ON THE MECHANISM OF PANCREATIC LIPOLYSIS OF GLYCERIDES

by

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During hydrolysis of triglycerides by pancreatic lipase an exchange of glyceride fatty acids and free fatty acids takes place *in vivo* as well as *in vitro*[1]. In the *in vitro* experiments corn oil containing a small amount of labelled free palmitic acid was hydrolysed by a mixture of bile and pancreatic juice from rat. During the course of the hydrolysis the fatty acids of the remaining glycerides became labelled. It remained unexplained why the specific activity of the fatty acids of the glycerides only reached about 50% of that of the free fatty acids.

In the present investigation this problem has been studied by repeating the earlier incorporation studies with triolein containing free labelled oleic acid as substrate and by following the incorporation of the labelled acids into the different glycerides formed during the course of the reaction.

This paper also includes studies of the effect of pH, Ca ions and bile acids on the course of the hydrolysis of glycerides and the exchange of fatty acids between free fatty acids and glyceride fatty acids during hydrolysis by pancreatic lipase *in vitro*.

EXPERIMENTAL

Preparations

Enzymes. A mixture of bile and pancreatic juice as well as pure pancreatic juice from rats was used as an enzyme source. The mixture of bile and pancreatic juice was collected as previously described[1]. For collection of pure pancreatic juice this procedure had to be modified. Firstly the bile was diverted by a cannula inserted into the first third of the bile duct and then the pancreatic juice was obtained from a cannula in the distal end of the lower two thirds of the bile duct.

The bile-pancreatic juice and the pancreatic juice used in these experiments were collected from several rats during several days for each animal. The juices were collected in vessels chilled with dry ice and stored at about —12°. The juice obtained each day was assayed for lipase activity and all active samples pooled and lyophilized after addition of the amount of 0.1 *N* hydrochloric acid required to neutralize the bicarbonate present in the juice. The dry substance obtained could then be stored at room temperature.

A sample of juice was considered active when 2.5 ml thereof mixed with 0.5 ml 0.1 *N* hydrochloric acid hydrolysed about 150 mg olive oil to more than 90% during 18 hours at 40°. This method of estimating the lipase activity is not very accurate, but the only intention at that time was to avoid samples with low activity and to ascertain the amount of juice that had to be taken with a certain amount of glyceride to obtain a practically complete hydrolysis inside 18 hours. Sometimes samples of juice were collected with very low lipase activities. It was observed that if a sample of such inactive juice was mixed with active lipase preparations their activity was lost. It appears likely that the inactivation is due to proteolytic activity.

The proportion between bile-pancreatic juice and substrate and pancreatic juice and substrate in each experiment is given later on.

Substrates. Triolein: analytical grade (Hormel).

Olive oil: commercial preparation.

References p. 504.

1.2-Diolein: this was prepared enzymically[2]. 10 ml of triolein were incubated with a mixture of 30 ml bile-pancreatic juice and 0.6 ml 1 *N* hydrochloric acid for 1 hour at 40° at an initial pH of 6.4. After acidification and extraction of the lipids with ether the glycerides were separated from the free fatty acids by passage through a column of IRA-400[3]. 6.83 g glycerides thus obtained were separated into tri-, di- and monoglycerides on a column of 50 g silicic acid containing 20 g filter acid, according to procedures described earlier[4]. From this separation 3.72 g of triglyceride and 2.45 diglyceride were obtained. The diglyceride prepared in this way is of the 1.2-configuration as was found earlier[2]. The purity of the 1.2-diglyceride was over 90% as calculated from figures obtained by oxidation with chromic acid[2].

Palmitic acid-1-^{14}C: was prepared as described by BERGSTRÖM, BORGSTRÖM AND ROTTENBERG[5].

Oleic acid-1-^{14}C*: was prepared according to the method of BERGSTRÖM, ROTTENBERG AND PÄÄBO[6].

Dihydroxystearic acid-1-^{14}C*: was obtained as an intermediary product from the preparation of the labelled oleic acid.

Decanoic acid-1-^{14}C* and *butyric acid-1-*^{14}C* were prepared according to standard procedures from their norbromides and $^{14}CO_2$.

Glycerol-1-^{14}C: was obtained from A.E.R.E., Harwell, England. 9.0 mg contained 0.1 mc.

Sodium-taurocholate: was prepared according to CORTESE[7].

General procedures

The substrate, usually about 250 mg, was weighed into 10 ml test tubes. The lyophilized bile-pancreatic juice was dissolved in phosphate buffer, pH 6.4, in an amount equal to that of the original volume of the juice. The appropriate amount of 0.1 *N* hydrochloric acid was added to make the pH about 6.4 and the volume made up with buffer to 120% of the original. Of this solution 6 ml were added to each test tube containing the substrate. The tubes were sealed in a flame and placed in a water bath at 40° with arrangements for rotating the tubes placed perpendicularly to the rotating axle at a rate of about 40 rpm.

As large amounts of fatty acids in proportion to the buffering capacity of the solution were liberated from the glycerides during the hydrolysis, the pH was not constant during the course of these experiments. After 24 hours incubation time, when the hydrolysis was practically complete, the pH was found to be inside the range 5.9 to 6.2. The initial pH value chosen for the hydrolysis, about 6.4, was considered to approximate the pH prevailing in the lumen of the small intestine during digestion.

In the experiments in which the effect of pH upon the rate of hydrolysis and incorporation of labelled free acids into the glycerides during hydrolysis were studied, only 50 mg olive oil containing labelled free acid substrate were used in each tube. A mixture of 4 ml buffer and 1 ml solution of lyophilized pancreatic juice containing the dry substance equal to 0.2 ml of the original juice was used as enzyme source. No bile or bile acids were added in these experiments. The incubation time was half an hour. Phosphate buffers were used in these experiments inside the pH range 6.0 to 8.6 and the universal buffer of STENHAGEN AND TEORELL[8] for higher and lower pH values.

The effect of Ca ions on the rate of hydrolysis and incorporation of free labelled fatty acids at different pH values was studied in similar experiments. Here the pH range 6.6 to 8.6 was studied and veronal/hydrochloric acid buffers used. The amounts of glyceride were 25 mg and the amount of pancreatic juice dry substance corresponded to 0.05 ml original pancreatic juice in each tube. The total volume in each tube was 5 ml. The amount of Ca ions added in the one series of experiments corresponded to about one Ca atom to each fatty acid that could be liberated and was added in the form of calcium chloride.

* These labelled acids have kindly been placed at my disposal by Professor S. BERGSTRÖM of this institute.

References p. 504.

In these latter experiments in which the amounts of glyceride in each tube were 25 and 50 mg the quantities of fatty acids liberated were so small that the pH of the solutions was not changed to any large extent during the course of the hydrolysis. The pH was measured before and after the completion of the experiments with a Beckman Model glass electrode pH meter.

At the end of the respective periods of incubation the reaction mixtures were rinsed into 100 ml flasks with water, and 5 ml 5 *N* hydrochloric acid added to stop the lipolysis. The volume was made up to about 50 ml with water and the lipids were extracted with ether.

Separation of the free fatty acids and the glycerides of the recovered lipids was performed on columns of the ion-exchanger IRA-400[3]. When a further separation of the glycerides into tri-, di- and monoglycerides was wanted this was made on columns of silicic acid as described by BORGSTRÖM[4]. The composition of the different lipid mixtures was calculated from the weights of the different fractions obtained.

The radioactivity of the different samples was assayed after direct plating of 1 mg of the substances on aluminium planchettes. A thin window G-M tube was used. The background was about 40 cpm and at least 1000 counts were counted for each sample.

Further experimental details are given in the text to the respective figures.

RESULTS

Hydrolysis of triolein containing free labelled oleic acid by bile-pancreatic juice

In this experiment the lipids recovered after the different times of incubation were separated into two fractions *viz.* free fatty acids and glycerides. In Fig. 1 the specific activities of the glyceride fatty acids are given in per cent of the specific activity of the fatty acids obtained after hydrolysis of the original lipid mixture, that is, the specific activity which would have been obtained if the free labelled fatty acids and the glyceride fatty acids had been completely mixed. The figures obtained in a similar experiment in which corn oil containing free labelled palmitic acid was used are also plotted for comparison. These latter figures are taken from an earlier investigation[1]. From a comparison of the curves in Fig. 1 it is apparent that no difference is found in the rate of incorporation of labelled oleic or palmitic acid into the glycerides of triolein or corn oil respectively during hydrolysis with pancreatic lipase. The differences in specific activity between the glyceride fatty acids and the free fatty acids found in the earlier investigation in which labelled palmitic acid dissolved in corn oil was used were also found when

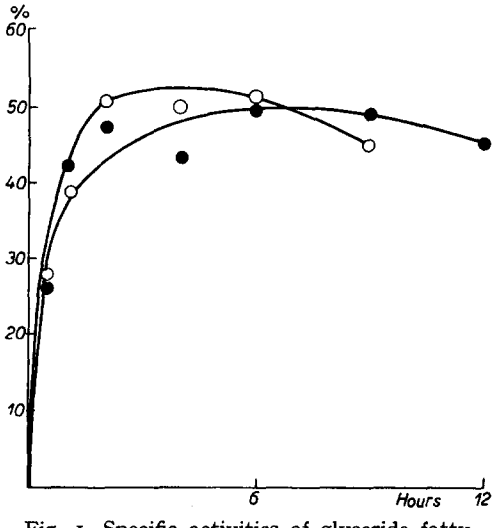

Fig. 1. Specific activities of glyceride fatty acids in per cent of specific activity of the total fatty acid mixture hydrolysed during digestion *in vitro* by rat pancreatic juice. Substrate: triolein containing 1.96% ^{14}C-labelled free oleic acid ●——●, and corn oil containing p.5% ^{14}C-labelled palmitic acid O——O. Initial pH 6.4.

References p. 504.

triolein containing free labelled oleic acid was used and do not appear to be due to enzyme specificity.

If the specific activities of the glyceride fatty acids are calculated in per cent of the specific activity of the free fatty acids at the different times of incubation, a curve is obtained which rapidly reaches a plateau and remains at practically the same level even after 24 hours of incubation. This indicates that an equilibrium is reached between the free fatty acids and the glyceride fatty acids. In this equilibrium, only part of the glyceride fatty acids can participate as the specific activity of the glyceride fatty acids is only about half that of the free fatty acids. The glycerides then must contain fatty acids which are in equilibrium with the free fatty acids and have the same specific activity as these, besides fatty acids which have not been exchanged with the free fatty acids and therefore remain inactive. To study this reaction further the specific activities of the different glyceride fractions formed during the hydrolysis in similar experiments have been investigated.

Specific activities of the fatty acids of tri-, di- and monoglycerides in relation to the specific activity of the free fatty acids during hydrolysis of triolein containing free labelled oleic acid

This experiment was similar to the foregoing except that the glyceride mixture obtained was separated into tri-, di- and monoglycerides on columns of silicic acid.

To the left in Fig. 2 the course of the hydrolysis is seen. From these curves it appears that the hydrolysis of the triglyceride proceeds practically to completion in 24 hours (more than 95% hydrolysis). During the hydrolysis di- and monoglycerides appear.

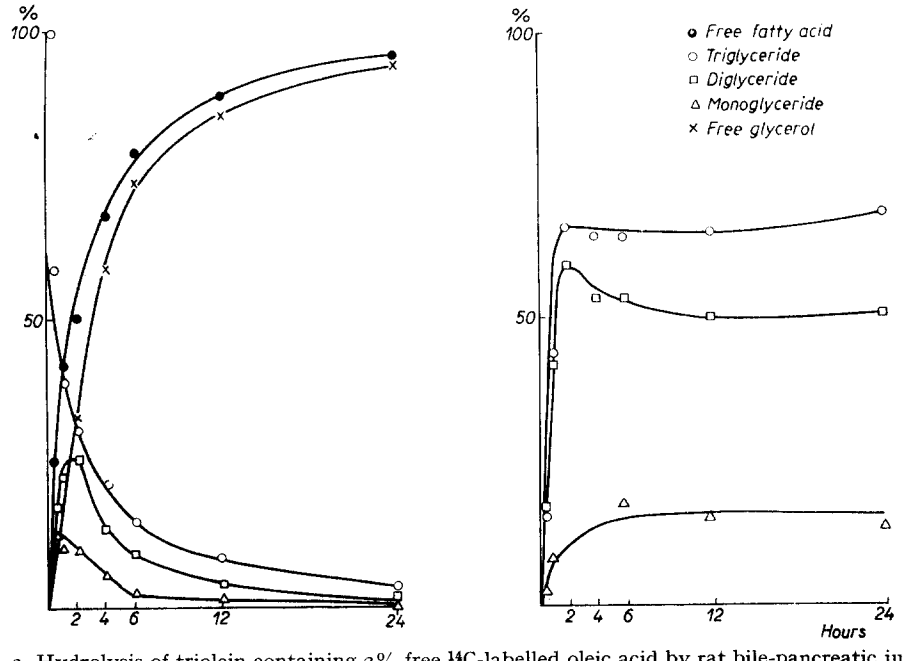

Fig. 2. Hydrolysis of triolein containing 2% free ^{14}C-labelled oleic acid by rat bile-pancreatic juice *in vitro*. Initial pH 6.4. To the left the course of the hydrolysis and to the right the specific activities of the fatty acids of the tri- di- and monoglycerides in per cent of the specific activities of the corresponding free fatty acids.

References p. 504.

Their absolute amounts are largest after about half an hour's hydrolysis for monoglycerides and two hours for diglycerides. During this time the triglycerides have been rapidly degraded and after about 4 hours' hydrolysis the remaining glyceride mixture is composed of about equimolar amounts of tri-, di- and monoglycerides which then slowly disappear at about the same rate. The maximum amount of monoglycerides found is a little above 10% calculated in molar units from 100 moles triglycerides present at the beginning. No accumulation of monoglycerides is found during the later part of the hydrolysis. During the hydrolysis glycerol is rapidly liberated so that after about 3 hours' hydrolysis under these conditions about half of the glycerol molecules of the original triglyceride are in the free form as calculated from the amounts of remaining glycerides and free fatty acids.

From the results of an earlier series of experiments[1], it was calculated that diglycerides did not seem to constitute a significant part of the glycerides formed during the first hours of hydrolysis *in vitro*. These calculations have now been found to be based on erroneous results owing to the unspecificity of the method used for determining monoglycerides in these experiments[4].

In the right part of Fig. 2 the specific activities of the fatty acids of the tri-, di- and monoglycerides in these experiments are given, calculated in per cent of the specific activities of the corresponding free fatty acids. From the curves in this figure it is seen that the specific activities of the triglyceride fatty acids rapidly reached about 65–70%, that of the diglyceride fatty acids about 50% and that of the monoglyceride fatty acids about 15–20% of the specific activities of the corresponding free fatty acids.

A possible explanation of the differences in specific activities of the fatty acids of the different glyceride fractions in this experiment is that the triglycerides contain two fatty acids which are in equilibrium with the free fatty acids and thus have a specific activity identical with that of the free fatty acids, and one fatty acid which remains inactive. In the same way the diglyceride fatty acids would be composed of equal amounts of fatty acids in equilibrium with the free fatty acids and inactive fatty acids. One fatty acid in the tri- and diglycerides then must have a special position in which it is not exchangeable with the free fatty acids. As the 1 and 3 position in the triglycerides must be supposed to be identical, then the 2-position must be the position of the inactive fatty acid in the glyceride molecules which is not exchanged with free fatty acids. If this interpretation of the differences in the specific activities of the fatty acids of the tri- and diglycerides found in this experiment is correct then the diglyceride formed during the hydrolysis with pancreatic lipase must be of the 1.2-configuration.

That the diglyceride formed during the hydrolysis of triglycerides by pancreatic lipase is mainly of the 1.2-configuration was then evidenced in experiments the results of which have been published separately[2].

This indicates that the fatty acids in 1- and 3-position in the glycerides rapidly come into equilibrium with the free fatty acids during hydrolysis by pancreatic lipase while the fatty acids in 2-position are not exchanged and therefore remain inactive under the same conditions.

The specific activities of the monoglyceride fatty acids in the above experiments, and also in similar experiments which are described below, rapidly reached about 10–20% of those of the corresponding free fatty acids. This indicates that the monoglyceride fraction formed during hydrolysis is a mixture of 1-monoglycerides, the fatty acids of which have a specific activity identical with that of the free fatty acids (relative

References p. 504.

specific activity = 100) and of 2-monoglycerides containing inactive fatty acids in the proportion of about one in five to ten. That the activity found in the monoglyceride fractions in these experiments is not the result of admixture of active tri- or diglycerides due to incomplete separations, was shown by the fact that rechromatography of the monoglyceride fractions on silicic acid columns did not result in any significant changes of their specific activity.

A direct estimation of the specific activities of the fatty acids of 1- and 2-monoglycerides formed during hydrolysis of olive oil containing free labelled palmitic acid was also undertaken.

1 ml of olive oil containing free labelled palmitic acid was incubated for two hours at 40° with 10 ml bile-pancreatic juice. After acidification with acetic acid the lipids were extracted with ether. The free fatty acids were separated from the glycerides by passage through a column of Amberlite IRA-400. The fatty acids of the 1- and 2-monoglycerides were then isolated, as earlier described, by counter-current distribution followed by oxidation of the 1-monoglyceride by periodic acid and chromatography on silicic acid[4]. 863 mg total lipids were recovered of which 77.1% were glycerides. From this glyceride mixture 8.1 mg fatty acids from 1-monoglycerides and 13.1 from 2-monoglycerides were isolated. The specific activities of the fatty acids of the 1-mono- and 2-monoglycerides were found to be 73.2 and 10.9% respectively of that of the free fatty acids. These figures clearly show that differences exist in the properties of the fatty acids in the 1- and 2-position in the glycerides and that the 1-monoglyceride found during the hydrolysis of triglycerides thus not only originates from isomerized 2-monoglyceride but, partly at least, has been formed directly from the hydrolysis of the 1.2-diglyceride by splitting off the ester bond in 2-position. The fatty acid in 1-position has a specific activity near to that of the free fatty acids while that of the fatty acid in 2-position is low. The figures obtained are only approximate since the separation of the fatty acids in 1- and 2-position is incomplete with the method used[4].

Hydrolysis of 1.2-diolein containing free labelled oleic acid by bile-pancreatic juice

This experiment was undertaken to determine whether the incorporation of labelled free acid into the glycerides during the course of hydrolysis by pancreatic lipase is due to a transacylation process or if a resynthesis of glyceride ester bonds occurs. For this reason enzymically prepared 1.2-diolein was incubated with bile-pancreatic juice in the presence of free labelled oleic acid. The results are seen in Fig. 3. To the left the composition of the glyceride mixture after different times of incubation is seen. It is apparent that during the hydrolysis of the 1.2-diglyceride by pancreatic lipase triglycerides have been synthesized from the diglycerides and free fatty acids. At most about 1/5 of the original diglyceride molecules have been converted into triglycerides by synthesis of new glyceride ester bonds.

This finding proves that the incorporation of free fatty acids into glycerides during the course of the hydrolysis by pancreatic lipase is due, partly at least, to a synthesis of new glyceride ester bonds. The high rate of incorporation of labelled free acids into the glycerides indicates, however, that an exchange of esterbond fatty acids and free fatty acids also takes place.

To the right in Fig. 3 the specific activities of the tri-, di- and monoglyceride fatty acids are given in per cent of the specific activities of the corresponding free fatty acids present at that time. These curves show about the same course as in the experiment

References p. 504.

where triglyceride containing free labelled acid was hydrolysed, but a gradual increase of the curves for all three glyceride fractions is more apparent. The explanation of this is at present not clear. The mutual relation between the curves is, however, the same as in the earlier experiment and indicates that the fatty acid in 2-position in the diglyceride is not exchanged with free labelled fatty acids. Probably a resynthesis from monoglyceride to diglyceride also takes place under the same conditions. If this is the case then a resynthesis from 1-monoglyceride is very unlikely to be of any greater importance for the specific activity of the fatty acids of the diglyceride then should not remain at a level of about 50% of that of the free fatty acids, as found in the foregoing experiment, but rapidly come into equilibrium with the free fatty acids.

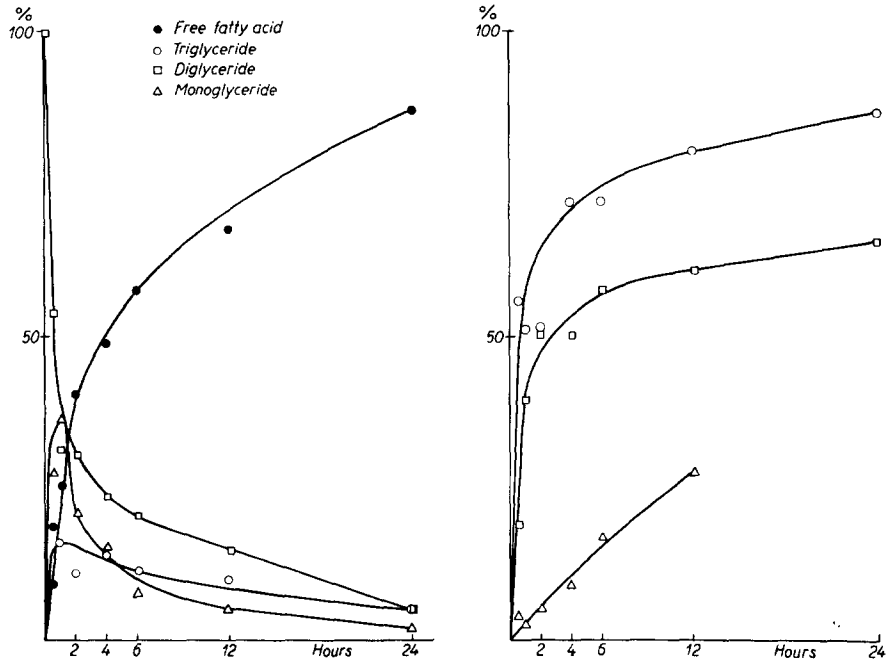

Fig. 3. Hydrolysis of enzymically prepared 1.2-diolein containing free labelled oleic acid by rat bile pancreatic juice. Initial pH 6.4. To the left the course of the hydrolysis calculated in molar units. To the right the specific activities of the fatty acids of the tri-, di- and monoglycerides in per cent of specific activities of the corresponding free fatty acids.

Hydrolysis of triglycerides in the presence of labelled free glycerol

This experiment was undertaken to investigate the extent to which a synthesis of glycerides from free fatty acids and glycerol takes place under the conditions of these experiments by the action of pancreatic lipase.

The reaction mixture used contained about 250 mg olive oil and 6 ml bile-pancreatic juice (pH 6.2) in which 7.2 mg ^{14}C-labelled glycerol had been added. The total activity in the free glycerol added was 720,000 cpm. After 6 hours' incubation at 40° 150 mg glyceride mixture was recovered with a specific activity of 8 ± 2 cpm and mg. The background was 42 cpm. The amount of glycerides synthesized from labelled glycerol and free fatty acids was very small, if any. The equilibrium for the reaction monoglyceride $\rightleftharpoons$ free fatty acid + glycerol then must be very far to the right. The overall

References p. 504.

hydrolysis of glycerides by pancreatic lipase under the conditions of these experiments must consequently go almost to completion in spite of the fact that simultaneously with the hydrolysis tri- and probably diglycerides are synthesized from di- and monoglycerides, respectively.

Hydrolysis of triolein containing free labelled oleic acid by pure pancreatic juice plus sodium tauro-cholate

From the curves in Fig. 3 it is apparent that at the pH used (6.4) no obvious differences exist in the course of the hydrolysis or in the formation of lower glycerides if pancreatic juice alone, or pancreatic juice together with tauro-cholic acid in a concentration of 0.3%, is used for the hydrolysis*. The course of the hydrolysis seen in these experiments with pure pancreatic juice and pancreatic juice in the presence of tauro-cholic acid is also principally the same as when a mixture of bile and pancreatic juice was used.

Lower relative s.a. of the monoglyceride fatty acids in the series where no bile acids were present seem to be the only differences observed. Possibly this may indicate that in the absence of bile acids the hydrolysis of 1.2-diglyceride to 2-monoglyceride is even more predominant.

Rate of incorporation of some different labelled acids into glycerides during hydrolysis

The labelled acids used in these experiments were ^{14}C-labelled butyric, decanoic and dihydroxistearic acid which were dissolved in olive oil in the concentrations given in Table I. After six hours' hydrolysis the lipids were extracted and separated into glycerides and free fatty acids as usual. In Table I the specific activities of the glyceride fatty acids are given in per cent of the specific activity of the corresponding free fatty acids. For comparison one similar experiment was also performed with triolein containing free labelled oleic acid as substrate. From the figures in Table I it is apparent that decanoic acid is built into the glycerides during hydrolysis to the same extent as is oleic acid. From the foregoing it is clear that the maximum extent of incorporation

TABLE I

Incorporation of different ^{14}C-labelled acids into glycerides during hydrolysis *in vitro* by rat bile-pancreatic juice. pH 6.4.

Substrate: 250 mg olive oil containing ^{14}C-labelled:	*% of labelled substance in substrate at the beginning*	*% hydrolysis after 6 hours*	*Relative specific activities* of the glyceride acids after hydrolysis*
Oleic acid	2	58.2	51.8
Decanoic acid	1	56.0	47.7
Decanoic acid	25	64.4	48.7
Butyric acid	9	51.5	0.9
Butyric acid not hydrolysed	9	—	0.02
Dihydroxystearic acid	0.14	60.7	26.4

* Relative specific activities of the glyceride fatty acids = specific activities of the glyceride fatty acids in per cent of the specific activities of the corresponding free fatty acid.

* After the completion of this investigation it has been found that at a pH of about 6.4, lower concentrations of taurocholic than 0.3% accelerates the rate of hydrolysis of olive oil by pancreatic lipase[20].

References p. 504.

into glycerides of a free fatty acid is reached when the specific activities of the fatty acids in the 1- and 3-positions in the glycerides are the same as those of the corresponding free fatty acids. This equilibrium apparently is reached inside 6 hours for oleic acid as for decanoic acid.

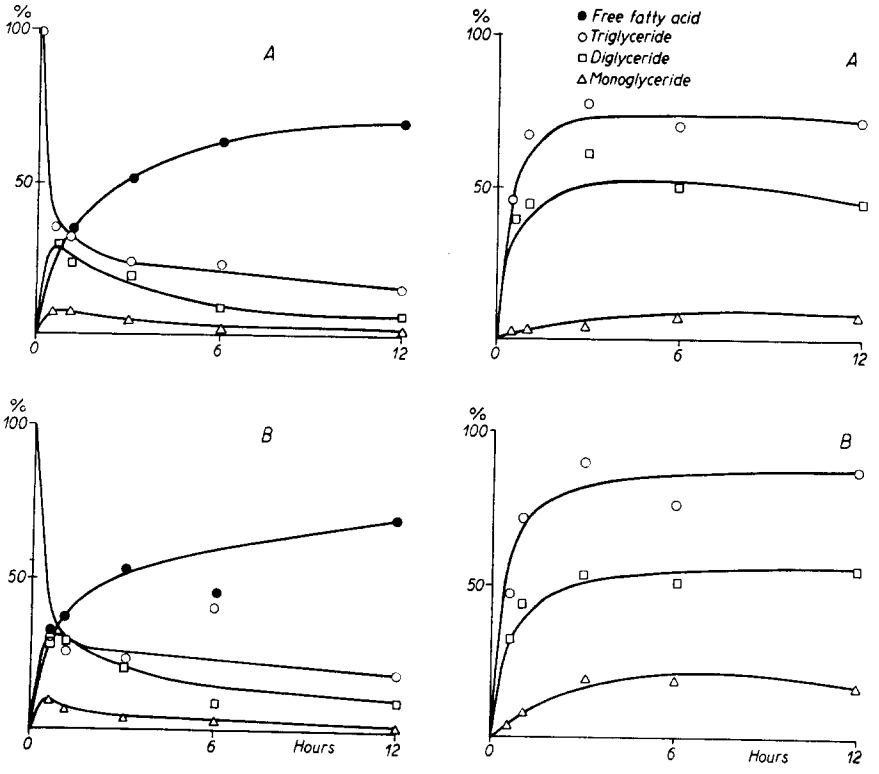

Fig. 4. Hydrolysis of olive oil containing 0.7% free ^{14}-C labelled palmitic acid by rat pancreatic juice and rat pancreatic juice in the presence of 0.3% synthetic taurocholic acid. 20 ml rat pancreatic juice were added with 20 ml water and 10 ml 0.1 *N* hydrochloric acid giving a pH of 6.4. Of this mixture 4 ml were taken to test tubes containing 220 mg substrate. To each tube was then added in series A 2 ml water and to series B 2 ml of a solution containing 18 mg Na taurocholate. To the left in the figure the course of the hydrolysis and to the right the specific activities of the glyceride fatty acids in per cent of the specific activities of the corresponding free fatty acids are seen. The symbols are the same as in Figs. 2 and 3

With butyric acid as the free labelled acid, on the other hand, practically no incorporation into glycerides was observed under the same conditions while dihydroxystearic acid was built into glycerides, but at a rate lower than that for decanoic and oleic acid so that after six hours' incubation time the relative specific activity of the glyceride fatty acids was only about half that reached for oleic and decanoic acid.

The effect of pH and Ca ions on the rate of hydrolysis and synthesis of glycerides by pancreatic lipase

In figure 5 the pH/activity curve of rat pancreatic lipase with olive oil as substrate is seen. The pH optimum is close to 8 and at pH values above 10 and below 5 the enzyme

References p. 504.

is inactivated. The values for the specific activities of the glyceride fatty acids in the same experiments calculated in per cent of the corresponding specific activities of the free fatty acids are also given in the same figure. The relative specific activities of the glyceride fatty acids must be assumed to be proportional to the rate of resynthesis of glyceride ester bonds during the hydrolysis and show a course parallel to that of the rate of hydrolysis, indicating that the hydrolysis and resynthesis are effected by the same enzyme system.

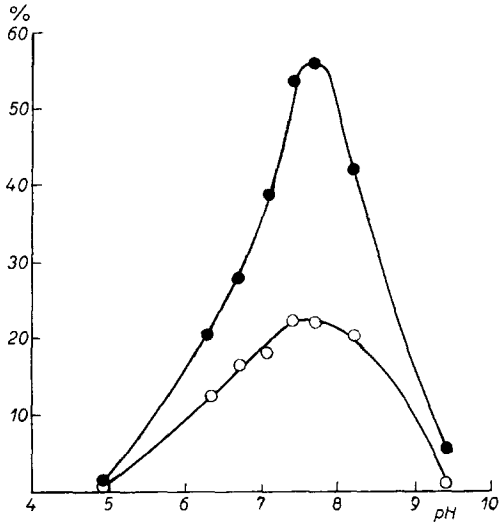

Fig. 5. Effect of pH on the rate of hydrolysis ●——● and specific activities of the glyceride fatty acids in per cent of the specific activities of the corresponding free fatty acids O——O during hydrolysis *in vitro* of olive containing 0.7% free labelled palmitic acid by rat pancreatic juice. No bile acids were present. Incubation time half an hour.

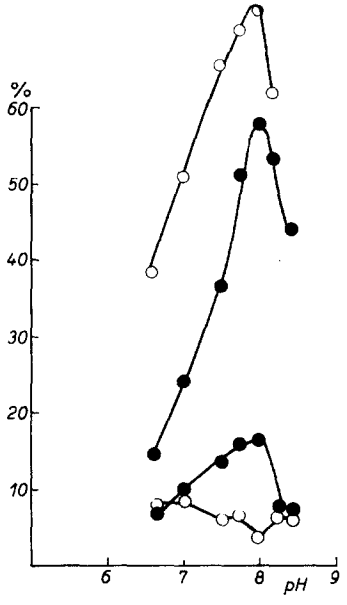

Fig. 6. Effect of Ca ions on the rate of hydrolysis (above), and specific activities of the glyceride fatty acids in per cent of the corresponding free fatty acids (below), during hydrolysis *in vitro* of olive oil containing 0.7% labelled palmitic acid by pancreatic juice. ●——● In the absence of Ca ions. O——O In the presence of one atom Ca for each fatty acid molecule that could be liberated.

Addition of Ca ions to the pancreatic juice accelerates the rate of hydrolysis of triglycerides at all pH values as can be seen from Fig. 6. At the same time a decreased rate of resynthesis of glyceride ester bonds is effected by the addition of Ca ions since the curve for the relative specific activities of the glyceride fatty acids is lower in the experiment in which Ca ions were added.

DISCUSSION

The previously demonstrated exchange of glyceride fatty acids and free fatty acids occurring during the hydrolysis of glycerides by pancreatic lipase[1] has in the present investigation been found to be, partly at least, due to a resynthesis of glyceride ester bonds taking place simultaneously with the hydrolysis.

The synthetic effect of pancreatic lipase *in vitro* has already been demonstrated by many workers[9,10]. These experiments have, however, generally been performed in or-

References p. 504.

ganic solvents in the presence of only minute amounts of water and with the alcohol in large excess. FABISCH[11] claimed to have demonstrated a synthesis of glycerides from glycerol and long chain fatty acids emulsified in water, by the action of pancreatic lipase. No glycerides were, however, isolated.

In our experiments *in vitro*, in conditions resembling those prevailing in the lumen of the small intestine during digestion, *i.e.* in the presence of large amounts of water, a synthesis of glycerides from glycerol and free fatty acids could not be demonstrated. Under these conditions the synthetic effect of the pancreatic lipase was found to be limited to an esterification of alcohol groups of partial glycerides with free fatty acids.

In the presence of an excess of water the reaction glycerol + free fatty acid $\rightleftharpoons$ monoglyceride + water must be so far to the left that this reaction for practical purposes can be considered as irreversible. Probably the lipase acts at the interface lipid phase/water phase and has a very low affinity for the water soluble free glycerol. Under the same conditions the reaction diglyceride + fatty acid $\rightleftharpoons$ triglyceride and probably the reaction monoglyceride + fatty acid $\rightleftharpoons$ diglyceride apparently must be such that a rapid exchange of glyceride fatty acids and free fatty acids takes place during hydrolysis.

The results obtained in this investigation indicate that only the fatty acids in the 1- and 3-position in the glyceride molecule come into equilibrium with the free fatty acids, while fatty acids in 2-position are not exchanged at all during the course of the hydrolysis by pancreatic lipase *in vitro*. From these results it seemed probable that the diglyceride formed during hydrolysis should be of the 1.2-configuration. That this is the case has also been evidenced in experiments the results of which have been published separately[2] and is in agreement with the suppositions put forward by SCHÖNHEYDER AND VOLQVARTZ[12] and by MATTSON, BENEDICT, MARTIN AND BECK[13].

The course of the further hydrolysis of the 1.2-diglyceride by pancreatic lipase has been difficult to establish owing to the limitations of the analytical methods available for the quantitative determination of 1- and 2-monoglycerides of a glyceride mixture, and to the readiness with which 2-monoglycerides are isomerized to 1-monoglycerides. MATTSON, BENEDICT, MARTIN AND BECK[13] found that a portion of the monoglyceride fraction formed during hydrolysis by pancreatic lipase is of the 2-configuration and they believe that almost all of the monoglyceride initially formed is the 2-isomer. After the isolation of the monoglyceride fraction formed during pancreatic lipolysis *in vitro* by counter-current distribution, BORGSTRÖM[2] determined that about 20% thereof was the 1-isomer. It was, however, impossible from these experiments to elucidate whether this 1-isomer was formed initially or by isomerization of 2-monoglycerides after the hydrolysis. The results of the present investigation indicate that the 1.2-diglyceride is degraded both to the 1- and 2-isomer but that about 5 to 10 times as many 2-monoglyceride as 1-monoglyceride molecules are initially formed on hydrolysis.

The resynthesis of glyceride ester bonds during hydrolysis of glycerides has as yet only been evidenced for the step 1.2-diglyceride to triglyceride but probably a synthesis also occurs from 2-monoglycerides. Our results indicate that a synthesis from 1-monoglycerides is unlikely to be of any importance.

That an exchange of glyceride fatty acids and free fatty acids, similar to that found *in vitro* also takes place *in vivo*, that is in the lumen of the small intestine during digestion, has been demonstrated earlier[1,14]. However, a continuous admixing of unchanged glycerides from the stomach and of lipids contained in the bile and other

References p. 504.

secretions to the intestinal content as well as absorption from the intestine make an interpretation of the course and the extent of this process *in vivo* difficult[14].

That no significant synthesis of glycerides from free fatty acids and glycerol takes place under the conditions in the lumen of the small intestine during digestion has been found earlier[1,15,16].

As the reaction monoglyceride $\rightleftharpoons$ glycerol + free fatty acid is practically irreversible *in vitro* in the presence of large amounts of water, the hydrolysis of triglycerides by pancreatic lipase must go to completion under these conditions *in vitro*. This is also indicated to be the case in our experiments where a mixture of bile and pancreatic juice from rats was used for the hydrolysis. The rate of hydrolysis is more rapid in the beginning than in the later part, apparently because an equilibrium is soon reached between synthesis and hydrolysis of tri- and diglycerides. The overall hydrolysis, however, slowly proceeds to completion as monoglycerides are hydrolysed but not resynthesized.

The pH/activity curve for rat pancreatic lipase was found to have a rather sharp maximum at a pH of about 8 in the absence of bile acids and with olive oil as substrate. This is in accordance with the results obtained by SCHÖNHEYDER AND VOLQVARTZ[17] for pig pancreas lipase. No effect of bile acids in a concentration of 0.3% ont the rate of hydrolysis of triglycerides was found at a pH of about 6.4. At other pH values and concentrations of bile acids, however, accelerating and inhibitory effects of tauro-cholic acid on the rate of hydrolysis have been found. These effects are under investigation[20].

The accelerating effect of Ca ions on the rate of hydrolysis of triglycerides by pancreatic lipase in the absence of bile acids as found in the present investigation, is similar to that found earlier by SCHÖNHEYDER AND VOLQVARTZ[17] and occurs both in alkaline and acid media. SCHÖNHEYDER AND VOLQVARTZ found that this effect of Ca ions could not be due to the formation of "complex adsorbates" as proposed by WILLSTÄTTER *et al.*[18] because addition of calcium soaps was found to have no effect on the rate of hydrolysis. The results of the present investigation show that the addition of Ca ions not only accelerates the rate of hydrolysis but also decreases the rate of resynthesis of glyceride ester bonds. Possibly the decreased rate of resynthesis effected by the Ca ions can, partly anyhow, be explained as the result of a removal of fatty acid as soaps from the oil/water interface in which the enzyme is probably situated.

It is apparent from the results of this investigation that the rate of resynthesis of glyceride ester bonds by pancreatic lipase is different for different acids. The determining factor for the rate of resynthesis is probably complex. That the short chain butyric acid is not resynthesized to any appreciable extent could be an indication that the extent of solubility in water of the acid is of importance. However, that it is not only the solubility in water or the "lipophility" of the acid which determines the rate of resynthesis is indicated by the fact that decanoic acid is incorporated to the same extent as the long chain oleic and palmitic acid in our experiments, while the rate of incorporation of dihydroxystearic acid is lower. In another investigation it has been found that the highly fat soluble unsubstituted cholanic acid is scarcely incorporated into glycerides at all by pancreatic lipase under the same conditions[19]. This indicates that enzyme specificity is also of importance.

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References p. 504.

SUMMARY

During hydrolysis of triglycerides of long chain fatty acids by pancreatic juice from rat an exchange occurs between glyceride fatty acids and liberated free fatty acids. In this exchange only fatty acids in the 1- and 3-position of the glyceride take part and these fatty acids rapidly come into equilibrium with the free fatty acids.

The exchange between the glyceride fatty acids and the free fatty acids is at least partly due to a resynthesis of glyceride ester bonds occurring simultaneously with the hydrolysis. This resynthesis has been evidenced for the step 1.2-diglyceride to triglyceride. Under the conditions of the experiments, resembling those prevailing during digestion in the lumen of the small intestine, no resynthesis takes place from glycerol and fatty acids by the action of pancreatic juice and the overall hydrolysis of glycerides accordingly proceeds to completion *in vitro*.

The results of this investigation indicate that the hydrolysis of glycerides by pancreatic juice enzyme proceeds via the 1.2-diglyceride to both 1- and 2- monoglycerides, the hydrolysis to the 2-monoglyceride prevailing.

The pH/activity curves for the hydrolysis and resynthesis of glyceride ester bonds by pancreatic juice enzyme with olive oil as substrate in the absence of bile acids are parallel and show a maximum at pH about 8.

Addition of Ca ions accelerates the rate of hydrolysis of triglycerides by pancreatic juice both in acid and alkaline media. The increased rate of hydrolysis brought about by Ca ions is parallel to a decreased rate of resynthesis of glyceride ester bonds.

Different fatty acids are built into glycerides during hydrolysis with pancreatic enzyme at different rates.

RÉSUMÉ

Pendant l'hydrolyse des triglycérides des acides gras supérieures par du suc pancréatique de rat il y a un échange entre les acides gras liés au glycérol et les acides hydrolysés. Seules les chaînes liées en position 1 et 3 du glycérol prennent part à cet échange; un equilibre s'établit rapidement entre eux et les acides gras libérés.

L'échange entre les acides gras du glycéride et les acides gras libres dépend au moins partiellement d'une résynthèse des esters du glycérol qui se forment en même temps que la scission a lieu. Cette résynthèse a été pour le passage du diglycéride-1.2 au triglycéride. Sous les conditions de nos expériences qui ressemblent à celles de l'intestin grêle, aucune synthèse n'a lieu à partir du glycérol et des acides gras sous l'influence du suc pancréatique et c'est pourquoi la scission *in vitro* est totale.

Les résultats de nos expériences montrent que l'hydrolyse des glycérides par le suc pancréatique précède en passant par les diglycérides1-1.2 aux monoglycérides-1 et -2, l'hydrolyse aboutissant aux monoglycérides-2 étant dominante.

Les courbes pH/activité pour l'hydrolyse et la résynthèse des liaisons éther-sel des glycérides par l'enzyme du suc pancréatique, avec l'huile d'olive comme substrat et en l'absence d'acides billaires, sont parallèles et montrent un maximum à environ pH = 8.

En présence des ions Ca^{+2}, l'hydrolyse des triglycérides par le suc pancréatique est accélérée aussi bien en milieu acide qu'alcalin. L'augmentation de la vitesse d'hydrolyse occasionnée par les ions Ca^{++} va de pair avec une diminution de la vitesse de résynthèse des liaisons éther-sel des glycérides.

Pendant l'hydrolyse par l'enzyme du suc pancréatique les différents acides gras sont incorporés dans les glycérides avec des vitesses différentes.

ZUSAMMENFASSUNG

Während der Hydrolyse von Triglyceriden langkettiger Fettsäuren durch Pankreassaft aus Ratten kommt es zu einem Austausch zwischen den Glyceridfettsäuren und abgespaltenen Fettsäuren in der 1- und 3-Stellung des Glycerinsteil. Sie kommen rasch in ein Gleichgewicht mit den freien Fettsäuren.

Der Austausch zwischen Glyceridfettsäuren is zumindest teilweise bedingt durch eine Resynthese der Glycerid-Ester-Bindung, die gleichzeitig mit der hydrolytischen Spaltung vor sich geht. Diese Resynthese wurde bewiesen für den Schritt vom 1.2-Diglycerid zum Triglycerid. Unter experimentellen Bedingungen, die denen, die im Dünndarm vorherrschen, gleichen, findet keine Resynthese aus Glycerin und Fettsäuren durch die Wirkung von Pankreassaft statt und demzufolge geht die totale Hydrolyse *in vitro* vollständig vor sich.

Die Ergebnisse dieser Untersuchungen zeigen, dass die Spaltung von Glyceriden durch Pankreassaft-Enzym über 1.2-Diglyceride zu 1- und 2-Monoglyceriden verlaüft. Die Aufspaltung zum 2-Monoglycerid herrscht vor.

References p. 504.

Die pH-Aktivitätskurven für die Hydrolyse and die Resynthese der Glycerid-Ester-Bindung durch Pankreassaft-Enzym mit Olivenöl als Substrat und in Abwesenheit von Gallensäuren verlauten parallel und zeigen ein Maximum ungefähr bei pH 8.

Zugabe von Ca-Ionen beschleunigt die Hydrolysengeschwindigkeit von Triglyceriden durch Pankreassaft in alkalischem und in saurem Milieu. Die zunehmende Hydrolysengeschwindigkeit, die durch die Ca-Ionen hervorgebracht wird, läuft parallel mit einer abnehmenden Resynthese der Glycerid-Ester-Bindung.

Während der Hydrolyse mit Pankreassaft-Enzym werden verschiedenartige Fettsäuren mit verschiedener Geschwindigkeit in die Glyceride eingebaut.

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