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THE EFFECT OF ETHANOL ON PHOSPHOLIPID METABOLISM IN RAT PANCREAS

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Abstract—The phospholipid effect involves agonist-induced breakdown of phosphatidyl inositol (or polyinositides) generating second messengers followed by increased incorporation of ^{32}P during the resynthetic phase of the cycle. Ethanol, an aetiological factor in pancreatitis, has been shown to have various effects on pancreatic secretion. In this study ethanol decreased the incorporation of ^{32}P into phosphatidyl inositol but had no effect on the stimulated breakdown of prelabelled phosphatidyl inositol.

However, in addition to recycling of phosphatidyl inositol stimulation of pancreatic tissue results in increased incorporation of precursors into other phospholipids. Cholecystokinin increased the incorporation of both [U-^{14}C] glucose and ^{32}P into phosphatidyl ethanolamine 3-fold but had no effect on ^{32}P incorporation into phosphatidyl choline. As well as increased incorporation of ^{32}P into phosphatidyl inositol (8-fold) cholecystokinin also increased the incorporation of [U-^{14}C] glucose into phosphatidyl inositol (4–5-fold) implying significant *de novo* synthesis of 1,2 diacyl glycerol in addition to the currently accepted recycling of the 1,2 diacyl glycerol back to phosphatidyl inositol.

Ethanol caused an inhibition of ^{32}P incorporation into total phospholipid of rat pancreas during basal and stimulated conditions. When individual phospholipids were separated ethanol was found to decrease the incorporation of ^{32}P into phosphatidyl choline under basal conditions and into all phospholipids during cholecystokinin stimulation. With [U-^{14}C] glucose as the precursor, ethanol inhibited its incorporation into phosphatidyl choline only. Ethanol did not alter the total ^{32}P radioactivity in the aqueous phase of the pancreatic extract nor the percent incorporated into nucleotides. This excluded decreased uptake of ^{32}P and incorporation into nucleotides as a mechanism for the differential inhibition of ^{32}P versus [U-^{14}C] glucose incorporation into phospholipids other than phosphatidyl choline under stimulated conditions.

That stimulation of the exocrine pancreas by secretagogues leads to changes in pancreatic phospholipid metabolism was first demonstrated some 30 years ago [1]. The earliest observation was an increase in the incorporation of ^{32}P or myo[2-^{3}H] inositol into phosphatidyl inositol (PI) [1–4]. Cleavage of the phosphorylated headgroup of PI by phospholipase C was shown to be the stimulus-responsive step with subsequent recycling of the 1,2 diacyl glycerol [5] and incorporation of ^{32}P and myo[2-^{3}H] inositol. This phospholipid recycling effect was a biochemical curiosity for many years, its physiological significance has only more recently been appreciated. Hydrolysis of the more highly phosphorylated inositide, phosphatidyl inositol diphosphate (PIP_2), by phospholipase C results in inositol triphosphate (IP_3) and 1,2 diacyl glycerol production which act as second messengers, raising the intracellular calcium and activating protein kinase C respectively [6–8]. PI has assumed a secondary role being the precursor for PI kinase to form PIP and PIP_2. Thus the disappearance of PI is thought to be consequent upon phosphorylation rather than hydrolysis. PI has 80% arachidonate at the second carbon position and simulation of pancreatic tissue with CCK or acetyl choline analogues has been shown to release stearic acid and arachidonic acid, the latter being a precursor of the prostaglandins [9]. While IP_3 may initiate pancreatic secretion there is preliminary evidence to suggest that arachidonate may be a factor responsible for sustained secretion [10]. Such breakdown of PI would demand biochemical pathways in addition to 1,2 diacyl glycerol recycling. Hokin in 1953 noted that in addition to ^{32}P, acetyl choline also increased the incorporation of ^{14}C glycerol into PI [11]. This *de novo* synthesis of PI has been further extended by other workers [12, 13]. More recently we have shown that secretagogues increase the incorporation of [U-^{14}C] glucose into a range of phospholipids, the increase into PI being the greatest [14, 15]. This pathway of increased *de novo* synthesis is more compatible with a model involving cleavage of the 1,2 diacyl glycerol releasing arachidonate.

Ethanol, an aetiological factor in pancreatitis, has been shown to alter pancreatic secretion but little attention has been focused on its effects on pancreatic phospholipid metabolism [16–19]. In this paper we compare the effect of cholecystokinin on the *in vitro* incorporation of ^{32}P and [U-^{14}C] glucose into phospholipid of rat pancreas and examine the effect of ethanol on these metabolic pathways.

MATERIALS AND METHODS

Female Sprague–Dawley rats, weighing 200–250 g, maintained on a regular chow diet prior to being fasted for 20 hr, were killed by cervical dislocation and exsanguination. The pancreas was excised, freed of adipose tissue and lobules prepared [20]. Pancreatic lobules were incubated in 5 ml of Krebs–

Table 1. Effect of ethanol on the incorporation of ^{32}P into phospholipids of rat pancreas

	Basal	CCK	CCK ethanol 340mM	N	% Inhibition
LPC/Sph	103 ± 35	261 ± 85	137 ± 74	5	47% $P < 0.05$
PC	3266 ± 541	2923 ± 1002	1296 ± 571	5	56% $P < 0.01$
PE	601 ± 135	1933 ± 835	1430 ± 847	5	26% $P < 0.01$
PS/PI	1502 ± 566	8866 ± 2834	6151 ± 2934	5	31% $P < 0.05$
PI	1158 ± 134	9004 ± 3007	5692 ± 3926	3	37% N.S.
PS	246 ± 112	1115 ± 39	698 ± 362	3	37% N.S.

Results expressed as (dpm/μg P) mean ± SD.

P, paired *t*-test; N, number of animal experiments; LPC, lysophosphatidyl choline; Sph, sphingomyelin; PC, phosphatidyl choline; PE, phosphatidyl ethanolamine; PS, phosphatidyl serine; PI, phosphatidyl inositol.

Ringer–Hepes buffer, pH 7.4, with glucose at 5.6 mM, [U-^{14}C] glucose (5 μCi), ^{32}P phosphate (10 μCi), ethanol and secretagogue added as indicated in 100% O_2 atmosphere at 37°. Unless otherwise indicated incubations were for 2 hr and were terminated by removing tissue from the incubation medium and washing in ice-cold buffer. Lipids were extracted by homogenizing tissue in 20 vol. of chloroform:methanol (2:1), washed once with 0.73% NaCl and three times with pure solvent upper phase [21]. No radioactivity was detectable in the last wash. Aliquots of washed extract were evaporated to dryness under nitrogen, Brays scintillation fluid [22] was added and radioactivity determined in a Packard Tri-Carb Liquid Scintillation Counter. Quench corrections were made using the external standard method [23]. Phospholipids were separated by TLC [22, 24]. Individual phospholipid bands were visualised using iodine, scraped into counting vials and scintillation fluid added. Recoveries were greater than 85%.

Incorporation of isotopes into lipids was linear over a 2 hr period.

Phosphorus determination was by the method of Bartlett [25].

The ^{32}P radioactivity in the aqueous phase of the tissue homogenate was determined by quantitating the radioactivity in an aliquot of the upper phase of the chloroform methanol extract after the salt wash.

Separation of ^{32}P nucleotide from free ^{32}P. Phenyl Boronate Agarose 60 (Amicon Corporation) columns were preequilibrated using 20 mM Hepes buffer, pH 8.8 containing 15 mM magnesium chloride. To characterise the binding properties, ^{32}P and ^{32}P ATP in the above buffer (0.5 ml) were applied to separate columns and washed with further buffer until negligible counts appeared in the eluent. The ^{32}P-labelled nucleotides retained by the column were eluted with water. To separate free ^{32}P from that incorporated into nucleotides a 1 ml aliquot of the aqueous phase of the tissue extract was diluted with 1.5 ml of buffer, mixed and then applied to the column. The column was washed with a further 3 ml of buffer and the total eluent pooled (5.5 ml). The bound radiolabelled nucleotides were eluted with 3 ml of water. Scintillant was added to aliquots of

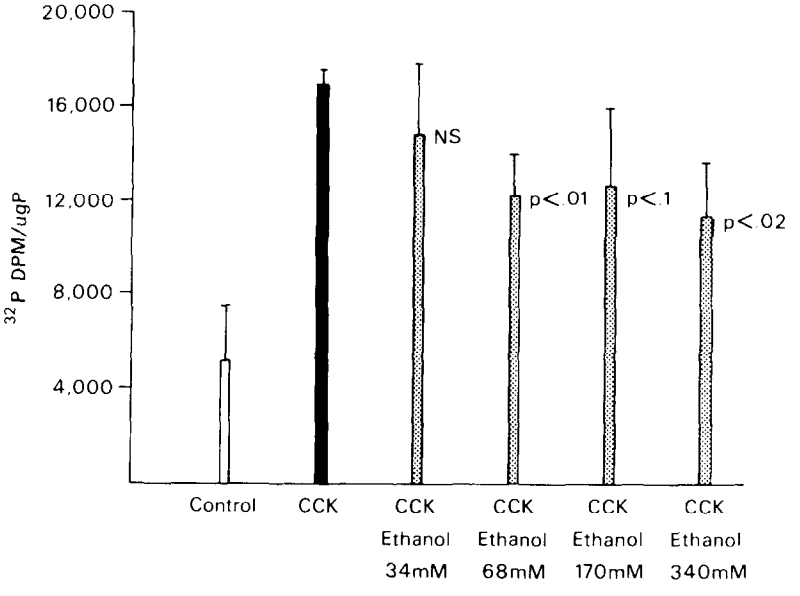

Fig. 1. The effect of increasing concentrations of ethanol on the incorporation of ^{32}P into total lipid of rat pancrease during stimulated conditions—cholecystokinin (CCK) 5 U/ml. The mean ± SD is given for 5 animal experiments. P value—paired *t*-test.

Table 2. The effect of ethanol on the incorporation (dpm/μg P) of [U-^{14}C] glucose into total lipid and phospholipids of rat pancreas *in vitro*

	Basal	CCK	CCK Ethanol 34 mM	CCK Ethanol 68 mM	CCK Ethanol 170 mM	CCK Ethanol 340 mM	N
Total lipid	16,108 ± 5697	20,686* ± 5630	21,182 ± 7408	21,366 ± 6075	21,625 ± 6780	18,709† ± 5328	6
LPC	146 ± 47	171 ± 60	195 ± 92	162 ± 66	150 ± 55	125 ± 47	5
Sph	113 ± 32	135 ± 57	186 ± 100	154 ± 87	149 ± 75	118 ± 56	5
PC	4103 ± 1321	5528 ± 1202	5091 ± 1304	4495 ± 1657	3801‡ ± 1237	3523‡ ± 964	5
PE	707 ± 279	2218 ± 963	2539 ± 1148	2372 ± 1112	2315 ± 848	2462 ± 850	5
PS/PI	661 ± 493	3604 ± 1778	4265 ± 2057	3873 ± 1696	3824 ± 1546	3896 ± 1612	5
PI	585 ± 45	2594 ± 220	—	—	—	2572 ± 187	3
PS	92 ± 31	308 ± 72	—	—	—	248 ± 51	3

CCK (cholecystokinin) 5 U/ml; incubation (2 hr).
Results expressed as dpm/μg P, mean ± SD.
P = paired *t*-test; N = number of animal experiments; LPC = lysophosphatidyl choline; PC = Phosphatidyl choline; PI = Phosphatidyl inositol; Sph = sphingomyelin; PS = phosphatidyl serine; PE = phosphatidyl ethanolamine.
* $P < 0.05$, CCK vs control; † $P < 0.05$, CCK vs CCK ethanol; ‡ $P < 0.02$, CCK vs CCK ethanol.

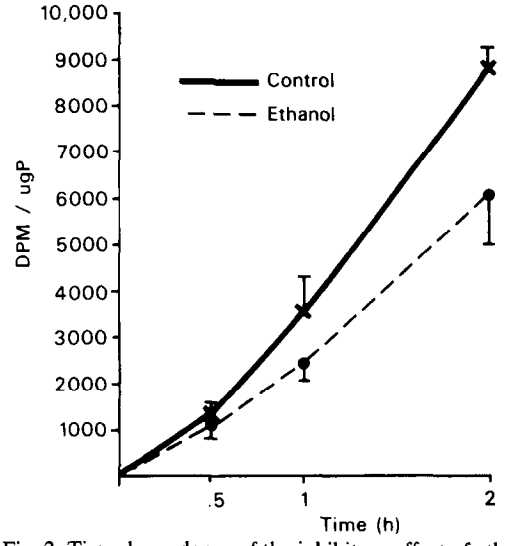

Fig. 2. Time dependence of the inhibitory effect of ethanol (340 mM) on the incorporation of ^{32}P into total lipid in rat pancreatic lobules. The mean ± SD is given for 3 animal experiments.

the eluent from the buffer and water wash and the radioactivity determined in a Parkard Tri-Carb Liquid Scintillation Counter.

In vivo *pulse*–in vitro *chase experiment*. Four fasted rats were injected i.p. with 50 μCi of myo[2-^{3}H] inositol, ^{32}P (100 μCi) and pilocarpine (10 mg/kg) and killed 4 hr later [26]. The pancreas was excised, lobules prepared and duplicate incubations carried out in a medium containing atropine 10^{-5} M, lithium 10 mM and inositol 2 mM with or without cholecystokinin ± ethanol (340 mM) for 30 min. Incubations were terminated, lipids extracted, washed and radioactivity measured as above.

Lithium was included in the incubation medium to inhibit myo-inositol 1-phosphate phosphatase thereby preventing reincorporation of any labelled phosphate molecules that had been cleaved [27].

Materials. [U-^{14}C] glucose (296 μCi/μmol) ^{32}P orthophosphate (Amersham) γ^{32}P (NE Nuclear) myo[2-^{3}H] inositol (specific activity 19.1 Ci/mmole) was obtained from Amersham; cholecystokinin-pancreozymin was obtained from Sigma Chemical Co. (St Louis, MO).

RESULTS

There was a differential effect of cholecystokinin (CCK) on the incorporation of ^{32}P and [U-^{14}C] glucose into the different phospholipids of rat pancreas. The incorporation of ^{32}P and [U-^{14}C] glucose into phosphatidyl inositol were increased 8-fold and 4.5-fold respectively, whereas the incoporation of ^{32}P into phosphatidyl choline was unchanged, and [U-^{14}C] glucose incorporation increased 1.25-fold. For phosphatidyl ethanolamine the incorporation of both isotopes was increased 3-fold (Tables 1 and 2). High concentrations of ethanol decreased the incorporation of [U-^{14}C] glucose into total lipid. Thin layer chromatography showed that this inhibition was specific to the phosphatidyl choline band (Table 2).

Table 3. The effect of ethanol on the incorporation of ^{32}P into phospholipids in rat pancreatic lobules

Ethanol	Nil	34 mM	68 mM	170 mM	340 mM
Total lipid DPM/μg P	7587 ± 1326	6170 ± 883 P < 0.01	5893 ± 1506 P < 0.1	5077 ± 631 P < 0.05	4542 ± 1251 P < 0.01
LPC	211 ± 120	—	—	—	133 ± 41
Sph	167 ± 147	—	—	—	84 ± 55
PC	4886 ± 1323	—	—	—	2043* ± 454
PS/PI	2094 ± 670	—	—	—	2005 ± 425
PE	750 ± 270	—	—	—	781 ± 305

Results expressed as dpm/μg P. The mean ± SD is given for 5 animal experiments.
P, paired *t*-test; LPC, lysophosphatidyl choline; PC, phosphatidyl choline; PI, phosphatidyl inositol; Sph, sphingomyelin; PS, phosphatidyl serine; PE, phosphatidyl ethanolamine.
* $P < 0.01$.

Ethanol caused an inhibition of ^{32}P incorporation into total lipid of rat pancreas during both basal and stimulated states (Fig. 1, Table 3). This inhibition was not apparent until the first hour of incubation (Fig. 2). In the unstimulated state only decreased incorporation of ^{32}P into phosphatidyl choline was observed (Table 3) whereas under stimulated conditions there was decreased incorporation of ^{32}P into all phospholipids (Table 1). Although ^{32}P incorporation into phosphatidyl inositol was reduced by 37% ethanol had no effect on the stimulated loss of PI prelabelled *in vivo* with either ^{32}P of ^{3}H-myo-inositol (Table 4).

Ethanol and cholecystokinin have been shown to decrease ATP levels in pancreatic tissue. Decreased incorporation of ^{32}P into nucleotides was therefore considered as a possible explanation for the inhibitory effect of ethanol on ^{32}P incorporation into phospholipids. However, ethanol did not alter the total radioactivity nor the ratio of free ^{32}P to that incorporated into nucleotides (Table 5), which would argue against decreased ATP as the mechanism for the inhibitory effect of ethanol on ^{32}P incorporation.

DISCUSSION

The existence of phosphatidyl inositol recycling has been known for many years [1–4], but the physiological significance of the effect has only recently been appreciated. Hydrolysis of the more highly phosphorylated inositide (PIP_2) by phospholipase C releases inositol triphosphate (IP_3) and 1,2 diacyl glycerol which act as second messengers to initiate the secretory process [6–8]. Loss of labelled PI is as a consequence of phosphorylation of PI by PI kinase to PIP_2 rather than hydrolysis of PI itself. The second messenger function of PI has been further extended in that stimulated breakdown of inositol phospholipids also results in increased release of arachidonate from the glycerol backbone. Arachidonic acid is a precursor of the prostaglandins which may sustain the secretory response [10]. Such an event requires a stimulus-induced synthetic pathway additional to the currently accepted recycling of the 1,2 diacyl glycerol backbone to PI.

Hokin *et al.* first described increased ^{14}C glycerol incorporation into pancreas with the agonist acetyl choline [11]. This work was extended by Calderon and coworkers who showed that carbamylcholine increased the incorporation of ^{32}P, ^{14}C glycerol and ^{14}C acetate into phosphatidyl inositol while the incorporation of the same precursors into phosphatidyl choline was decreased [13]. More recently we have shown that secretagogues increased the incorporation of [U-^{14}C] glucose into a range of phospholipids the increase being greatest for PI [14, 15]. The [U-^{14}C] glucose label is incorporated into the glycerol backbone (80–85%) but there was also agonist induced increased incorporation of [U-^{14}C] glucose into the glyceride fatty acid [14].

In this study cholecystokinin resulted in an 8-fold increased incorporation of ^{32}P into PI and a 4–5-fold increased incorporation of [U-^{14}C] glucose (Tables 1 and 2) which is in agreement with Calderon *et al.*

Table 4. Percentage disappearance of phosphatidyl inositol prelabelled with ^{32}P and Myo[2-^{3}H] inositol

	Cholecystokinin	
Labelled PI	Control	Ethanol (340 mM)
Myo[2-^{3}H] inositol	27% ± 2.9	28% ± 5.4 N.S.
^{32}P	28% ± 5.1	28% ± 15 N.S.

Three animals were studied.
Rat pancreas was prelabelled *in vivo* for 4 hr (see Methods) – *in vivo in vitro* pulse chase.
Lobules were then incubated for 1 hr *in vitro* in presence of cholecystokinin (5 U/ml).

Table 5. The effect of ethanol (340 mM) on ATP synthesis and breakdown

Radioactivity in the aqueous phase	Control	Ethanol
^{32}P Total dpm/μg P	10,034 ± 2149	9,590 ± 1352
% Nucleotides	15.7 ± 4.0	16.8 ± 4.6

The mean ± SD is given for 5 animal experiments.
The total radioactivity of an aliquot from the chloroform–methanol extract was determined and the Free ^{32}P separated from that incorporated into nucleotides on a baronate column (see Methods).

[13] who found a 7-fold increase in incorporation of ^{32}P and a 4.7-fold increase in incorporation of ^{14}C glycerol. Such findings indicate that *de novo* synthesis of PI is significant and is in keeping with the further cleavage of 1,2 diacyl glycerol.

Ethanol caused a dose-dependent decreased incorporation of ^{32}P into total lipid during basal and stimulated conditions (Table 3, Fig. 1). However, in the basal state this inhibition was specific for phosphatidyl choline. During the stimulated cycle there was decreased incorporation of ^{32}P into all phospholipids including PI (Table 1). Stimulated loss of prelabelled PI may be via direct breakdown of labelled PI or via phosphorylation to PIP_2 prior to hydrolysis [28]. Ethanol did not affect the loss of labelled PI that occurred with stimulation. Thus although ethanol inhibited the resynthesis of PI, the phase involved in stimulus-transduction was not affected. This finding is consistent with our previous observation that ethanol even in high concentrations does not inhibit exocytosis of amylase nor the release of nascent pulse-labelled proteins from rat pancreatic lobules [29]. Orego-Matte *et al.* reported decreased incorporation of ^{32}P into total lipid of rat pancreas *in vivo* after both acute and chronic ethanol administration [16], but because of the *in vivo* nature of the study and the rapid exchange of the phospholipids one cannot be sure that the phospholipids measured were synthesised in the pancreas [17]. This study confirms the observation of Orego Matte *et al.* that ^{32}P incorporation into rat pancreatic total lipid is inhibited by ethanol. However, the *in vitro* conditions of this study preclude their hypothesis that interference with cholinergic transmission is the mechanism for the inhibitory effect of ethanol [16].

Somer *et al.* [17] found that under basal conditions ethanol increased the incorporation of ^{14}C acetate and [U-^{14}C] glucose into triglyceride of rat pancreas and the incorporation of both isotope into phosphatidyl choline was decreased whereas Calderon *et al.* [18] found that ethanol did not affect ^{14}C acetate incorporation into phosphatidyl choline. In this study ethanol also decreased [U-^{14}C] glucose incorporation into phosphatidyl choline under stimulated conditions but only at high concentrations (Table 2). With the knowledge that ethanol and cholecystokinin acutely decrease ATP levels in pancreatic tissue [13, 30], and that there was inhibition of ^{32}P incorporation into all phospholipids versus only decreased incorporation into phosphatidyl choline for [U-^{14}C] glucose, lead us to consider that ethanol might inhibit the incorporation of ^{32}P into nucleotides. The uptake of radioactivity in the aqueous phase was not reduced by ethanol nor was the per cent incorporation into nucleotides vs free altered, which would argue against decreased incorporation of ^{32}P into nucleotides as the mechanism for the inhibitory effect of ethanol. It is possible that during the stimulated cycle newly synthesised nucleotides (ATP) may be diverted for exocytosis rather than synthesis. Alternatively ethanol may relatively increase the incorporation of [U-^{14}C] glucose into the glycerol backbone or fatty acid side chain which would account for the increased $^{14}C/^{32}P$ ratio observed in phospholipids (with exception of phosphatidyl choline) under stimulated conditions (Tables 1 and 2). Calderon *et al.* found that ethanol increased the incorporation of [U-^{14}C] glucose into the glycerol moiety but not the fatty acid of glycerolipids under basal conditions, but the effect of ethanol on the stimulated pancreas was not studied [18]. Clearly, phosphatidyl choline synthesis is the most sensitive to the inhibitory effects of ethanol. This could be explained by inhibition of the synthetic pathway of phosphatidyl choline beyond 1,2 diacyl glycerol which is a common precursor for both phospholipid and triglyceride synthesis.

In summary, we have shown that ethanol decreases the incorporation of ^{32}P into PI and other phospholipids of rat pancreas but that PI breakdown which is involved in initiating the secretory process is unaffected. This general inhibitory effect of ethanol on phospholipid synthesis may in part explain the complex effects of ethanol on membrane function [31].

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REFERENCES

1. M. R. Hokin and L. E. Hokin, *J. biol. Chem.* **203**, 969 (1953).
2. L. E. Hokin and M. R. Hokin, *J. biol. Chem.* **233**, 805 (1958).
3. L. E. Hokin and D. Huebner, *J. Cell Biol.* **33**, 521 (1967).
4. P. De Camilli and J. Meldolesi, *Life Sci.* **15**, 711 (1974).
5. J. F. Dixon and L. E. Hokin, *J. biol. Chem.* **259**, 14418 (1984).
6. H. Streb, R. F. Irvine, M. J. Berridge and I. Schulz, *Nature, Lond.* **306**, 67 (1983).
7. Y. Takai, A. Kishimoto, M. Inoue and Y. Nishizuka, *J. biol. Chem.* **252**, 7603 (1977).
8. M. J. Berridge, *Biochem. J.* **220**, 345 (1984).
9. R. L. Geison, M. W. Banschbach, K. Sadeghian and M. Hokin-Neaverson, *Biochim. biophys. Res. Commun.* **68**, 343 (1976).
10. S. J. Pandol, N. F. Kondratenko and M. S. Schoeffield, *Gastro.* **90**, 1577 (1986).
11. L. E. Hokin and M. R. Hokin, *J. biol. Chem.* **233**, 805 (1958).
12. P. Calderon, J. Furnelle and J. Christophe, *Biochim. biophys. Acta* **574**, 391 (1979).
13. P. Calderon, J. Furnelle and J. Christophe, *Biochim. biophys. Acta* **574**, 404 (1978).
14. H. Patapanian, R. C. Pirola and J. B. Somer, *Biochem. biophys. Res. Commun.* **99**, 319 (1981).
15. B. A. Chapman, J. S. Wilson, P. E. Colley, R. C. Pirola and J. B. Somer, *Biochem. biophys. Res. Commun.* **115**, 771 (1983).
16. H. Orrego-Matte, E. Navio, A. Feres and L. Costamaillere *Gastro.* **56**, 280 (1969).
17. J. B. Somer, G. Thompson and R. C. Pirola, *Alcoholism Clin. exp. Res.* **49**, 341 (1980).
18. P. Calderon-Attas, J. Furnelle and J. Christophe, *Biochim. biophys. Acta* **620**, 387 (1980).
19. M. C. Geokas, *Med. Clin. N. Amer.* **68**, 57 (1984).
20. G. A. Scheele and G. E. Palade, *J. biol. Chem.* **250**, 2660 (1975).
21. J. Folch, M. Lees and G. H. Sloane-Stanley, *J. biol. Chem.* **226**, 497 (1957).
22. F. Snyder, *Analyt. Biochem.* **9**, 183 (1964).
23. Y. Masuzawa, Y. Osawa, K. Inove and S. Nojima, *Biochim. biophys. Acta* **326**, 339 (1973).

24. V. P. Skipski, R. F. Peterson and M. Barclay, *Biochem. J.* **90**, 374 (1964).
25. G. R. Bartlett, *J. biol. Chem.* **234**, 466 (1959).
26. D. Gerber, M. Davies and L. E. Hokin, *J. Cell. Biol.* **56**, 736 (1973).
27. M. J. Berridge, C. P. Downes and M. R. Hanley, *Biochem. J.* **206**, 587 (1982).
28. L. E. Hokin, *Ann. Rev. Biochem.* **54**, 205 (1985).
29. B. A. Chapman and N. R. Pattinson, *Biochem. Pharmac.* **36**, 3353 (1987).
30. N. Solomon, T. E. Solomon, E. D. Jacobsen and L. L. Schanbour, *Dig. Dis.* **19**, 253 (1974).
31. E. Rubin and H. Rottenberg, *Fedn. Proc. Fedn Am. Socs. exp. Biol.* **4**, 2465 (1982).