# Digestion of the mono- and diesters of hexane-1,6-diol by pancreatic lipase

**F. H. Mattson and R. A. Volpenhein**

Procter & Gamble Company, Miami Valley Laboratories, Cincinnati, Ohio 45239

**Abstract** The digestion in vitro by pancreatic lipase (EC 3.1.1.3) of the mono- and dioleate esters of hexane-1,6-diol has been studied. Under the conditions employed, the pathways for the lysis of these materials are proposed to be a hydrolysis step

diester → monoester + free fatty acid

and a transesterification step

2 monoesters → diester + free alcohol.

If only the diester is present initially, it is hydrolyzed at a continuously decreasing rate with an accumulation of monoester. When the ratio of bulk concentration of diester to monoester i approximately 2.5 to 1, the diester and monoester are lysed at the same rate. As digestion continues, the amount of diester decreases but the amount of monoester remains constant. This behavior is attributed to the greater surface activity of the monoester, which causes the accumulation of this species at the oil–water interface.

**Supplementary key words** fatty acid ester hydrolysis · transesterification · hexanyl-1,6-dioleate · hexanyl-1-oleoyl-6-ol · pancreatic lipase · interfacial reactions · surface activity

Pancreatic lipase (EC 3.1.1.3) is active only at an interface (1) and hydrolyzes specifically esters of primary alcohols (2). Thus, the ester linkages at the 1- and 3-positions of a water-insoluble triglyceride are hydrolyzed, but the ester linkage at the 2-position is not (3). In the course of our studies of the digestion of various esters by this enzyme, triglycerides, of all the substrates investigated, were found to be hydrolyzed most rapidly. Others (4) have reported that triglycerides are hydrolyzed more rapidly than are methyl esters. These observations suggested the likelihood that the 1- and 3-positions of triglycerides were particularly susceptible to attack by this enzyme, possibly because of the spatial relationships of the ester linkages in a triester of glycerol. To investigate this possibility, the hydrolysis of a number of model compounds is being studied. The effect of alkyl and acyl chain length on the hydrolysis of esters of *n*-alcohols has been reported (5).

Another series of compounds in this investigation are esters of alcohols of the general structure

$$HOC(CH_2)_nCOH.$$

The natural occurrence and physical and chemical properties of such alcohols and their esters have been reviewed (6). However, little is known about the metabolism, including the digestion and absorption of such compounds. Long et al. (7) have reported that propylene glycol distearate is hydrolyzed by steapsin, a preparation of pancreatic tissue. Hofmann and Borgström (8) found that the monooleate ester of ethylene glycol was hydrolyzed by rat pancreatic juice. Neither report gives any details of the mechanism. We are reporting the results of our experiments with one member of this series, namely the mono- and diesters of hexane-1,6-diol, because of the unexpected pathway of lysis of these compounds.

## METHODS

Oleic acid was isolated from olive oil. [1-$^{14}$C]Oleic acid was purchased from Tracerlab, Waltham, Mass., and the hexane-1,6-diol from Matheson Coleman & Bell, Norwood, Ohio. After purification, these materials were analyzed by thin-layer and gas–liquid chromatography and were found to be better than 99% pure. The mono- and diesters were prepared by reacting the appropriate amount of alcohol with oleoyl chloride (9). The products were purified by crystallization from acetone at −35°C and by silica gel column chromatography (benzene was used for elution of the diester, and benzene–ethyl ether 90:10 for the monoester) until thin-layer chromatography (developing solvents were ethyl ether–hexane

15:85 for the diester fraction and 50:50 for the monoester fraction) showed the presence of only the desired ester.

The lipolytic enzymes, other than lipase, in rat pancreatic juice were inactivated by keeping a pH 9 solution of reconstituted juice at 40°C for 1 hr. The methods of obtaining, storing, and treating the pancreatic juice have been described previously (10).

Digestions were carried out in flasks mounted on a wrist-action shaker. The contents of the flasks were 0.1 M Tris, 1 M NaCl, 330 μmoles of $CaCl_2$, substrate, and, unless otherwise indicated, 1.2 mg of treated pancreatic juice powder in a total volume of 55 ml at a pH of 8.0 and at 25°C (10). At the end of the digestion period, the contents of the flasks were acidified to pH 2 with HCl and the lipids were extracted with ethyl ether. The free fatty acid content of a portion of the sample was determined by electrometrically-monitored titration in chloroform–ethanol 3:1 to which was added one drop of saturated aqueous NaCl. From the remainder of the sample, free fatty acids were removed by the ion-exchange resin IRA-400, and the esters then were fractionated by chromatography on a silica gel column. Purity of the fractions was confirmed by thin-layer chromatography. The amounts of mono- and diesters eluted from the column were determined by weighing. Experiments with model mixtures gave recovery values of 95%. The amount of free alcohol was obtained by subtracting the sum of the mono- and diesters present after digestion from the amount of esterified alcohol present initially. Since the errors of both the mono- and diester determinations accumulated in this value for the free alcohol, the figure was confirmed by comparing the loss in ester groups with the increase in free fatty acid. Radioactivity was determined by liquid scintillation counting on a Packard model 3375B counter after dissolving the sample in 10 ml of ethanol–toluene 1:1 with added POPOP (0.4%) and PPO (0.05%).

In the tables and in Figs. 1–3, the values reported are as fatty acid equivalents for the changes in the amounts of starting materials and of their digestion products. Consequently, the μmoles of diester are one-half the fatty acid value that is given; the values for μmoles of monoester and its fatty acid equivalent are, of course, equal. In these figures, the number in parentheses on each line is the slope for the linear portion of that line in μmoles of fatty acid per minute. A minus sign preceding the number indicates that material to be decreasing during the course of the digestion.

## RESULTS

The lipid compositions after various periods of hydrolysis of hexanyl-1,6-dioleate are shown in Fig. 1.

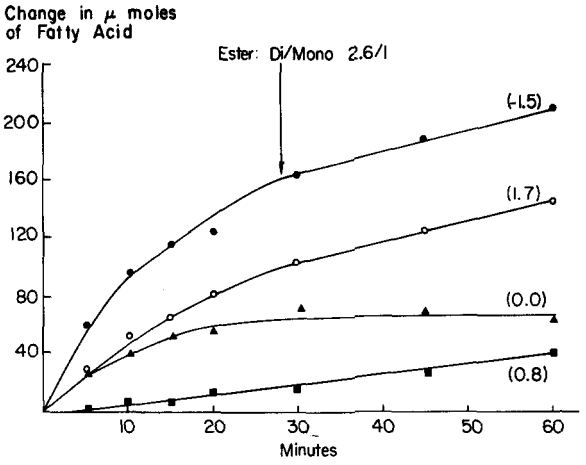


FIG. 1. Products of the lysis of hexanyl-1,6-dioleate by 1.2 mg of lyophilized pancreatic juice. The initial amount of dioleate ester was 225 μmoles, equivalent to 450 μmoles of fatty acid. Values shown are for change in fatty acid equivalents. Each number in parentheses is the slope of that line in μmoles fatty acid/min between 30 and 60 min. ●, diester; ▲, monoester; ○, free fatty acid; ■, free alcohol.

The linear portions of the lines to which the rate values refer are from 30 to 60 min. The rate of disappearance of the diester was initially rapid but then became slower, attaining a constant value of 1.5 μmoles as fatty acid (0.8 μmole diester) per minute. The amount of monoester initially increased and then remained unchanged at about 70 μmoles. Although the amount became constant, monoester was being lysed throughout almost the entire digestion period, if the constant rate (0.8) of appearance of free alcohol is a criterion of monoester disappearance.

Mixtures containing various proportions of diester and of fatty acid-labeled monoester, but with the total μmoles of the two compounds being the same, were digested for 20 min. The changes in the composition of the lipids and in the distribution of radioactivity among the lipids are shown in Table 1. When only monoester was added to the digest, 32 μmoles on the basis of weight or 21 μmoles on the basis of radioactivity of fatty acid was present as diester after the digestion. Even where there was no net accumulation of diester, the synthesis of this compound is demonstrated by the presence of labeled fatty acid in the diester fraction. The moles o labeled fatty acid appearing as free fatty acid were constant and were approximately the same as the moles of labeled fatty acid appearing in the diester fraction. This relationship held regardless of the total amount of free fatty acid that was formed.

Mixtures containing 250 μmoles each of unlabeled diester and of fatty acid-labeled monoester were digested for various times. The changes in lipid composition and in the distribution of the labeled fatty acid are shown in Table 2. As the time of digestion increased, decreasing

TABLE 1. Change in equivalent μmoles of fatty acid after 20-min digestion of oleate diester and [1-$^{14}$C]oleate monoester of hexane-1,6-diol

| | Digest, μmoles (as FA), Di/Mono | | | | |
|---|---|---|---|---|---|
| | 0/498 | 248/374 | 496/249 | 744/124 | 992/0 |
| | *equivalent μmoles of fatty acid* | | | | |
| Diester | | | | | |
| Unlabeled[a] | (11) | −19 | −50 | −99 | −188 |
| Labeled | 21 | 23 | 24 | 21 | 0 |
| Total | 32 | 4 | −26 | −78 | −188 |
| Monoester | | | | | |
| Unlabeled[a] | (14) | 22 | 36 | 56 | 82 |
| Labeled | −66 | −61 | −53 | −42 | 0 |
| Total | −52 | −39 | −17 | 14 | 82 |
| Free fatty acid | | | | | |
| Unlabeled[a] | (6) | 18 | 34 | 59 | 110 |
| Labeled | 19 | 19 | 19 | 14 | 0 |
| Total | 25 | 37 | 53 | 73 | 110 |
| Summation of total values | −52 | −39 | −43 | −78 | −188 |
| by sign | +57 | +41 | +53 | +87 | +192 |

[a] Unlabeled values were obtained by Total minus Labeled and hence accumulated the errors in the two determinations. Values in parentheses are obviously impossible and represent summed analytical error. The extent of this error is indicated by the summation of the total values by sign, the algebraic sum of which should equal zero.

amounts of mono- and diester remained. Although the total amount of diester decreased, there was a progressive increase in the amount of labeled fatty acid in this fraction. Thus, fatty acids that were initially present as monoester were subsequently found in the diester fraction. This increase was approximately the same as the increase in labeled free fatty acid. The total amount of monoester, although decreasing, showed an accumulation of unlabeled fatty acids, which were originally present as diester.

TABLE 2. Change in equivalent μmoles of fatty acid on digesting for various times a mixture of 250 μmoles each of oleate diester and [1-$^{14}$C]oleate monoester of hexane-1,6-diol

| | Digestion Time (min) | | | |
|---|---|---|---|---|
| | 5 | 10 | 15 | 30 |
| | *equivalent μmoles of fatty acid* | | | |
| Diester | | | | |
| Unlabeled[a] | −15 | −20 | −28 | −58 |
| Labeled | 3 | 8 | 12 | 20 |
| Total | −12 | −12 | −16 | −38 |
| Monoester | | | | |
| Unlabeled[a] | 12 | 14 | 12 | 32 |
| Labeled | −10 | −16 | −19 | −44 |
| Total | 2 | −2 | −7 | −12 |
| Free fatty acid | | | | |
| Unlabeled[a] | 2 | 12 | 14 | 30 |
| Labeled | 3 | 7 | 11 | 21 |
| Total | 5 | 19 | 25 | 51 |
| Summation of total values | −12 | −14 | −23 | −50 |
| by sign | +7 | +19 | +25 | +51 |

[a] See footnote to Table 1.

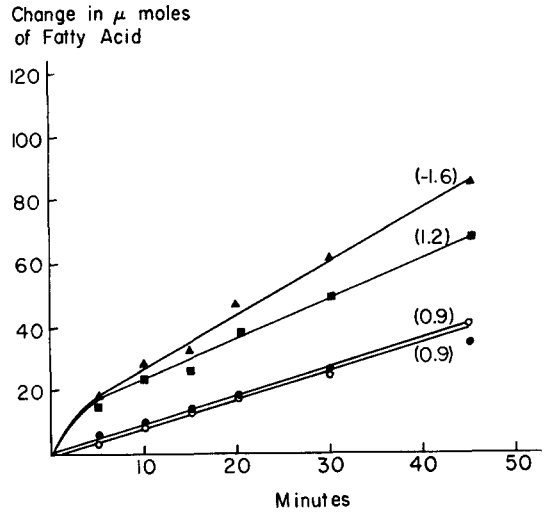


FIG. 2. Lysis products of hexanyl-1-oleoyl-6-ol. The initial amount of monooleate ester was 500 μmoles. Values shown are for change in fatty acid equivalents. Each number in parentheses is the slope of that line in μmoles fatty acid/min between 10 and 45 min. See Fig. 1 for identification of symbols.

Digests containing initially only fatty acid-labeled monoester (500 μmoles) were hydrolyzed for various periods of time. The changes in the composition of the lipids are shown in Fig. 2. The formation of diester can be seen in these experiments as well. The rates of change in the level of the monoester and of the three products were constant between 10 and 45 min. The ratio of these rates of change for monoester decrease, free alcohol increase, free fatty acid increase, and diester (as diester) increase is approximately 4:3:2:1.

500 μmoles of monoester, to which had been added 42 μmoles of labeled free oleic acid, was digested for 60 min. The composition of the lipids and the distribution of the labeled acid are given in Table 3. The 72 μmoles of fatty acids in the diester could have come from either the monoester or the added free fatty acid. As shown by the level of labeled acid, only 2 μmoles were originally present as free fatty acid. If the diester was formed by esterification of monoester, no more than 5%, 2/36, of the esterifying acid was supplied by the added free fatty acid; the main portion, 95%, came from other monoesters.

A mixture of 250 μmoles each of unlabeled monoester and of methyl [1-$^{14}$C]oleate was digested for various periods of time. The changes in lipid composition and in the distribution of the labeled acid are shown in Fig. 3. Hydrolysis of the methyl oleate is shown by the presence of labeled free fatty acid; at 60 min, this amounted to 38 μmoles of the 105 μmoles of total free fatty acid. Of particular importance is the presence in the diester fraction of labeled acid; at 60 min, 15 of the 67 μmoles of fatty acid as diester were labeled acids. In the preceding paragraph it was pointed out that added free

TABLE 3. Composition in equivalent μmoles of fatty acids after digesting for 60 min a mixture of 500 μmoles of the oleate monoester of hexane-1,6-diol and 42 μmoles of [1-$^{14}$C]oleic acid

| | Total | Labeled |
|---|---|---|
| | *equivalent μmoles of fatty acid* | |
| Diester | 72 | 2 |
| Monoester | 385 | 0.3 |
| Free fatty acid | 108 | 37 |

fatty acid was not incorporated into the diester. However, if a fatty acid was present as an ester, either methyl oleate (Fig. 3) or the monooleate ester of hexane diol (Table 1 and Fig. 2), it could participate in the formation of hexanyl dioleate.

Digests initially containing 128 or 490 μmoles of hexanyl-1,6-dioleate were exposed to the action of 1.2 mg of the enzyme preparation. The composition of the lipids was determined after various periods. The patterns of change were similar to those shown in Fig. 1. Thus, there was an initial rapid decrease in diester and an increase in monoester. As the digestion progressed, the rate of hydrolysis of the diester slowed and then became constant in spite of the subsequent, further decrease in the amount of diester. At the time the rate of diester disappearance became constant, the amount of monoester became constant. As shown in Table 4, the rates of change in the amounts of diester and of products were essentially the same in all three experiments. The constant amounts of monoester that were attained depended on the initial content of diester. These levels were realized after 15, 30, and 40 min of digestion, where the initial level of diester was 128, 225, and 490 μmoles, respectively.

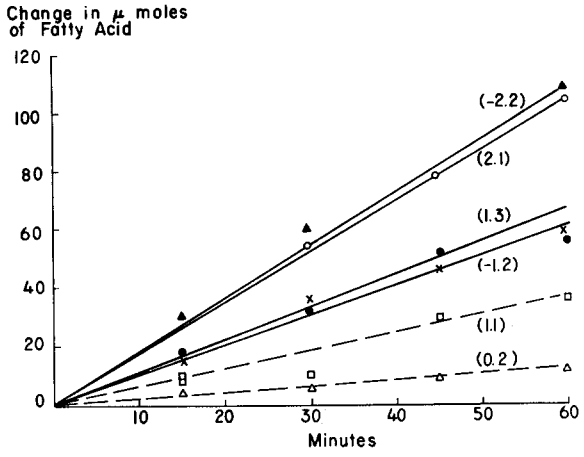


FIG. 3. Lysis products of a mixture of hexanyl-1-oleoyl-6-ol and methyl [1-$^{14}$C]oleate. Digest initially contained 250 μmoles of each ester. Values shown are for change in fatty acid equivalents. Each number in parentheses is the slope of that line in μmoles fatty acid/min between 0 and 60 min. By difference, the rate of appearance of unlabeled diester is 1.1 μmoles of fatty acid/min and of unlabeled free fatty acid is 0.9 μmole/min. □, labeled free fatty acid; △, labeled diester; ×, methyl oleate; see Fig. 1 for identification of other symbols.

TABLE 4. Effect of amount of substrate on the hydrolysis of hexanyl-1,6-dioleate[a]

| Initial Diester | Rate of Change per Minute: Diester | Rate of Change per Minute: Free FA | Rate of Change per Minute: Free Alcohol | Monoester Level Attained | Di/Mono[b] |
|---|---|---|---|---|---|
| *μmoles* | *μeq FA* | *μeq FA* | *μmoles* | *μmoles* | |
| 128 | −1.4 | 1.4 | 0.7 | 30 | 2.8 |
| 225 | −1.5 | 1.7 | 0.8 | 68 | 2.6 |
| 490 | −1.7 | 1.6 | 0.8 | 145 | 2.2 |

[a] The indicated amounts of diester were digested with 1.2 mg of lyophilized pancreatic juice. Values for rate of change are during the period when the level of monoester was constant. Fig. 1 is a plot typical of that obtained from the data for these experiments and is for the experiment in which the initial amount of substrate was 225 μmoles.

[b] Ratio of the amounts of di- and monoester present at the time the amount of monoester became constant.

TABLE 5. Effect of amount of enzyme on the hydrolysis of 225 μmoles of hexanyl-1,6-dioleate[a]

| Enzyme | Rate of Change per Minute: Diester | Rate of Change per Minute: Free FA | Rate of Change per Minute: Free Alcohol | Monoester Level Attained | Di/Mono[b] |
|---|---|---|---|---|---|
| *mg* | *μeq FA* | *μeq FA* | *μmoles* | *μmoles* | |
| 0.6 | −1.3 | 1.1 | 0.5 | 74 | 2.4 |
| 1.2 | −1.5 | 1.7 | 0.8 | 68 | 2.6 |
| 1.8 | −2.8 | 2.8 | 1.5 | 79 | 2.3 |

[a] See footnote to Table 4. Fig. 1 is a plot of the data obtained in the experiment in which 1.2 mg of enzyme was used.

[b] See footnote to Table 4.

A similar series of experiments was carried out in which the level of enzyme was varied but the initial substrate level was constant at 225 μmoles of diester. The results obtained with 1.2 mg of enzyme are shown in Fig. 1. The pattern of the formation of products was similar when 0.6 or 1.8 mg of enzyme was used. The rates of change, once the level of monoester became constant, are shown in Table 5. These values increase with increasing levels of enzyme, but in the three experiments the ratios diester/free fatty acid/free alcohol are all approximately 1/1/0.5. In all three instances, a constant level of monoester, approximately 75 μmoles, was attained.

## DISCUSSION

Pancreatic lipase catalyzes the hydrolysis of triglycerides to partial glycerides and free fatty acids or the reverse reaction, esterification of partial glycerides by free fatty acids. Borgström (11) has shown these reactions to be pH dependent; at an alkaline pH, hydrolysis is

favored, whereas at an acid pH, esterification of partial glycerides by free fatty acid predominates.

The studies described here were carried out at pH 8. Although there was a disappearance of ester groups from both the mono- and diesters, the results are not consistent with stepwise hydrolysis, namely

$$\begin{array}{ccccc} \text{diester} & \rightarrow & \text{monoester} & \rightarrow & \text{free alcohol} \\ & & + & & + \\ & & \text{fatty acid} & & \text{fatty acid} \end{array}$$

nor with simply the reverse of these reactions. We have considered a number of possible mechanisms and find the results to agree best with the following pathways. One of these is hydrolysis

$$\text{diester} \rightarrow \text{monoester} + \text{free fatty acid}$$

and the other is transesterification

$$2 \text{ monoesters} \rightarrow \text{diester} + \text{free alcohol.}$$

The bases for proposing this sequence of reactions are as follows.

There is a synthesis of diester taking place. Thus, in the experiments reported in Figs. 2 and 3 and in the 0/498 digest in Table 1, no diester was present initially. Exposure of the monoester to the enzyme resulted in the formation of diester. Moreover, as shown in Figs. 2 and 3, the quantity of diester was still increasing at the end of 60 min, by which time an appreciable portion of the monoester had disappeared.

The results presented in Tables 1 and 2 show the formation of fatty acid-labeled diester from fatty acid-labeled monoester. This formation took place in the presence of the diester that was present initially. Although diester was being formed, there was no net increase, because hydrolysis was taking place at the same time. The hydrolysis of the unlabeled diester, which was present initially, resulted in unlabeled fatty acids appearing in approximately equal amounts in the monoester and free fatty acid fractions. This ratio is in conformity with the hydrolysis of diester to monoester plus free fatty acid but would not be compatible with the complete hydrolysis of diester to free alcohol and free fatty acid.

The fatty acid for the formation of diester could be supplied by the monoester (Tables 1 and 2 and Fig. 2) or by methyl ester (Fig. 3). However, as shown in Table 3, when labeled free fatty acid was added to the digest it was incorporated into neither diester nor monoester. Thus, the donor fatty acid must be present as an ester—free fatty acid will not serve.

As discussed above, the appearance of equal amounts of fatty acid, which came from the diester, in the monoester and free fatty acid fractions (Tables 1 and 2) demonstrates the formation of monoester from diester. The constant levels of monoester that were attained (Fig. 1 and Tables 4 and 5), after a short period of exposure to the enzyme, must be due to the lysis of monoester at the same rate that it is being formed from diester.

The lysis of the mono- and diesters of hexane-1,6-diol has four characteristics. First, the diester is hydrolyzed to monoester and free fatty acid. Second, over a considerable range of diester concentration, the monoester is lysed as rapidly as it is formed, i.e., at the same rate as the diester is converted to monoester. Third, the free alcohol formed as the result of digestion must arise as an end-product of the lysis of monoester, regardless of the mechanism of this lysis. Fourth, a portion of the fatty acids of the monoesters appears as diesters. As shown in Tables 4 and 5, the relative rates of change of diester (as diester) decrease, free fatty acid increase, and free alcohol increase are 1:2:1. These four characteristics of the reaction and the relative rates of change of the various components are all consistent with the pathways shown in Fig. 4. The value used in this figure for the diester is for the diester itself and hence is one-half the corresponding "as fatty acid" values in Tables 4 and 5.

The lysis of the mono- and diesters occurs by two different mechanisms. The diester is hydrolyzed to monoester and free fatty acid, whereas two monoesters transesterify, with the products being a diester and free alcohol. The occurrence of such transacylation reactions that do not require an energy source has been demonstrated in other systems. Erbland and Marinetti (12, 13) showed that rat liver homogenates could cause an acyl transfer between two molecules of lysolecithin, with the products being lecithin and glycerolphosphorylcholine. This reaction has been demonstrated in a variety of tissues (14). A similar reaction, although actually an alcoholysis, is the esterification of cholesterol by the transfer of a fatty acid from the 2-position of lecithin (15). Such transferase activity is not unusual for hydrolytic enzymes (16).

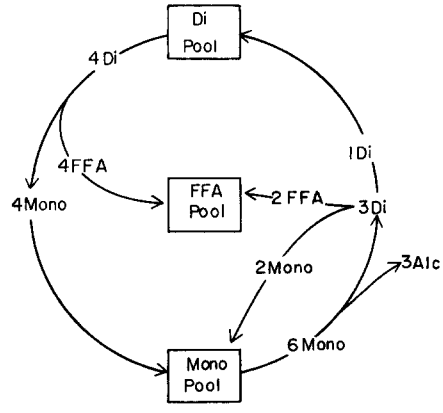


FIG. 4. Proposed mechanism for the lysis of hexanyl-1,6-dioleate (*Di*) and hexanyl-1-oleoyl-6-ol (*Mono*) to the end-products, oleic acid (*FFA*) and hexane-1,6-diol (*Alc*).

It is difficult to say whether such transfers, in this instance two hexanyl monoesters forming a hexanyl diester, are attributable to a specific characteristic of the enzyme or whether they are the result of the reaction taking place at an oil–water interface. The former mechanism could obtain if the reaction involved the formation of a complex consisting of the enzyme and two molecules of monoester with the resulting reaction being the transfer of an acyl group from one monoester to the other. An alternative mechanism is one in which the immediate product of the splitting of the ester is a fatty acid–enzyme complex. The fate of the fatty acid, once this complex disrupts, depends on whether it is ionized or not and on the availability of an acceptor group. The effect of dissociation has been shown in studies (11) in which the acceptor group was not changed. There it was found that at pH 6, where the acids would be mainly undissociated, esterification was favored, whereas at an alkaline pH, and the resulting presence of dissociated acid, hydrolysis predominated. Note that this does not relate to the $pK_a$ of the fatty acid, for we (17), as well as others, have shown that the dissociation of an acid at an oil–water interface is not relatable to its $pK_a$ in the monomeric form. Of equal importance in determining the nature of the reaction is the availability of the fatty acid acceptor. Thus, we (18) have demonstrated that even if the reaction is predominately hydrolysis, formation of hexyl esters takes place as well, if hexanol is added to the system. The amount of esterification, under these conditions, depends on the chain length of the alcohol and is apparently a function of its distribution between the oil and water phases.[1] In the case of the hexanediol monoester, its orientation at the oil–water interface may be such that it is optimally located to act as the fatty acid acceptor at the site where another monoester is cleaved. Indeed, we have noted that the addition of heptane to the digests, which probably alters the structure of the interface, results in some hydrolysis and a decrease in transesterification. Until suitable methods for adequately characterizing an oil–water interface become available, one can only speculate as to the mechanisms of these reactions.

Pancreatic lipase is operative only at an oil–water interface (1). The rate of the reaction is a function of the surface area of the interface (18, 19). The presence of two competing substrates, such as is the case when triglyceride is partially hydrolyzed and the resulting diglyceride is also a substrate, makes meaningful rate studies difficult, if not impossible. Since it is an interfacial reaction, one of the rate-determining factors is the concentrations of the two competing substrates at the interface, rather than their concentrations in the bulk phase. In the case of glyceride hydrolysis there is the further complication that monoglycerides are formed, which, being the most surface-active of the three glyceride species, will concentrate preferentially at the oil–water interface. Similar problems exist in the studies reported here, but they can be more easily identified because of the presence of only two ester species. That the relative rates of disappearance of the mono- and diesters do not depend on their bulk concentration can be seen, for example, in Fig. 1. In the period between 30 and 60 min, the total amount of diester decreased by 28 μmoles, while the amount of monoester was constant at 68 μmoles. During this same period, the relative rates of disappearance of the diester and of the monoester (appearance of free alcohol) were unchanged, which must mean that the relative surface concentration of the two species also was unchanged. This constancy of relative surface concentration was maintained in spite of the change in relative bulk concentration.

The competition of two substrates at an interface is shown also by the results in Tables 4 and 5. When the initial amount of diester was the same, even though the amount of enzyme was changed, the level of monoester attained was about 75 μmoles (Table 5). However, as shown in Table 4, the constant level of monoester that was realized increased from 30 to 68 to 145 μmoles as the initial amount of substrate was increased. In each of these experiments there was an initial rapid decrease of diester and an increase of monoester. When, as indicated by the arrow in Fig. 1, the ratio of diester to monoester attained approximately 2.6 to 1, the level of monoester became constant. The values for this ratio in the other experiments are shown in Tables 4 and 5. That all are about 2.5 is probably due to the bulk concentration of monoester becoming constant at the time the relative surface concentration of the mono- and diesters also becomes constant.

Unfortunately, it is not possible to determine the surface concentration of the two ester species at these high bulk concentrations. The interfacial tensions (Fig. 5) of these two esters and mixtures of them were measured

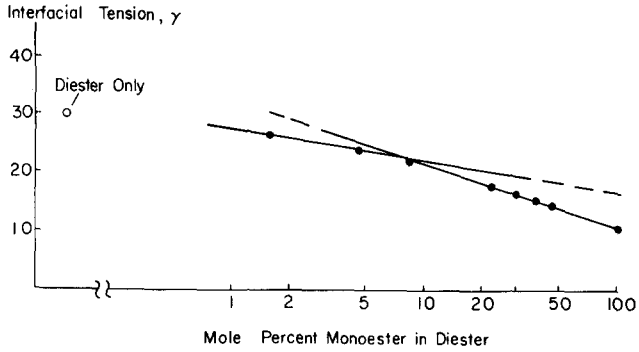


FIG. 5. Interfacial tension of water vs. hexanyl-1-oleoyl-6-ol in hexanyl-1,6-dioleate.

[1] Mattson, F. H., and R. A. Volpenhein: Unpublished experiments.

against water by the pendant drop technique (20, 21). The value for the diester was 29.8 dynes/cm² and for the monoester 10.7. Thus, there would be a surface excess of monoester, if both mono- and diesters were present. An explanation for the change in slope at 8 mole % of monoester is not certain, but it could represent a phase transformation. At a di- to monoester ratio of 2.5 to 1 (approximately 30 mole % monoester), the interfacial tension was 16 dynes/cm². Although it is not possible to calculate the relative interfacial concentration of the mono- and diesters at these bulk concentrations, it seems likely that an appreciable portion of the surface is monoester in spite of the diester being the predominate species in the bulk phase. The nature of an adsorption isotherm is such that where there is a considerable surface excess of a component, a marked increase in the bulk concentration of that component is needed to bring about an appreciable increase in the surface concentration. This relative constancy of surface composition would explain the unchanged rates of mono- and diester hydrolysis in spite of the marked changes in the ratio of the bulk concentration of these two components.

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