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THE LIPOLYTIC ENZYMES OF RAT PANCREATIC JUICE

R. G. H. MORGAN*, J. BARROWMAN, H. FILIPEK-WENDER AND B. BORGSTRÖM
Division of Physiological Chemistry, Chemical Center, University of Lund, Lund (Sweden)

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SUMMARY

The lipolytic activities of lyophilized rat pancreatic juice have been studied after separation of the proteins by gel filtration, ion exchange chromatography and differential inactivation at several pH's.

Two enzymes are found which attack glycerides. They are readily separated by all three techniques. Gel filtration studies give molecular weights of approx. 40 000 and 65 000 for these enzymes. The former, which attacks emulsified triglyceride and monoglyceride, appears to be lipase, (glycerol ester hydrolase, EC 3.1.1.3). The other enzyme attacks micellar solutions of mono- and triglycerides and micellar solutions of cholesterol esters. The enzymic activity against cholesterol esters was always found with that against micellar monoglyceride regardless of the technique used for separation of the two enzymes. Activity against β-naphthyl acetate was found in association with the enzyme splitting micellar monoglyceride and micellar cholesterol esters, while β-naphthyl laurate was split by lipase. The specificities of these two enzymes have been investigated with particular reference to the physical state of the substrate.

A small amount of phospholipase activity, probably a phospholipase A_2 was found on gel filtration, having an approximate molecular weight of 18 000.

INTRODUCTION

Under suitable conditions, pancreatic juice shows enzymic activity against the ester bond found in a variety of lipids, and partly on the basis of this activity, has been considered to contain a number of lipolytic enzymes. Thus, pancreatic juice will hydrolyse emulsified long chain triglycerides[1], cholesterol esters[2,3], and some phospholipids[2], activities usually thought to be due to glycerol ester hydrolase (EC 3.1.1.3), sterol ester hydrolase (EC 3.1.1.13) and phospholipase (EC 3.1.1.4), respectively. In addition, activity against water-soluble esters has been reported[4–6] possibly due to the presence of carboxylic ester hydrolase (EC 3.1.1.1), and an enzyme active against esters of secondary alcohols has also been suggested[7].

Besides the activity against lipid classes described above, pancreatic juice also

* Present address: Gastro-Intestinal Research Unit, Mayo Clinic, Rochester, Minnesota, U.S.A.

hydrolyses some substrates which do not readily fall into any of these classes. Thus, the insoluble long chain esters of β-naphthol are split[8,9] and micellar solutions of monoglyceride are also attacked[10]. In both cases, hydrolysis is considered to be due to glycerol ester hydrolase, but the long chain esters of β-naphthol involve an alcohol other than glycerol, while micellar monoglycerides, although esters of glycerol, are not emulsified.

It is apparent that some of these substrates show similarities in chemical structure (*e.g.* triglycerides and phosphatidyl choline) or in physical form (*e.g.* water-soluble esters and micellar monoglyceride). It seemed possible, therefore, that lipolytic enzymes in pancreatic juice might show cross specificity, or that one enzyme might be involved in the hydrolysis of lipids of more than one class. In view of this, rat pancreatic juice was fractionated by gel filtration and the fractions tested against a variety of esters to identify the lipolytic enzymes, and to determine whether cross specificity was present. Besides a phospholipase, two major peaks of enzymic activity were found, both of which showed activity against several ester types. The substrate specificity of these enzymes was investigated. The juice was also fractionated by ion exchange chromatography in an attempt to separate further each lipolytic peak, and the effect of pH on activity against a number of lipid classes was determined. A preliminary report of some of this work has been published[11].

MATERIALS AND METHODS

Bile salts were prepared by conjugation of cholic and deoxycholic acids with taurine, following the procedure of HOFMANN[12]. Their purity was checked by thin layer chromatography and they were found to be at least 97% pure. Triolein (glycerol trioleate, B.D.H.), monoolein (Myverol, glycerol monooleate, Eastman Kodak Co.) and cholesterol oleate (B.D.H.) were purified by chromatography on alumina columns. The purity of the products was checked by thin-layer chromatography. β-Monoolein was synthesised from 1:3 benzylidine glycerol according to the method of MARTIN[13] and was stored in heptane at −20°. The purity of this product was checked by thin-layer chromatography immediately prior to use; less than 10% of the α-isomer was present. Egg lecithin was purified by chromatography on silica acid columns. Cyclohexanol laurate, *n*-propyl oleate and iso-propyl oleate were synthesised from the acid chlorides. The purity of all these materials was checked by thin-layer chromatography. β-Naphthyl acetate and β-naphthyl laurate (Dajac Laboratories) were purified by recrystallisation from ethanol.

Pancreatic enzymes

Rat pancreatic juice, free of bile, was obtained by cannulating the pancreatic duct after draining the bile with a separate cannula; the juice was collected over dry ice and lyophilized. About 25 mg of powder, consisting of approx. 80% protein, were obtained per ml of juice.

Gel filtration

Sephadex G-100 (Lot No. TO. 5218) and G-200 (Lot No. TO. 4762) were used. The gel was packed in columns of diameter 15 mm, total volume 120 ml. 10, 20 or 25 mg of lyophilized rat pancreatic juice dissolved in 1 ml of 0.15 M NaCl were applied to the

column and eluted with 0.15 M NaCl at 4°. Blue dextran (mol.wt. 500 000) was used to determine the void volume of the gel. Fractions of approx. 2 ml were collected at flow rates of between 6 and 10 ml per h. The elution of the protein was determined by measurement of the ultraviolet absorption at 280 mμ of the effluent. The columns were calibrated with caeruloplasmin (Kabi), bovine serum albumin (crystallised, B.D.H.), egg albumin (Rudolph Grave) and cytochrome *c* (horse heart, type III, Sigma).

Ion exchange chromatography

DEAE Sephadex was used. Cationic proteins were eluted with 0.05 M Tris–HCl buffer (pH 7.4) and anionic proteins by elution with a gradient of 0.05 M to 1 M NaCl in this buffer. Between 10 and 20 mg of lyophilized pancreatic juice were applied to columns of 2.5 cm × 25 cm and fractions of approx. 2.5 ml were collected.

Enzyme assays

The composition and incubation conditions of the principal substrates used are given in Table I. A number of other substrates were used on a few occasions. These will be described in the section of results.

TABLE I

THE COMPOSITION AND INCUBATIONS OF THE PRINCIPAL SUBSTRATES USED

Emulsified triolein	Triolein Sodium taurodeoxycholate	20 12	μmoles/ml phosphate buffer (0.15 M in Na), pH 5.8	5 min at room temp.
Micellar monoolein	Monoolein Sodium taurodeoxycholate	12 12	μmoles/ml phosphate buffer (0.15 M in Na), pH 5.8	60 min at room temp.
Emulsified cholesterol oleate	Cholesterol oleate Octadecane Sodium taurocholate	16 7.8 16	μmoles/ml glycyl–glycine buffer (0.05 M), pH 9.0	5 min at 25°
Micellar lecithin	Lecithin Sodium taurocholate	4 4	μmoles/ml glycyl–glycine buffer (0.05 M), pH 9.0	2 h at 37°

The incubations of emulsified triolein and micellar monoolein were carried out in a total volume of 2 ml. The mixture contained 1 ml of substrate solution, 100 μl of enzyme source in the case of triolein lipolysis and 200 μl in the case of monoolein lipolysis and the remainder made up with 0.15 M NaCl. The reactions were stopped by the addition of 6 ml of ether–heptane–ethanol (1:1:1, v/v), the free fatty acids being extracted into the upper phase after vigorous shaking. 2-ml aliquots of this phase were taken to dryness, dissolved in 3 ml of ethanol and titrated with 0.02 M NaOH using Nile Blue as indicator.

In the case of emulsified cholesterol oleate and micellar lecithin, aliquots taken were generally 200 μl and 1 ml, respectively; the reactions were stopped by the addition of 4 ml of isopropanol: 1.5 M H_2SO_4 (40:1, v/v). The free fatty acids were extracted with heptane and titrated with 0.02 M NaOH according to the method of Dole as modified by TROUT, ESTES AND FRIEDBERG[14].

Micellar triolein was prepared by insonating in an ice bath for 30 min, a mixture containing 3 mg of lecithin, 4 μmoles triolein and 5.4 μmoles sodium taurodeoxycholate

per ml, after the method of VAHOUNY, WEERSING AND TREADWELL[15]. This mixture was slightly turbid, but a clear solution of micellar triolein was obtained by filtration for 48 h with a Millipore filter of pore size 500 Å in chambers of the type described by BORGSTRÖM[16]. This substrate was incubated for 30 min at room temperature in a total volume of 2 ml composed of 1 ml of substrate, 200 μl of enzyme source and 800 μl of 0.15 M NaCl. Thereafter, the reaction mixture was treated in the same way as the micellar monoolein or emulsified triolein substrates.

Emulsified monoolein was prepared by shaking 1 ml of a solution of 12 μmoles/ml monoolein in heptane with 1 ml of 0.15 M phosphate buffer (pH 5.8) containing an aliquot of the enzyme source. Incubations were carried out in sealed ampoules at 37° for 1 h. In some experiments, sodium taurodeoxycholate 6 μmoles/ml was added to the aqueous phase.

Studies with esters of β-naphthol

The hydrolysis of β-naphthyl acetate and laurate was measured by the method of NACHLAS AND BLACKBURN[17]. β-Naphthyl acetate formed a water-clear solution, and β-naphthyl laurate a cloudy dispersion. The aliquots of enzyme source taken in this assay varied between 10 and 100 μl. β-Naphthyl stearate was also used in two early experiments, but it proved difficult to use due to clumping of the dispersed substrate. Activity was usually measured in the absence of bile salts, but in a few cases, sodium cholate (10 μmoles/ml) was added.

RESULTS

Sephadex chromatography. When rat pancreatic juice was fractionated on Sephadex G-100 and G-200, hydrolytic activity against micellar monoolein was well separated from activity against emulsified triolein (more than 30 separations). The apparent molecular weight of the enzyme attacking micellar monoolein was approx. 65 000, based on the $K_{av.}$ (ref. 18) of the peak of activity while that of the enzyme splitting emulsified triolein was about 40 000.

Hydrolytic activity against cholesterol oleate closely paralleled activity against micellar monoolein (11 separations) and could not be separated from it even when fractions as small as 1 ml were collected. A small hydrolytic activity against ovolecithin occurred predominantly in fractions collected late in the separations and distinct from either of the other two lipolytic enzymes (3 separations). An approximate molecular weight of 18 000 was calculated. This enzyme appears to be a phospholipase A_2 (ref. 19), since when β-[^{3}H]acyl lecithin was used as a substrate, the released fatty acid was highly labelled and the lysolecithin produced showed only slight activity.

Besides the major peaks, minor peaks of activity against all substrates were also found. Thus, a small and variable amount of activity against micellar monoolein, cholesterol oleate and emulsified triolein was found at the void volume in most separations. A third, usually small, peak of activity against triolein was occasionally eluted later than the main peak. With ovolecithin, small peaks of hydrolytic activity, corresponding to the peaks active against micellar monolein and emulsified triolein were found.

These separations are shown graphically in Figs. 1 and 2 (a) and the $K_{av.}$'s are presented in Table II, together with the approximate recoveries.

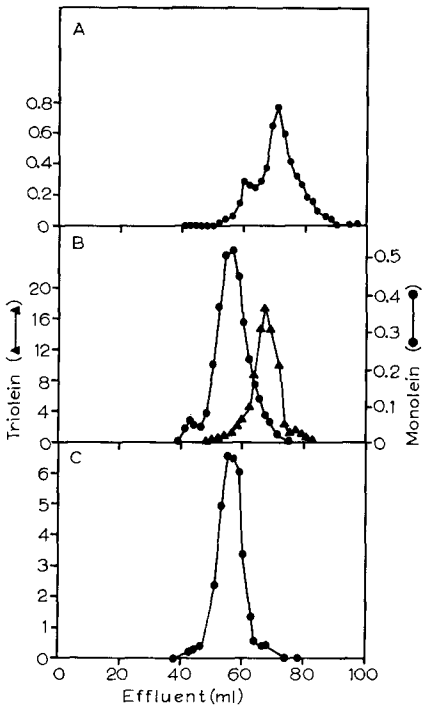

Fig. 1. Chromatography of lyophilized rat pancreatic juice on Sephadex G-100. Temp. 4°. Eluted with 0.15 M NaCl. Flow rate approx. 8 ml/h. Void volume: 42 ml. Total volume: 120 ml. a. Absorbance at 280 mμ of the eluate. b. ●—●, hydrolytic activity against micellar monoglyceride; ▲—▲, hydrolytic activity against emulsified triglyceride; c. Hydrolytic activity against cholesterol oleate. The enzymic activities are expressed as μequiv fatty acid released/min per fraction.

TABLE II

K_{av} VALUES (MEANS AND RANGES), AND RECOVERIES OF LIPOLYTIC ENZYMES OF RAT PANCREATIC JUICE SEPARATED ON SEPHADEX G-100 AND G-200

8 samples of lyophilized pancreatic juice from 6 rats were used. 23 separations were performed on G-100 and 4 on G-200. K_{av} for enzymes splitting emulsified triolein and micellar monoolein were estimated on all separations. Values for cholesterol oleate hydrolysis were obtained from 8 separations on G-100 and 3 on G-200. Two estimations giving the same value were obtained for lecithin hydrolysis. Recoveries are expressed as a mean percentage and range of the applied activity. *, not estimated; ×, present on 4 occasions.

Substrate	K_{av}.		*Approx. Mol. wt.*	*Per cent recovered*	
	G-100	*G-200*			
Emulsified triolein (1)	0.0	0.0	>800 000		
Emulsified triolein (2)	0.32 (0.29–0.35)	0.63 (0.52–0.66)	40 000	30 (18–60)	(7 expts.)
Emulsified triolein (3)	0.49× (0.46–0.50)	*	20 000		
Emulsified cholesterol oleate	0.19 (0.16–0.20)	0.40 (0.38–0.44)	65 000	62 (30–93)	(6 expts.)
Micellar monoolein	0.19 (0.16–0.25)	0.38 (0.33–0.43)	65 000	64 (46–74)	(7 expts.)
Ovolecithin	0.56	*	18 000	138 (127–150)	(2 expts.)

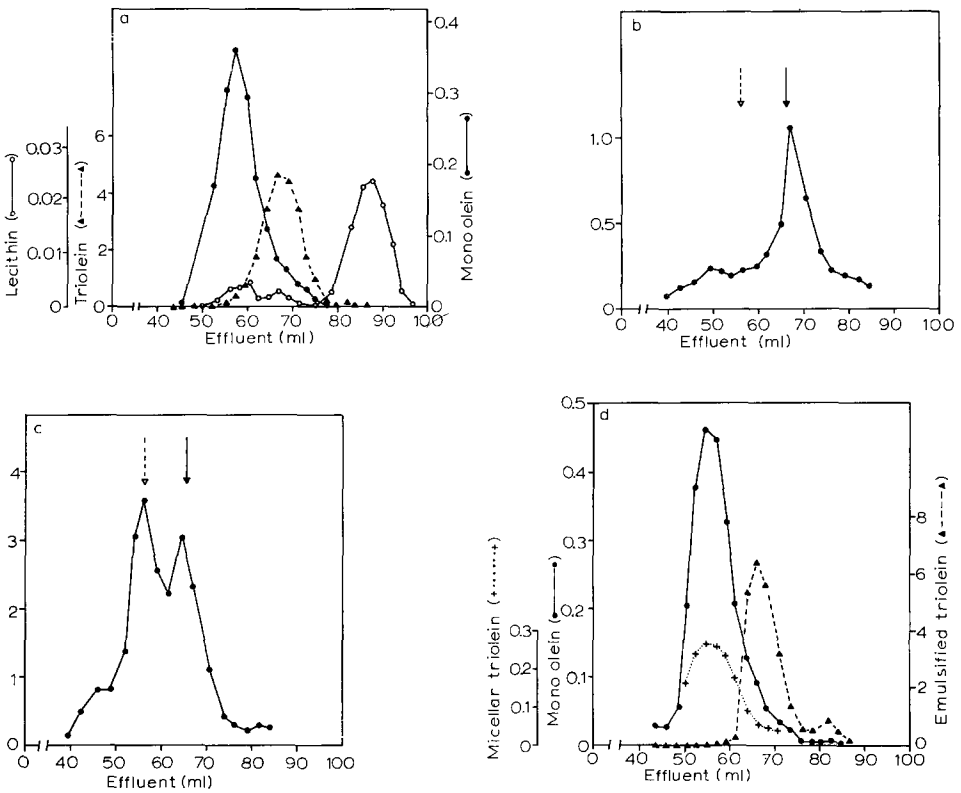

Fig. 2. Chromatography of samples of lyophilized rat pancreatic juice on Sephadex G-100. Temp. 4°. Eluted with 0.15 M NaCl. Flow rate 8–10 ml/h. Total volume: 120 ml. Void volumes: approx. 40 ml. The ordinates give the enzymic activities expressed as μequiv fatty acid released per min per fraction. a. Activities against micellar monoolein (●—●), emulsified triolein (▲— — —▲), and micellar lecithin (○—○). b. Activity against emulsified monoolein in heptane. c. Activity against emulsified monoolein in heptane with 6 mM sodium taurodeoxycholate in the aqueous phase. d. Activities against micellar monoolein (●—●), emulsified triolein (▲— — —▲), and micellar triolein (+ · · · +). In Figs. 2b and 2c the broken arrow represents the elution volume of peak enzymic activity against micellar monoolein and the solid arrow represents the elution volume of peak enzymic activity against emulsified triolein.

Ion exchange chromatography

Five separations of four samples of rat pancreatic juice were performed, using DEAE-Saphadex at pH 7.4. Protein was eluted in two peaks, a small, fairly symmetrical cationic fraction and a larger anionic fraction showing several components. The principal activity against emulsified triolein was found in the cationic fraction which contained activity against β-naphthyl laurate; the cationic peak was eluted with 0.05 M buffer. Activity against micellar monoolein appeared as a very sharp peak early in the anionic protein peak. Activity against cholesterol oleate and β-naphthyl acetate followed the micellar monoolein peak almost exactly.

In two experiments, where the two lipolytic activities were separated by ion exchange chromatography at pH 8.0 on DEAE-cellulose, the activities against an emulsion of tributyrin at pH 8.5 were studied. The incubation mixture contained 2% tributyrin and released fatty acid was titrated continuously. The cationic fractions

TABLE III

RETENTION OF HYDROLYTIC ACTIVITY TOWARD VARIOUS SUBSTRATES BY PANCREATIC JUICE MAINTAINED PRIOR TO ASSAY AT THE pH INDICATED FOR 1 h AT 40°

pH	*Emulsified triolein (%)*	*Micellar monoolein (%)*	*Cholesterol oleate (%)*	*β-Naphthyl acetate (%)*
4	0	60	75	72
5	90	93	100	100
6	100	100	100	100
7	100	96	100	100
8	100	95	100	100
9	83	0	0	0

were found to contain strong hydrolytic activity towards this ester while little or no activity was found among the anions.

pH stability of the enzymes

The stability of enzyme activity was studied when pancreatic juice proteins were kept in buffers at various pH values at 40°; the technique was similar to that used by MATTSON AND VOLPENHEIN[7]. Activity against cholesterol oleate, micellar monoolein, β-naphthyl acetate and emulsified triolein was studied. The results are shown in Tables III and IV. It will be seen that activity against emulsified triolein was rapidly lost at pH 4.0, but activity against other substrates fell only slightly. On the other hand, activity against triolein was stable at pH 9.0 but activity against the other substrates was rapidly lost. In general, activity against micellar monoolein, cholesterol oleate and β-naphthyl acetate varied in parallel.

Specificity of enzymes: physical state of the substrate

The fact that micellar monoolein and emulsified triolein were split by different enzymes could be due to the difference in the chemical configuration of the substrate. However, it seemed more likely that differences in the physical state of the substrate

TABLE IV

RETENTION OF HYDROLYTIC ACTIVITY TOWARD VARIOUS SUBSTRATES BY PANCREATIC JUICE MAINTAINED PRIOR TO ASSAY AT 40° FOR THE TIMES INDICATED

Time (min)	*Emulsified triolein (%)*	*Micellar monoolein (%)*	*Cholesterol oleate (%)*	*β-Naphthyl acetate (%)*
Buffer pH 4.0				
0	100	100	100	100
30	0	68	85	83
60	0	60	75	60
120	0	50	75	72
Buffer pH 9.0				
0	100	100	100	100
30	91	17	18	0
60	83	0	0	0
120	80	0	0	0

were of more importance in deciding which enzyme attacked the glyceride ester bond. Monoolein was therefore prepared in an emulsified form and activity tested against this. It was found that emulsified monoolein was rapidly hydrolysed, but only by fractions showing activity against emulsified triolein, with little or no hydrolysis by fractions active against micellar monoolein (Fig. 2b). When 6 mM sodium taurodeoxycholate was present in the aqueous phase in studies with emulsified monoolein, hydrolysis showed two peaks, corresponding to the enzymes active against micellar monoolein and emulsified triolein (Fig. 2c). Presumably in these studies with bile salts, a micellar and an emulsified phase coexist.

In two experiments where triolein was prepared in micellar form by the method of VAHOUNY, WEERSING AND TREADWELL[15], it was hydrolysed by fractions showing activity against micellar monoolein (Fig. 2d). With this substrate, fatty acid could also have been released from the lecithin used to solubilize the triglyceride, but this seems unlikely in view of the low lecithinase activity found in the fractions showing maximal activity against the micellar triolein substrate (Fig. 2a). Furthermore, in one experiment, triolein containing 9,10[^{3}H]oleic acid uniformly in the 1, 2 and 3 position was used in preparing the micellar substrate. In this case, the released fatty acid was highly labelled and showed approximately the same specific activity as the substrate.

Studies with esters of β-naphthol also confirmed that the physical state determined which enzyme attacked the substrate. Thus water-insoluble β-naphthyl laurate and stearate were split only by the enzyme active against emulsified glycerides, while water-soluble β-naphthyl acetate was hydrolysed by the larger molecular weight enzyme. Activities against both β-naphthyl acetate and laurate were markedly stimulated in the presence of 10 mM sodium cholate.

An apparent inconsistency in the concept that the enzyme of mol.wt. 65 000 attacked only a solubilized substrate was the fact that this enzyme was active against cholesterol oleate. However, it was recognised that cholesterol oleate would have a slight micellar solubility in the bile salts used in preparing the substrate, and it seemed possible that only the cholesterol oleate in the micellar phase was hydrolysed. To determine whether this was so, the cholesterol esterase substrate was prepared with [^{3}H]labelled cholesterol oleate. This substrate was then filtered through Millipore filters of 500 Å pore size[16]. The clear micellar filtrate contained significant amounts of radioactivity, from which it was calculated that about 5% of the cholesterol ester was in micellar solution at the start of each incubation. In a further study, 1 ml of the micellar phase separated from 4 ml of emulsified cholesterol oleate was used as substrate for pancreatic juice. It was found that approximately equal amounts of fatty acid were released from the filtered micellar phase as from unfiltered emulsified substrate. This suggested that it is only the micellar cholesterol oleate which was hydrolysed.

Specificity of enzymes: chemical nature of ester bonds

Besides the substrates already discussed, the activity of both major lipolytic enzymes was tested against several long chain fatty acid esters differing in the configuration of the ester bond. In these studies, *n*-propyl oleate, isopropyl oleate and cyclohexanol laurate in heptane solution were tested as substrates both in the presence and absence of 6 mM sodium taurodeoxycholate, while β-monoolein was tested as a micellar solution in 6 mM sodium taurodeoxycholate and as a heptane emulsion in the absence of bile salts.

TABLE V

Activity: — none detected: (+), slight or doubtful; +, small; ++, moderate; +++, strong.

Substrate	*Enzyme of approx. 65 000 mol. wt.*	*Enzyme of approx. 40 000 mol. wt.*	*Phospholipase mol. wt. 18 000 approx.*
β-naphthyl acetate	+++	—	—
Micellar α-monoolein	+++	—	—
Micellar β-monoolein	+	—	—
Micellar triolein	++	—	—
Cholesterol oleate	+++	—	—
Emulsified α-monoolein	—	+++	—
Emulsified β-monoolein	—	+	—
Emulsified triolein	—	+++	—
Emulsified tributyrin	++	—	—
β-naphthyl laurate	—	+++	—
Ovolecithin	(+)	(+)	+++

In the presence of bile salts, all the above substrates were hydrolysed by the enzyme of molecular weight 65 000, (*n*-propyl > isopropyl > cyclohexanol) the β-monoolein (better than 90% β-isomer) at a rate equal to about 30% the rate found with commercial monoolein (approx. 88% α-isomer). None of these substrates was hydrolysed by the enzyme of mol.wt. 40 000 at the enzyme concentrations used, nor were they hydrolysed by either enzyme in the absence of sodium taurodeoxycholate. Table V summarises the substrate specificities of lipolytic enzymes examined in this study with respect to the principal compounds tested.

DISCUSSION

These studies show that rat pancreatic juice contains at least two enzymes capable of hydrolysing esters of glycerol and long chain fatty acids, although their relative importance in the digestion of long chain triglycerides in the normal animal is not known.

The enzyme which hydrolysed emulsified triolein was almost certainly glycerol ester hydrolase (EC 3.1.1.3), and the molecular weight calculated for it in the present study from gel filtration data is in agreement with that found in similar studies elsewhere[20–22]. The substrate specificity found for the enzyme here also agrees in general with that found for purified glycerol ester hydrolase. Thus the enzyme showed an absolute requirement for an emulsified substrate and would attack only the primary ester bond of glycerides[23,24]. However, it also readily hydrolysed β-naphthyl laurate and β-naphthyl stearate, in which the fatty acid is esterified to a tertiary alcohol. The use of long chain esters of β-naphthol to study glycerol ester hydrolase histochemically[25,26] is thus justified.

The peak of activity against triolein which was eluted later than the main glycerol ester hydrolase peak had a molecular weight of about 20 000. This might, therefore, be a monomeric form of a normally dimeric enzyme. However, it might represent glycerol ester hydrolase which has been partly destroyed by proteolytic activity, leaving the active site intact. Certainly, the smaller enzyme appeared to be

formed from the larger enzyme on storage, since juice fractionated within a few days of collection usually showed no mol.wt. 20 000 peak, while a sample of juice repeatedly fractionated over several weeks showed a progressive decrease in the mol.wt. 40 000 enzyme together with a progressive increase in amounts of the smaller enzyme. A third possibility is that the 20 000 mol.wt. peak results from the activation of small amounts of glycerol ester hydrolase by a co-factor present in these fractions. Some preliminary experiments suggest that such a co-factor may be present in this area. It appears to be partially separated from glycerol ester hydrolase during gel filtration which might explain the low recoveries of glycerol ester hydrolase after Sephadex chromatography (Table II). Low recoveries of enzymes might also be explained by proteolytic digestion during gel chromatography.

The enzyme which attacked water-soluble esters or micellar solubilized lipids showed a very low substrate specificity, requiring only that the substrate be in solution. This enzyme showed both sterol ester hydrolase (EC 3.1.1.13) and carboxylic ester hydrolase (EC 3.1.1.1) activity. The possibility that several enzymes of the same molecular weight were present in these fractions was thoroughly investigated by repeated Sephadex chromatography, by DEAE-Sephadex chromatography and by studies of enzyme stability at different pH values. However none of these techniques showed any differentiation of enzymic activity towards micellar monoolein, cholesterol oleate, or β-naphthyl acetate. On the basis of this, it is concluded that sterol ester hydrolase and carboxylic ester hydrolase are identical, at least in rat pancreatic juice. This finding is in agreement with earlier work based on inhibition studies with these enzymes[6,27,28]. The finding that it is only the micellar solubilized cholesterol oleate which is attacked also confirms earlier work[15].

Phospholipase has been frequently reported in pancreatic tissue[29,30] and duodenal mucosa and contents[31,32] but only rarely in pancreatic juice[3,33]. Pancreatic lipase has been shown to attack, the α-fatty acid of lecithin[34]. The fact that phospholipase could be readily detected in the present study may be a result of tryptic activation of prophospholipase during column chromatography. The reconstituted juice applied to the column showed no tryptic activity, but a peak of high tryptic activity was recovered after chromatography (unpublished observations). Recently it has been shown that phospholipase is present in pancreatic juice as a prophospholipase which is strongly activated by trypsin[35]. The recovery of more than 100% of the applied phospholipase activity (Table II) may reflect this activation.

Recently, MATTSON AND VOLPENHEIN have suggested that rat pancreatic juice contains several lipolytic enzymes on the basis of inactivation studies[7]. In some respects the studies reported in this paper are in agreement, but they differ in a number of details. Thus, MATTSON AND VOLPENHEIN identified an enzyme active against esters of secondary (and other) alcohols, which might be identical with sterol ester hydrolase. This would appear to correspond to the enzyme of 65 000 separated on gel filtration. However, they report that this enzyme shows an absolute requirement for bile salts. In the present study, an absolute requirement for bile salts was found with a number of substrates (*e.g.* monoglycerides, propyl esters, cyclohexanol laurate) but almost certainly this was only because bile salts were needed to solubilize the substrate. When the substrate was itself water-soluble, or at least could be solubilized in an aqueous system in the absence of bile salts (*e.g.* naphthyl acetate), it was readily split. This enzyme therefore has the characteristics of a carboxylic ester hydrolase, and has been

so designated in the present study. Using dissolved methyl butyrate as a model substrate, MATTSON AND VOLPENHEIN were unable to demonstrate any hydrolysis in the absence of bile salts (confirmed in the present study). They therefore concluded that carboxylic ester hydrolase was not present in pancreatic juice. In relation to the knowledge derived from the present experiments it is obvious that the hydrolysis of monoglyceride in the experiments by HOFMANN AND BORGSTRÖM[10] was due to a combination of the two lipolytic enzymes now shown to be present in the pancreatic juice of rats.

The nomenclature of the enzyme separated by gel filtration is complicated by the complex specificity which these enzymes show. Phospholipase appears to have a high specificity, and its designation as phospholipase (probably A_2) is relatively simple. However, glycerol ester hydrolase attacks esters of both glycerol and naphthol, while sterol/carboxylic ester hydrolase attacks a variety of alcohols and long and short chain fatty acids. A nomenclature based on the chemical nature of the ester bond split by these latter two enzymes is therefore difficult to establish, as has been pointed out elsewhere[24]. It is possible that the physical state of the substrate might form a basis for nomenclature, as this property appears to play an important role in determining the specificity of the enzymes[7,24].

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