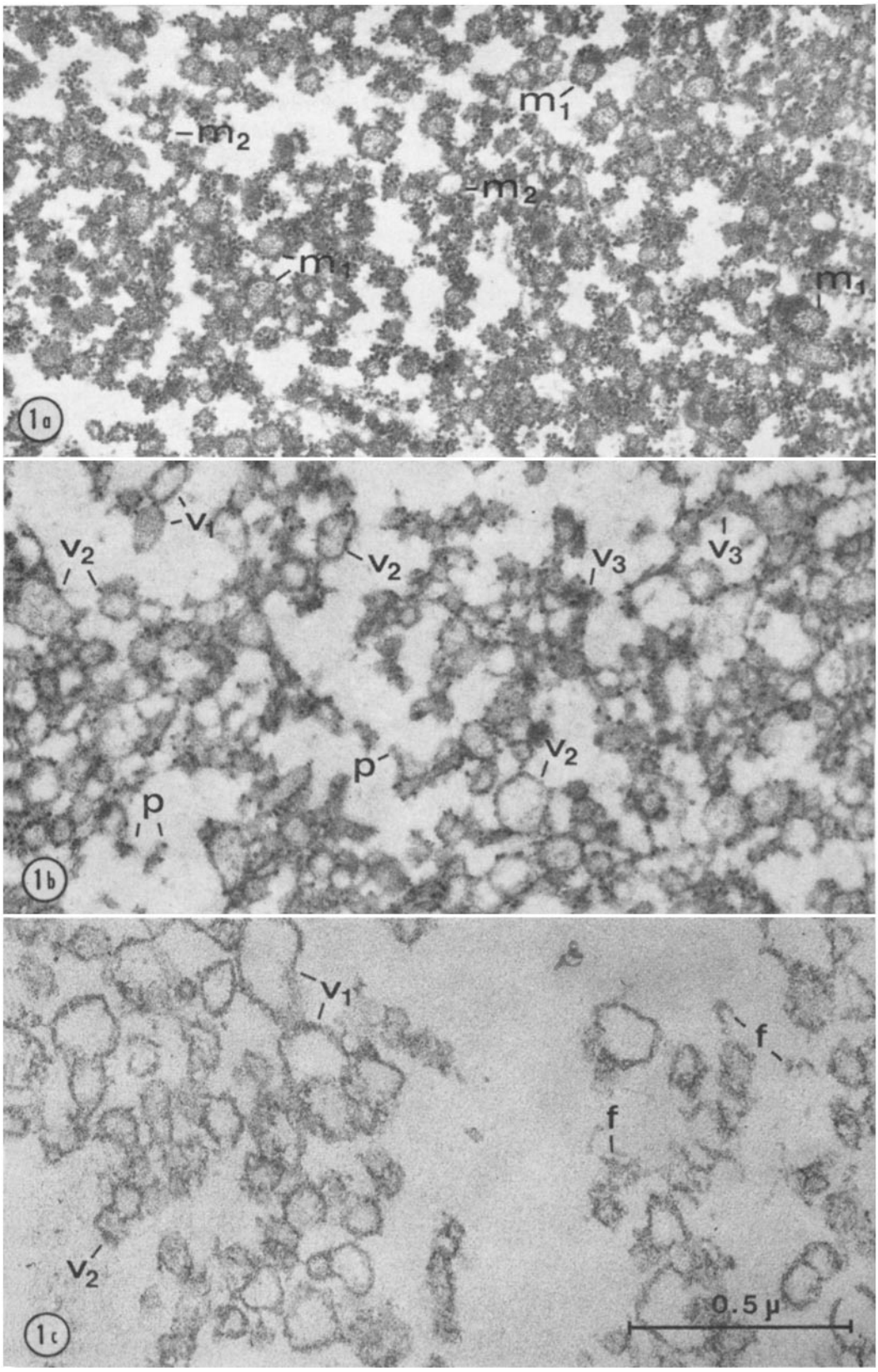
It is evident that the high salt-puromycin treatment releases most of the bound RNA and also ~80% of the α-amylase. In agreement with these biochemical results, the morphological study reveals that the number of attached ribosomes is drastically reduced with respect to that found in untreated rough microsomes (compare Figs. 1 *a* and *b*). In most of the vesicles the homogeneous content is no longer visible, while in some others it appears only partially extracted or completely preserved. The unit membrane structure of membranes appears always well preserved.

Washing of the KCl-puromycin-extracted microsomes with $NaHCO_3$, pH 7.8, results in the virtually complete extraction of the remaining α-amylase and RNA. As shown in Fig. 1 *c* for rough microsomal membranes, the final pellets contain only membranes usually organized in closed vesicles, which are bounded by a unit membrane and have no visible content. Comparable results have been obtained with the smooth microsomal membranes. The membrane subfractions contain the bulk of the PLP present in the original microsome fractions and 30–35% of the proteins.

Another possible source of nonmembrane proteins present in microsome fractions is the cell sap, since soluble proteins are known to be absorbed onto the outer surface of cellular membranes upon homogenization. We have checked the effectiveness of the two procedures used for isolating membranes, i.e. KCl-puromycin incubation and $NaHCO_3$ extraction, in releasing radioactive proteins absorbed onto microsomes upon homogenization of fresh pancreas tissue in the presence of a radioactive final supernatant fraction. The latter had been obtained by centrifuging at high speed the homogenate derived from pancreas slices labeled in vitro with L-leucine-^{14}C. As shown in Table II, both treatments turn out to be very effective in removing absorbed TCA-insoluble radioactivity, thus suggesting that our final membrane subfractions should be practically devoid of any contamination by cell sap proteins.

Effect of In Vitro Stimulation of Secretion on the Incorporation of ^{32}Pi into Microsome PLP and of L-Leucine-^{14}C into Microsome Membrane Proteins

In previous studies we have reported that in the guinea pig pancreas all microsomal PLP are located in microsomal membranes (23). The study of the incorporation of ^{32}Pi into membrane PLP was therefore carried out with unfractionated rough and smooth microsomes.



In agreement with the classical results by the Hokins (7–15), we observed that incubation of pancreas slices in the presence of a cholinergic drug (carbachol) at a concentration known[4] to greatly stimulate secretion of digestive (pro)enzymes elicits a large increment of the ^{32}Pi incorporation into microsomal PLP. The effect was evident both in rough and in smooth microsomes and was particularly marked in the latter fraction, where the increase was nearly four fold (Table III). Since, in the pancreas, rough microsomes are known to originate from fragmented and resealed RER cisternae while smooth microsomes are primarily contributed by Golgi elements, our results are consistent with the previous radioautographic findings by Hokin and Huebner (13) which showed increased labeling in the cellular areas occupied by these two structures.

The effect of stimulation of secretion on the incorporation of L-leucine-^{14}C into protein was investigated in membrane subfractions isolated from rough and smooth microsomes as well as in other subfractions derived therefrom, i.e., the KCl-puromycin–released and the $NaHCO_3$-extracted subfractions. Results are shown in Table IV. At variance with the situation found with PLP, no significant changes appeared upon stimulation in the rate of incorporation of L-leucine-^{14}C into the proteins of the membranes, as well as into those of the other subfractions. As expected, the specific radioactivity of the $NaHCO_3$-extracted proteins (which appear to be accounted for nearly exclusively by exportable [pro]enzymes) was found to be four- to sevenfold higher than that found in the membranes, while the activity of the KCl-puromycin released proteins, containing secretory (pro)enzymes as well as ribosomal and soluble proteins, was intermediate. At the time point investigated (30 min), smooth membrane proteins were consistently more labeled than those of the rough counterpart.

[4] From the literature as well as from our preliminary experiments.

DISCUSSION

Isolation of the Microsomal Membrane Fractions

The isolation from microsomes of fractions containing pure membranes or the purification of membrane-bound proteins should be considered a prerequisite to the study of the synthesis of membrane proteins. Results obtained with unfractionated microsomes can not be extrapolated to membranes since most of the TCA-insoluble radioactivity recovered in microsomes may be contributed by nonmembrane proteins.

In the present research we have isolated from rough and smooth microsomes of the guinea pig pancreas morphologically recognizable membrane fractions. Such fractions contain only trace amounts of nonmembrane proteins, as judged from their low RNA content, α-amylase activity, and recovery of absorbed radioactive proteins. We believe therefore that only a small proportion of the TCA-insoluble radioactivity found in membrane preparations should be attributed to contaminations, particularly by secretory proteins, while most of it should be regarded as due to membrane synthesis.

Another question which might be asked is whether membrane components are also extracted during the purification procedures. To get some insight into this problem, we have investigated the

FIGURE 1 (*a*) Rough microsome fraction. The fraction consists of rough microsomes of varied size and content density. Most of them have a dense content (m_1); some are partially extracted (m_2). (*b*) Rough microsome fraction after treatment with KCl-puromycin. The fraction contains vesicles, bounded by a unit membrane (v), and membrane pieces with free edges (p). The number of bound ribosomes is drastically reduced with respect to the original rough microsome fraction. Many vesicles appear completely (v_1) or partially (v_2) stripped of them, while some others (v_3) still exhibit many ribosomes attached to their membranes. Most of the vesicles are swollen and totally or partially extracted, while some others retain a moderately dense content. (*c*) Rough microsome fraction after treatment with KCl-puromycin and milk alkaline extraction with $NaHCO_3$. The fraction consists of empty swollen and distorted (v_1), frequently ruptured (v_2) microsomal vesicles bounded by a recognizable unit membrane. Membrane fragments (f) are seen scattered among the vesicles. × 70,000.

TABLE II

Releasing Effect of the KCl-Puromycin Extraction (A) and of the Mild Alkaline Extraction with $NaHCO_3$ (B) on Cell Sap Proteins Absorbed onto Microsomes Isolated from the Guinea Pig Pancreas

The effect is estimated by comparing the recovery of absorbed TCA-insoluble radioactivity found in microsomes washed with 0.3 M sucrose (controls) with that found in microsomes extracted with either KCl-puromycin (A) or 0.2 M $NaHCO_3$, pH 7.8 (B). The gross chemistry of the fraction is also given.

Treatment of isolated microsomes	Protein	PLP	TCA-insoluble radioactivity	% of release
	mg	*μmoles*	*dpm*	
A.				
0.3 M sucrose	3.41	0.56	1234	
KCl-puromycin	1.58	0.51	243	80.3
B.				
0.3 M sucrose	3.25	0.52	1303	
$NaHCO_3$	1.37	0.45	99	93.7

In order to prepare radioactive cell sap proteins, sets of pancreas slices were labeled in vitro for 1 hr in Krebs-Ringer bicarbonate solution supplemented with amino acids and glucose and containing L-leucine-^{14}C (0.3 μCi/ml; 0.4 mM). The slices were gently homogenized in 10 vol of 0.3 M sucrose and centrifuged at 50,000 rpm for 60 min in a Spinco 50 Ti rotor in order to separate the cell sap (final supernatant) from any particulate matter.

Pieces of fresh pancreas, weighing 0.5 g, were homogenized in 10-ml aliquots of such radioactive cell sap, and total microsomes were isolated as described (20).

In A, control microsomes were resuspended in 0.3 M sucrose and immediately reisolated, whereas the experimental samples were resuspended in 1 M KCl, 0.005 M $MgCl_2$, 0.0005 M puromycin, pH 6, and incubated for 4 hr at 4°C. After addition of concentrated sucrose to a final concentration of 2 M and gradient centrifugation as described under Methods, the floating band was collected and analyzed.

In B, isolated microsomes were resuspended in either 0.3 M sucrose (controls) or in the NaCl-$NaHCO_3$ mixture and immediately re-isolated.

Values given are the averages of two highly consistent experiments.

TABLE III

Carbachol Stimulation of Pancreatic Slices In Vitro. Effect on the Incorporation of ^{32}Pi into the Phospholipids or Rough and Smooth Microsomes

Guinea pig pancreas slices were preincubated in vitro for 20 min either with or without carbachol (10^{-4} M), then washed, transferred to flasks containing radioactive medium, and labeled for 30 min.

	Specific radioactivity (cpm/μmole of lipid P)		
	Control	Stimulated	% of increase
Rough microsomes	200 *130–370*	536 *446–626*	+168
Smooth microsomes	444 *328–560*	2051 *1870–2232*	+363

Values given are the averages of two experiments. Ranges are in italics.

distribution of several enzymes[5] (which, from our previous studies [23], are known to be bound to the microsomal membranes of the guinea pig pancreas) among the subfractions obtained by our purification procedures. The results (not shown in tables) indicate that while most of these enzymes, such as NADH-cytochrome *c* reductase, ATPase, 5′ nucleotidase, and β-leucylnaphthylamidase, are recovered quantitatively in the membrane subfractions, up to 50% of the NADPH-cytochrome *c* reductase activity was solubilized and recovered in the KCl-puromycin–released subfractions.[6] On the other hand, it should be emphasized that isolated membranes retain their morphological unit membrane configuration, contain the bulk of the PLP originally present in microsomes, and have PLP/protein ratios comparable with those found in membrane fractions isolated from other sources (see reference

[5] Enzyme assays were made as described previously (32).

[6] The result is not surprising since in other tissues, such as the liver, the NADPH cytochrome *c* reductase is known to be easily solubilized by treatment with pancreatic digestive enzymes such as lipase and trypsin (33, 34). In our experiments these enzymes are probably extracted from the lumen of pancreas microsomes during incubation with KCl-puromycin (Table I and Fig. 1 *b*). The solubilization is likely to be due to lipase, which needs no activation and is active in the cold, rather than to the proteases which are stored in microsomes as zymogens.

TABLE IV

Carbachol Stimulation of Pancreatic Slices In Vitro. Effect on the Incorporation of L-Leucine-^{14}C into the Proteins of Rough and Smooth Microsomal Subfractions

Guinea pig pancrease slices were preincubated in vitro for 20 min either with or without carbachol (0.0001 M), then washed, transferred to flasks containing radioactive medium, and labeled for 30 min.

Microsomal subfractions	Specific radioactivity (dpm/mg protein)			
	Rough microsomes		Smooth microsomes	
	Control	Stimulated	Control	Stimulated
KCl-puromycin released	12,950 ± 3,928	13,867 ± 2,152	16,410 ± 2,500	16,402 ± 1,371
$NaHCO_3$ extracted	33,775 ± 5,590	37,725 ± 3,038	37,965 ± 5,002	45,850 ± 5,111
Membranes	6,477 ± 983	5,525 ± 1,069	11,465 ± 692	10,757 ± 629

Values given are the averages of four experiments ± SE.

35). We can conclude, therefore, that a large scale removal of membrane components is unlikely, even if it is probable that some membrane-bound proteins are solubilized during purification. With this limitation in mind, we believe that our membrane fractions may be considered suitable for studying the synthesis of membrane proteins.

Effect of Stimulation of Enzyme Secretion on Microsome Membrane Synthesis

Two mechanisms have been suggested to explain how secretory cells remove from the apical portion of the plasmalemma the excess membrane resulting from the insertion of the limiting membranes of secretory granules during exocytosis. The first is based on morphological evidence obtained both in the guinea pig pancreas (1) and in the rat parotid (36), whose acinar cells are similar to those of the pancreas, and suggests that intact patches of membranes may be withdrawn from the plasmalemma and eventually reutilized in the intracellular transport of secretory material. Since, according to this scheme, membranes are preserved throughout the secretory cycle, no net increase of the rate of membrane synthesis is required. Another possibility, which has been envisaged, is that the granule membrane, after fusing with the plasmalemma, is broken down to its molecular components (6). In this case the cell would necessarily increase the synthesis of new membranes to substitute for the membranes which have been degraded (6). Alternatively, as suggested recently by Hokin, granule membranes would be disassembled to yield macromolecular subunits, which would then be reassembled into complete membranes (13–15). The cellular sites where resynthesis or reassembly of membranes might take place are the RER and the Golgi apparatus, since the increase of ^{32}Pi incorporation into PLP brought about by secretagogues (PI effect) has been localized in these structures by light microscope radioautography (13).

Our work was carried out with the aim of contributing to the understanding of this problem by testing whether, in the acinar cells of the guinea pig pancreas, in vitro stimulation of secretion brings about a change of the rate of synthesis of the membrane components in both the RER and the Golgi apparatus. By means of established cell fractionation procedures (22, 23) we have first isolated the rough microsomes, whose vesicles are nearly exclusively derived from the RER, and the smooth microsomes, which in the guinea pig pancreas are known to be composed primarily of Golgi vesicles and cisternae (22); we have found that in both these fractions stimulation results in a marked increase in the rate of incorporation of ^{32}Pi into membrane PLP, thus confirming the radioautographic results of Hokin and Huebner (13). However, the incorporation of L-leucine-^{14}C into the proteins of the purified membranes isolated from rough and smooth microsomes was unchanged. Since the in vitro stimulation of secretion does not seem to bring about any changes of the intracellular pool of leucine (5, 20), this observation indicates that the synthesis of the two major membrane components may be differently affected by the stimulation of secretion and rules out the possibility that exocytosis is necessarily coupled with an increase in the rate of synthesis of complete microsomal membranes.

Recent results from the Hokins' laboratory

show that the correlation between the PI effect and enzyme secretion is not very strict, since the two processes show different dose responses (37) and poor correlation in time (14); furthermore, in vitro stimulation in the absence of Ca^{++} does not result in enzyme secretion, although the PI effect still takes place (38). Our conclusion is in agreement with recent results by Jamieson and Palade (4, 5) which clearly demonstrate that in the acinar cell stimulated in vitro in the presence of cyclohexamide a complete cycle of granule membrane insertion into the plasmalemma and withdrawal can be carried out although protein synthesis is inhibited by ~95%. Since granule discharge is followed by a marked increase in the number and size of Golgi elements, it was concluded that the excess membranes of the latter are most likely not newly synthesized but either brought back as intact membrane from the cell surface or assembled from a pool of preexisting macromolecular precursors (5). In the first case the PI effect might not be related to membrane synthesis but rather to different metabolic events, yet unidentified, triggered by the stimulation of secretion, whereas in the latter case it could be dependent on the assembly of new membranes, i.e., the PI might be inserted as "cement" between membrane "subunits" (13–15) or macromolecules.

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