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INHIBITION OF GASTRIC ($H^+ + K^+$)-ATPase BY UNSATURATED LONG-CHAIN FATTY ACIDS

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Arachidonic acid and unsaturated C_{18} fatty acids at concentrations near 10^{-5} M markedly inhibited ($H^+ + K^+$)-ATPase in hog or rat gastric membranes. Arachidonic acid was a more potent inhibitor than unsaturated C_{18} fatty acids, but the involvement of the metabolites of arachidonic acid cascade was ruled out. Linolenic acid inhibited the formation of phosphoenzyme and the K^+-dependent *p*-nitrophenylphosphatase activity of the hog ATPase. Treatment with fatty acid-free bovine serum albumin abolished only the inhibitory effect of the fatty acid on the phosphatase activity without restoring the overall ATPase action. These data suggest the existence of at least two groups of hydrophobic binding sites in the gastric ATPase for unsaturated long-chain fatty acids which affect differentially the catalytic reactions of the ATPase. ($H^+ + K^+$)-ATPase in rat gastric membranes was found more susceptible to the fatty acid inhibition and also more unstable than the ATPase in hog gastric membranes. The presence of a millimolar level of lanthanum chloride or ethylene glycol bis(β-aminoethyl ether)-*N*,*N*′-tetraacetic acid stabilized the rat ATPase probably via the inhibition of Ca^{2+}-dependent phospholipases in the gastric membranes.

Introduction

Gastric microsomal membranes from various species contain K^+-dependent ATPase [1–7]. Further studies have established the ATPase as an electroneutral H^+/K^+ exchange pump [8–10] and its involvement in the terminal steps of the gastric acid secretory process [11]. The partial reactions of the ATPase have been shown to include H^+-dependent formation and K^+-dependent breakdown of a phosphorylated intermediate of the ATPase [12–14]. These catalytic reactions of the ATPase, an integral membrane protein, are generally expected to be influenced to a certain extent by its interactions with the surrounding membrane phospholipids and possibly their metabolites. It has been recently reported that ($H^+ + K^+$)-ATPase activity in hog gastric membranes decreased markedly upon partial digestion of the membrane lipids by phospholipase A_2 or C or upon limited extraction of the lipids with ethanol [15–18]. In particular, free fatty acids released from the membrane lipids by the action of phospholipase A_2 have been reported to reversibly inhibit K^+-dependent *p*-nitrophenylphosphatase activity of hog ($H^+ + K^+$)-ATPase [15]. The phosphatase activity, copurified with the ATPase, presumably represents its dephosphorylation reaction [19–22]. During preliminary studies, we have also observed that arachidonic acid at 10^{-5} M markedly inhibited ($H^+ + K^+$)-ATPase in rat gastric mem-

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Abbreviation: EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*′-tetraacetic acid.

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branes. This study was initiated, therefore, to examine the effect of unsaturated long-chain fatty acids on gastric $(H^+ + K^+)$-ATPase. It has been found that the fatty acids inhibited not only the K^+-*p*-nitrophenylphosphatase activity, but also the formation of phosphoenzyme. The inhibitory effect on the overall ATPase action was irreversible, although the K^+-*p*-nitrophenylphosphatase activity was fully recovered by the action of fatty acid-free bovine serum albumin as reported previously [15]. By contrast, we have observed that $(Na^+ + K^+)$-ATPase, an analogous membrane ATPase, was not noticeably affected by the fatty acids.

Materials and Methods

$(H^+ + K^+)$-ATPase preparation

From the rat. A tissue fraction enriched in gastric parietal cells was obtained typically from twenty male Sprague-Dawley rats weighing about 230 g according to the method of Robert et al. [23]. The mucosal tissues were suspended in 10 volumes of a solution containing 250 mM mannitol, 2 mM $MgCl_2$, and 2 mM Hepes-Tris, pH 7.4 (mannitol buffer), and homogenized in a Sorvall Omni-Mixer for 3 min. The homogenate was centrifuged at $20\,000 \times g$ for 15 min. The supernatant was collected and centrifuged at $170\,000 \times g$ for 30 min to obtain the microsomal membranes. The microsomes were further resolved by sedimentation through a sucrose density gradient consisting of 30 and 40% (w/w) sucrose solutions containing 2 mM $MgCl_2$ and 2 mM Hepes-Tris, pH 7.4. After centrifugation at $100\,000 \times g$ for 60 min, the light microsomal membrane band above 30% sucrose was collected and stored under liquid nitrogen until use. For a typical preparation, the specific activity of $(H^+ + K^+)$-ATPase, determined as the difference in ATP hydrolysis in the absence or presence of KCl (20 mM), was 9.5 μmol/h per mg protein in the homogenate, 25 μmol/h per mg protein in the microsomes and 112 μmol/h per mg protein in the light microsomal membranes obtained from the density gradient. The ATP splitting activity in the absence of KCl was 5, 10 or 38 μmol/h per mg protein for the respective preparations in the same order given above. It should be noted that the whole procedure was completed within 3 h at 4°C, since prolonged preparation time often resulted in a significant loss of $(H^+ + K^+)$-ATPase in the light microsomal membranes.

From the hog. The mucosal scrapings from hog stomachs were prepared as reported by Saccomani et al. [6]. The rest of the procedure was identical as described above. The specific activity of $(H^+ + K^+)$-ATPase in the hog gastric membranes thus prepared ranged from 90 to 110 μmol/h mg protein.

$(Na^+ + K^+)$-ATPase preparation

The microsomes enriched in $(Na^+ + K^+)$-ATPase were prepared from the inner medulla of dog kidneys according to the method of Kyte [24]. To disrupt the sidedness of the membrane vesicles and enhance the accessibility of substrates to the catalytic sites, the microsomal membranes were treated with deoxycholate at a final concentration of 0.6 mg/ml. The detergent was removed by centrifugation. The specific activity of $(Na^+ + K^+)$-ATPase in the microsomes was 53 μmol/h per mg protein.

Assay procedures

$(H^+ + K^+)$-ATPase activity was determined in 1-ml incubation media containing 40 mM Tris-acetate pH 7.4, 2 mM $MgCl_2$, 2 mM ATP with or without 20 mM KCl, and gramicidin (5 μg/ml). In some experiments, 20 mM KCl and gramicidin were replaced with 7 mM KCl and 7 mM NH_4Cl [21]. Identical results were obtained. Typically, about 50 μg membrane protein were incubated at 37°C for 5 min. The reaction was terminated by adding 1 ml of ice-cold 10% trichloroacetic acid and 0.1 g of HCl washed charcoal. The charcoal was then removed by centrifugation and passage through glass wool filters. The amount of inorganic phosphate in the filtrates was determined by the method of Tsi et al. [25]. $(Na^+ + K^+)$-ATPase activity was measured in 1 ml of incubation media containing 120 mM NaCl, 7 mM KCl, 0.5 mM EDTA, 4 mM $MgCl_2$, 4 mM ATP, and 5 mM Hepes-Tris, pH 7.4, with or without 1 mM ouabain. The rest of the procedures was the same as described above.

K^+-Dependent *p*-nitrophenylphosphatase was assayed in 1 ml incubation media containing 10 mM Tris, pH 7.5, 5 mM *p*-nitrophenyl phosphate

with or without 10 mM KCl [26]. The reaction was terminated with 1.5 ml 0.5 M NaOH after incubation at 37°C for 5 min. The mixture was centrifuged briefly and the absorbance of the supernatant, at 410 nm, was measured. In all the assays performed under these conditions, reaction rates did not deviate from linearity with respect to reaction time and protein concentrations.

The formation of phosphoenzyme at steady state was measured with $(H^+ + K^+)$-ATPase in hog gastric membranes. One milliliter of incubation medium contained about 50 μg membrane proteins, 40 mM Tris-acetate, pH 7.4, 2 mM $MgCl_2$, and 10 μM[γ-^{32}P]ATP. The reaction was at 22°C. The termination of the reaction and the filtration of the reaction mixture over a Millipore filter (3-μm pore size) were carried out exactly as described [14]. The ^{32}P-radioactivity of the filters was counted in 10 ml of Instagel scintillation cocktail (Packard) using a Tricarb spectrometer. A time-course study indicated the level of phosphoenzyme was steady from 15 to 30 s. In some assays, unsaturated fatty acids and their analogs in methanolic solution were added. The level of methanol never exceeded 2% and was maintained constant in all assay tubes.

To analyze membrane proteins, polyacrylamide disc gel electrophoresis (5.6% gel, 1% sodium dodecyl sulfate) of protein samples (~ 20 μg) were carried out as described by Fairbanks et al. [27]. Protein was determined by the method of Lowry et al. [28] using bovine serum albumin as a standard.

Materials

Free fatty acids, diacylglycerols, and linolenyl alcohol were obtained fron Nu-Chek Prep Inc., Elysion, MN. Linolenyl methyl ester, lysophosphatidylcholine, and essentially fatty acid free-bovine serum albumin were purchased from Sigma. Prostaglandins were from The Upjohn Company. All other materials were of reagent-grade quality and obtained from standard sources.

Results

Arachidonic acid, unsaturated C_{18} fatty acids, and few analogs of linolenic acid were compared for their inhibitory potency on $(H^+ + K^+)$-ATPase in hog or rat gastric membranes (Table I). Arachidonic acid was the most potent inhibitor of gastric $(H^+ + K^+)$-ATPase among those free fatty acids tested. To eliminate possible effects of the various metabolites of arachidonic acid on the ATPase, the hog membranes were treated with indomethacin (cycloxygenase inhibitor) or 5,8,11,14-eicosatetraynoic acid (lipoxygenase inhibitor) at 10^{-4} M. The treatment, however, did not alter the dose of arachidonic acid to produce 50%inhibition of the ATPase activity (ED_{50}), $3 \cdot 10^{-5}$ M. Also several prostaglandins (prostaglandin D_2, E_2, $F_2\alpha$, and I_2) at millimolar concentrations showed no inhibitory effect on the gastric ATPase. These observations indicate that the metabolites of arachidonic acid cascade were not involved in the fatty acid inhibition of the ATPase.

A series of unsaturated C_{18} fatty acids were also inhibitory to the ATPase in hog gastric membranes, but their ED_{50} values, 6 to $8 \cdot 10^{-5}$ M, were about twice as high as that of arachidonate. The number or the type (*cis* or *trans*) of double

TABLE I

COMPARISON OF INHIBITORY POTENCY OF UNSATURATED LONG-CHAIN FATTY ACIDS AND THEIR ANALOGS ON $(H^+ + K^+)$-ATPase IN RAT OR HOG GASTRIC MEMBRANES

The rat or hog gastric membranes (~ 50 μg) were preincubated in the presence or absence of fatty acids for 2 min at 37°C. The reaction was initiated by adding ATP and lasted for 5 min at 37°C. The fatty acid dose to produce 50% inhibition of the ATPase activity, ED_{50}, was obtained from a typical dose-response curve. Standard errors were found less than 20% from several experiments in which data in triplicate were available.

	ED_{50} (μM)	
	Hog $(H^+ + K^+)$-ATPase	Rat $(H^+ + K^+)$-ATPase
Arachidonic acid	30	15
Linolenic acid	58	30
Linoleic acid	60	30
Linoelaidic acid	76	30
Oleic acid	80	30
Linolenyl alcohol	240	60
Linolenyl methyl ester	No inhibition	No inhibition
Lysophosphatidylcholine, oleoyl	75	75

bonds within the C_{18} series produced only a marginal effect on their potency. Linolenyl alcohol was much less potent than linolenic acid, and its methyl ester was not inhibitory at all. Therefore, the carboxylic anion appeared to play an important role in the fatty acid inhibition of the ATPase. The same trends were observed from similar studies with $(H^+ + K^+)$-ATPase in rat gastric membranes, although the rat ATPase was found more sensitive to the fatty acids; the ED_{50} values of the fatty acids were roughly one-half of those observed with the hog enzyme. On the other hand, oleoyllysophosphatidylcholine, a powerful detergent, displayed the same inhibitory potency on rat and hog gastric $(H^+ + K^+)$-ATPase; and its ED_{50} value, $7.5 \cdot 10^{-5}$ M, was higher than most of the unsaturated fatty acids tested.

Because of the facile metabolism of arachidonic acid in biological membranes, linolenic acid was employed in characterizing further the mode of action of unsaturated long-chain fatty acids on gastric $(H^+ + K^+)$-ATPase. Fig. 1 shows the dose-response curves for linolenic acid action on $(Na^+ + K^+)$-ATPase from canine renal medulla in comparison with $(H^+ + K^+)$-ATPase in rat or hog gastric membranes. At the concentrations above 20 μM, linolenic acid failed to show an evident

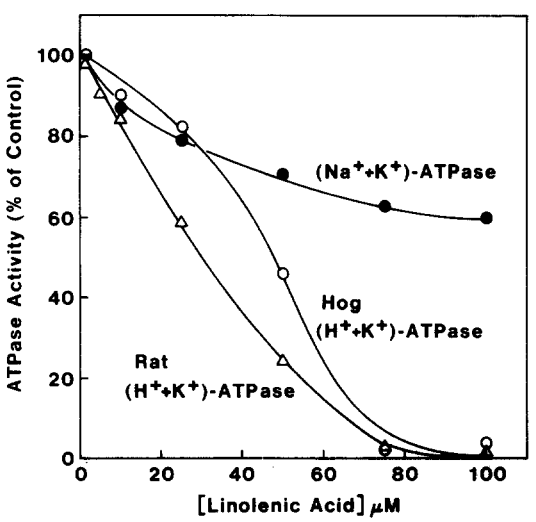

Fig. 1. Dose-response profiles for linolenic acid action on $(Na^+ + K^+)$-ATPase from canine renal medulla and $(H^+ + K^+)$-ATPase in rat or hog gastric membranes. About 50 μg of membrane proteins were employed in all assays to maintain consistent ratios of linolenic acid to membrane proteins.

dose-dependent effect on $(Na^+ + K^+)$-ATPase. Almost 70% of $(Na^+ + K^+)$-ATPase activity still survived at 100 μM linolenic acid, at which concentration gastric $(H^+ + K^+)$-ATPase was completely inhibited.

The effect of linolenic acid on the formation of phosphorylated intermediate of $(H^+ + K^+)$-ATPase in hog gastric membranes was examined at 10 μM [γ-^{32}P]ATP. The steady-state level (20 s) of the phosphoenzyme was 851 pmol/mg protein and was reduced to 149 pmol/mg protein in the presence of 80 μM linolenic acid. An Mg^{2+} chelating agent, (1,2-cyclohexylene-dinitro)tetraacetic acid (CDTA), at 5 mM produced a similar reduction of the phosphoenzyme level (150 pmol/mg protein). K^+-*p*-Nitrophenylphosphatase activity of the gastric membranes was also inhibited by linolenic acid. The dose-response profile for linolenic acid action on the phosphatase activity was identical to that on the ATPase activity. These observations can be interpreted to mean that linolenic acid inhibited both the phosphorylation and dephosphorylation reaction of gastric $(H^+ + K^+)$-ATPase.

The reversibility of the inhibitory effect of linolenic acid on $(H^+ + K^+)$-ATPase was tested. As shown above, hog gastric membranes in the presence of 100 μM linolenic acid lost more than 90% of K^+-*p*-nitrophenylphosphatase and $(H^+ + K^+)$-ATPase activities. When the membranes treated with the fatty acid (100 μM) were washed in the presence of 1% fatty actid-free bovine serum albumin, almost 60% of K^+-*p*-nitrophenylphosphatase activity but no significant amount of $(H^+ + K^+)$-ATPase activity were restored. It should be noted that the wash of the native membranes with the bovine serum albumin alone led to 20 and 30% reduction of the $(H^+ + K^+)$-ATPase and K^+-*p*-nitrophenylphosphatase activities, respectively. The reversible inhibition of K^+-*p*-nitrophenylphosphatase activity of hog $(H^+ + K^+)$-ATPase by free fatty acids has been also reported by Saccomani et al. [15].

During this study we have observed that $(H^+ + K^+)$-ATPase and K^+-*p*-nitrophenylphosphatase activities of rat gastric membranes were quite unstable at 37°C. Both activities decreased simultaneously with an apparent half-life of 33 min (Fig. 2), whereas the same activities in the hog

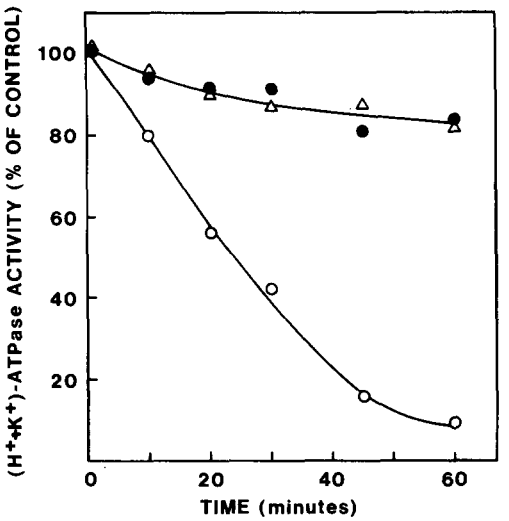

Fig. 2. Time-course profiles of $(H^+ + K^+)$-ATPase activity in rat gastric membranes. The gastric membranes in mannital buffer (3 mg protein/ml) were incubated at 37°C in the absence (○) or presence of 1 mM EGTA (●) or lanthanum chloride (△). An aliquot of 15 μl was withdrawn at the indicated time points and was assayed for K^+-dependent ATPase activity.

membranes were hardly affected. The possibility of proteolytic digestion of $(H^+ + K^+)$-ATPase in rat gastric membranes has been ruled out since SDS gel electrophoresis patterns of the membranes before or after inactivation of 90% $(H^+ + K^+)$-ATPase activity showed no noticeable differences. For instance, no changes were observed in the intensities of the most prominent band at 100 kDa representing $(H^+ + K^+)$-ATPase [29] or the second leading band at 50 kDa or other minor bands at 150, 95, 68, 45, and 30 kDa. The gel pattern was similar to that reported by Shaltz et al. [7]. The presence of 1 mM lanthanum chloride or ethylene glycol bis(β-aminoethyl ether)-*N*,*N*′-tetraacetic acid (EGTA) almost completely prevented the decay of the ATPase activities in rat gastric membranes (Fig. 2). Probably the preventive effect of La^{3+} or EGTA can be attributed to the inhibition of Ca^{2+}-dependent phospholipases in the rat membranes. The abundance of various phospholipases and lipases in rat gastric mucosa has been reported [31–34]. In addition, the extreme sensitivity of $(H^+ + K^+)$-ATPase in rat gastric membranes to unsaturated long-chain fatty acids was shown in this study.

Discussion

In this study we have examined the interaction of gastric $(H^+ + K^+)$-ATPase with unsaturated long-chain fatty acids. Since the fatty acids are anionic amphiphiles, their effects on the ATPase could arise from disordering its surrounding membranes lipids as well as some specific interaction with the ATPase. Several observations made in this study favor the latter as a predominant mode of action between gastric $(H^+ + K^+)$-ATPase and unsaturated long-chain fatty acids. First of all, the dose-dependent inhibition of the gastric ATPase was observed at fairly low concentrations (10^{-5} M) of the fatty acids. $(Na^+ + K^+)$-ATPase, an analogous membrane ATPase, was hardly affected at these levels of the fatty acids and Ca^{2+}-ATPase has been reported to be rather stimulated [35,36]. Since the lipid composition of the hog gastric membranes has been found not remarkably different from that of other mammalian plasma membranes [15,18], the differential effect of the fatty acids on gastric $(H^+ + K^+)$-ATPase should be attributed to some unique molecular interaction between them. Secondly, some structural variations of unsaturated fatty acids drastically altered their inhibitory potency of the gastric ATPase; for example, linolenic acid was much more potent than linolenyl alcohol or methyl ester. This effect is not likely correlated to the improved detergent property of the anionic species since lysophosphatidylcholine, a superior detergent, was less effective in inhibiting the ATPase activity than most of unsaturated long-chain fatty acids. Finally, the fatty acids inhibited irreversibly $(H^+ + K^+)$-ATPase activity, but reversibly its K^+-*p*-nitrophenylphosphatase activity. This selective recovery of the phosphatase activity of the gastric ATPase (with bovine serum albumin washing) suggests that the ATPase has at least two groups of hydrophobic binding sites of different affinity for the fatty acids, each affecting the catalytic function of the ATPase differentially. Separation of the phosphatase activity from gastric $(H^+ + K^+)$-ATPase or $(Na^+ + K^+)$-ATPase activity has also been reported by selective modification of certain sulfhydryl group(s) of the ATPase with thimerasol [26,27].

Physiological significance of the fatty acid in-

hibition of gastric ($H^+ + K^+$)-ATPase is not apparent at the present time. In vivo, the level of unsaturated long-chain free fatty acids is not likely high enough to produce a significant effect on gastric ($H^+ + K^+$)-ATPase. It is still possible that the fatty acids merely minic a more potent biological regulator yet unknown but structurally similar to the fatty acids. Under some pathological conditions where phospholipases are activated abnormally, however, the fatty acids themselves could be an important factor affecting gastric ($H^+ + K^+$)-ATPase function. Similar conditions may be encountered not infrequently during studies with isolated membranes which lack cellular enzymes to metabolize the products of phospholipases. For instance, we have ascribed the inactivation of ($H^+ + K^+$)-ATPase in rat gastric membranes to the action of Ca^{2+}-dependent phospholipases through their hydrolysis products.

In conclusion, we have shown some specific molecular interaction between gastric ($H^+ + K^+$)-ATPase and unsaturated long-chain fatty acids and have suggested the harmful effect of phospholipases on ($H^+ + K^+$)-ATPase in isolated rat gastric membranes.

References

1 Ganser, A.L. and Forte, J.G. (1973) Biochim. Biophys. Acta 307, 169–180
2 Ganser, A.L., Tanisawa, A.S. and Forte, J.G. (1974) Ann. N.Y. Acad. Sci. 242, 255–267
3 Spenney, J.G., Saccomani, G., Spitzer, H.L. and Sachs, G. (1974) Arch. Biochem. Biophys. 161, 456–471
4 Ray, T.K. and Forte, J.G. (1974) Biochim. Biophys. Acta 363, 320–339
5 Forte, J.G., Ganser, A.L., Beesley, R. and Forte, T.M. (1975) Gastroenterology 69, 175–189
6 Saccomani, G., Stewart, H.B., Shaw, D., Lewin, M. and Sachs, G. (1977) Biochim. Biophys. Acta 465, 311–330
7 Shaltz, L.J., Bools, C. and Reimann, E.M. (1981) Biochim. Biophys. Acta 673, 539–551
8 Lee, J., Simpson, G. and Scholes, P. (1974) Biochem. Biophys. Res. Commun. 60, 825–832
9 Sachs, G., Chang, H.H., Rabon, E., Schackmann, R., Lewin, M. and Saccomani, G. (1976) J. Biol. Chem. 251, 7690–7698
10 Sachs, G., Chang, H.H., Rabon, E., Schademann, R., Saran, H.M. and Saccomani, G. (1977) Gastroentrology 73, 931–940
11 Fellenius, E., Berglindh, T., Sachs, G., Olbe, L., Glander, B., Sjoslrand, S.-E. and Wallmark, B. (1981) Nature 290, 159–161
12 Ray, T.K. and Forte, J.G. (1976) Biochim. Biophys. Acta 443, 451–467
13 Wallmark, B. and Mardh, S. (1979) J. Biol. Chem. 254, 11899–11902
14 Stewart, B., Wallmark, B. and Sachs, G. (1981) J. Biol. Chem. 256, 2682–2690
15 Saccomani, G., Chang, H.H., Spisni, A., Helander, H.F., Spitzer, H.L. and Sachs, G. (1979) J. Supramol. Struct. 11, 429–444
16 Sen, P.C. and Ray, T.K. (1979) Biochem. J. 182, 637–640
17 Sen, P.C. and Ray, T.K. (1980) Arch. Biochem. Biophys. 202, 8–17
18 Schrijen, J.J., Omachi, A., Van Groningen-Luyben, W.A.H.M., DePont, J.J.H.H.M. and Bonting, S.L. (1981) Biochim. Biophys. Acta 649, 1–12
19 Forte, J.G., Forte, G.M. and Saltman, P. (1967) J. Cell. Physiol. 69, 293–304
20 Forte, J.G., Ganser, A.L. and Tanisawa, A.S. (1974) Ann. N.Y. Acad. Sci. 242, 255–267
21 Sachs, G., Spenney, J.G. and Lewin, M. (1978) Physiol. Rev. 58, 106–173
22 DePont, J.J.H.H.M. and Bonting, S.L. (1981) in New Comprehensive Biochemistry (Bonting, S.L. and DePont, J.J.H.H.M., eds.), Vol. 2, pp. 209–234, Elsevier, Amsterdam
23 Robert, A., Bayer, R.B. and Nezamis, J.E. (1963) Gastroenterology 45, 740–751
24 Kyte, J. (1971) J. Biol. Chem. 246, 4157–4165
25 Tsai, C.M., Chen, K.-Y. and Canellakis, E.S. (1975) Biochim. Biophys. Acta 401, 196–212
26 Forte, J.G., Poulter, J.L., Dykstra, R., Rivas, J. and Lee, H.C. (1981) Biochim. Biophys. Acta 644, 257–265
27 Fairbanks, G., Steck, T.L. and Wallach, D.F.H. (1971) Biochemistry 10, 2606–2626
28 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 164–275
29 Saccomani, G., Shah, G., Spenney, J.G. and Sachs, G. (1975) J. Biol. Chem. 250, 4802–4809
30 Forte, J.G., Ganser, A.L. and Ray, T.K. (1976) in Gastric Secretion (Kasbekar, D.K., Sachs, G. and Rehm, W., eds.), Vol. 13, pp. 302–330, Marcel Dekker, New York
31 Wassef, M.K., Lin, Y.N. and Horowitz, M.I. (1979) Biochim. Biophys. Acta 528, 318–330
32 Lin, Y.N., Wassef, M.K. and Horowitz, M.I. (1979) Arch. Biochem. Biophys. 193, 213–220
33 Wassef, M.K. and Horowitz, M.I. (1981) Biochim. Biophys. Acta 665, 234–243
34 Levy, E., Goldstein, R., Freier, S. and Shafrir, E. (1981) Biochim. Biophys. Acta, 664, 316–326
35 Swoboda, G., Fritzsche, J. and Hasselbach, W. (1979) Eur. J. Biochem. 95, 77–88
36 Cheah, A.M. (1981) Biochim. Biophys. Acta 648, 113–119
37 Henderson, G.R. and Askari, A. (1977) Arch. Biochem. Biophys. 182, 221–226