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PHOSPHOLIPID TURNOVER AND ULTRASTRUCTURAL CHANGES IN RESTING AND SECRETING BULLFROG GASTRIC MUCOSA

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SUMMARY

1. Ultrastructural changes and $^{32}P_i$ incorporation into phospholipids were studied in resting and histamine-stimulated bullfrog gastric mucosae.

2. The relatively smooth apical surface of the resting oxyntic cell becomes engrossed with elaborate microvilli with the onset of acid secretion induced by histamine. These membranous alterations occur presumably at the expense of the abundant smooth endoplasmic reticulum present in the oxyntic cells. Our observations thus confirm the previous findings of other investigators.

3. The ultrastructural changes were accompanied by an increase in the incorporation of $^{32}P_i$ into the phospholipid fraction of the histamine-stimulated mucosae, but did not involve a significant net synthesis of lipids.

4. There was a 2–5-fold enhanced incorporation of $^{32}P_i$ into phosphatidyl choline and phosphatidyl ethanolamine, and a smaller change in incorporation into phosphatidic acid and phosphatidyl inositol in the mucosae stimulated by histamine. Phosphatidyl serine and sphingomyelin showed relatively little $^{32}P_i$ incorporation.

5. Thiocyanate, an inhibitor of acid secretion, abolished the enhanced $^{32}P_i$ incorporation into the specific phospholipids.

6. Changes in $^{32}P_i$ incorporation into ATP and acid-soluble phosphate ester fraction of the stimulated mucosae were variable. However, even the greatest change observed was not sufficient to account for the large changes observed in specific phospholipids.

7. [2-^{14}C]Acetate was incorporated to varying degrees into different phospholipids; the greatest incorporation occurred in phosphatidyl choline. However, histamine stimulation did not significantly alter the incorporation of ^{14}C into any of the phospholipids.

8. The increased turnover of the polar groups of phospholipids thus appears to be correlated with alterations and movements of membranous structures concomitant with the histamine-induced acid secretion in gastric mucosa.

INTRODUCTION

Isotopic evidence has been used to demonstrate an increased turnover of specific phospholipids in certain tissues stimulated to secretory activity by appropriate

hormones. Thus, HOKIN AND HOKIN[1] have demonstrated an increased turnover of $^{32}P_i$ into phosphatidic acid, and of $^{32}P_i$ and [2-^{3}H]inositol into phosphatidyl inositol in avian salt gland slices following stimulation with acetylcholine. In addition, the stimulation of acinar cells of pancreas by acetylcholine, causes a marked increase in the turnover of phosphatidyl inositol as measured by the incorporation of $^{32}P_i$, [1-^{14}C]glycerol and [2-^{3}H]inositol[2]. A similar increased turnover of phospholipids appears to be associated with cellular activity involving movement and alteration of the membranous structure. KARNOVSKY[3] has presented evidence indicating an increased turnover of individual phospholipids in polymorphonuclear leucocytes during phagocytosis. The incorporation of $^{32}P_i$ into three of the acidic phospholipids, namely phosphatidyl inositol, phosphatidyl serine and phosphatidic acid, is greater than that of the resting non-phagocytosing cell.

While a similar phospholipid effect was observed by EGGMAN AND HOKIN[4] when gastric mucosae were stimulated by acetylcholine to secrete pepsin, they reported[5] the absence of any such effect on stimulation of the mucosae to acid secretion by histamine. They concluded from these studies that secretion of HCl by the gastric mucosa was, therefore, probably brought about by a mechanism different from that involved in the secretion of NaCl and many organic substances in other tissues.

However, ultrastructural evidence has been presented by several workers which shows that the apical secretory membranes of the oxyntic or acid-secreting cells of the gastric mucosa undergo profound structural changes dependent upon the state of secretory activity[6–8]. In the unstimulated or inhibited preparations the apical microvilli of the secretory surface are relatively short and sparse, and in some oxyntic cells they appear to be completely absent. For gastric mucosae secreting H^+ at a brisk rate, long filamentous microvilli appear at the secretory surface. It has been postulated that the microtubular components of the abundant smooth-surfaced endoplasmic reticulum represent the source of the membranous transformations which appear to produce the extensive microvillar elaboration concomitant with the secretory process[9–10]. One of the problems with the ultrastructural work on frog gastric tissue has involved the difficulty of obtaining *in vitro* resting preparations which might be compared to those stimulated by histamine or gastrin to secrete HCl.

The purpose of this work was to explore both the ultrastructural changes and the phospholipid turnover using a gastric mucosal preparation whose secretory rates could be controlled by the addition of appropriate agents[11]. We have found ultrastructural evidence showing membrane transformations at the apical secretory surface of the oxyntic cells which is in general agreement with the results described and predicted by others, and in keeping with these structural alterations, we have found an enhanced turnover of several phospholipid components in the gastric tissue as the secretory activity is increased.

METHODS

Preparation of resting isolated frog gastric mucosae

Gastric mucosa was dissected from the bullfrog, *Rana catesbeiana*, and divided longitudinally into two approximately equal halves. Each half was mounted onto a polyethylene tube, secured in place by an O-ring and bathed in histamine-free frog Ringer's nutrient solution[12]. The secretory side was bathed in unbuffered 120 mM

NaCl and both solutions were bubbled with O_2–CO_2 (95:5, v/v). The rate of acid secretion was measured by titrating the secretory solution to a constant pH end point of 4.5 using standardized 0.02 M NaOH. After noting the initial rate of spontaneous acid secretion, the mucosae were incubated overnight for 16–17 h in order to obtain resting preparations as described earlier[11]. The following morning, they were washed three times at 10–15-min intervals with histamine-free frog Ringer's solution on the serosal side and with 120 mM NaCl on the secretory side, and the rates of acid secretion, if any, were noted. These mucosae usually secreted no acid and were therefore designated as resting mucosae.

Electron microscopy

For the ultrastructural comparison of resting and secreting oxyntic cells, one of the two resting halves was stimulated to secrete acid by adding 0.1 mM histamine to the nutrient side while the other half was used as a control. The rates of acid secretion were monitored for the next 30–120 min after which period the mucosae were removed and prepared immediately for electron microscopy. The gastric tissue was placed in a pool of 3 % glutaraldehyde buffered with 0.1 M Na_2HPO_4–NaH_2PO_4 at pH 7.4 and cut into approximately 1-mm squares. These small pieces of mucosa were prefixed in fresh 3 % glutaraldehyde for 1.5 h at 4°. The tissue was then washed for 0.5 to 1.0 h in three separate changes of 0.1 M phosphate buffer at 4° and post-fixed in buffered 1 % OsO_4 for 1 h at 4°. Dehydration of the tissue was carried out for 5 min each through 70, 85 and 95 % ethanol at 4°. The tissue was then transferred through three separate changes of absolute ethanol at room temperature. The fixed, dehydrated tissue was infiltrated overnight with Epon and then embedded in fresh Epon at 60° for 2–3 days. Thin sections (600–900 Å) were examined with the Siemens Elminskop I operated at 80 kV.

Phospholipid turnover

The resting mucosae, after preliminary washes as described above, were transferred twice at 15-min intervals to phosphate-free Ringer's solution and then to Ringer's solutions containing 10–20 μC $^{32}P_i$ per ml. Histamine, 0.1 mM final concentration, was added to one half while the other half served as a control. The interval between exposure of the mucosae to $^{32}P_i$ and addition of histamine varied from 5 to 60 min, while the total period of $^{32}P_i$ exposure for both mucosal halves varied from 90 to 150 min in different studies. The rates of acid secretion were continually monitored during this period. At the end of the exposure period the mucosae were removed from the bathing media and, after a quick rinse in Ringer's solution, they were blotted gently between filter papers and dropped into a tared beaker containing chilled 0.3 M $HClO_4$, homogenized and the homogenate centrifuged at 30000 $\times$ g for 5 min. The precipitate was washed once with 0.3 M $HClO_4$ and saved for phospholipid extraction. The supernatant liquid and wash were pooled, neutralized with KOH and used for a crude analysis of ATP by the column chromatography procedure described by GLYNN AND CHAPPELL[13].

The precipitate was washed 4 times: once with 0.3 M $HClO_4$ containing 1 mM P_i; with 0.3 M $HClO_4$ containing 0.5 mM P_i; and twice with phosphate-free 0.3 M $HClO_4$. It was then extracted with $CHCl_3$–ethanol–0.1 M HCl, as described by

HOKIN AND HOKIN[3] with the modification that the chloroform extract was washed once again with ethanol–0.1 M HCl.

The $CHCl_3$ extract was used for the separation of lipids by two-dimensional thin-layer chromatography. Plates were prepared with 0.075-mm silica gel H and the solvent systems were adapted from SKIDMORE AND ENTENMAN[14] and ROUSER, GALLI AND KRITCHEVSKY[15]. The following solvents were used: Solvent I: $CHCl_3$–CH_3OH–7 M NH_4OH (60:35:5, by vol.); Solvent II: $CHCl_3$–CH_3OH–7 M NH_4OH (35:60:5, by vol.); Solvent III: $CHCl_3$–CH_3COCH_3–CH_3OH–CH_3COOH–H_2O (10:4:2:2:1, by vol.). Using Solvents I and II in the vertical and horizontal directions, respectively, resulted in good separation of phosphatidyl choline, phosphatidyl ethanolamine, sphingomyelin and a resolvable but somewhat streaked spot of phosphatidic acid. Phosphatidyl inositol and phosphatidyl serine tended to overlap in this sytem. The latter problem was circumvented to some extent by using Solvents I and III where phosphatidyl serine was clearly separated, but there often remained some overlap between phosphatidyl inositol and sphingomyelin. The system using Solvents I and III was also effective in producing a well defined spot for phosphatidic acid.

The individual lipid spots were detected both by iodine vapor stain and autoradiography. Identification of the spots was accomplished with the aid of standard lipids run on parallel plates, as well as with various specific detection reagents[14]. The silica gel containing the spots was scraped and the scrapings were extracted two times with each of the solvents used for running the chromatogram and finally with a 2:1 mixture of $CHCl_3$–CH_3OH. By this procedure we were able to extract 90–95 % of the total lipid applied to the silica gel. An aliquot of the extract was taken to dryness in a liquid-scintillation vial, the dry lipid taken up in the scintillation fluid and counted in a Packard Tricarb spectrometer. Total phosphate in the remaining portion of extract was assayed by the method of BARTLETT[16] after digestion in H_2SO_4.

In a few experiments, both [2-^{14}C]acetate and $^{32}P_i$ were used simultaneously. After extraction and separation of lipids, ^{14}C and ^{32}P were counted on separate channels for radioactivity.

In studies of the effect of SCN^- inhibition of acid secretion on ^{32}P turnover, freshly dissected mucosal halves were allowed to reach steady secretory rates in phosphate-free Ringer's solution containing 0.1 mM histamine. One half was treated with 10 mM NaSCN and the other with 10 mM NaCl. 30 min later, equal amounts of $^{32}P_i$ were added to the nutrient media of both the mucosae. The incubations were continued for 2 h, after which the mucosae were extracted for lipids as described above.

RESULTS

Ultrastructure of resting and histamine-stimulated oxyntic cells

Fig. 1 is an electron micrograph showing a cross-section through a gastric gland of the non-secreting stomach half. The secretory surface of the resting oxyntic cell is relatively smooth with occasional club-like microvilli extending into a patent lumen. The apical portion of the oxyntic cell consists almost entirely of components of the smooth-surfaced endoplasmic reticulum. These membranes appear as long tubular elements which are very regular in diameter (500–600 Å). The uniformity of tubule

diameter and the vast number found in the cytoplasm result in a geometry which suggests hexogonal packing of these structures; this is seen more clearly in the insert shown in Fig. 1.

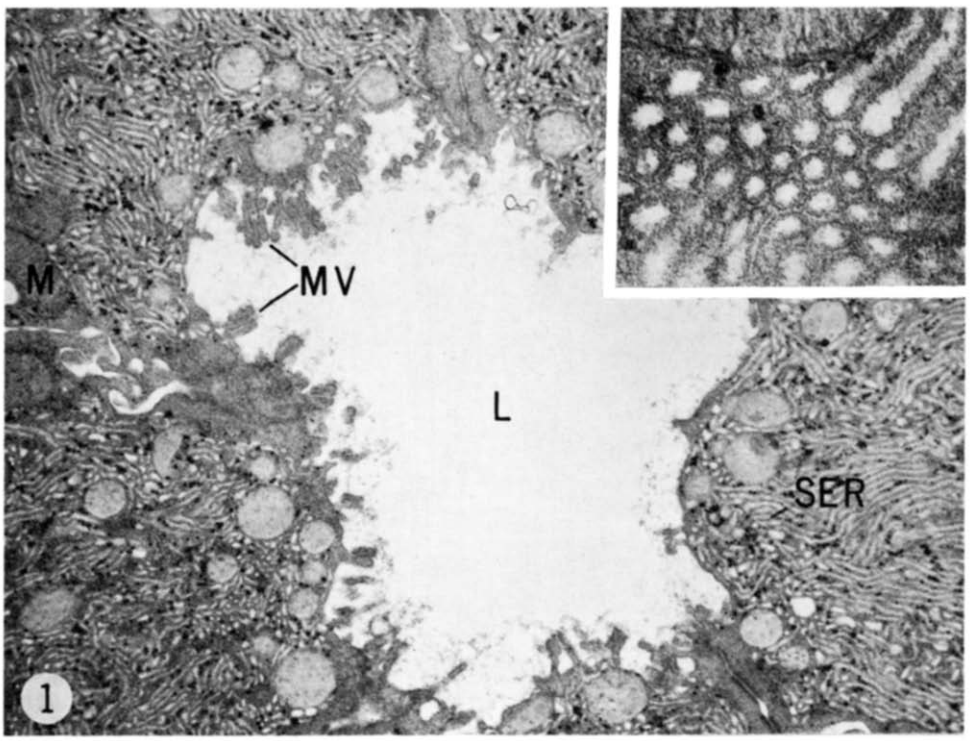

Fig. 1. Cross-section through a gastric gland of a non-secreting bullfrog gastric mucosa. The apical portions of the oxyntic cells contain numerous tubular components of the smooth-surfaced endoplasmic reticulum (SER), while mitochondria (M) are more basally located. The apical secretory membrane has a few relatively short microvilli (MV) extending into the glandular lumen (L). Magnification, 12000 ×. The insert shows the smooth-surfaced endoplasmic reticulum in greater detail. The close packing of these tubular membranous components within the cell produces a hexagonal array of the cross-sectional profiles. Magnification, 80000 ×.

A section through oxyntic cells of the histamine-stimulated half of the stomach is shown in Fig. 2. A profound change has occurred at the apical surface of the cells. Long, often anastomosing, plasma membrane extensions project into the lumen of the gland. Since tubules of the smooth-surfaced endoplasmic reticulum are seen in close proximity to the plasma membrane, it is suggestive that during HCl secretion membranous components in the apical cytoplasm may contribute to the increased surface area of the secretory plasma membrane.

The lipid profile of frog gastric mucosa

Fig. 3 shows a typical thin-layer chromatography profile of the chloroform-extractable lipids from frog gastric mucosa as detected with iodine vapor and as observed from radioautography of ^{32}P-containing phospholipids. With the aid of standard lipids as well as various detection reagents phosphatidyl choline, phospha-

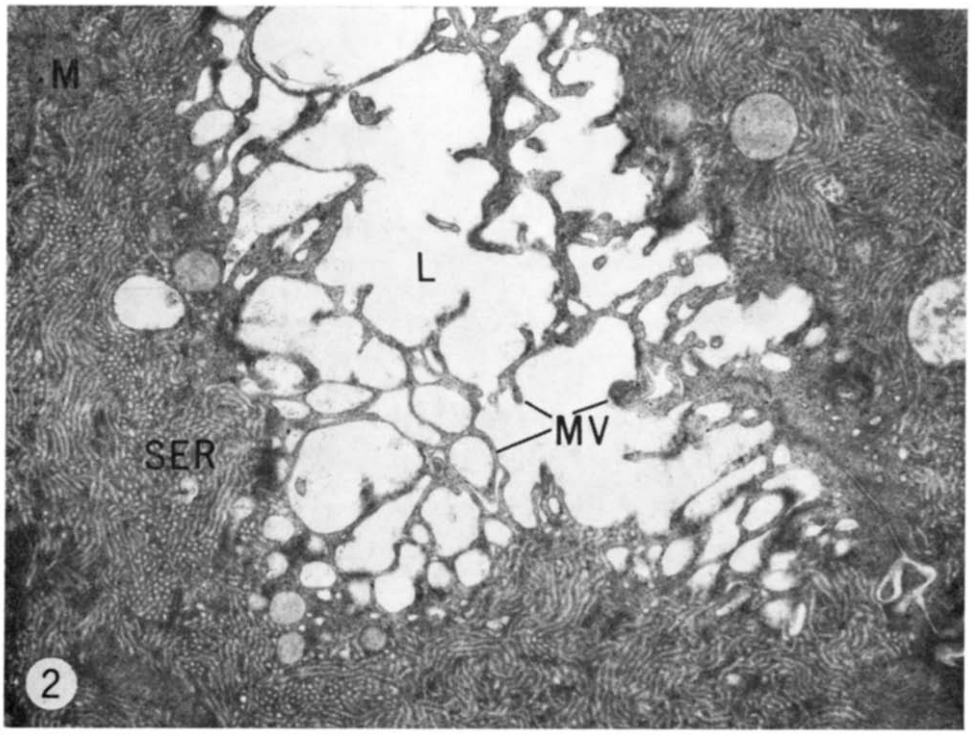

Fig. 2. Electron micrograph of oxyntic cells in the portion of gastric mucosa stimulated by 0.1 mM histamine to secrete acid (3.6 μequiv H^+/cm^2 per h). The most profound change observed in these cells, compared to the non-secreting ones shown in Fig. 1, is that the apical secretory surfaces containlong filamentous extensions (MV) which project into the glandular lumen (L). Magnification, 12000 ×.

tidyl ethanolamine, phosphatidyl serine, phosphatidyl inositol, sphingomyelin, lysophosphatidyl choline and phosphatidic acid were identified.

Of the total mucosal phospholipid P, almost 50 % was composed of phosphatidyl choline and phosphatidyl ethanolamine, the former accounting for about 35 % of the total (Table I). About 65 % of the total extractable phospholipid P could be accounted for by the phospholipids identified and shown in Table I.

TABLE I

CONCENTRATION OF VARIOUS PHOSPHOLIPIDS IN BULLFROG GASTRIC MUCOSA

Numbers in parentheses refer to number of mucosae used to determine the concentration of the phospholipid.

Phospholipids	*μmoles P_i/g wet tissue ± S.E.*
Phosphatidyl choline (8)	3.42 ± 0.20
Phosphatidyl ethanolamine (8)	1.10 ± 0.10
Phosphatidyl serine (3)	0.74 ± 0.17
Phosphatidyl inositol (3)	0.14 ± 0.04
Sphingomyelin (5)	0.68 ± 0.07
Lysophosphatidyl choline (5)	0.14 ± 0.03
Phosphatidic acid (5)	0.04 ± 0.01
Total $CHCl_3$ extract (8)	9.9 ± 0.49

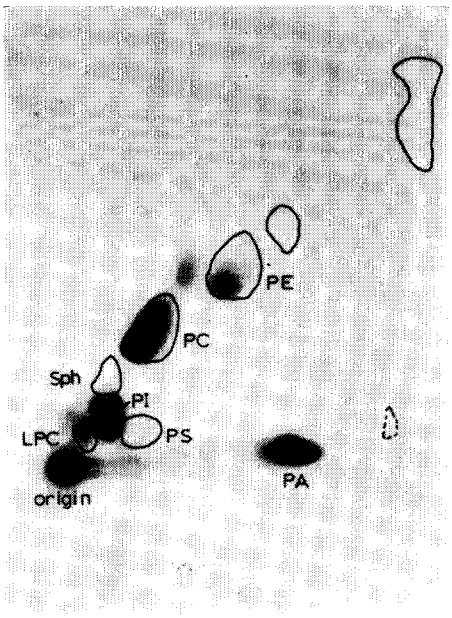

Fig. 3. Thin-layer chromatogram of lipids extracted from bullfrog gastric mucosa. The radioautogram with dense ^{32}P spots has been superimposed over a tracing of the lipid spots detected by iodine staining (solid lines). Those lipids identified were phosphatidyl ethanolamine (PE), phosphatidyl choline (PC), phosphatidyl serine (PS), phosphatidyl inositol (PI), sphingomyelin (Sph), lysophosphatidyl choline (LPC) and phosphatidic acid (PA). Cholesterol and fatty acids moved near the solvent front. Several unidentified spots of radioactivity and iodine-staining properties are apparent. An unidentified brownish spot is shown (dotted line) which did not stain with iodine, contained no detectable P_i or radioactivity, and on isolation was found to have an absorption maximum of 400 mμ in $CHCl_3$–CH_3OH. See text for chromatographic solvent composition. Vertical direction: Solvent I. Horizontal direction: Solvent III.

In a preliminary study the mucosa was scraped with a glass slide and the total phospholipid P estimated separately in the scraped cells and in the submucosa. It was observed that almost 75 % of phospholipid P was contained in the former fraction; the phospholipid pattern of both fractions, however, appeared to be very similar to that of the whole mucosa.

Significant amounts of $^{32}P_i$ were incorporated into phosphatidyl choline, phosphatidyl enthanolamine, phosphatidic acid and phosphoinositide. Incorporation of $^{32}P_i$ into phosphatidyl serine was usually low, but variable, while sphingomyelin always showed negligible activity. An unidentified component(s) of relatively high specific activity remained at the origin, and may very likely be a $^{32}P_i$ contamination in the extract.

Effect of histamine stimulation on acid secretion and phospholipid turnover

There was a slight increase in the mean phospholipid content of mucosae stimulated by histamine as compared to resting tissues (Table II) but a *t*-test on the differences between paired mucosal halves was below the 10 % level of significance. However, all mucosae which secreted acid in response to histamine also showed an enhanced incorporation of $^{32}P_i$ into the phospholipid fraction. A comparison of the specific activity of various phospholipid components in resting and histamine-stimu-

lated paired mucosal halves is shown in Table III. The individual lipid components showing the most marked increase in specific activity were phosphatidyl choline and phosphatidyl ethanolamine. There was a 2–5-fold increase in $^{32}P_i$ incorporation into these major structural lipids of the histamine-stimulated preparations. There were also consistently observed increases in $^{32}P_i$ incorporation into phosphatidic acid and phosphatidyl inositol. With the solvent systems that we used early in this work the phosphatidyl inositol and phosphatidyl serine were not completely separable, but in more recent experiments where complete separations of these two components were achieved, we have found the specific activity of phosphatidyl inositol to be 20–50 times greater than that of phosphatidyl serine (*cf.* Table V). It is noteworthy that for most mucosae, both resting and stimulated, the specific activity of the phosphatidyl choline was greater than that of phosphatidyl ethanolamine, while in a few instances (three out of thirteen experiments) the situation was reversed. The relative increase in incorporation of $^{32}P_i$ into phosphatidyl ethanolamine appeared to be typical of gravid female frog mucosae, and we have no explanation at the present time for this phenomenon. It is possible that an altered hormonal state and other unknown factors could account for these relative differences.

TABLE II

COMPARISON OF PHOSPHOLIPID CONTENT IN RESTING AND HISTAMINE-STIMULATED PAIRED GASTRIC MUCOSAL HALVES

Data are given for 5 paired mucosal halves as μmoles P_i/g wet tissue ± S.E.

Fraction	*Paired gastric mucosal halves*	
	Resting	*Histamine stimulated*
Phosphatidyl choline	3.36 ± 0.26	3.67 ± 0.30
Phosphatidyl ethanolamine	0.99 ± 0.20	0.99 ± 0.14
Total $CHCl_3$ extract	9.0 ± 0.8	10.3 ± 0.3

TABLE III

COMPARISON OF UPTAKE OF $^{32}P_i$ INTO VARIOUS PHOSPHOLIPID COMPONENTS IN RESTING AND HISTAMINE-STIMULATED GASTRIC MUCOSAL PAIRS

R = resting mucosa, S = stimulated by 0.1 mM histamine. All values for phospholipids are given as ^{32}P counts/min × 10^{-4} per μmole phospholipid phosphate.

	Gastric mucosal pair No.											
	1		*2*		*3*		*4*		*5*		*6*	
	R	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*
H^+ secretory rate (μequiv/cm^2 per h)	0	3.1	0	3.1	<0.1	3.3	<0.1	2.8	0	3.1	0	2.7
Phosphatidyl choline	1.6	4.0	0.3	0.6	0.6	1.3	0.2	0.8	0.03	0.2	0.6	2.5
Phosphatidyl ethanolamine	0.3	1.6	0.1	0.3	0.2	0.4	1.6	5.0	0.3	0.6	0.2	0.5
Phosphatidyl serine *plus* phosphatidyl inositol					6.7	7.5	1.5	2.7	0.4	1.6	6.3	13.5
Phosphatidic acid					65.0	82.0	48.0	87.0	4.6	17.2	81.2	100.0
Total phospholipid-P	2.8	5.0	1.1	1.5	1.9	2.9	1.1	2.4	0.4	0.9	1.8	4.1

In experiments using [2-^{14}C]acetate, a considerable amount of radioactivity was incorporated into the chloroform-extractable fraction; however, the differences between the resting and histamine-stimulated mucosal pairs were quite small compared to those observed with ^{32}P. The results of an individual experiment showing the incorporation of both ^{32}P and ^{14}C into various phospholipid components is shown in Table IV. The choline phosphatides accounted for about 25 % of the total ^{14}C in the chloroform extract, which was very much greater than that of any other phospholipid component. The specific activities given in Table IV based on μmoles of P_i, also reflect the relatively rapid rate of ^{14}C incorporation into phosphatidyl choline. The

TABLE IV

CHANGES IN UPTAKE OF [^{32}P]PHOSPHATE AND [2-^{14}C]ACETATE INTO VARIOUS PHOSPHOLIPID COMPONENTS OF GASTRIC MUCOSA ASSOCIATED WITH STIMULATION OF H^+ SECRETION BY HISTAMINE

Values for specific activity of ^{32}P and ^{14}C all $\times 10^{-4}$.

Components	*Resting mucosa*		
	μmoles P_i/g wet tissue	*^{32}P counts/min per μmole P_i*	*^{14}C counts/min per μmole P_i*
Phosphatidyl choline	3.74	0.2	3.0
Phosphatidyl ethanolamine	1.65	1.6	0.9
Phosphatidyl serine *plus* phosphatidyl inositol	1.22	1.5	0.9
Phosphatidic acid	0.03	48.0	1.7
Sphingomyelin	0.55	0.02	1.0
Total $CHCl_3$ extract	10.60	1.10	3.9
	Histamine-stimulated		
	μmoles P_i/g wet tissue	*^{32}P counts/min per μmole P_i*	*^{14}C counts/min per μmole P_i*
Phosphatidyl choline	3.64	0.8	3.1
Phosphatidyl ethanolamine	1.30	5.0	0.9
Phosphatidyl serine *plus* phosphatidyl inositol	0.99	2.7	0.8
Phosphatidic acid	0.03	87.5	1.2
Sphingomyelin	0.52	$<$0.02	1.3
Total $CHCl_3$ extract	10.8	2.40	4.7

absence of ^{14}C in lysophosphatidyl choline and its relatively large incorporation into phosphatidyl choline suggests a rapid turnover of a single fatty acid residue in the latter. While analogous lyso derivatives and their acylations are known to occur for phosphatidyl ethanolamine and phosphatidic acid in various tissues, their metabolic or functional significance is not clear. In this instance, however, these reactions, unlike the turnover of the polar groups of these phospholipids, do not appear to participate directly in the sequence of events leading to acid secretion.

Effect of SCN^-

Inhibition of acid secretion by 10 mM NaSCN resulted in diminished incorporation of $^{32}P_i$ into the phospholipids of gastric mucosa (Table V). The most profound

decrease in uptake and, hence, specific activity of $^{32}P_i$, occurred in phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl inositol and phosphatidic acid components. In general the patterns of $^{32}P_i$ incorporation in the SCN^--inhibited and normally secreting mucosae were comparable to those of the resting and histamine-stimulated mucosae, respectively.

Miscellaneous observations

Incorporation of $^{32}P_i$ into the acid-soluble phosphate ester fraction and into the crude ATP fraction of the histamine-stimulated mucosae showed variable increases over those of the resting mucosae. These findings are shown in Table VI and are essentially in agreement with those of EGGMAN AND HOKIN[5]. The average increase in

TABLE V

CHANGES INDUCED BY NaSCN IN GASTRIC ACID SECRETION AND INCORPORATION OF ^{32}P (SPECIFIC ACTIVITY) INTO VARIOUS PHOSPHOLIPIDS

The specific activities of all phospholipid are given as ^{32}P counts/min $\times 10^{-4}$ per μmole phospholipid phosphate.

Component	*Expt. 1*		*Expt. 2*	
	Secreting mucosa	*10 mM NaSCN*	*Secreting mucosa*	*10 mM NaSCN*
H^+ secretory rate (μequiv H^+/cm^2 per h)	3.1	0.4	5.0	<0.1
Phosphatidyl choline	2.20	0.78	2.81	0.60
Phosphatidyl ethanolamine	0.51	0.44	0.28	0.04
Phosphatidyl serine	0.18	0.14	6.23*	1.68*
Phosphatidyl inositol	15.40	6.83		
Sphingomyelin	0.34	0.12	0.13	0.19
Phosphatidic acid	104.00	37.00	89.40	39.40
Total $CHCl_3$ extract	3.25	1.53	2.97	0.74

* Phosphatidyl serine and phosphatidyl inositol were not completely separated and therefore the data have to be given as combined values.

TABLE VI

COMPARISON BETWEEN RESTING AND HISTAMINE-STIMULATED MUCOSAE OF THE SPECIFIC ACTIVITIES OF ACID-SOLUBLE PHOSPHATE ESTER FRACTIONS AND OF ATP

R = resting mucosa. S = mucosa stimulated by 0.1 mM histamine.

Acid-soluble phosphate esters			[^{32}P]*ATP*		
Counts/min per mg wet wt.		*% Change*	*Counts/min per mμmole adenine*		*% Change*
R	*S*		*R*	*S*	
34 873	48 395	+ 38	7987	6 937	— 14
21 613	30 231	+ 39	3283	7 445	+ 126
52 973	60 281	+ 13	7025	8 634	+ 19
37 483	62 696	+ 67	8122	12 988	+ 60

* Based on absorbance at 257 mμ and molar extinction coefficient of 14.7 $mM^{-1} \cdot cm^{-1}$ for adenine.

incorporation of $^{32}P_i$ into ATP induced by histamine stimulation was about 50% above that observed for resting mucosae. This increase probably reflects an increased metabolic activity of the tissue associated with H^+ secretion. However, these changes were not nearly as pronounced as the increase in specific activity of some of the phospholipid components.

DISCUSSION

Evidence has been presented to show that gross changes in membrane structure of oxyntic cells are correlated with H^+ secretory activity in gastric mucosa of frog[7,9,10,17] and other species[8,18]. Our present findings have confirmed these earlier results and by comparing resting preparations of gastric mucosa with those stimulated by histamine we have direct measurements of physiological secretory performance to correlate with the ultrastructural data. The oxyntic or acid-secreting cell contains abundant smooth-surfaced endoplasmic reticulum, in the form of microtubular elements, the apical portion of the cytoplasm. The apical surface membrane is relatively smooth in the resting, non-secreting, stomach with only a few short microvilli into the glandular lumen. Administration of histamine produces an elaboration of numerous microvilli at the apical secretory surface. It has been postulated that the microtubular elements of the apical cytoplasm are utilized to increase the surface area of the cells during secretory activity[9,10]. The exact function of the smooth-surfaced endoplasmic reticulum in the H^+ secretory process is unknown. It is possible that H^+ is pre-secreted in the microtubules as the result of metabolic activity and that histamine (or other secretagogues) act as a stimulus for the surface contact and deposition of H^+. Alternatively the microtubules may act as a membrane reserve which, under histamine stimulation, serves for the elaboration of apical microvilli where the metabolically dependent translocation of H^+ occurs. These alternative mechanisms would simply separate chronologically the actual ionic translocation from the membranous transformations known to accompany the H^+ secretory process. Whatever the detailed mechanism of HCl secretion, the overall process appears to involve the migration and interconversion of microtubular elements and surface plasma membrane, and possibly a breakdown and resynthesis of phospholipid constituents of the membrane. This increased breakdown and synthesis, in our estimate, is an important factor contributing to the observed increase in $^{32}P_i$ turnover of lipid components, especially in the major structural phospholipids, phosphatidyl choline, and phosphatidyl ethanolamine. The absence of a large difference in the incorporation of [2-^{14}C]acetate in various phospholipids between resting and secreting mucosae may be indicative of the importance of the polar groups rather than the acyl constituents in the observed membrane transformations.

One might also explore the possibility that there was no difference in the rate of phospholipid turnover in the resting and secreting preparations, but that the observed changes in specific activity were the result of an enhanced uptake of the trace $^{32}P_i$ or an increased turnover of ATP in histamine-stimulated mucosa. If the specific activity in ATP were to increase, this would contribute to an increase in ^{32}P labeling of phospholipids. However, the increase in radioactivity of the acid-soluble phosphate ester fraction, as well as that of the crude ATP fraction in the case of stimulated mucosae, although variable, was never large enough to account for the observed

changes in specific phospholipids. This observation coupled with the fact that there is no significant difference in ATP levels in the resting and stimulated mucosa[11] suggest that there is not a 2–4-fold change in specific activity of ATP which would be required to explain phospholipid changes. Barring a situation in which large changes in specific activity of ATP could occur in a unique compartment, one is tempted to rule out the possibility that the changes observed in specific activity of phosphatidyl choline and phosphatidyl ethanolamine are the result of an increased ATP turnover; and to suggest that there is, in fact, a concomitant increase in the rate of turnover of these phospholipids in the histamine-stimulated mucosa.

There may be several reasons for the apparent discrepancy between our results of phospholipid turnover and those of EGGMAN AND HOKIN[5]. Firstly, the poor labeling of phosphatidyl ethanolamine in their experiments may have resulted from a small normal uptake of P_i by gastric mucosa from the medium. In our experiments we used P_i-free frog Ringer's, except for the radioactive $^{32}P_i$ of high specific activity, and this modification permitted a significant uptake of radioactivity, thus enabling reasonable incorporation into various phospholipid components. Secondly, it is to be pointed out that the isolated mucosae of *R. pipiens*, like those of *R. catesbeiana* ordinarily secrete acid spontaneously in the absence of exogenous histamine and the addition of histamine to these preparations results in only small increases in the secretory rate. In the experiments of EGGMAN AND HOKIN, both the control and histamine-stimulated preparations may have been secreting H^+ at almost maximal rates, as is evidenced from their data. The maximal increment in the rate of acid secretion on addition of histamine in their preparation was of the order of 0.6 μequiv/h per 100 mg mucosa. In the present studies the resting preparations secreted little, or no acid and, on stimulation with 0.1 mM histamine, the rate increased to an average of 3 μequiv/cm^2 per h (1 cm^2 mucosa weighs approximately 100 mg). In one of our experiments for no apparent reason the gastric mucosa was refractory to histamine and it is significant that in this instance, there was no difference in uptake of ^{32}P into phospholipids between the control and histamine-treated mucosal pair.

The present observations, therefore, show that there is an increased turnover of phospholipids in gastric mucosa when transport is stimulated. The mucosa differs from other secretory tissues showing the "phospholipid effect" that phosphatidyl choline and phosphatidyl ethanolamine are the lipids affected most. Although the changes in specific phospholipids vary somewhat with the particular tissue, the widespread occurrence of the effect in many secretory or ingestive phenomena (refs. 1–4; F. DIES quoted by LOTSPEICH[19]) suggests that the changes in phospholipid turnover may be characteristic of membrane migration and alterations associated with translocation phenomenon. Thus the effect appears not to be limited to secretion of a specific ion or organic hydrophilic substance with or without cationic groups, as suggested by EGGMAN AND HOKIN[5].

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