Effects of dietary fibers and cholestyramine on the activity of pancreatic lipase in vitro\textsuperscript{1-3}

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ABSTRACT Most experiments were conducted in the presence of human gallbladder bile; colipase and pancreatic lipase were purified using porcine pancreas. The adsorption of bile salts, phospholipids and cholesterol from the bile, together with that of pancreatic lipase was measured on wheat bran, cellulose, hemicellulose (xylan), slightly methylated pectin (42%) and cholestyramine. In contrast to cholestyramine which intensively binds biliary lipids (61.7-81.7%) and pancreatic lipase (47.5%), the fibers studied only had a low adsorbent power. The direct influence of these fibers and of cholestyramine at concentrations ranging from 0-5% on lipase activity was measured at constant pH, using two conventional assay systems, long chain triglycerides and tributyrin. In the presence of human bile and colipase, a drastic reduction in triglyceride hydrolysis by lipase was observed with cholestyramine (loss of 66-82%) and wheat bran (loss of 77-94%) at 1% concentration. The other fibers did not have any marked effects on enzyme activity. The use of a radio labeled lipase made it possible to demonstrate that the inhibitory effect of bran on enzyme activity was independent of adsorption phenomena on bran. The fraction of bran that can be solubilized in the aqueous phase, in fact, induced this reduction in activity. The presence of protein inhibitor in bran may be responsible for the reduction in pancreatic lipase activity. \textit{Am J Clin Nutr} 1985;42:629-638.

KEY WORDS Dietary fiber, bran, pectin, cholestyramine, pancreatic lipase, biliary lipids, in vitro

Introduction

A considerable amount of work has been devoted to the study of the effects of the various dietary fibers on lipid metabolism. The hypocholesterolemic effects of pectin (1, 2) and guar gum (3) have been firmly established, but the possible changes in the metabolism and transport of triglycerides by these fibers or cereal brans remain controversial (4, 5). The lack of knowledge about the effects of fibers on the mechanisms directly implicated in digestion and intestinal absorption of lipids, makes it particularly difficult to give a global analysis of work that has already been done.

Studies relative to the influence of dietary fibers on physiochemical and enzyme processes in fat assimilation, have only been underway for a very short period of time. It is known that various dietary fibers present in food can induce the following: an adaptation in pancreatic lipase secretion (6, 7, 8, 9); a smaller or greater degree of sequestration of bile salts (10, 11, 12) and products resulting from triglyceride lipolysis (13); morphological and ultrastructural changes in intestinal mucosa (14); and modification of fat absorption (15, 16) by the small intestine. Several authors have reported an increase in fecal excretion of fats both in animals (17) and in man (18, 19, 20) resulting from fiber-rich diets.

Hydrolysis of dietary fats by pancreatic lipase in the small intestine is one of the critical phases in their assimilation. Some initial studies dealing with the effects of different dietary fibers on the enzyme activity of pancreatic li-

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Bile, pancreatic juice, and dietary fat and cholesterol are all important components in the digestive process. However, the role of bran in this process is less understood. A study by Schneeman et al. (21) showed that bran does not lower the proportion of lipase active in the soluble phase, while Isaksson et al. (22, 23) reported that the presence of pectin or wheat bran may cause the enzyme to become partly inactive in the duodenal juice. If changes in the pH or the viscosity of the medium can account for the inactivating effects of pectins (23), the mechanisms by which bran can lessen pancreatic lipase activity still remain unknown.

An in vitro study was therefore undertaken of the direct effects of wheat bran, cellulose, hemicellulose, slightly methylated pectin and cholesteramine as reference, on pancreatic lipase activity via a vis short- and long-chain emulsified triglycerides. This study was conducted in media containing human gallbladder bile, since it has been revealed that biliary lipids play an important role in the action mechanism of pancreatic lipase (24, 25).

Materials and methods

Materials

The wheat bran came from ARIA Laboratories (Paris, France); two variants differentiated by particle size were used—coarse bran with a mean particle size of 540 μ, and finely ground bran, with a mean particle size of 85 μ. Total dietary fiber content of the bran was 42.5%, of which cellulose represented 10.8%, hemicellulose 28.4%, and lignin 3.3%. For some experiments coarse bran was hydrated to saturation point using distilled water, then baked in a 150°C oven for an hour and a half, and finally totally dehydrated before being used. Pectin (type LM 27 NAND) was obtained from UNIPECTINE SA (Paris, France). It showed a degree of methyl esterification of 42%. Xylan (hemicellulose prepared from Larchwood) was procured from Sigma (St Louis, MO). The cellulose and cholesteramine used for the purposes of this study came from Serva (Heidelberg, Germany). The last three constituents were dried before use.

Human bile was obtained from gallbladders removed during surgical operations. Once samples had been taken with a sterilized syringe, 0.2% sodium azide was added, and they were then stored at 4°C. Several samples of bile obtained in this manner were pooled and centrifuged at 15,000 × g, 4°C for 15 min to eliminate sediments. Bile salt concentrations, phospholipid, and cholesterol concentrations were measured. So as to ensure that experimental conditions could be reproduced, bile solutions with bile salts/phospholipids molar ratios equal to 5.7 were used; this was obtained by appropriately diluting the bile pools with a Tris-HCl 10 mM, NaCl 150 mM, pH 7.5 buffer solution, or with this buffer solution containing conjugated purified bile salts close in composition to human bile (glycocholate 29%, glycochenocholate 40%, taurocholate 8%, taurodesoxycholate 23%) (26). Depending on the origin of the gallbladder bile used, the phospholipid/cholesterol molar ratios ranged between 2.3 and 2.7.

Pancreatic lipase was purified (27) using delipidated porcine pancreas powder ORGANOY (St. Denis, France). Traces of residual cofactor were removed as previously described (28). The specific activity of the lipase Ia and LB mixture was 3170 units/mg protein (29). 5-Thio-2-nitro[14C]benzoyl lipase was prepared as previously described (30). Specific radioactivity of the labeled enzyme was 2.38 × 10^7 dpm/mg of enzyme, and its specific activity measured with tributyrin was 5650 units/mg. The pancreatic colipase used was a mixture of porcine colipases I and II, prepared as described elsewhere (31). Specific activity was 5480 units/mg of protein.

Binding measurements

The following procedure was used to determine the binding of biliary lipids and pancreatic lipase on fibers: 50 mg (1%), 100 mg (2%), or 250 mg (5%) of each type of fiber were added to centrifuge tubes containing either 5 ml of diluted bile solution, or 5 ml of purified bile salt solution, or 5 ml of Tris-HCl 10 mM, 150 mM NaCl, pH 7.5 buffer solution. For all experiments conducted using diluted bile or purified bile salts, bile salt concentrations were always 8 mM. When deemed necessary, 15 μl of the [14C]lipase solution (15 μg), with or without colipase, were first added to 5 ml of the various solutions and incubated for 10 min at 25°C. These mixtures were then added onto the fibers. The final pancreatic lipase concentration was 0.6 × 10^{-7} M and that of colipase when present, was 1.2 × 10^{-7} M. This cofactor/enzyme molar ratio was very close to that measured in human duodenal contents (32).

The incubation of the mixtures with the fibers was carried out by gentle shaking in a 37°C water bath for 15 min. It was verified that this length of time allowed for maximum adsorption under the conditions used. The tubes were then centrifuged at 17,000 × g for a period of 15 min at 4°C; pectin required 100 μl of a 40% CaCl₂ solution to be added to each tube before it was centrifuged, for sedimentation of a thick gel to occur.

In all cases, the supernatant was collected and homogenized; 100 μl were sampled for determination of total biliary acids which was done by enzyme procedure (33). Two hundred microliters of supernatant were extracted per 2 ml of chloroform/methanol (2:1, v/v); the phosphorus in the phospholipids was assayed from the inferior phase, once mineralization had taken place, using automatic colorimetric procedure (34). Five hundred microliters of supernatant were extracted per nine vol of isopropl alcohol, and the cholesterol measured by colorimetric procedure (Boehringer, Mannheim, Germany). An aliquot of 500 μl of supernatant (in one experiment 100 mg of pellet) was added to 10 ml of Unisolve (Kock Light, Cilnbrook Bucks, England) to evaluate radioactivity by means of liquid scintillation. All experiments were carried out three times and assays twice. A control condition was set up without added binding substances according to the same protocol. Binding was determined as the difference between the quantity of each lipid or lipase added and that found in the supernatant after incubation. The vol of solution that was required to hydrate each type of fiber was determined after being centrifuged under our experimental conditions. This was taken into account in the calculations.
so as to determine real absorption of each constituent independently of the water-holding capacity of each studied fiber.

**Lipase activity measurements**

Pancreatic lipase activity was determined in the presence or absence of fibers by means of two conventional procedures. The first used olive oil long-chain triglycerides emulsified by gum arabic (29) as the substrate. Assay conditions were as follows: 10 ml of emulsion containing 800 mg of triglycerides were topped up to make a final vol of 30 ml with 10 mM Tris–HCl pH 7.5 buffer. Where necessary, 2 ml of the four purified conjugated 120 mM bile salt solutions (see above) or gallbladder bile were added. In both cases, the final bile salt concentration was 8 mM. Varying quantities of the studied fibers and cholestyramine (0–1500 mg) were added to the medium and incubated for 15 min by gentle magnetic shaking at a temperature of 25°C. Fixed quantities of lipase (4.5 μg) and colipase (1.8 μg) were added to the medium, giving concentrations of 0.4 × 10⁻⁶ M and 0.8 × 10⁻⁶ M respectively. Enzyme activity was measured for 15 min at pH 9.0 and a temperature of 25°C, using a TTI pH Stat titrator (Radiometer, Copenhagen, Denmark).

The second assay procedure used a short-chain triglyceride as the substrate—here tributyrin—emulsified by shaking (35). 500 μl of tributyrin (Fluka, Buchs, Switzerland) were topped up to make a final vol of 15 ml with 150 mM NaCl adjusted to pH 7.5. When necessary, purified bile salt solutions or gallbladder bile solutions were added, giving a final bile salt concentration of 8 mM. Varying quantities of fibers (0–750 mg) were added to the medium and incubated as previously described. Fixed quantities of lipase (4.5 μg) and colipase (1.8 μg) were added, giving concentrations of 0.6 × 10⁻⁶ M and 1.2 × 10⁻⁶ M respectively. Activity was measured for 15 min at pH 7.5 and a temperature of 25°C using a pH Stat titrator. In both assay procedures, one lipase unit was defined as 1 μ equivalent fatty acid titrated per minute at 25°C.

**Results**

**Binding of pancreatic lipase and biliary lipids**

As pancreatic lipase is an enzyme which acts at the lipid/water interface, all factors that may modify its interaction with the interface are capable of influencing its enzyme activity. Consequently, it was of the utmost importance to begin this investigation by measuring the amount of pancreatic lipase adsorption on various dietary fibers as is shown in Table 1. Under physiological conditions, i.e., in the presence of bile and colipase, pancreatic lipase was only moderately sequestered by wheat bran. Maximum binding (34.5%) was measured with finely ground bran, while baking the bran tended to reduce binding with coarse bran from 22.5% to 15.2%. Cellulose and hemicellulose (here in the form of xylan), the two major constituents of wheat bran, had very negligible binding power. Binding with pectin was not as strong as that observed with bran; only 5.8% of the enzyme was trapped. On the other hand, cholestyramine revealed a high binding power since approximately half (47.5%) of the lipase present in the aqueous phase was sequestered by this agent. In the presence of a 8 mM purified bile salt solution, binding of pancreatic lipase with coarse wheat bran was lower (9.7%) than that measured in the presence of bile, whereas the contrary was observed with cholestyramine (68%). When the medium was solely made up of 10 mM Tris HCl buffer containing 150 mM of NaCl, binding rates comparable to those obtained in the presence of 8 mM of bile salts were obtained, i.e., 11.7% with coarse wheat bran and 65% with cholestyramine respectively.

As biliary lipids play an important role in the action mechanism of pancreatic lipase (25, 36), it was equally important to measure their interaction with dietary fibers. Table 2 shows the binding percentage of bile salts, phospholipids and cholesterol in human bile on the various fibers studied. Under physiological concentration conditions, it could be observed that the binding of the three types of biliary lipids on wheat bran was low, irrespective of particle size; however, the bile salts tended to become slightly more adsorbed (maximum 14%) than the phospholipids (maximum 9.3%). Cellulose and xylan had only a marginal adsorbent power and binding with pectin remained very low. Cholestyramine, on the
Table 2: Binding of human bile lipids on various kinds of fiber

<table>
<thead>
<tr>
<th>Binding substance</th>
<th>Bile salts</th>
<th>Phospholipids</th>
<th>Cholesterol % bound</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coarse wheat bran</td>
<td>12.7 ± 1.4*</td>
<td>7.0 ± 0.6</td>
<td>9.1 ± 0.8</td>
</tr>
<tr>
<td>Fine wheat bran</td>
<td>14.0 ± 1.3</td>
<td>9.3 ± 0.6</td>
<td>9.2 ± 0.7</td>
</tr>
<tr>
<td>Baked coarse wheat bran</td>
<td>8.5 ± 0.7</td>
<td>4.2 ± 0.3</td>
<td>8.6 ± 0.8</td>
</tr>
<tr>
<td>Cellulose</td>
<td>1.2 ± 0.5</td>
<td>4.3 ± 0.2</td>
<td>0.9 ± 0.6</td>
</tr>
<tr>
<td>Xylan</td>
<td>1.8 ± 0.6</td>
<td>2.1 ± 0.3</td>
<td>1.0 ± 0.5</td>
</tr>
<tr>
<td>Pectin</td>
<td>5.7 ± 0.4</td>
<td>4.1 ± 0.4</td>
<td>2.3 ± 0.6</td>
</tr>
<tr>
<td>Cholestyramine</td>
<td>81.7 ± 3.4</td>
<td>61.7 ± 2.7</td>
<td>73.1 ± 3.2</td>
</tr>
</tbody>
</table>

Diluted human bile containing 8 mM bile salts, 1.4 mM phospholipids and 0.6 mM cholesterol was incubated in the presence of 2% binding substances. Binding was measured after centrifugation and controls were done without added fiber.

* Each value is the mean ± SEM for 3 replicates.

Other hand, sequestered the three types of biliary lipids to a very large degree in an overall comparable manner, by retaining about three-quarters of the lipids originally present in solution.

Pancreatic colipase is a protein cofactor that is essential for the interaction of pancreatic lipase with lipids in the presence of detergents and therefore for the expression of enzyme activity (37, 38). Research was undertaken to examine whether colipase could intervene in fiber interaction with lipase. As is shown in Table 3, the rate of adsorption of pancreatic lipase on wheat bran was highly affected by colipase, since with the cofactor absent, the binding rate was approximately 6 to 8 times lower than if it was present, in a molar ratio of 2:1 in relation to the enzyme. This effect was observed whether bile salts (8 mM) were present or not, as well as in the presence of diluted bile. This colipase effect was much less pronounced when pancreatic lipase was bound with cholestyramine: a reduction of only half the amount of bound lipase was measured when the cofactor was absent and diluted bile present, and this effect disappeared when the medium contained bile salts solution or buffer solution.

Dietary fiber and lipase activity on long-chain triglycerides

The direct effects of dietary fibers on the enzyme activity of pancreatic lipase were then evaluated according to two assay procedures, one with long-chain triglycerides (olive oil) as the substrate, the other with a short-chain triglyceride (Tributyrin). Figure 1 shows the effects of the fibers at concentrations varying from 0–5% on the enzyme velocity of pancreatic lipase in the presence of bile on the long chain triglycerides. While cellulose and hemicellulose (xylan) remained without effect, wheat bran exerted a very strong inhibitory effect on enzyme activity, an effect which increased as the size of the particles decreased. As indicated in Figure 2C, finely ground bran concentrations of 0.4% and 1% led to activities representing only 64% and 23%, respectively, of those activities measured in the absence of fibers. A comparable inhibitory effect was measured in the presence of bile salts in a micellar solution (Fig 2B), whereas in the presence of a buffer solution much higher bran concentrations (2–5%) were necessary to reach

Table 3: Effect of colipase on the adsorption of pancreatic lipase on wheat bran and cholestyramine under various conditions

<table>
<thead>
<tr>
<th>Binding substance</th>
<th>Colipase</th>
<th>Human bile</th>
<th>Bile salts solution</th>
<th>Buffer solution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% bound</td>
<td>3.7 ± 0.3*</td>
<td>3.2 ± 0.4</td>
<td>2.8 ± 0.5</td>
</tr>
<tr>
<td>Fine wheat bran</td>
<td>none</td>
<td>32.5 ± 1.7</td>
<td>19.7 ± 1.1</td>
<td>21.7 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>added</td>
<td>23.6 ± 1.8</td>
<td>63.6 ± 3.2</td>
<td>61.5 ± 2.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>47.1 ± 2.2</td>
<td>63.7 ± 4.1</td>
<td>63.5 ± 3.8</td>
</tr>
</tbody>
</table>

* Each value is the mean ± SEM for 3 replicates.
FIG 1. Activity of pancreatic lipase on olive oil long-chain triglycerides at pH 9.0 and 25°C, in the presence of increasing amounts of dietary fibers and cholestyramine. The assay medium contained diluted bile and colipase. Each point represents the mean of 3 replicates.

FIG 2. Activity of pancreatic lipase on olive oil long-chain triglycerides in the presence of colipase and increasing amounts of fine wheat bran and cholestyramine, expressed as a percentage of the activity measured in the absence of binding substances. Assay medium: A—10 mM Tris HCl, 150 mM NaCl; B—8 mM purified bile salts mixture; C—diluted human bile. Each point represents the mean of 3 replicates.
a comparable inhibitory level (Fig 2A). By comparison, cholestyramine (Figs 1 and 2) showed a strong inhibitory effect on enzyme activity, equivalent to that of wheat bran in the presence of bile, whereas there was no inhibitory effect in the presence of bile salts, and only a moderate one (35%) in buffer solution.

Dietary fiber and lipase activity on short-chain triglycerides

When lipase activity was measured in the presence of human bile in the second assay system with Tributyrin acting as the substrate, higher levels of enzyme inhibition could be measured. Cellulose had no effect on enzyme activity up to 5% concentration and xylan exerted only a moderate inhibitory effect on concentrations higher than 2%. Wheat bran demonstrated a very strong inhibitory effect on pancreatic lipase, that was all the stronger as particle size decreased. Fine bran and coarse bran induced residual lipase activities of 34% and 73%, respectively, when bran concentration was 0.12%; bran concentration of 0.33% revealed activities of 14% and 50% respectively. The inhibition level of enzyme activity was greater than 90% in concentrations of 2% and above for both types of bran. An inhibitory effect comparable to bran was obtained with cholestyramine, which inhibited 92% of lipase activity at 1% concentration. Under these assay conditions, pectin showed more original effects: when present in concentrations lower than 1%, it led to a maximum increase in enzyme activity of 45% for a concentration of 0.33%, whereas for concentrations higher than 2% it caused a moderate inhibition of enzyme activity (reduction of 31% for a 5% pectin concentration).

In the presence of a micellar solution of purified bile salts or a buffer solution, wheat bran exerted a powerful inhibitory effect on pancreatic lipase activity, as in the presence of bile. Contrary to its effect observed in the presence of bile, 1% pectin strongly inhibited the activity of pancreatic lipase in the presence of purified bile salts (93% reduction) or buffer solution (87% reduction). Under the latter two conditions, 1% cholestyramine had no more than a moderate inhibitory effect on enzyme activity (50% and 30% respectively).

Inhibition mechanism of pancreatic lipase by wheat bran

To begin with, the comparison of the data resulting from the measurements of lipase adsorption on the tested fibers and that concerning enzyme activities measured using two types of substrate, did not suggest that the adsorption of pancreatic lipase on dietary fibers was the major phenomenon responsible for certain drastic reductions in its activity. This point was studied more precisely with regard to wheat bran. [14C]Lipase was incubated with 1% coarse wheat bran in the presence of human bile; the pellet and the supernatant were separated by centrifugation and the quantities of enzymes present in the two phases along with the corresponding enzyme activities were measured simultaneously on tributyrin. The smaller fraction (27.6%) of the enzyme adsorbed on wheat bran only showed a residual 3.8% activity which could be explained by the incapacity of the lipase thus adsorbed to hydrolyze an emulsified substrate. However, the greater part (72.4%) of the enzyme, present in the supernatant from incubation with bran, likewise only exhibited a 7.7% residual activity; total residual enzyme activity (supernatant plus pellet) was therefore 11.5%, as has already been observed during preceding experiments of the overall measurement of lipase activity in the presence of wheat bran. The results indicate that there was no relationship between the amount of lipase adsorbed or present in the supernatant and the corresponding enzyme activities and would therefore strongly suggest the inhibitory role played by the soluble compounds of bran extracted in the supernatant. This was confirmed further in an additional experiment. The effect of bran incubated in buffer solution was measured by adding to the lipase assay medium in presence of human bile, either all the incubation (pellet plus supernatant), or separately adding the supernatant of the bran incubation corresponding to 1% concentration in the medium. It was thus observed that the supernatant from the incubation of raw wheat bran was alone responsible for a very powerful inhibitory effect on lipase which only retained 6% of its activity. The use of prebaked bran (150°C, 1.5 h) under identical experimental conditions showed that
the supernatant from incubation lost some of its inhibitory power after heat treatment; lipase residual activity was 38% in that case. Moreover, previously treating the supernatants of 1% wheat bran incubations with pronase (30 min at 25°C; 4 mg/ml), would lead to a total loss of the lipase inhibitory effect of wheat bran.

Discussion

Mechanisms of the inhibition of pancreatic lipase activity by dietary fibers

For the purposes of this in vitro study, the quantities of dietary fibers usually consumed by humans were used as reference to determine experimental conditions, and give physiological significance to the results. The recently analyzed data on the total amounts of dietary fibers consumed in 38 countries (39) revealed that approximately 22–40 g/day are consumed in Western Europe, North America, and Japan; 51–71 g/day in Eastern Europe; and 42–93 g/day in South America. Between one-third and two-thirds of intake comes from cereals, whereas twice as many dietary fibers are ingested by vegetarians in industrialized countries than by nonvegetarians (40). Given the amounts of fibers and lipids ingested and with a known average quantity of intestinal lipid concentrations (41) or various digestive secretions, the dietary fiber concentrations in the duodenum can be evaluated as varying between 0.10% and 2.10%, depending on diet. In this study work evolved around a 0–5% fiber concentration zone. The biliary lipid and triglyceride concentration zones used also corresponded to those found in the small human intestine (41), as did the molar ratio of colipase and pancreatic lipase (32).

Given the important role played by the various biliary lipids in the action mechanism of pancreatic lipase (24, 25, 36), it was necessary to initially measure the degree of binding of these lipids on dietary fibers. Although such measurements have been carried out extensively for purified bile salts (10, 11, 12) and for phospholipids and cholesterol using synthetic mixtures (42), no adsorption rate values for these lipids on dietary fibers using human bile were as yet available. With the exception of cholestyramine which intensively bound bile salts, phospholipids and cholesterol from bile (Table 2) as has already been shown with synthetic mixtures (42), the other studied dietary fibers had only a very low adsorbent power. Pectin, cellulose, and hemicellulose only bound a few percent of the biliary lipids present, while wheat bran had the capacity to bind approximately 14% of bile salts and 10% of phospholipids and bile cholesterol. These values obtained on human bile were close to those already obtained with purified lipid synthetic solutions (42).

This study differed from previous ones (21–23, 43) in that the influence of various dietary fibers on pancreatic lipase activity was measured at the very level of enzyme reaction. The effect of slightly methylated pectin was only measured during assays where tributyrin was the substrate, since in the other system the presence of gum arabic in the olive oil emulsion might interfere with the effect of pectin.

In the presence of bile, pectin inhibited only moderately the lipase activity. In previous studies (21, 43) a 2.5% or 5% pectin concentration did not show any activating or inhibiting effect in relation to lipase activity with substrate absent, but in the presence of human duodenal content, Isaksson et al (22) reported an inactivation of pancreatic lipase of approximately 95% with slightly methylated pectin, and 80% with highly methylated pectin at 1% concentration. These authors postulated that the inactivating effect of slightly methylated pectin was due to a decrease in the pH of the medium, while a strong increase in the viscosity of the duodenal juice was responsible for the effect of highly methylated pectin (23). The effect shown by slightly methylated pectin may not have been visible as the measurements of activities were taken with a pH Stat at constant pH (pH 7.5) in this study, which might probably explain this difference in the results obtained. The possible inhibition of lipase activity by slightly methylated pectin cannot however be explained by pectin sequestering lipase, as results (Table 1) reveal that this binding was only marginal (5.8%). This was equally true for biliary lipids.

When the substrate consisted of emulsified olive oil long-chain triglycerides, wheat bran had a strong inhibitory effect on pancreatic lipase activity in the presence of bile. This
drastic reduction in activity, even when bran concentrations were less than 1%, increased even more as particle size decreased. A comparable, though even more obvious effect, was obtained when another conventional assay system was used with tributyrin as substrate. Both systems showed that the inhibitory effect of wheat bran was comparable to cholestyramine within the concentration range studied (0–5%). Cellulose and hemicellulose (here in the form of purified commercial preparations), the two principle constituents of wheat bran, did not, on the other hand, significantly change enzyme activity any more than they bound the enzyme (Table 1). In the absence of substrate, these inactivations of pancreatic lipase remained unobserved by Dunaf and Schnee man (43) whereas Isaksson et al (22, 23) reported 40–50% losses in lipase activity with 1.5% wheat bran in human duodenal juice. Given that the two assay systems used in our study were very different, the major role of direct interaction between bran and the substrate/water interface can be dismissed in the inhibition observed. The adsorption measurements of lipase on wheat bran (Table 1) suggest that binding with fibers may occur, and the greater the surface area of the bran, correlated to the fineness of the particles, the greater the phenomenon. Although the adsorption rate of lipase on wheat bran was not negligible (22.5–34.5% for a 2% bran concentration), it cannot account for the very drastic inhibitions of activity measured, ie, 80% and 90% in one or other of the assay systems for a 2% fine bran concentration.

As already indicated, biliary lipids only adsorbed very moderately on wheat bran, and it therefore does not seem likely that their interactions with lipase and pancreatic colipase (44) or with triglycerides (24, 25) can be strongly modified by the presence of bran and implicated in the results obtained. In fact, the very great inhibitory effect on enzyme activity observed with bran in the presence of bile was equally measured in the absence of bile, either in the presence of a buffer solution, or a purified bile salt solution. However, as cholestyramine bound approximately three-quarters of the biliary lipids present (Table 2), and half the pancreatic lipase (Table 1), the combined effect of high enzyme sequestration and the majority of biliary lipids being rendered inaccessible to the substrate, may account for the drastic reduction of enzyme activity induced by this resin. The inhibition of lipase activity by cholestyramine was indeed much more moderate when the medium was other than bile.

A complementary experiment using a radiolabeled enzyme, moreover, made it possible to directly confirm that the greater fraction of the enzyme not adsorbed by bran loses the greatest part of its activity after incubation with bran, in agreement with previous lipase inactivation measurements (22, 23). The confirmation of this independence of the inactivation and adsorption on bran for the most part was able to be shown definitively, because the addition of the supernatant obtained after incubation of bran in the aqueous phase to the medium, directly and drastically inhibited the activity of pancreatic lipase. According to findings of Isaksson et al (23), 1.5% wheat bran concentrations do not cause any significant changes in the viscosity or the pH of human duodenal content.

The results obtained with wheat bran that had undergone high-heat treatment (150°C for 1½ h) equally showed that this treatment led to a moderate decrease in the adsorption of lipase on bran (Table 1) and to a heavy decrease in the loss of the enzyme activity, both vis a vis the insoluble fraction and the soluble one obtained after preliminary incubation of bran in the aqueous phase. All the results therefore led to suggest that a wheat bran constituent, highly soluble in an aqueous medium and partially inhibited by heat treatment, was responsible for the essential part of the inactivating effect of bran vis a vis pancreatic lipase. In fact, the treatment of aqueous extracts of wheat bran by pronase led to total loss of the inactivating effect of wheat bran vis a vis pancreatic lipase. It therefore seems reasonable to put forward the hypothesis that a proteinic type inhibitor could be present in wheat bran and thus be responsible for the greatest part of the inhibitory effect of wheat bran with regard to pancreatic lipase. A proteinic inhibitor of pancreatic lipase has already been revealed and purified from soya (45, 46).

The results obtained in vitro could be discussed in the light of known data obtained from physiological studies on animals and humans. Several studies (5, 47–53) have previ-
ously shown that fasting serum triglyceride values could be lowered when plant fibers were added to the diets. These lowering effects appear more clearly when postprandial triglyceride values are measured (5, 54). In addition, decreases in triglyceride hepatic storage were reported in rats fed a bran-enriched diet (15, 55). In some cases, diet supplements of bran might increase the fecal fat output (17–20). It is unlikely that these cases of fat assimilation impairment could be attributed to the effect of bran on the intestinal mucosa ultrastructure (14), or to the binding of triglyceride lipolysis products by bran particles (13). Thus, the observed decrease in fat assimilation and accumulation induced by cereal fibers could be explained by an inhibition of intestinal lipolysis. It should be emphasized that these effects may be more important in certain pathological cases where pancreatic lipase levels are drastically reduced, as in congenital pancreatic lipase deficiency (56), chronic pancreatitis (57) or cystic fibrosis.

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