Phospholipide Turnover in Microsomal Membranes of the Pancreas during Enzyme Secretion

By COLVIN M. REDMAN and LOWELL E. HOKIN, M.D.

(From the Department of Physiological Chemistry, University of Wisconsin, Madison)

(Received for publication, March 23, 1959)

ABSTRACT

After incubation of pigeon pancreas slices with P^{32} and isolation of various fractions by differential centrifugation the deoxycholate extract of the microsome fraction was found to account for over half of the phospholipide P and over half of the P^{32} incorporated into the phospholipides. The remaining phospholipide P and P^{32} were fairly evenly distributed in the nuclei, zymogen granules, mitochondria, microsomal ribonucleoprotein particles, and the soluble fraction.

When enzyme secretion was stimulated with acetylcholine about two-thirds of the increment in radioactivity in the total phospholipides was found in deoxycholate soluble components of the microsome fraction. The remainder of the increment was distributed in the other fractions. This indicates that the cellular component in which the increase in phospholipide turnover occurs on stimulation of secretion is a membranous structure. Evidence is presented which indicates that the increment in radioactivity in the non-microsomal fractions on stimulation of secretion is due to contamination of these fractions with fragments of the stimulated membranous structure.

The distribution of P^{32} radioactivity in each of the chromatographically separated phospholipides in the various fractions from unstimulated tissue paralleled the distribution of radioactivity in the total phospholipide fraction, indicating that individual phospholipides are not concentrated in different fractions but are associated together in the membranous structures of the microsome fraction. The major proportion of the stimulation of the turnover of the individual phospholipides also occurred in the microsome fraction.

The distribution of radioactivity from glycerol-1-C^{14} in the total phospholipides and in the individual phospholipides in the various fractions was similar to the distribution of P^{32}. In the microsome fraction acetylcholine stimulated the incorporation of glycerol-1-C^{14} in each phospholipide which showed a stimulation of P^{32} incorporation.

The significance of the turnover of phosphatides in microsomal membranes in relation to the mechanism of secretion is discussed.

INTRODUCTION

In previous studies a relationship was shown to exist between phospholipide turnover and secretion in a variety of exocrine and endocrine glands (1–8). In these earlier studies phospholipide turnover was followed in whole cells. Knowledge as to the cellular component in which the increased turnover of phosphatides occurs when secretion is stimulated would aid greatly in determining the significance of the phospholipide effect.

In the present studies the cellular site of the phospholipide effect has been investigated by the technique of differential centrifugation of homogenates of pancreas tissue after incubation with labelled lipide precursors in the presence and absence of the secretogogue, acetylcholine. Most of the phospholipide P, the lipide radioactivity, and the increment of radioactivity on stimulation of secretion was in deoxycholate-soluble material in the microsome fraction. This fraction has previously been shown to consist of membranous material, primarily vesicles derived from the endoplasmic reticulum (9).

The significance of this finding in relation to the role of phospholipides in secretion is discussed.

Materials and Methods

Incubation of Tissues.—Equal portions of pancreas slices from two pigeons were placed in each of two vessels containing 11.5 ml. of bicarbonate saline, 0.5 ml. of 5 per cent glucose, and the indicated radioactive precursor. The quantities and specific activities of the radioactive precursors were the same as in a previous study (6). All P^{32} activities are corrected to a specific activity for the inorganic phosphate of 20 μc./μM phosphate. The C^{14} activities were not corrected. The quantity of tissue per vessel ranged from about 700 to 1200 mg. A final concentration of 10^{-5} M acetylcholine chloride and 10^{-4} M eserine sulfate were added to the appropriate vessel to stimulate secretion. The vessels were gassed with 5 per cent CO_2 in O_2 and incubated for 2 hours at 37°C.

Fractionation of the Tissues.—After incubation, the slices from each vessel were placed in 10 ml. of cold 17 per cent sucrose and homogenized in a motor-driven Potter-Elvehjem all glass homogenizer (10) for about 90 seconds. The homogenate was strained through cheese-cloth. The filtrate was made up to a volume of 10 ml. with 17 per cent sucrose, and a 1 ml. aliquot was removed for various analyses.

As reported in an earlier study (11) preliminary washing of the particulate material with sucrose allowed better separation of the various cell fractions. The following procedure, which was a slight modification of previously published methods (11–13), was followed. The homogenate was centrifuged for 30 minutes in the model L Spinco centrifuge at 105,000 *g*. The sediment was rehomogenized in 17 per cent sucrose and again centrifuged at 105,000 *g* for 30 minutes. The two supernatant fractions were pooled to make up the soluble fraction. The sediment was rehomogenized in cold 17 per cent sucrose and centrifuged at 600 *g* for 10 minutes in a Lourdes refrigerated centrifuge. The sediment is referred to as the nuclear fraction. The supernatant fluid was centrifuged at 1,200 *g* for 10 minutes, and the sediment obtained is referred to as the zymogen granule fraction. The mitochondrial fraction was obtained by centrifuging at 8,200 *g* for 15 minutes. The supernatant fluid obtained from this centrifugation was discarded. In those experiments in which particulate fractions were divided into deoxycholate soluble and insoluble fractions (14), the fractions were suspended in an appropriate volume of 17 per cent sucrose, and an aliquot was removed for various analyses. The remainder was mixed with an appropriate amount of 1 per cent sodium deoxycholate in 0.4 M glycyl glycine buffer. The final pH was 8.0. After standing for 5 minutes at 0° the deoxycholate-treated suspensions were centrifuged. The treated microsomes were centrifuged at 105,000 *g* for 3 hours, and the other treated fractions were centrifuged at 8,500 *g* for 30 minutes.

Isolation and Counting of the Phospholipides.—The various fractions were suspended in sucrose, and trichloroacetic acid was added to give a final concentration of 5 per cent. The total phospholipides were isolated and aliquots were chromatographed as described previously (6). The phospholipide P was determined on other aliquots (6). All values for phospholipide P are based on 1 gm. of original tissue. The phospholipide spots on the chromatograms were identified by autoradiography and by staining with rhodamine G (15). The chromatograms containing C^{14}-labelled lipides required contact with x-ray film for 1 month to obtain satisfactory autoradiograms. After this time all of the lipides showed radioactive spots which coincided with the stained spots. Both the P^{32}-labelled spots and the C^{14}-labelled spots were cut out and counted in a Nuclear Chicago "micromil" counter. Although over two-thirds of the radioactivity from the C^{14} was absorbed by the paper, counting the spots directly eliminated the necessity for elution, and gave more reproducible results, especially with those phosphatides which elute rather poorly (6). To correct for absorption on paper a sample of C^{14}-labelled lipides was plated at infinite thinness. Standard spots of varying sizes were also prepared on the chromatogram paper. From this data and the weight of the spots cut out from the chromatograms the C^{14} counts were corrected to infinite thinness.

RESULTS

Distribution of Phospholipide P in the Pancreas Cell.—In earlier studies, using large quantities of dog pancreas, it was possible to isolate a zymogen granule fraction fairly free of contamination (12), but this was not possible with small quantities of pigeon pancreas. The purity of the zymogen granule fraction from dog pancreas was indicated by its very low RNA content and its low phospholipide content as compared with the other fractions; the zymogen granule fraction from pigeon pancreas contained as much phospholipide as the nuclear and mitochondrial fractions and much more RNA than was previously observed with the zymogen granules of dog pancreas. The greater purity of the zymogen granules from dog pancreas was, in all likelihood, due to the very much larger amounts which could be isolated and purified by repeated centrifugation at 1000 *g*. Siekevitz and Palade (16) showed with the electron microscope that the mitochondrial and zymogen granule fractions of the pancreas from the guinea pig were inhomogeneous, being contami-

TABLE I

Incorporation of P^{32} into the Total Phospholipides in Various Cellular Components of Non-Secreting and Secreting Pancreas Slices

Fraction	Average μg. phospholipide P per fraction	Specific activity *counts/min. per μg. P*		Total radioactivity per fraction		Increment in total radioactivity per fraction
		U	S	U	S	
Homogenate (13)	542	191	778	103,500	442,000	338,500
Nuclei (13)	43	168	527	7,220	22,600	15,380
Zymogen granules (7)	46	136	433	6,250	20,000	13,750
Mitochondria (11)	49	159	453	7,790	22,200	14,410
Microsomes (13)	219	177	851	38,800	186,000	147,200
Soluble (11)	48	241	695	11,600	33,400	21,800

The tissues were incubated and fractionated after incubation as described under Experimental. The data are averages of several experiments; the figure in parentheses after each fraction represents the number of individual experiments. In each experiment the total phospholipide P in each fraction is the average of the phospholipide P in the samples from the unstimulated and stimulated tissues. The total radioactivity was calculated from the phospholipide P and the specific activity. *U* = unstimulated; *S* = stimulated.

nated primarily with microsomal elements. On the other hand, these authors found that the microsome fraction contains primarily membranous vesicles with adherent ribonucleoprotein particles, being derived chiefly from the endoplasmic reticulum.

Over half of the phospholipide P of the pancreas cell was in the microsome fraction (Table I). Each of the other fractions contained about one-fourth to one-fifth as much phospholipide P as the microsome fraction. The total recovery of phospholipide P was only 75 per cent, suggesting some breakdown of phospholipide during the prolonged fractionation procedure—in spite of the fact that the temperature was maintained between 0° and 4°. The pancreas is known to contain powerful phospholipases (17). The lower total phospholipide P as compared to a previous report (6) was due to retention of fragments of tissue on straining through gauze.

Incorporation of P^{32} into the Total Phospholipides of Various Fractions.—The specific activities of the total phospholipides in the various particulate fractions did not show any great differences (Table I). The specific activities of the phospholipides in the soluble fraction were greater. Accordingly, with the exception of this fraction the total radioactivity of the phospholipides in each fraction paralleled the total phospholipide P (Table I), being by far the greatest in the microsome fraction.

When enzyme secretion was stimulated with acetylcholine the major increment in radioactivity in the phospholipides occurred in the microsome fraction (Table I). This increment was 43 per cent of the increment in the homogenate and 69 per cent of the increment calculated by summation of the increments in all of the fractions. The remainder of the increment was distributed fairly evenly in the other fractions.

Incorporation of P^{32} into the Phospholipides in Deoxycholate-Soluble and Insoluble Fractions.—Palade and Siekevitz (9) showed that treatment of the microsome fraction of guinea pig pancreas with about 0.3 per cent sodium deoxycholate solubilized the membranous fragments and vesicles, leaving the ribonucleoprotein particles essentially intact. Most of the phospholipide in the microsome fraction was solubilized by this treatment. As shown in Table II, treatment of the microsome fraction from incubated pigeon pancreas slices with 0.4 per cent sodium deoxycholate solubilized over 80 per cent of the phospholipide. In experiments not included in the Tables almost all of the RNA was found in the deoxycholate-insoluble, or ribonucleoprotein particle fraction. It can be concluded that most of the phospholipide in the pigeon pancreatic acinar cell is contained in membranous structures sedimenting in the microsome fraction. This is undoubtedly due to the presence of an extensive

TABLE II

Incorporation of P^{32} into the Phospholipides of the Deoxycholate-Soluble and Insoluble Fractions from Microsomes of Secreting and Non-Secreting Pigeon Pancreas Slices

Fraction	Phospholipide P μg. P per gm. tissue	Specific activity of phospholipides counts/min. per μg. P			Radioactivity of phospholipides counts/min. per fraction		
		U	S	S/U	U	S	Δ
Untreated microsomes	201	135	669	4.9	27,200	134,300	107,100
Deoxycholate-soluble fraction	166	114	615	5.4	18,900	102,900	84,000
Deoxycholate-insoluble fraction	26.3	106	556	5.2	2,780	14,600	11,820

The tissues were incubated and the microsomes were prepared as described under Experimental. The microsomes were treated with final concentrations of sodium deoxycholate ranging from 0.2 to 0.4 per cent. The figures are averages of 7 separate experiments. Δ = increment in radioactivity of phospholipides.

endoplasmic reticulum in the acinar cell, as revealed by electron microscopy (18–20).

The distribution of radioactivity in the deoxycholate-soluble and insoluble fractions of the microsomes paralleled the distribution of the phospholipide P (Table II). Since the specific activities in the two fractions were the same, it is probable that the small amount of phospholipide in the ribonucleoprotein particle fraction was due to incomplete extraction by deoxycholate. In fact, higher concentrations of deoxycholate dissolved essentially all of the phospholipide P, but these concentrations of deoxycholate also solubilized some of the RNA. It is likely that the ribonucleoprotein particles contain little if any phospholipide.

Almost all of the increment in P^{32} radioactivity in the phospholipides on stimulation of secretion with acetylcholine was in the deoxycholate-soluble fraction of the microsomes (Table II). This indicates that the site of the phospholipide effect on stimulation of secretion is in some membranous structure whose fragments sediment primarily in the microsome fraction. The percentage stimulation of P^{32} incorporation into the phospholipides was the same in the deoxycholate-soluble and insoluble fractions. This is further suggestive evidence that the phospholipide in the microsomal ribonucleoprotein particle fraction is due to incomplete removal by deoxycholate treatment of membranous material.

The finding of Palade and Siekevitz (16) that the non-microsomal fractions contained membranous fragments is the most likely explanation for the small increments in radioactivity in these fractions on stimulation of secretion. This is further supported by the fact that treatment of each of the non-microsomal particulate fractions with deoxycholate removed most of the radioactivity, leaving behind material which showed a negligible increment of radioactivity on stimula-

TABLE III

Incorporation of P^{32} into the Total Phospholipides of the Deoxycholate-Soluble and Insoluble Fractions of Particulate Components of Secreting and Non-Secreting Pigeon Pancreas Slices

	Phospholipide P μg./gm. tissue	Radioactivity counts/min. per fraction		
		U	S	Δ
Nuclei				
Untreated	31.0	3,610	9,870	6,260
Deoxycholate-soluble	20.6	2,480	8,570	6,090
Deoxycholate-insoluble	6.2	565	992	427
Mitochondria + Zymogen granules				
Untreated	60.0	4,660	20,135	15,470
Deoxycholate-soluble	46.1	4,025	17,190	13,160
Deoxycholate-insoluble	9.0	738	1,094	356
Microsomes				
Untreated	196	17,200	135,300	118,100
Deoxycholate-soluble	149	14,600	93,700	79,100
Deoxycholate-insoluble	13.0	2,190	10,200	8,010

The tissues were incubated, and the various fractions were isolated as described under Experimental. All fractions were treated with 0.4 per cent sodium deoxycholate, pH 8.0. The figures are averages of 2 separate experiments.

TABLE IV

Incorporation of P^{32} into Individual Phospholipides in Various Fractions from Non-Secreting and Secreting Pigeon Pancreas Slices

Cell fraction		Total radioactivity, *counts/min. per fraction*					
		Phosphoinositide B	Phosphoinositide A	Unknown	Lecithin	Phosphatidyl ethanolamine	Phosphatidic acid
Homogenate	U	1,130	10,930	3,770	15,220	4,480	5,730
	S	13,790	114,800	7,310	23,000	12,250	11,180
Nuclear + zymogen granules	U	95	925	235	1,090	275	328
	S	1,064	6,990	378	1,220	552	487
Mitochondria	U	91	779	213	977	331	310
	S	1,110	8,995	428	1,670	917	592
Microsomes	U	597	5,260	1,900	8,300	2,560	3,190
	S	3,620	48,110	4,170	12,930	7,680	4,590
Soluble	U	166	1,390	556	2,830	769	767
	S	1,132	9,080	1,090	2,940	1,540	1,470

The tissues were incubated, the various fractions were isolated, and the phospholipides were separated and counted as described under Experimental.

tion of secretion (Table III). In two experiments the average ratio of radioactivity of the phosphatides of the stimulated to the unstimulated tissue in the nuclear and mitochondrial (plus zymogen granule) fractions was 2.7 and 4.3 respectively. After extraction with 0.4 per cent deoxycholate the ratios were 1.8 and 1.5, respectively. This shows that the phospholipide remaining after extraction with deoxycholate is poorer in stimulated material, indicating that the membranous fragments are more susceptible to extraction by deoxycholate than are some of the phospholipides in the mitochondria and nuclei. The deoxycholate-extracted phospholipide showed a percentage increase in radioactivity on stimulation of secretion more closely approaching that of the microsome fraction, which is what would be expected if this deoxycholate-soluble material were membranous fragments which sediment primarily in the microsome fraction.

The increment in radioactivity in the soluble fraction is probably due to traces of low density microsomal material in this fraction.

Incorporation of P^{32} and Glycerol-1-C^{14} into the Individual Phosphatides in Various Fractions of Non-Secreting and Secreting Pancreas Slices.— The major incorporation of P^{32} into each of the individual phosphatides, and the major increment in this incorporation on stimulation of secretion was in the microsome fraction (Table IV). The distribution of radioactivities of the individual phosphatides in the various cellular fractions thus parallels that of the total phosphatide fraction. This indicates that the turnover of each of the individual phosphatides occurs predominantly in the membranous components of the microsome fraction. As reported previously, the greatest percentage stimulation was in the phosphoinositides, with lesser stimulations in the other phosphatides.

As with P^{32} incorporation, most of the incorporation of glycerol-1-C^{14} into the individual phosphatides and the increment in this incorporation on stimulation of secretion were in the microsome fraction (Table V). The percentage stimulation of glycerol-1-C^{14} incorporation into the various phosphatides was less than that of P^{32} incorporation, as reported previously (6). However, a sufficient number of experiments were carried out in the present series to demonstrate that significant stimulations of glycerol-1-C^{14} incorporation did occur in all of those phosphatides which showed a stimulation of P^{32} incorporation. This was particularly evident in the microsome fraction, which generally showed a greater stimulation of glycerol-1-C^{14} incorporation than the whole homogenate.

TABLE V

Incorporation of Glycerol-1-C^{14} into Individual Phospholipides in Various Fractions from Non-Secreting and Secreting Pigeon Pancreas Slices

Cell fraction		Total radioactivity, *counts/min. per fraction*					
		Phosphoinositide B	Phosphoinositide A	Lecithin	Phosphatidyl ethanolamine	Phosphatidic acid	Non-P lipide
Homogenate	U	652	1,300	4,440	780	1,190	16,990
	S	757	5,890	6,340	1,746	1,550	17,630
Nuclear + zymogen granules	U	17	160	310	58	73	680
	S	32	243	327	75	156	696
Mitochondria	U	157	130	269	55	71	477
	S	140	322	446	109	116	843
Microsomes	U	353	799	2,870	556	652	3,200
	S	550	2,810	4,230	675	728	3,370
Soluble	U	154	134	486	85	251	7,420
	S	203	476	815	198	348	8,430

The tissues were incubated, the various fractions were isolated, and the phospholipides were separated and counted as described under Experimental. The data in this Table are from the same Experiment as that in Table IV.

The discrepancy in the percentage stimulation between glycerol-1-C^{14} incorporation and P^{32} incorporation is probably due to more than one pathway for formation of a particular phosphatide. For instance, phosphatidic acid is formed by two pathways, only one of which is stimulated by acetylcholine (11). The pathway which is not stimulated probably contributes most of the glycerol-1-C^{14} to phosphatidic acid, masking the stimulation of glycerol-1-C^{14} incorporation by the other pathway.

A highly radioactive non-phosphorus–containing lipide fraction (6) contained a very high proportion of the total glycerol-1-C^{14} which was incorporated into the lipides. The incorporation of glycerol-1-C^{14} into this non-phosphorus lipide fraction did not appear to be increased on stimulation of secretion. Most of this radioactivity was in the soluble fraction (Table V). Of the particulate fractions the microsome fraction showed the greatest radioactivity.

DISCUSSION

The data presented here show that the membranous fragments of the microsome fraction contain most of the phospholipide P of the cell, as well as most of the radioactivity incorporated into the phospholipides. The major finding of this paper is that the increase in phosphatide turnover on stimulation of enzyme secretion is in this membranous fraction. Some cellular component, whose fragments are contained in this fraction, appears to be the site of stimulation of phosphatide turnover on stimulation of secretion. Palade and Siekevitz (9) have shown that the microsome fraction prepared in 0.88 M sucrose from unincubated pancreas consists almost exclusively of rough surfaced membranes of the endoplasmic reticulum. According to Palade and Siekevitz (21–24) the endoplasmic reticulum is concerned with the transport of the newly formed enzymes from the attached ribonucleoprotein particles across the lipoprotein membranes into the cisternal spaces bounded by the endoplasmic reticulum. The enzymes are then segregated in the cisternal spaces, where they form what these authors call intracisternal granules. These granules then move to the Golgi region, where they are invested with a membrane and form a mature zymogen granule. The zymogen granules then migrate to the apical region of the acinar cell; on stimulation of secretion the membranes of the zymogen granules fuse with the plasma membrane, and their contents are discharged into the

lumen of the duct. According to this scheme the digestive enzymes traverse a membrane only once; *i.e.*, when they are segregated in the cisternal spaces bounded by the rough surfaced membranes of the endoplasmic reticulum.

Recent studies by Eggman and Hokin (25) indicate an additional mechanism of secretion. These studies have shown that 85 per cent of the amylase in the pancreas of pigeons which have been fed *ad lib*, and which may thus be regarded as being in the steady state of synthesis and secretion of enzymes, is in the soluble form. Furthermore, 86 per cent of the amylase which is extruded from the pancreas on stimulation of secretion *in vitro* with acetylcholine is derived from soluble amylase. It, therefore, appears that in the pigeon pancreas in the steady state, amylase is not extensively segregated into zymogen granules but remains in the soluble form in the cytoplasm and is extruded as such. This mechanism of secretion receives further support from the studies of Grossman and his associates (26–29). After several hours of repeated stimulation of pancreatic secretion with parasympathomimetic drugs the acinar cells were found to be virtually depleted of zymogen granules; yet the cells continued to secrete large quantities of enzyme, and a linear dose response curve was obtained as in the fasted pancreas. Lin and Grossman (29) concluded that "the storing up of zymogen granules is not required for large enzyme secretory activity." The results of Palade and Siekevitz and those of Grossman and his associates and Eggman and Hokin can thus best be interpreted as indicating two mechanisms of secretion: (1) secretion of enzymes in the form of zymogen granules, and (2) secretion of enzymes in the soluble form. The formation of zymogen granules would be expected to occur on fasting, so that mechanism (1) would predominate on stimulation of secretion immediately after a period of fasting. Mechanism (2) would predominate under conditions of continual feeding or parasympathomimetic stimulation. It is also possible that the destructive enzymes are parceled into zymogen granules, while the nondestructive enzymes, such as amylase, remain predominantly in the soluble form. This problem is being investigated. It is also likely that there may be species differences in the relative contribution of the two mechanisms.

The question arises as to which step in either of the postulated secretion mechanisms the phospholipide effect is concerned. Since this effect occurs on stimulation of secretion in tissues such as the salt gland (30) and the adrenal medulla (31)—structures which do not synthesize proteins for secretory purposes and which do not contain rough surfaced membranes (32, 33)—it can be concluded that the phospholipide effect is not concerned with the synthesis of proteins and is not exclusively concerned with the secretion of proteins. Rather, the effect appears to be associated with the extrusion of a wide variety of water soluble secretory products from the cell. The finding of a phospholipide effect in the salt gland in association with sodium chloride transport (30) also excludes the effect from being associated with the intracellular transport or extrusion of secretory granules.

Since the microsome fraction contains essentially rough surfaced membranes of the endoplasmic reticulum (9) and since the phospholipide effect occurred in this fraction the most likely role of the phospholipide effect is in the transport of enzymes across the membranes of the endoplasmic reticulum. The cisternal spaces bounded by these membranes may be regarded as being outside the cell proper, since there is evidence that at least some of the membranes of the endoplasmic reticulum are infoldings of the plasma membrane (20). The fact that most of the amylase in the pigeon pancreas in the steady state is present in the cell in the soluble form and the fact that this soluble enzyme is secreted directly without being first segregated into zymogen granules suggests that the soluble enzyme is secreted across the membranes of the endoplasmic reticulum and diffuses through the cisternae to the lumen of the duct.

The presence of an extensive intracellular smooth membranous system in the salt gland (32) indicates that the phospholipide effect can also occur in smooth surfaced membranes. These smooth surfaced membranes can be seen to represent infoldings of the plasma membrane. Therefore, a sharp functional distinction should not be made between the smooth and the rough surfaced membranes with regard to the phospholipide effect and secretion. That some cells contain rough surfaced membranes appears to be due to the fact that they synthesize and secrete proteins and thus contain ribonucleoprotein particles attached to the endoplasmic reticulum.

In the secretory cycle postulated by Palade and and Siekevitz the transport of newly synthesized

protein from the ribosomes to the intracisternal spaces may also require the phospholipide mechanism. However, a 3-fold stimulation of protein synthesis in pancreas slices does not increase the turnover of phospholipides (1). This lack of increase in phospholipide turnover on stimulation of protein synthesis is open to several interpretations: (1) A secretory stimulus may also be required to transport newly synthesized enzymes across the membranes of the endoplasmic reticulum. (2) Under the conditions of enzyme synthesis *in vitro* the newly formed enzyme may be released from the ribosomes into the soluble phase of the cytoplasm. (3) The newly synthesized enzymes adherent to the ribonucleoprotein particles may not require a phospholipide mechanism for their transport across the membranes of the endoplasmic reticulum into the intracisternal spaces. These various possibilities are under investigation.

In conclusion it appears to the authors that the most likely interpretation of the phospholipide effect which can account for the observations in the pancreas and in the other secretory tissues so far studied is that it is concerned with the secretion across lipoid membranes of substances present in the cell in the soluble form. It may also be concerned in the transport of proteins directly from the ribonucleoprotein particle adherent to the membranes. The lipoid membranes are assumed to represent infoldings of the surface membrane. Whether these membranes contain attached ribonucleoprotein particles or not depends on the type of secretory product elaborated or transported by the cell. A mechanism whereby phosphatidic acid and phosphoinositide function as carriers in the transport of water-soluble substances across lipoid membranes has been presented elsewhere (34, 35).

Bibliography

1. Hokin, M. R., and Hokin, L. E., *J. Biol. Chem.*, 1953, **203,** 967.
2. Hokin, L. E., and Hokin, M. R., *Biochim. et Biophysica Acta*, 1955, **18,** 120.
3. Hokin, L. E., and Hokin, M. R., Symposium on the Chemistry and Physiology of Phospholipids, *Canad. J. Biochem. and Physiol.*, 1956, **34,** 349.
4. Hokin, L. E., and Hokin, M. R., *J. Physiol.*, 1956, **132,** 442.
5. Hokin, L. E., and Sherwin, A. L., *J. Physiol.*, 1957, **135,** 18.
6. Hokin, L. E., and Hokin, M. R., *J. Biol. Chem.*, 1958, **233,** 805.
7. Hokin, M. R., Hokin, L. E., Saffran, M., Schally, A. V., and Zimmermann, B. U., *J. Biol. Chem.*, 1958, **233,** 811.
8. Hokin, M. R., Benfry, B. G., and Hokin, L. E., *J. Biol., Chem.*, 1958, **233,** 814.
9. Palade, G. E., and Siekevitz, P., *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** 671.
10. Potter, V. R., and Elvehjem, C. A., *J. Biol. Chem.*, 1936, **114,** 495.
11. Hokin, L. E., and Hokin, M. R., *J. Biol. Chem.*, 1958, **233,** 822.
12. Hokin, L. E., *Biochim. et Biophysica Acta*, 1955, **18,** 379.
13. Weiss, S. B., Acs, G., and Lipmann, F., *Proc. Nat. Acad. Sc.*, 1958, **44,** 189.
14. Palade, G. E., and Siekevitz, P., *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** 171.
15. Marinetti, G. V., Erbland, J., and Kochen, J., *Fed. Proc.*, 1956, **15,** 837.
16. Siekevitz, P., and Palade, G. E., *J. Biophysic. and Biochem. Cytol.*, 1958, **4,** 203.
17. Wittcoff, H., The Phosphatides, New York, Reinhold Publishing Co., 1951.
18. Dalton, A. F., *Am. J. Anat.*, 1951, **89,** 109.
19. Porter, K. R., *J. Histochem. and Cytochem.*, 1954, **2,** 346.
20. Palade, G. E., *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** 85.
21. Palade, G. E., *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** 417.
22. Palade, G. E., *Fed. Proc.*, 1959, **18,** to be published.
23. Siekevitz, P., and Palade, G. E., *J. Biophysic. and Biochem. Cytol.*, 1958, **4,** 309.
24. Siekevitz, P., and Palade, G. E., *J. Biophysic. and Biochem. Cytol.*, 1958, **4,** 557.
25. Eggman, L. D., and Hokin, L. E., to be published.
26. Almeida, A. L., and Grossman, M. L., *Gastroenterology*, 1952, **20,** 554.
27. Wang, C. C., Grossman, M. I., and Ivy, A. C., *Am. J. Physiol.*, 1948, **154,** 358.
28. Kalser, M. H., and Grossman, M. I., Gastroenterology, 1954, **26,** 189.
29. Lin, T. M., and Grossman, M. I., *Am. J. Physiol.*, 1956, **186,** 52.
30. Hokin, L. E., and Hokin, M. R., *Nature*, in press.
31. Hokin, M. R., Benfey, G. B., and Hokin, L. E., *J. Biol. Chem.*, 1958, **233,** 814.
32. Doyle, W. L., personal communication.
33. Wetzstein, R. Z., *Z. Zellforsch*, 1957, **46,** 517.
34. Hokin, L. E., and Hokin, M. R., *J. Biol. Chem.*, 1959, **234,** 1387.
35. Hokin, L. E., and Hokin, M. R., *Gastroenterology*, 1959, **36,** 368.