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Research Article

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DEMONSTRATION OF AN INTESTINAL MONOGLYCERIDE LIPASE: AN ENZYME WITH A POSSIBLE ROLE IN THE INTRACELLULAR COMPLETION OF FAT DIGESTION *

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The concept is well established that digestion of foodstuffs that occurs within the lumen of the intestine serves to convert macromolecules to smaller ones that may be more assimilable and available for transport across the mucosal cell. Not until recently has it become widely appreciated that this intraluminal digestion is often incomplete and that additional digestion of the partially split fragments may actually occur within the mucosal cell during the process of absorption. Thus, it has been shown recently (2) that dipeptides liberated from ingested proteins by the action of pancreatic proteolytic enzymes may be absorbed by the cell and split by mucosal dipeptidases (3). Similarly, it has been demonstrated that after the intraluminal digestion of starch to disaccharides, these sugars are hydrolyzed within the intestinal epithelial cell by specific disaccharidases (4–6). In fact, these functions appear to be carried out at the surface of the epithelial cells by enzymes localized at the microvilli or brush borders of the cells (3, 6).

In the case of fat digestion, the ingested lipid, most of it triglyceride, is hydrolyzed in the lumen of the gut by action of pancreatic lipase. This enzyme attacks specifically the ester bonds joining the fatty acyl chain to the two primary (α and α') hydroxyl groups of the glycerol molecule (7). The result is a liberation of fatty acids, but there is also a significant accumulation of unhydrolyzed monoglyceride, especially β-monoglyceride. Although some of this β-monoglyceride may be isomerized to an α-monoglyceride and then be subject to further attack by pancreatic lipase, considerable amounts of monoglycerides appear to escape hydrolysis and remain available for intestinal absorption.

The present study demonstrates that monoglycerides absorbed by the intestinal mucosal cell may undergo further cleavage within it by the action of an intestinal lipase. Although intestinal lipases were postulated as early as 1892 by Schiff (8) and have recently been demonstrated more convincingly (9, 10), the present results emphasize that under reasonably physiologic conditions the intestinal lipase is most active towards monoglycerides. Data are also presented concerning the properties of the enzyme system and its localization within the cell compartments. The intestinal monoglyceride lipase may have a significant role in fat absorption, especially in facilitating the completion of fat digestion within the mucosal cell.

MATERIALS AND METHODS

Mono-, di-, and tripalmitin were obtained from Dr. F. H. Mattson,[1] as were α-monolinolein and the monoglyceride isomers α- and β-mono-olein, α- and β-monopalmitin, and α- and β-monostearin. A series of DL-α-monoglycerides of varying chain length in the fatty acid portion was synthesized from the acyl chlorides and isopropylidine glycerol under conditions established by Baer and Fischer (11). Acyl chlorides were prepared by refluxing the fatty acids [2] butyric, caproic, caprylic, capric, lauric, myristic, palmitic, and stearic with excess thionyl chloride, and purified by distillation under reduced pressure of 10 to 15 mm of mercury obtained by a water aspiration system. The DL-isopropylidine glycerol was made by exhaustive reflux of dry acetone and glycerol

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[1] Biochemical Research Division, Proctor & Gamble Company, Cincinnati, Ohio.

[2] Nutritional Biochemicals Corporation, Cleveland, Ohio.

with petroleum ether (boiling range, 38° to 58° C) through a column of glass helices over into a total-reflux condenser fitted with a Barrett water trap (12), followed by distillation of the product. The monoglycerides were purified by recrystallization and silicic acid chromatography. A similar process was used to synthesize DL-α-monopalmitin labeled in the glycerol portion by starting with glycerol-1(3)-C^{14} [3] diluted to a specific activity of 0.06 μc per μmole. Tri- and diglycerides labeled in the glycerol portion were prepared by direct acylation of glycerol-C^{14} in dry pyridine with acyl chlorides, or with the free acids in trifluoroacetic acid anhydride (13), and were purified by elution from columns of silicic acid. All solvents were of certified reagent quality [4] and were freshly redistilled before use; glycerides were checked finally for purity on thin layer plates of silicic acid.

The glyceride substrates were suspended variously in aqueous solutions of bovine serum albumin,[2] Tween 80,[5] or sodium taurocholate.[6] Crude pancreatic lipase was obtained commercially.[7]

Female albino rats [8] weighing 200 to 300 g and fed on Purina chow were ordinarily fasted overnight before use. After cervical dislocation, the abdomens were opened, and the small intestine was rinsed *in situ* with Ringer's solution at room temperature and then chilled to 0° in 0.278 M mannitol solution buffered to pH 7.0 with 0.01 M Tris-maleate. After the epithelial cells were gently scraped free, 14 ml of mannitol solution per g of scraped cells was added and an homogenate prepared with a Potter-Elvehjem tissue grinder, with a Teflon pestle. After filtration through a double layer of absorbent gauze, nuclei and cell debris were spun down as sediment at $1,400 \times g$ for 10 minutes, mitochondria, at $5,900 \times g$ for 15 minutes, and the microsomal fraction, at $105,000 \times g$ for 60 minutes. The tissue fractions were resuspended in appropriate volumes of isotonic KCl buffered to pH 7.0 with 0.01 M phosphate, and proteins were measured by the biuret reaction or the Lowry method (14). Isolated brush border preparations were made as described by Miller and Crane (6). Tissue suspensions boiled for 15 minutes before mixture with substrate and medium were used for control experiments in which inactivated enzyme preparations were desired.

Assay of the lipolytic activities of the various suspensions was carried out by two methods: *1*) liquid scintillation counting of glycerol-C^{14} and lower glycerides produced by hydrolysis of the glycerides labeled in the glycerol portion, and *2*) direct titration of fatty acids released at a constant pH in an automatic glass electrode titrating device.[9] In the first method, free glycerol was separated from the glyceride substrates by the chloroform-methanol extraction technique of Folch, Lees, and Sloane Stanley (15), the glycerides remaining in the lower, chloroform phase, and the glycerol entering the upper, aqueous phase. Samples of the upper phase were used for chemical and radioactive assay of the free glycerol.

1. *Radioactive assay of free glycerol-C^{14} and glycerol-labeled glycerides.* To avoid certain difficulties, both practical and theoretical, in the chemical assay of liberated glycerol from glycerides, glyceride substrates were synthesized with the glycerol portion labeled with C^{14} in the 1- or 3-position, as described above. Glycerides were suspended in bovine serum albumin solution, 100 mg per ml of 0.01 M potassium phosphate buffer at pH 7.4, with the aid of small amounts of diethyl ether for initial dissolving of the glycerides and a glass homogenizing vessel with a rotating Teflon pestle. During homogenization, the temperature was gradually raised to 40° C by immersion of the vessel in a beaker of warm water, until all of the ether was driven off and a uniform suspension of 1 mg glyceride per ml of albumin solution remained. The incubation media consisted of 1 ml of the glyceride-albumin suspension; 0.4 ml of fivefold concentrated, low calcium, Krebs-Ringer phosphate buffer at pH 7.4 (16); and 0.1 to 0.5 ml of the intestinal tissue suspensions in isotonic buffer of the same type, which was also used to make the final volumes to 2.0 ml. Incubations of the intestinal cell homogenates and resuspended subcellular fractions were carried out in conical Pyrex centrifuge tubes for 30 to 60 minutes at 38° C in air, with occasional shaking. In some experiments, sodium taurocholate was added to the media to a final concentration of 20 mM per L.

Incubations were terminated by addition of 15 ml of ice-cold methanol, followed by 30 ml of chloroform. After 30 minutes or more at room temperature, 10 ml of 0.05 M KCl was added and the phases were allowed to separate overnight at 4° C. The upper phase was separated and samples were taken for counting of the glycerol-C^{14}. The glycerides in the lower, chloroform phase were recovered after removal of the solvent under a stream of nitrogen, then redissolved in chloroform and applied in spots on silicic acid thin-layer plates. After ascending chromatography in a solvent system 30 per cent (by volume) diethyl ether, 69 per cent hexane, and 1 per cent glacial acetic acid, spots were located by brief exposure of the plates to iodine vapor and identified by comparison with standard glycerides. The light brown glyceride spots were marked by needle scratches on the surrounding silicic acid, and the iodine was allowed to disappear completely by sublimation. Careful scraping of the silicic acid areas bearing the lipids was done by razor blade, and the scrapings from the glass plates were transferred quantitatively into counting bottles. Scintillation counting fluid was added, made up in 5 ml of 95 per cent ethanol and 10 ml of toluene containing 0.3 per cent 2,5-diphenyl-oxazole and 0.01 per cent *p*-bis-2-(5-phenyl-oxazolyl)benzene,[10] and counting was carried out in a

[3] Nuclear-Chicago Corporation, Chicago, Ill.
[4] Fisher Scientific Corporation, Boston, Mass.
[5] Polyoxyethylene sorbitan mono-oleate.
[6] Organon, Incorporated, West Orange, N. J.
[7] Sigma Chemical Corporation, St. Louis, Mo.
[8] Charles River Laboratories, Boston, Mass.
[9] Model TTT-1, Radiometer Corporation, Copenhagen.
[10] Pilot Chemicals, Inc., Waltham, Mass.

TABLE I

*Formation of lower glycerides and free glycerol from glycerides by rat-gut microsomes**

Substrate	Labeled product isolated			
	Triglyceride	Diglyceride	Monoglyceride	Glycerol
	% original substrate radioactivity			
Tripalmitin-C^{14}	97.4	1.9	0.4	0.3
Dipalmitin-C^{14}	0.3	96.6	1.5	1.6
Monopalmitin-C^{14}	0.5	0.3	7.3	91.9

* Incubation conditions as described in Figure 1.

Packard liquid scintillation spectrometer. Recovery of counts from lipids applied to silicic acid plates or from chromatographed lipid spots was within 5 per cent of the expected radioactivity.

In subsequent experiments, the preparation of the tissue was varied to permit assay of activity in whole intestinal epithelial cell homogenates, nuclei, mitochondria, microsomes, and cell sap, as well as in isolated brush borders and the supernatant fluid derived from them by the technique of Miller and Crane (6). Other series of experiments were performed at pH levels from 5.0 to 9.5, with 0.125 M Tris-maleate or potassium phosphate buffer.

2. *Titration of fatty acids released.* Suspensions of subcellular particles or pancreatic enzyme preparations were incubated at a constant temperature of 38° C, and at a pH maintained at 7.0 by the automatic addition of 0.2 to 1 mM NaOH by a radiometer glass-electrode titrating mechanism. The volume of base required to maintain the pH per unit of time was observed, and this reflected the rate of acid release. Although several systems were tried as suspending media, including solutions of albumin, gum arabic, and Tween 80, the most reproducible medium was found to be a solution of 0.005 M sodium taurocholate in 0.002 M potassium phosphate buffer at pH 7.0. This medium permitted reasonably good dispersion of the substrates and good sensitivity to pH changes without undue instability of pH in control experiments. Series of experiments were carried out with various glyceride substrates, including monoglycerides with varying fatty acyl chain lengths, synthesized as described above. Comparisons were made of the rates of fatty acid release from the α- and β-isomers of several monoglycerides, of intestinal tissue preparations and crude pancreatic lipase preparations, and of other pH levels. Results were expressed as mμ equivalents of acid released per minute per milligram protein. Similar techniques of continuous titration at constant pH have been previously described (17), and have the advantage of more closely estimating the true initial reaction velocity of the ester-bound hydrolytic reaction.

RESULTS

Assay of glyceride hydrolysis with radioactive substrates. In order to measure the lower glyceride products of the reaction as well as free glycerol, labeled glycerides were used in which the glycerol portion contained C^{14} in the 1- or 3-carbon. When suspensions of these radioactive glycerides were incubated with subcellular fractions from intestinal epithelial cells, it was seen that the monopalmitin-C^{14} was almost entirely hydrolyzed, whereas very little hydrolysis of the tripalmitin-C^{14} or the dipalmitin-C^{14} occurred. When the glycerides obtained after incubation in these experiments were separated on thin-layer plates of silicic acid, it was observed (Table I) that very little degradation had occurred of the tripalmitin to di- or monopalmitin, or of dipalmitin to monopalmitin. As might be expected in view of these results, it appeared that much of the monopalmitin produced from dipalmitin lipolysis was further split to free glycerol and palmitic acid.

Repeated localization studies revealed consistently that of the cell fractions, the highest specific activity in monoglyceride lipolysis was found in the microsomes and the mitochondria, which were several times more active than the whole homogenate or the cell sap. Because of the large amount of the cell protein in the cell sap, however, the total activity in this fraction was appreciable. When isolated brush borders were prepared, the supernatant mixture resulting from sedimentation of the brush borders resembled the whole homogenate in its activity, whereas the activity in the brush borders was slight. The specific and total activities are compared in Table II.

TABLE II

*Intracellular localization of monoglyceride lipase in rat intestinal mucosa**

Fraction	Specific activity	Protein in fraction	Total activity in fraction
	mμmoles hydrolyzed/min/mg protein	*% of whole homogenate*	
Whole homogenate	32.5	100.	100.
Nuclei, unwashed	26.0	28.2	22.6
Mitochondria	77.9	5.9	14.6
Microsomes	67.5	10.3	21.4
Cell sap	10.3	55.6	17.7
Sum of fractions			76.5
Isolated brush borders (prepared separately)	21.0	2.0	1.3

* Each incubation was carried out for 10 minutes at 38° C with 2 μmoles of monopalmitin-C^{14} (112,000 cpm) and 0.4 to 1.0 mg tissue protein in 5 per cent albumin at pH 7.0 in 0.15 M potassium phosphate, in a total volume of 2.0 ml.

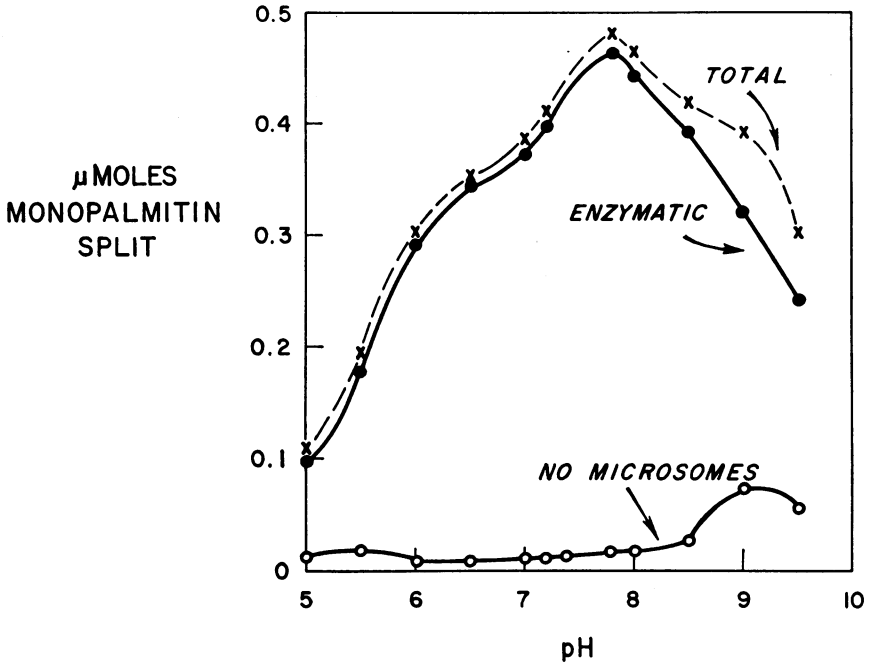

FIG. 1. CURVE OF MONOGLYCERIDE LIPASE ACTIVITY OF INTESTINAL MUCOSAL MICROSOMES AS A FUNCTION OF pH. Each point represents an incubation carried out for 30 minutes at 38° C at the indicated pH, with 0.7 μmole of mono-palmitin-C^{14} (40,000 cpm) and 0.56 mg microsomal protein in 0.125 M Tris-maleate and 0.125 M potassium phosphate buffers. The total volume was 2.0 ml.

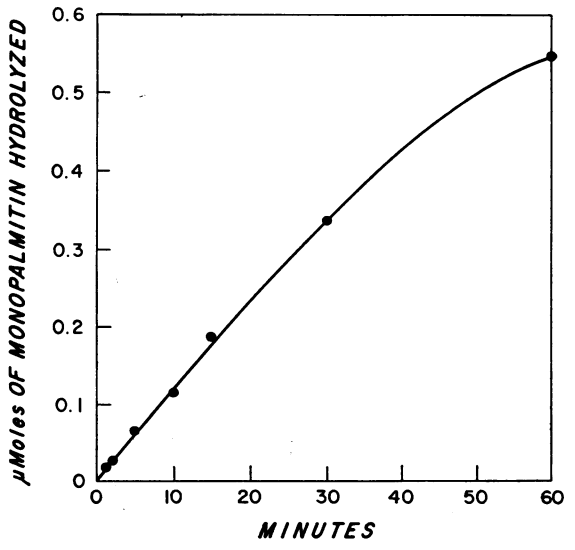

FIG. 2. RATE OF HYDROLYSIS OF MONOPALMITIN BY RAT INTESTINAL EPITHELIAL CELL MICROSOMES. Each incubation was carried out for the indicated time at 38° C, with 1 μmole of monopalmitin-C^{14} (56,000 cpm) and 0.4 mg microsomal protein in 5 per cent albumin and 0.15 M potassium phosphate buffer, pH 7.0. The total volume was 2.0 ml.

Although purification and isolation of the monoglyceride lipase from the cell fractions was not extensively carried out, rough characterization of some of the properties of the enzyme in these crude fractions was attempted. The pH optimum for the splitting of monopalmitin by intestinal epithelial cell microsomes was found to be about 7.8 (Figure 1), although appreciable hydrolysis occured between pH 7.0 and 8.5. Parallel experiments using no tissue preparation were carried out in order to estimate the relative amount of nonenzymatic hydrolysis; at the more alkaline pH values, increasing nonenzymatic hydrolysis by saponification was noted. The addition of 20 mM sodium taurocholate did not produce a marked shift in the pH optimum of the microsomal monoglyceride lipase, although it is known (18) to lower the optimal pH of pancreatic lipase by about 2 pH units.

The rates of lipolysis were fairly linear over a variable range of enzyme concentrations when the amount of monopalmitin-C^{14} substrate was constant. A time study (Figure 2) revealed that

the rate of monopalmitin-C^{14} hydrolysis by intestinal microsomes was linear for about 20 minutes. The temperature stability of the microsomal monoglyceride lipolytic activity was noteworthy. It was repeatedly observed that tissue suspensions could be kept for several days at 0° to 4° C without appreciable loss of activity, that only slight loss of activity occurred after 24 hours at room temperature, and that temperatures above 60° C were required for inactivation.

Addition of 20 mM sodium taurocholate produced no enhancement of monoglyceride lipase activity in microsomes, mitochondria, or brush borders from rat intestinal epithelial cells. In fact, some 30 to 40 per cent inhibition of the microsomal activity was observed at this concentration of the conjugated bile salt. Studies using tri- and dilinolein-C^{14} (labeled in glycerol portion) revealed that they were as resistant to lipolysis by microsomes as were the saturated higher glycerides, despite the much clearer dispersions yielded by the unsaturated glycerides in albumin solution.

Study of glyceride hydrolysis by titration of fatty acids released. In order to follow the lipolysis of glycerides over a period of time without terminating the reaction, it was feasible to measure the fatty acids released by using an automatic titrating device at constant pH, measured by a glass electrode. This second, independent assay permitted quantitation of the fatty acids released from ester linkage under initial reaction conditions, since the pH of the medium was maintained constant by addition of enough NaOH to neutralize the fatty acids liberated. Under these conditions, there was no accumulation of free, unionized fatty acids and H^+ that would tend to reverse the reaction. This technique permitted assay of a much wider range of substrates, since labeled glyceride substrates were not necessary. Thus it was also possible to compare rates of hydrolysis of the series of synthetic monoglycerides with varying fatty acyl chain lengths. Titration could not be carried out in albumin solution, presumably because of the strong buffering capacity of the protein. In the search for suitable media, 10 per cent gum arabic was rejected because of pH instability in the absence of any added enzyme; cell sap was found to have a low but definite lipolytic activity of its own; and Tween 80, an oleic acyl monoester of polyoxyethylene sorbitans, itself acted as a substrate. A dilute sodium taurocholate solution weakly buffered by phosphate was found to provide reasonable dispersions of substrates, nonenzymatic pH stability, and sufficient sensitivity to permit titration of the fatty acids released.

At pH 7.0 and a constant temperature of 38° C, the microsome-catalyzed hydrolysis of monoglycerides was found to be maximal (Figure 3) when the fatty acyl chain length was 10 carbon atoms (monocaprin). Monoglycerides of shorter or longer side-chain lengths showed decreased rates of splitting. Unsaturation of the side-chain seemed to have the same effect as shortening a saturated chain, a double bond having roughly the effect of two fewer methylene groups. Thus, rates were comparable for monolinolein and monomyristin, and for mono-olein and monopalmitin.

The effect of position of the fatty acyl group was estimated by comparison of the rates of hydrolysis of the α- and β-isomers of monopalmitin, mono-olein, and monostearin. Although these rates appeared to be about equal in suspending media consisting of dilute cell sap, in experiments with taurocholate in the medium, the β-isomers appeared to be hydrolyzed more slowly. Possible isomerization of the β- to α-isomers during preparation and assay of the substrates was not excluded. Addition of crude pancreatic lipase, how-

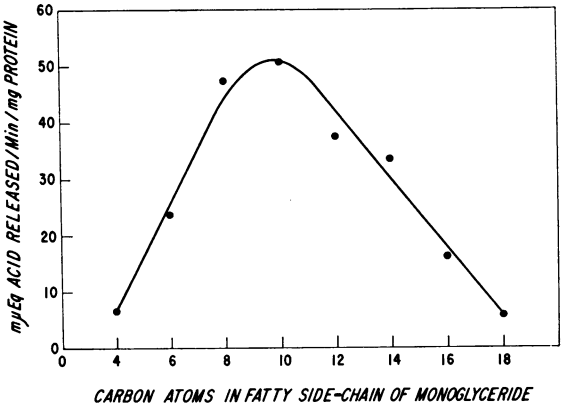

FIG. 3. RELATIVE RATES OF HYDROLYSIS OF MONOGLYCERIDES OF VARYING FATTY ACYL CHAIN LENGTH BY INTESTINAL MUCOSAL CELL MICROSOMES. In each titration at 38° C and constant pH 7.0, there were present 6 μmoles of monoglyceride, 1.7 mg microsomal protein, 0.005 M sodium taurocholate, and 0.002 M potassium phosphate, in an initial volume of 6 ml.

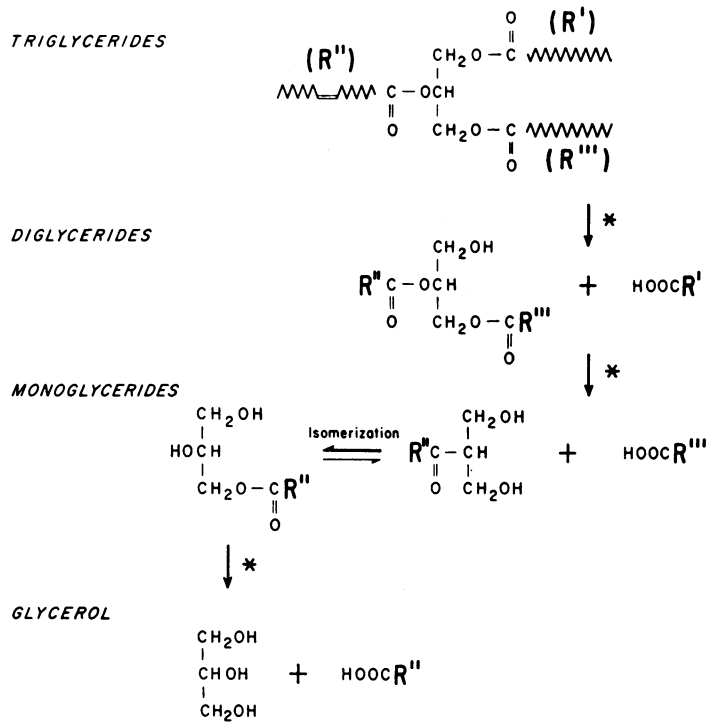

FIG. 4. SCHEME OF THE PROGRESSIVE HYDROLYSIS OF TRIGLYCERIDES BY PANCREATIC LIPASE. The fatty acyl side-chains are symbolized by the zigzag lines and by the alkyl abbreviation **R**. The asterisks indicate reactions thought to be catalyzed by pancreatic lipase.

ever, accelerated the splitting of α-monoglycerides but not β-isomers under similar conditions.

Results of inhibition studies revealed that the microsomal monoglyceride lipase was essentially uninhibited by 0.01 M disodium EDTA, but only slightly inhibited by potassium fluoride at the same concentration. Because of the very crude enzyme preparations, no substrate-inhibitor curves were attempted. No acceleration of the rate of monoglyceride hydrolysis was observed when calcium ions were added in a concentration of 0.005 M per L.

DISCUSSION

The question of whether an intestinal lipase actually exists has been raised for many years, and indeed, the studies Schiff (8) reported 70 years ago on pancreatectomized dogs suggested that there probably was such an enzyme. The great abundance of highly active pancreatic lipase bathing the intestinal mucosa has previously obscured the demonstration of a separate intestinal lipase. Evidence for such an enzyme has continued to accumulate, however, and recently some of the most convincing work has shown pronounced lipolytic activity in subcellular particles obtained from hog duodenum (9). In those studies, the intestinal lipase was distinctly different from pancreatic lipase in its substrate specificity and response to inhibitors, and considerable care was taken to avoid contamination of the intestinal tissue preparations with pancreatic fluid material. Other studies by Tidwell and Johnston (10), using everted sacs of hamster small gut in buffered albumin media containing various suspended glycerides, have indicated that preferential splitting of the monoglycerides occurred, with much less hydrolysis of the di- and triglycerides.

During digestion of dietary fats, pancreatic lipase has been shown to attack triglycerides most rapidly, diglycerides slightly less rapidly, and to have a sluggish effect on the monoglycerides formed (19). Furthermore, pancreatic lipase has been found to catalyze hydrolysis of the ester bonds at the outer, primary (α and α') alcohol groups of the glycerol, and to yield β-monoglycer-

ides as a most important product (20). Although isomerization of the β- to α-monoglycerides can occur spontaneously, allowing pancreatic lipase to complete the hydrolysis, this third stage proceeds much more slowly, and considerable amounts of monoglycerides are therefore available for intestinal absorption. This sequence is summarized in Figure 4.

Evidence that monoglycerides may actually be absorbed has been provided by the double-label studies of monoglycerides and triglycerides in which the glycerol portion and fatty acid portion were labeled with different isotopes (21, 22). Whereas some of the absorbed monoglycerides appeared in the lymph as triglycerides after re-esterification by the intestinal mucosa, considerable amounts of the monoglycerides appeared to be split, as indicated by a fall in the ratio of glycerol to fatty acid isotope in the lymph triglycerides, compared to the original monoglyceride isotopic ratios. Furthermore, *in vitro* splitting of monoglycerides by intestinal epithelial cell preparations has recently been observed in the course of experiments on esterification of monoglycerides (23, 24).

In the present series of experiments, it was hoped that methods could be developed to demonstrate, under conditions reasonably approximating those in the mammalian body, whether an intestinal lipase distinct and different from pancreatic lipase exists. Therefore, unphysiologic media, such as those containing synthetic detergents or high degrees of alkalinity, were avoided, as were assays depending on such difficult-to-interpret techniques as clearing of turbidity. Efforts were made to exclude any contamination by traces of pancreatic lipase, insofar at least as this could be accomplished by washing the mucosa and then the subcellular particles before assay. It must be admitted that some pancreatic lipase could adhere to the cell fractions and especially to the brush borders, but the latter contained a negligible amount of the total lipolytic activity of the gut epithelial cells. Furthermore, the most highly washed fractions, namely, microsomes and mitochondria, showed the highest specific activities and had a substrate specificity distinct from that of pancreatic lipase.

Results by both of the methods used in the present experiments were in agreement, and indicated that indeed a lipase exists within the intestinal epithelial cells and has a great specificity for monoglycerides. Pancreatic lipase, on the other hand, has a maximal activity towards triglycerides, somewhat less towards diglycerides, and is least active with monoglycerides, especially the β-monoglycerides as substrates. If indeed pancreatic lipase acts strictly upon the primary alcohol ester bonds, this substrate specificity would be expected, for the triglycerides have two such bonds, the α-β-diglycerides one, and β-monoglycerides none. These isomers of the lower glycerides are those actually formed during digestion of triglycerides by pancreatic lipase (20, 25).

The intracellular localization of maximal specific activity of the intestinal monoglyceride lipase to the microsomes and mitochondria was a consistent finding; whether or not the lower level of enzyme activity in the cell sap represented an artifactual "solubilization" of the enzyme from the membranous structures during preparation and differential centrifugation could not be determined. The unimpressive degree of activity of the isolated brush borders was another indication of the difference between digestion of fat and that of carbohydrates and proteins. In the latter, the products of intraluminal digestion are water-soluble, and have been shown to undergo final digestion at the surface of the intestinal cells by disaccharidases and oligopeptidases localized in the brush border (3, 6). The major products of fat digestion, however, free fatty acids and monoglycerides, are not water-soluble to any large extent, and it is not surprising that other mechanisms may exist for the handling of these substances. In this connection, it is pertinent that several of the enzymes necessary for chemical transformations of the absorbed fatty acids and monoglycerides to higher glycerides seem to be localized primarily in the microsomal fraction of the intestinal epithelial cell, and to some extent in the mitochondria (23, 24, 26).

Better characterization of the properties of the intestinal monoglyceride lipase will perhaps be possible when isolation and purification of the enzyme from the crude tissue fractions can be accomplished. The definition of this enzyme as a lipase depends on its substrate specificity, although it could be argued that in view of the maximal hydrolytic specificity for monocaprin, it

should be called an esterase. The truly water-soluble, short-chain monoglycerides, however, were less rapidly split than the intermediate-length monoglycerides and the long-chain unsaturated monoglycerides.

The data presented correlate with observations of Tidwell and Johnston (10) and with the very recent studies from the same laboratory in which Pope, Askins, and McPherson (27) have described a lipase in hamster intestinal homogenates with a definite monoglyceride specificity.

Whether the low activity of the intestinal lipase towards tri- and diglycerides is a function of the poor solubility of these compounds in aqueous media has not been resolved by the present studies. In contrast to our findings, DiNella, Meng, and Park (9) found hog intestinal mucosal lipase to hydrolyze ester bonds of tri- and diolein as rapidly as mono-olein. Those studies, however, were carried out under quite different conditions, i.e., with media containing Span 20 [11] at pH 9.0. The crucial questions yet to be settled relate to the importance of the physical state of the substrate and the conditions existing in the medium. It is noteworthy that pancreatic lipase, which has been purified and characterized (28) to a much greater degree than intestinal lipase, shows higher activity when its substrate is water-insoluble, seems to act at lipid-aqueous interfaces, and in fact is inhibited if its substrate is put into true solution.

Whereas the physiologic importance of pancreatic lipase in intraluminal fat digestion is well established, the role and significance of the intestinal lipase is yet to be determined. It seems reasonable, however, that the presence of an enzyme system in the cell active in the hydrolysis of monoglycerides provides a mechanism for completing the digestion of glycerides after their absorption from the lumen of the intestine. In view of the present findings and the previous demonstration of the ability of the intestinal cell to convert monoglycerides to higher glycerides (24), a balance appears to exist within the mucosal cell between monoglyceride hydrolysis and esterification to di- and triglycerides. The mechanism(s) regulating these two pathways remains to be elucidated.

[11] Sorbitan monolaurate.

SUMMARY

An intestinal lipase has been demonstrated in homogenates and subcellular particles of rat intestinal epithelial cells. This lipase exhibits prominent specificity for monoglycerides, and is much less active upon the di- or triglycerides of long-chain fatty acids. A maximal hydrolytic activity was found for monoglycerides of intermediate chain length, i.e., from 8 to 12 carbon atoms in the saturated fatty acyl chain. Unsaturated long-chain monoglycerides were split at rates comparable to those for saturated monoglycerides of shorter chain length. A survey of the cell fractions showed the monoglyceride lipase activity to be concentrated primarily in the microsomal and mitochondrial fractions, with considerably lower specific activities present in the cell sap and isolated brush borders. Fluoride and EDTA (ethylenediamine tetraäcetic acid) did not inhibit the enzyme activity.

The intestinal monoglyceride lipase was found to be distinct and different from pancreatic lipase in *a*) its specificity for monoglycerides rather than triglycerides, *b*) its ability to catalyze hydrolysis of ester bonds on the β- as well as the α-hydroxyl groups of glycerides, and *c*) its activity upon dissolved rather than suspended substrates. Other differences appear to exist in the effect of inhibitors, pH optima, and chain-length specificity, but further studies are necessary when purer enzyme preparations are obtained.

The physiologic importance of the intestinal monoglyceride lipase is not yet known. It may, however, function in the intracellular completion of fat digestion before the synthesis of chylomicrons by the intestinal mucosa.

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