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

JACOPO MELDOLESI and DARIO COVA

From the Department of Pharmacology, University of Milan, and the Center of Cyto-
pharmacology of the Consiglio Nazionale delle Ricerche, Milan, Italy

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 dur-
ing 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 synthesized 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 ressealed RER cisternae) and from smooth microsomes (primarily con-
tributed 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 ex-
traction with 0.2 M NaHCO₃. Such treatments release the majority of nonmembrane com-
ponents 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 centrifuga-
tion. 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 macro-
molecules, 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 exo-
cytosis (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 mech-
anism of removal of excess membrane from the
acinocr 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. Alter-
natively these membranes may be degraded as
suggested originally by Fawcett (6), new mem-
branes being synthesized at other sites in the cell.

The results of a series of papers by the Hokins
Abbreviations used: ATP, adenosine triphosphate; NADH and NADPH, nicotinamide adenine dinucleotide and dinucleotide phosphate, reduced forms; Pi, inorganic phosphate; PLP, phosphatidyl-inositol; PLP, phospholipids; RER, rough-surfaced endoplasmic reticulum (RER) and the Golgi apparatus, are involved in the process (13).

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 carbachol, a cholinergic drug) on the incorporation of L-leucine-14C 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 Sigurta Drug Co., Milan, Italy) weighing 450-500 g were fasted overnight. They were stunned by a blow to the head and bled by cardiospection. The pancreata were quickly removed and immersed in ice-cold incubation medium. Tissue slices were prepared and incubated under 95% O2-5% CO2 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. 32P monophosphate was used at the concentration of 80 µCi/ml (1.16 mCi); L-leucine-14C at the concentration of 0.75 µCi/ml (0.133 mCi).

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 microsomal 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 microsomal fractions were resuspended in 1 ml of 0.3 M KCl, 0.005 M MgCl2, 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 MgCl2, and overlaid with 1 ml of 1.8 M sucrose-1 M KCl-0.005 M MgCl2. 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.
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 NaHCO3, 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 NaHCO3-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 + NaHCO3 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). a-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 suspended in 0.2 M K phosphate at pH 6.9, followed by a second 2 hr extraction. The precipitate was dissolved in hyamine 10 X and mixed with a dioxane-toluene-base liquid scintillation fluid.

All counting rates given are corrected for background. 14C 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 a gift of Farmitalia Labs. for Basic Research, Milan, Italy. 1-leucine-14C (uniformly labeled; SA:260 mCi/mmole) and K monophosphate32p (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 fractions 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 Nomomura (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, 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 NaHCO3, 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 + NaHCO3 treatments is shown in Fig. 1.

---

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 (mg/g pancreas (wet weight)) | PLP (µmoles of lipid P/mg protein) | RNA (µg/mg protein) | α-Amylase (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           |
| NaHCO₃ 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 36.1 6.1                    | 68.4 71.5                         | 18.2 69.0           |
| NaHCO₃ 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 NaHCO₃, 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 30-55% 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 NaHCO₃ 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-¹⁴C. 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 ³²P into Microsome PLP and of L-Leucine-¹⁴C 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 ³²P 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 to greatly stimulate secretion of digestive (pro)enzymes elicits a large increment of the 32Pi 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-14C 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 NaHCO3-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-14C into the proteins of the membranes, as well as into those of the other subfractions. As expected, the specific radioactivity of the NaHCO3-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

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 (m1); some are partially extracted (m2). (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 (v1) or partially (v2) stripped of them, while some others (v3) 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 NaHCO3. The fraction consists of empty swollen and distorted (v1) vesicles frequently ruptured (v2) microsomal vesicles bounded by a recognizable unit membrane. Membrane fragments (f) are seen scattered among the vesicles. X 70,000.
**TABLE II**

Releasing Effect of the KCl-Puromycin Extraction (A) and of the Mild Alkaline Extraction with NaHCO₃ (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 NaHCO₃, pH 7.8 (B). The gross chemistry of the fraction is also given.

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-leucine¹⁴C (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 MgCl₂, 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-NaHCO₃ mixture and immediately re-isolated.

Values given are the averages of two highly consistent experiments.

| Treatment of isolated microsomes | Protein | PLP TCA-insoluble radioactivity | % of release |
|----------------------------------|---------|-----------------------------|--------------|
|                                   | mg      | μmole | 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                      |
| NaHCO₃                           | 1.37    | 0.45  | 99 93.7                   |

**TABLE III**

Carbachol Stimulation of Pancreatic Slices In Vitro. Effect on the Incorporation of ³²P 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 | Control | Stimulated | % of increase |
|------------------------|---------|------------|--------------|
| Rough microsomes       | 200     | 396        | +168         |
| Smooth microsomes      | 444     | 2051       | +963         |

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

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

Guinea pig pancreas 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) |
|-------------------------|---------------------------------------|
|                         | Control | Stimulated | Control | Stimulated |
| KCl-puromycin released   | 12,950 ± 3,928 | 13,867 ± 2,152 | 16,410 ± 2,500 | 16,402 ± 1,371 |
| NaHCO3 extracted         | 33,775 ± 5,590 | 37,725 ± 3,038 | 37,965 ± 5,002 | 45,850 ± 5,111 |
| Membranes                | 6,477 ± 983  | 5,925 ± 1,069 | 11,465 ± 692  | 10,757 ± 629   |

Values given are the averages of four experiments ± SE.

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 32P 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 32P into membrane PLP, thus confirming the radioautographic results of Hokin and Huebner (13). However, the incorporation of L-leucine-14C 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.

We are grateful to Doctors G. E. Palade and J. D. Jamieson for helpful suggestions and encouragement. Received for publication 8 March 1971, and in revised form 15 June 1971.

REFERENCES

1. Palade, G. E., 1959. In Subcellular Particles. T. Hayashi, editor. The Ronald Press Company, New York. 64.
2. Caro, L., and G. E. Palade. 1964. J. Cell Biol. 20:473.
3. Ichikawa, A. 1965. J. Cell Biol. 24:369.
4. Jamieson, J. D., and G. E. Palade. 1971. J. Cell Biol. 48:503.
5. Jamieson, J. D., and G. E. Palade. 1971. J. Cell Biol. 50:135.
6. Fawcett, D. W. 1962. Circulation. 26:1105.
7. Hokin, M. R., and L. E. Hokin. 1953. J. Biol. Chem. 203:967.
8. Hokin, L. E., and M. R. Hokin. 1956. J. Physiol. (London). 132:442.
9. Hokin, L. E., and M. R. Hokin. 1955. Biochim. Biophys. Acta. 18:102.
10. Hokin, L. E., and M. R. Hokin. 1958. J. Biol. Chem. 233:305.
11. Hokin, L. E., and M. R. Hokin. 1965. J. Histochem. 13:113.
12. Redman, C. M., and L. E. Hokin. 1959. J. Biophys. Biochem. Cytol. 6:207.
13. Hokin, L. E., and D. Huesbner. 1967. J. Cell Biol. 21:521.
14. Hokin, L. E. 1969. Ann. N.Y. Acad. Sci. 165:995.
15. Hokin, L. E. 1967. In Handbook of Physiology, Section 6. C. F. Code, editor. American Physiological Society, Washington, D. C. 2935.
16. Poort, G., and A. N. Sangster. 1963. Biochim. Biophys. Acta. 76:471.
17. Schuchner, E. 1954. Ph.D. Thesis, McGill University, Quebec, Canada.
18. Webster, P. D. 1968. Gastroenterology. 55:375.
19. Webster, P. D., and M. P. Tyor. 1966. Amer. J. Physiol. 211:157.
20. Meldolesi, J. 1970. Brit. J. Pharmacol. 40:721.
21. Siekevitz, P., and G. E. Palade. 1960. J. Biophys. Biochem. Cytol. 7:519.
22. Jamieson, J. D., and G. E. Palade. 1967. J. Cell Biol. 34:577.
23. Meldolesi, J., J. D. Jamieson, and G. E. Palade. 1971. J. Cell Biol. 49:109.
24. Falch, J., M. Lees, and G. H. Sloane-Stanley. 1957. J. Biol. Chem. 226:497.
25. Ames, B. N. 1966. In Methods in Enzymology. E. F. Neufeld and V. Ginsburg, editors. Academic Press Inc., New York. 8:115.
26. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. J. Biol. Chem. 193:265.
27. Meijbaum, W. 1939. Z. Phys. Chem. (Leipzig). 258.117.
28. Berenfeld, P. 1955. In Methods in Enzymology. S. P. Colowick and N. O. Kaplan, editors. Academic Press Inc., New York. 1:49.
29. Sabatini, D. D., G. Blobel, Y. Nomura, and M. R. Adelman. In Advances in Cell Biology and Cytopharmacology. F. Clementi and B. Ceccarelli, editors. Raven Press, Hewlett, N. Y. In press.
30. Adelman, M. R., G. Blobel, and D. D. Sabatini. 1970. J. Cell Biol. 47:3 a. (Abstr.)
31. Blobel, G., and D. D. Sabatini. 1971. Proc. Nat. Acad. Sci. U.S.A. 68:390.
32. Meldolesi, J., J. D. Jamieson, and G. E. Palade. 1971. J. Cell Biol. 49:150.
33. Williams, C. H., and H. Kamin. 1962. J. Biol. Chem. 237:587.
34. Omura, T., P. Siekevitz, and G. E. Palade. 1967. J. Biol. Chem. 242:2389.
35. Meldolesi, J., J. D. Jamieson, and G. E. Palade. 1971. J. Cell Biol. 49:150.
36. Amsterdam, A., I. Ohad, and M. Schramm. 1969. J. Cell Biol. 41:753.
37. Hokin, M. R. 1968. Arch. Biochem. Biophys. 124:280.
38. Hokin, L. E. 1966. Biochim. Biophys. Acta. 115:219.