Activation of Pancreatic Lipase by Pharmaceutical Bile Products

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The relative effectiveness of pharmaceutical bile products to activate pancreatic lipase was compared using an assay technique which attempts to reflect *in vivo* conditions of human intestinal digestion.

POTENTIATION OF pancreatic lipase by bile has been known for seven decades (1). However, the mechanism of action remains obscure (2). Contributing factors to this lack of knowledge have been the unavailability until recently (3) of preparations of high purity pancreatic lipase and the diverse, nonspecific methodology used for lipolysis measurements. The same factors have militated against clear-cut ranking of the relative effectiveness of commercial bile acids and their conjugated salts as activators of pancreatic lipase.

EXPERIMENTAL

Materials.—The lipase preparations and bile derivatives used were commercial products manufactured in our laboratory. Dehydrated, defatted preparations of swine pancreas of equal lipase content were used throughout the study. The bile products tested included desiccated ox and hog biles; ox bile extract N.F., which constitutes the alcohol soluble portion of bile; sodium cholate, dehydrocholate, desoxycholate, and hyodesoxycholate, all of purity approaching 100%; and sodium taurocholate, 70% pure, consisting primarily of taurine conjugates of cholic and, to a small extent, of desoxycholic acids.

Methods.—Two assay methods were utilized. The first was the procedure of Tietz, *et al.* (4), which employs an olive oil substrate pre-emulsified with gum arabic (*Method A*). This technique was used to test the effect of prior substrate emulsification on lipolytic activity. The remainder of the investigation—the comparison of lipolytic activation by pharmaceutical bile products—was carried out using *Method B*. The latter is an adaptation (5) of the procedure of Sammons, *et al.* (6). In the method, attempting to reflect *in vivo* conditions, the common dietary lipid olive oil is the substrate. Emulsification is brought about by the addition of physiological concentrations of bile constituents, intestinal motility is reproduced by agitation, and the reaction is carried out at 37° and pH 7.8. In both *Methods A* and *B*, fatty acids liberated during the digestion period are titrated and the extent of lipolysis expressed as the amount of alkali consumed.

The first series of experiments was designed to show the role of substrate emulsification in activation of lipase by bile using *Method A*. The point was studied further employing *Method B*. Polysorbate emulsifiers[1] were aded to the incubation medium and the effect of further addition of ox bile extract determined. In the second series of experiments, carried out with *Method B*, the relative lipase activating effect of bile derivatives was determined. A weighed amount of lipase was homogenized in a Waring Blendor, model 702A, with a suitable amount of water; aliquots representing 0.5 mg. of enzyme were incubated with the substrate containing the bile preparations dissolved in the 5 ml. of buffer required by the procedure (5). In this manner the total volume of the reaction mixture was kept constant. After a 30-minute digestion period, the reaction was stopped by the addition of 5 drops of concentrated HCl, the liberated fatty acids were extracted into benzene, and titrated with alcoholic NaOH with phenolphthalein as indicator (5).

Results.—Addition of ox bile extract increased the enzymatic release of fatty acids from a pre-emulsified oil substrate (Table I). The same effect was demonstrated when utilizing a nonemulsified olive oil substrate, since, while the addition of polysorbate emulsifiers resulted in an increase in the rate of lipolysis, added ox bile extract again produced a further increase (Table II). The level of polysorbates added corresponded to a very narrow region in which potentiation of lipase activity could be demonstrated, presumably by substrate emulsification. In agreement with Minard (7), larger amounts of added emulsifiers had an inhibitory effect.

Table III summarizes the relative effectiveness of bile derivatives as lipase activators. In the first series two chemically distinct biles, desiccated hog and ox biles, proved to be similarly effective when tested at a concentration of 0.5%; the partially purified ox bile extract was slightly more potent.

TABLE I.—EFFECT OF BILE ON THE LIPOLYSIS OF A STABLE OLIVE OIL EMULSION BY PANCREATIC LIPASE

Lipase (mg./ Digestion Flask)	Ox Bile Extract N.F. (mg./ Digestion Flask)	Lipolytic Activity[a] (ml. 0.05 *N* NaOH/ Digestion Flask)
0.123	None	1.5
0.308	None	3.4
0.616	None	5.4
0.616	25	12.5

[a] *Method A*, employing lipase lot 109302.

TABLE II.—COMPARATIVE EFFECTIVENESS OF BILE AND POLYSORBATE EMULSIFIERS IN THE ACTIVATION OF PANCREATIC LIPASE

% Concn. of Additive in 5 ml. of Buffer Added to Digestion Mixture			Lipolytic Activity[a] (ml. 0.05 *N* NaOH/mg. Enzyme)
Emulsifier		Ox Bile Extract N.F.	
None		None	0.8
None		0.5	2.9
Polysorbate 80	0.01%	None	1.1
Polysorbate 80	0.05%	None	1.5
Polysorbate 80	0.20%	None	0.7
Polysorbate 80	3.20%	None	0.2
Polysorbate 80	6.40%	None	0.1
Polysorbate 80	0.05%	0.5	2.6
Polysorbate 65	0.05%	None	1.3
Polysorbate 65	0.05%	0.5	2.8

[a] *Method B*, employing lipase lot 109104.

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[1] Marketed as Tween 65 and Tween 80 by the Atlas Powder Co., Wilmington, Del.

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TABLE III.—COMPARATIVE ACTIVATION OF PANCREATIC LIPASE BY BILE DERIVATIVES

% Concn. of Bile Derivative in 5 ml. of Buffer Added to Digestion Mixture		Lipolytic Activity[a] (ml. 0.05 N NaOH/mg. Enzyme)
None	...	1.0
Hog bile powder	0.5	2.2
Ox bile powder	0.5	2.5
Ox bile extract N.F.	0.2	2.4
Ox bile extract N.F.	0.5	2.9
Ox bile extract N.F.	1.0	3.3
Ox bile extract N.F.	4.0	4.5
Ox bile extract N.F.	8.0	5.4
Ox bile extract N.F.	16.0	6.0
Sodium cholate	0.1	2.9
Sodium cholate	0.25	3.3
Sodium cholate	0.5	3.7
Sodium cholate	2.0	4.2
Sodium cholate	4.0	3.8
Sodium desoxycholate	0.1	3.5
Sodium desoxycholate	0.25	3.8
Sodium desoxycholate	0.5	5.0
Sodium desoxycholate	1.0	5.5
Sodium desoxycholate	2.0	6.2
Sodium desoxycholate	5.0	5.9
Sodium cholate + Sodium desoxycholate	0.25 + 0.1	3.4
Sodium hyodesoxycholate	0.1	3.5
Sodium taurocholate	0.25	1.7
Sodium taurocholate	0.5	2.9
Sodium dehydrocholate	0.25	0.8

[a] *Method B*, employing lipase lots 109104, 112753, and 115037.

Sodium cholate at a level of 0.25% and sodium desoxycholate at a level of 0.1% showed equivalent effects. Sodium cholate, the sodium salt of cholic acid (3,7,12-trihydroxycholanic acid), represents about 50% of dry ox bile and sodium desoxycholate, the sodium salt of desoxycholic acid (3,12-dihydroxycholanic acid), accounts for 20%. The activating effect of 0.5% ox bile extract, therefore, can be accounted for by either of its constituent bile acids, no additive effects of the latter being apparent. This was confirmed by a separate experiment in which the two pure bile salts were added simultaneously in the same concentrations present in 0.5% ox bile extract. The sodium salt of dehydrocholic acid, a chemically modified bile acid prepared by oxidation of cholic acid and lacking the characteristic hydroxyl groups of native bile acids, was without effect.

On an absolute weight basis, desoxycholate was the most potent activator—as much as a sixfold increase in activity was obtained. Within certain limits, the effect was correlated with added bile acid with a linear logarithmic relationship prevailing over a twentyfold range in concentration (Fig. 1). Similar results were obtained when lipolytic potentiation by ox bile extract, which contains desoxycholate, was tested (Table III).

In a further series of tests, the predominant and characteristic bile acid of hog bile, hyodesoxycholic acid (3,6-dihydroxycholanic acid), was compared to its isomeric counterpart of ox bile, desoxycholic acid. Both acids produced significant enzymatic activation of approximately the same magnitude at equal concentrations in the reaction mixture.

Since bile acids occur only as the glycine or taurine conjugates in native bile (2), the effect of one such compound was tested in a third series. Sodium taurocholate was chosen because this bile salt has become something of a standard for study in this field (2, 8–10). This bile salt did not achieve the degree of activation produced by the unconjugated bile acids in our study. Interpretation of the results is complicated because the material employed, representative of commercially available preparations, was only 70% pure.

DISCUSSION

Sobotka (1) has reviewed the early literature concerning the effect of bile acids in pancreatic lipolysis, including the pioneer work of Japanese investigators which established that different bile acids may differ in their ability to activate lipase. Independently, workers have claimed to achieve as much as a fourteenfold increase in the extent of the reaction by the use of bile, while others have observed no such activation under their test conditions (11). Confusion has probably arisen from the lack of a sufficiently pure pancreatic lipase which leads to investigations with diverse and heterogeneous pancreas preparations and from the complex methodology encompassing titrimetric, manometric, nephelometric, and colorimetric procedures with substrates which have varied from simple aliphatic esters to long-chain mixed triglycerides (12). Thus, some authors have attributed the accelerating effect of bile acids to mere substrate emulsification (13); others held that bile facilitates adsorption of the enzyme onto emulsified fat droplets (reviewed in *Reference 2*); Borgstrom has concluded that the level of taurocholate employed influences the pH optimum of the lipolytic reaction and hence its rate (10). More recently, Desnuelle attributed activation by taurocholate to a conversion of lipolysis by the latter from a non-temperature dependent reaction to a faster temperature dependent one (14).

This last observation was confirmed by the work of De Moerloose (15). This author employed "pancreatic lipase preparations" not further characterized and a titrimetric procedure based on the use of a pre-emulsified olive oil substrate. Using the sodium salts of three bile acids, a descending order of activation was observed for desoxycholic, cholic, and taurocholic acids. The activation was attributed to a facilitation of contact between enzyme and substrate independent of the surface-active or solubilizing properties of the bile salts employed.

Our observations indicate that the physical state of the substrate plays a role in the over-all reaction, shown by the potentiating effect of added polysorbate emulsifiers. However, the increased and

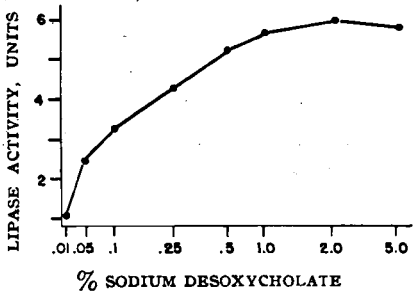

Fig. 1.—Effect of sodium desoxycholate on activation of pancreatic lipase (*Method B*).

additive effect of bile shows that this cannot be the entire explanation. Similar increases in activity produced by added taurocholate were reported by Goldman, *et al.* (8), who employed emulsified fats of extremely small droplet size as substrate—and more recently, by Constantin and co-workers (9), utilizing an emulsified olive oil substrate and a pancreatic lipase claimed to be homogeneous.

In the present study both ox and hog bile proved to be excellent activators of lipolysis, in spite of their distinct chemical make-up. Ox bile consists primarily of cholic and desoxycholic acids, with conjugation divided about equally between glycine and taurine; hog bile contains chiefly hyodesoxycholic and hyocholic acids, with exclusive glycine conjugation (1, 2). The dihydroxy acids, desoxycholic and hyodesoxycholic, had the greatest potentiating activity, with the trihydroxy acid, cholic, somewhat less. Dehydrocholic acid, lacking hydroxyl groups, did not show an effect *in vitro*, although *in vivo* it would be expected to exert a pronounced activating effect mediated by bile acids made available through its potent choleretic action (1). The foregoing structural specificity hints at an actual involvement of bile acids in lipolysis by some yet undescribed mechanism.

SUMMARY

Crude and purified bile derivatives showed activating properties on the course of pancreatic lipolysis *in vitro* when employing assay methods utilizing both emulsified and nonemulsified olive oil substrates. Of the salts of purified bile acids tested, desoxycholate was the most potent—a sixfold increase in lipolysis was achieved. Considerable activation was also obtained with hyodesoxycholate, cholate, and taurocholate and with ox and hog biles. In contrast, dehydrocholate did not have *in vitro* effect.

ADDENDUM

Recently an article appeared [Fritz, P. J., and Melius, P., *Can. J. Biochem. Physiol.*, **41**, 719(1963)], which advanced the theory that bile salts activate lipolysis by splitting the enzyme-diglyceride complex, thus increasing the action of lipase on the triglyceride ester. The activating effect was thought *not* to be due to simple emulsification. Glyco and taurocholate were superior to desoxycholate in contrast to the present findings and those of others (1, 15).

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Ceric Sulfate as Permanganate Replacement in Pharmacopeial Assays

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Standard 0.1 *N* potassium permanganate was replaced by standard 0.1 *N* ceric sulfate and orthophenanthrolene T. S. indicator in the official assay of ferrous sulfate U.S.P., hydrogen peroxide U.S.P., and sodium perborate N.F. Statistical analysis of the results using the *t* test shows no sufficient reason to doubt that the two methods yield the same mean value at the 10 per cent significance level.

ONE OF THE tests of a rational philosophy is that it contain no unnecessary axioms. One of the tests of a rational system of quantitative analysis might well be that it contain no unnecessary reagents. The "United States Pharmacopeia" and "National Formulary" do not meet this criterion.

This paper focuses attention on the use of strong oxidizing agents as standard solutions in titrimetry. Experience has shown the utility of three agents: ceric ion, dichromate, and permanganate. Of these, dichromate has slowly lost its status, being used now only as a precipitating agent in the determination of quinacrine. With the change in assay of most of the calcium preparations from oxidation of the precipitated oxalate with standard permanganate to a complexometric titration, the need for a standard permanganate solution has decreased. Materials requiring standard permanganate solution may be divided into five classes: (*a*) ferrous sulfate U.S.P., exsiccated ferrous sulfate U.S.P., hydrogen peroxide solution U.S.P., and sodium perborate N.F.; these are titrated directly. (*b*) Dibasic calcium phosphate U.S.P. and cherry juice U.S.P.; these require titration of precipitated calcium oxalate. (*c*) Lead monoxide N.F., lead subacetate solution N.F., and diluted lead subacetate solution N.F.; these require titration of oxalic acid left in the filtrate after precipitation of lead oxalate. (*d*) Sodium nitrite U.S.P. which requires a residual titration involving both standard permanganate and standard oxalic acid.

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