iodoacetamide, N-ethylmaleimide, N-(4-dimethylamino-3,5-dinitrophenyl)maleimide, 5,5′-dithio-bis(2-nitrobenzoic acid)[14–16] and methyl methanethiosulfonate[17]. The all-of-the-sites reactivity of some of the reagents mentioned (iodoacetic acid, iodoacetamide) remained both for holo- and apo-GAPDH[14–16]. As indicated by our results, 4-bromophenyl isothiocyanate should belong to this group of reagent involving a non-cooperative fashion. The site of action of this group of reagents is localized at the nicotinamide subsite, whereas reagents showing the anticipated half-of-the-sites reactivity (cooperative fashion) towards apo-GAPDH have their site of action localized to the adenine subsite[15].

The above-mentioned properties of ITC are kept also by water-insoluble polyisothiocyanates, this being evaluated not only for immobilization of thiol enzymes[18] but also in covalent chromatography of thiols[19] and thiol enzymes[20].

Inhibitory effect of isothiocyanates and some further thiol reagents on D-glyceraldehyde-3-phosphate dehydrogenase and their reactivity towards glutathione

Number	Compound	I_{50} (μM)	$\log k^*_{SH}$	Type of reaction***
I	C_6H_5NCS	300	2.98	Ad_N
II	BrC_6H_4NCS	180	3.14	Ad_N
III	$C_6H_5CH_2NCS$	150	2.52	Ad_N
IV	$BrC_6H_4CH_2NCS$	20	2.55	Ad_N
V	$(CH_3)_3SiO_2C(CH_2)_3NCS$	90	2.95	Ad_N
VI	$[HOOCC_6H_3(NO_2)S]_2$	10	4.82**	Red-ox
VII	$HOHgC_6H_4COONa$	20	8.60**	S_R
VIII	ICH_2COOH	40	−0.01**	S_N

* Rate constants $k_{SH}(M^{-1}s^{-1})$ of reactions with glutathione; ** As reported in Jocelyn[2] and Friedman[3]; *** Ad_N nucleophilic addition, Red-ox: reduction-oxidation, S_R: radical substitution, S_N: nucleophilic substitution.

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Effect of clofibrate on the phospholipid biosynthesis in rat liver

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Summary. The effect of clofibrate on rat liver phospholipid biosynthesis was studied using ^{32}P as a precursor. Phospholipid classes, levels and specific radioactivity were evaluated. Significant increases in levels of phosphatidylethanolamine and phosphatidylcholine were found and could account for the observed increase in total phospholipids. Specific activity of phosphatidylserine increased and that of phosphatidylethanolamine decreased. This fact suggests that clofibrate seems to alter the systems engaged in the transformation occurring within the different classes of phospholipids but not the de novo biosynthesis.

The biochemical mechanism(s) of ethyl-p-chlorophenoxy-isobutyrate (CPIB) in lipid metabolism is not fully understood, in spite of the numerous investigations on this subject since the drug was applied as a hypolipidemic agent in mammals[2]. Among the several hypotheses put forward to explain the biochemical mechanism underlying the action of the hypolipidemic drugs, alterations in the synthesis of lipoproteins have been postulated[3].

Because phospholipids are important constituents of lipoproteins and the liver is the main site of formation of lipids, an analysis of the effect of CPIB on the synthesis of liver phospholipids seems to be interesting in order to obtain more data on the alteration of liver mobilization caused by this drug.

Materials and methods. Male Wistar rats (weight 150 g) were kept on a standard laboratory diet. Experimental animals received 150 mg/kg of CPIB by a stomach tube once a day for 2 weeks. Controls were treated similarly

but CPIB was not given. Animals were stunned by a blow to the head, exsanguinated and the livers obtained were immediately processed. Livers were homogeneized with a Potter-Elvehjem fitted with a pestle of teflon in 0.25 M sucrose and centrifuged at 480 × g for 20 min.

Incubation was carried out essentially as described[4]. The incubation medium used for the homogenate contained: 100 mM sucrose, 10 mM $MgCl_2$, 10 mM fructose-1,6-P, 1 mM CMP, 5 mM NADH, streptomycin 200 µg/ml, 100 µCi ^{32}P-orthophosphate (carrier-free disodium ^{32}P-phosphate; Radiochemical Centre, Amersham). The pH was 7.4 and the total volume was 2 ml. Tubes were incubated for 60 min at 37 °C. Following incubation, the reaction was stopped by the addition of 2.1 vol. of cold methanol.

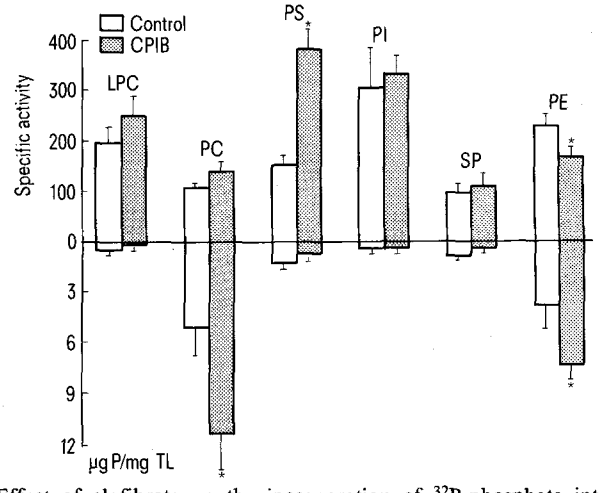
Total lipids were extracted by the procedure of Bligh and Dyer[5]. The combined chloroform extracts were washed with 0.75% NaCl solution and concentrated in vacuo. The lipids were taken up in chloroform-methanol (2:1); 3 mg of the lipid solution were chromatographed on 0.3 mm thin-layer plates of silica gel G. Solvents used were chloroform-methanol-7 M ammonium hydroxyde (90:54:11; by vol.) as the 1st solvent and chloroform-methanol-acetic acid-water (90:40:12:2; by vol.) in the 2nd direction. Lipids were visualized by placing the plates in iodine vapour, and following evaporation of the iodine, the spots were carefully removed and used for P determination and radioactivity estimation. Phosphorous was estimated according to the procedure of Bartlett[6]. For the radioassay of individual phospholipids, the spots from the thin-layer plates were transferred to scintillation vials containing 10 ml of scintillation solution. The scintillation solution was PPO 10.5 g; methyl-POPOP 0.45 g; naphthalene 150 g; dioxane to 1500 ml; and water to 1800 ml. Radioactivity was measured in a Packard 2425 liquid scintillation spectrometer.

Student's t-test[7] was used to evaluate statistical significance. Means and their SE are presented in the figure. Differences were regarded to be significant if p was 0.05 or less.

Results and discussion. Results are given in the figure. Changes in the levels of phospholipids (as µg P/mg total lipids) are indicated in the lower part of the figure and variations in specific activities are indicated in the upper part of the same figure. Estimated total phospholipids were 1.16 mg P/g liver (control group) and 1.26 mg P/g liver (CPIB-treated group); radioactive incorporation of ^{32}P into total liver phospholipids was 53 cpm/g liver for control group and 64 cpm/g liver for CPIB-treated rats. We must point out that data on phospholipid variations produced by CPIB are scarce. The effects of CPIB on experimental nephrotic syndrome have been studied by Galli et al.[8] who showed that CPIB normalized the raised serum phospholipid concentration by greatly reducing the posphatidylcholine levels. The CPIB action on enterohepatic circulation and biliary system resulted in a marked increase in the concentration of phosphatidylcholine in the bile of dogs[9]. More interesting for comparison purposes, are the results obtained by Saito[10] who found a similar increase in liver total phospholipids in rats fed 2 kinds of diets containing CPIB. Our findings are in agreement with these results on total phospholipids, and one can imagine that the increase of phosphatidylethanolamine and phosphatidylcholine could account for the rise in total phospholipids.

As can be seen in the figure, the patterns of levels and activities are very different, a fact that could indicate that CPIB does not affect the de novo biosynthesis but alters the systems engaged in the transformation occurring between the different classes of phospholipids[11]. Such action could explain, for example, the main variations shown in the figure, such as the appearance of phosphatidylserine and phosphatidylcholine through exchange of nitrogenous bases and methylation reactions[12,13].

It has been demonstrated that acidic phospholipids appear to play a critical role in hormonal activation of adenylate cyclase, and particularly phosphatidylserine is effective in restoring glucagon responsiveness to soluble adenylate cyclase[14,15]. Since it has been found that a decrease on both cyclic AMP-dependent protein kinase activity and cyclic AMP levels in rat liver occur after treatment with CPIB[16], the CPIB-mediated increase in the synthesis of phosphatidylserine may be independent of the cyclic AMP system. The possibility that phosphatidylserine plays a fundamental role in the action of CPIB needs further study.



Effect of clofibrate on the incorporation of ^{32}P-phosphate into phospholipids (upper part) and on the levels of phospholipids (lower part) in rat liver. Asterisks indicate statistically significant changes from the control level. LPC = lysophosphatidylcholine, PC = phosphatidylcholine, PS = phosphatidylserine, PI = phosphatidylinositol, SP = sphingomyelin, PE = phosphatidylethanolamine.

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