Specificity and Other Properties of Lysosomal Lipase of Rat Liver*

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SUMMARY

The pH profile obtained for enzymes in rat liver homogenate which hydrolyze glycerol esters of decanoic acid shows two distinct peaks of activity, one at pH 5 to 5.2 and the other at pH 8.6 to 9. The activity at acid pH is associated with lysosomes, and that at alkaline pH with microsomes. Glycerol trioleate, 1,2-dioleate, and 1-monodecanoate are hydrolyzed by lysosomes in decreasing order of activity, and by microsomes in increasing order of activity. When soluble lysosomal lipase hydrolyzed glycerol tri(oleate-1-14C), the percentage of radioactivity recovered in the products was as follows: oleic acid, 40%; glycerol 1,2-dioleate, 52%; glycerol 1,3-dioleate, 8%; and glycerol monooctyl, 0%. Complete solubilization of lysosomal lipase is achieved by suspending the lysosomal pellet in 0.05 M sodium phosphate buffer, pH 6.8. Lysosomal lipase is strongly inhibited by certain sulfhydryl reagents. A comparison of lipase activity in liver lysosomes with that in Triton WR 1339-filled liver lysosomes, as well as the limitations imposed by the use of Triton-filled lysosomes, is discussed.

LYSOSOMES of rat liver and kidney and of rabbit polymorphonuclear leukocytes (1, 2) have various lipolytic activities toward β-naphthyl acetate (3), lecithin (4), medium chain triacylglycerols (1, 5, 6), long chain triacylglycerols (7), and chylomicron triacylglycerol (2, 8). However, the hydrolysis of partial acyl esters of glycerol and the degradation products of triacylglycerol produced by action of lysosomes are not known. Earlier reports (3, 5, 6, 8) indicate that the major lipolytic activity found in lysosomes is associated with the membrane; no successful method of solubilization of the activity has been available. In this paper, the identification of metabolic products of triacylglycerol hydrolysis by lysosomal lipase and the solubilization and properties of the enzyme of lysosomes from Triton-injected and from normal rats are reported.

EXPERIMENTAL PROCEDURE

Materials—All common chemicals used in the experiments were reagent grade. Glycerol tridecenoate was obtained from Eastman. Triton WR 1339 (oxyethylated tert-octylphenol polyethylene polymer) was from Reger Chemical Company, Inc. (Irvington-on-Hudson, New York). Triton X-100 (octyl phenoxypolyethylenoxyethanol) was from Sigma. The p-nitrophenol esters of various fatty acids were obtained from Nutritional Biochemicals. Glycerol trioleate and 1,3-dioleate were from the Hormel Institute (University of Minnesota, Austin, Minnesota). Glycerol 1,2-dioleate was obtained from Supelec, Inc. (Belfonte, Pennsylvania). Glycerol tri(oleate-1-14C), with a specific activity of 35 mCi per mmole, was obtained from Nuclear-Chicago. Gum arabic was obtained from Sigma. Precast glass plates (Silika Gel P-204, 250 μ, 20 x 20 cm) for thin layer chromatography were obtained from Brinkmann Instruments, Inc. (Westbury, New York). Disopropyl fluorophosphate was obtained from Boots Pure Drug Company, Ltd., Nottingham, England, and sodium diethyldithiocarbamate was obtained from Eastman. Cab-O-Sil (thixotropic gel) was obtained from Beckman. 2,5-Diphenyloxazole (PPO) and 1,4-bis[2-(4-methyl-5-phenyloxazolyl)]benzene (dimethyl-POPOP) were from Packard Instrument Company. Hydroxylapatite was synthesized according to the method of Tiselius, Hjorten, and Levin (9).

Glycerol 1-monodecanoate and glycerol 1-monooctyl were synthesized from 1,2-isopropylideneglycerol (2, 3-dimethyl-1, 3-dioxolane-4-methanol) by the method of Hartman (10) as modified by Anfinson and Perkins (11), except that the final crystallization and further recrystallizations were carried out with 10 volumes of petroleum ether (b.p. 30-60°). Glycerol 1,2-didecanoate was synthesized according to the method of Haws and Malmkn (12). Hydrogenation was carried out in a Parr bomb (Thomas) under a pressure of 40 p.s.i., with palladium black (Sargent) as catalyst. Subsequent crystallization and recrystallizations were done with 5 volumes of petroleum ether at −20°. All of the glycerides used were chromatographically pure (>95%).

Tissue Fractionation—The fractionation of rat liver into the principal subcellular fractions and the purification of liver lysosomes were done according to the method of Ragab et al. (13). Lysosomes prepared by this method purify lysosomal enzymes 25 times over the homogenate; the lysosomes are at best 85% pure. For the preparation of Triton WR 1339-filled liver lysosomes, the livers were homogenized and fractionated according to the method of de Duve et al. (14), and the isolation of lysosomes of about 90% purity was done according to the procedure of Wettiaux, Wibo, and Baudhuin (15).

Fractionation of Lysosomes and Solubilization of Lipase—Lysosomes from normal rats were suspended in a 0.05 M sodium phosphate buffer, pH 6.8 (approximately 10 mg of protein per
amount of radioactive glycerol tri(oleate-1-14C) dissolved in about 50% stream of nitrogen through the dispersion while warming it to temperature, were blended with ice-cold 10% gum arabic solution 30 mM. Solid substrates were dissolved in a small volume of chloroform was mixed with unlabeled glycerol trioleate, also in the same manner. The chloroform was evaporated by passing a stream of nitrogen. Exactly 0.2 ml of chloroform were added, the contents were vigorously mixed with a vortex mixer for 60 sec, and the phases were allowed to separate. A 5-ml aliquot of the chloroform phase was transferred to a conical centrifuge tube, 2 ml of water were added without mixing, and the tube was centrifuged for a few minutes. After removal of all water, exactly 2.0 ml of the chloroform phase (suitably diluted) were mixed with 1.0 ml of 0.1% sodium diethyldithiocarbamate solution in n-butyl alcohol, and the absorbance at 440 nm was read.

This modified method gave essentially the same values as those obtained with the original method (19), and the time required for an assay (5 to 10 min per tube) was only one-third of that required by the original method. This method is not suitable for measurement of fatty acids in the presence of high protein concentration, owing to extensive foam formation in the presence of gum arabic. Because gum arabic causes turbidity and interferes with p-nitrophenol measurement, the hydrolysis of p-nitrophenol esters of long chain fatty acids was estimated by measuring fatty acid released, by the same modified method as described above. Since very little of the liberated p-nitrophenol, whose absorption maximum lies at about 400 nm, comes into the chloroform phase under the experimental conditions, its influence on the assay results was negligible. The estimation of protein was obtained by the method of Miller (20). Specific activity was expressed as millimicro moles of fatty acid released per mg of protein per min.

Radioactivity was measured as follows. When glycerol trioleate-1-14C was used as substrate, the reaction was stopped with 0.25 ml of 1 N HCl, followed by the addition of 25 volumes of a mixture of chloroform and methanol, 2:1 (21). Following extraction for 2 hours at room temperature, the two phases were separated by the addition of 10 ml of 0.05 M KCl. The methanol layer was carefully removed with a micropipette, and the chloroform phase was evaporated to a small volume by use of a rotary evaporator. The sample was then transferred to a 3 ml sample bottle and the chloroform was completely evaporated under a stream of nitrogen. Exactly 0.2 ml of chloroform was added to each bottle, and an appropriate amount of sample was applied to
a thin layer chromatogram, previously spotted with a small amount of carrier which contained 10 µg each of glycerol 1-mono-
oblate, 1,2-dioleato, and 1,3-dioleato. The plate was developed twice in the same direction with the same solvent system: petroleum ether, diethyl ether, and formic acid (75:25:1.5). After development and drying, spots were visualized with iodine vapor and were marked. After evaporation of iodine, each spot corresponded to the standard mixture of oleic acid and glycerol 1-
mono-, 1,2-di-, 1,3-di-, and trioleate was scraped off directly into counting vials (22). Following the addition of 15 ml of a thio-
tropic gel solution (3.5%, Cab-O-Sil, 0.5%, 2,5-diphenyloxazole, and 0.03% 1,4-bis[2-(4-methyl-5-phenyloxazolyl)]benzene in tolu-
enum, w/v), radioactivity was measured with a Beckman model
LS-100 liquid scintillation counter. The recovery of radioactivity
was 90 to 95%.

RESULTS AND DISCUSSION

Occurrence and Localization of Glyceride-hydrolyzing Systems in Rat Liver—Fig. 1A illustrates that rat liver homogenates exhibit two different lipolytic activities, one with optimum activity at pH 5 and the other at pH 8.8 to 9.0. With glycerol tridecanoate as substrate, the specific activity at acid pH was more than twice that exhibited at the alkaline pH optimum. The presence of two different lipolytic activities in rat liver homogenate was first demonstrated by Vavrivkova and Mosinger (23). They found major activity at pH 5, but their investigation was limited to the smaller activity at pH 8. A recent paper by Biale, Gorn, and Shafrir (24) reported rat liver homogenate lipase to have only an alkaline pH optimum. Hepatic lipases with acid pH optimum reported to date are: chylomicron triacylglycerol lipase (8), the lipase reported by Stoffel and Greten (7), and the acid lipase described by Mahadevan and Tappel (5). Lipases with an alkaline pH optimum are: lipase found in the soluble fraction of rat liver (25), lipase in microsomes (21), and lipase in mitochondria (26).

Localization of acid lipase in lysosomes of rat hepatic cells has been found by other workers. There have been reports on acid lipase (1) and chylomicron triglyceride lipase (2) of rabbit polymorphonuclear leukocyte lysosomes and on acid lipase of rat kidney (5).

When glycerol 1-monodecanoate was employed as substrate for rat liver homogenate, only one major peak of activity (pH optimum 8.8 to 9.0) was observed. A small shoulder consistently appeared at pH 4.5 to 5.5. Only after the isolation of soluble lysosomes could a single peak at pH 4.5 to 5.5 be obtained. Rat liver was reported by Belfrage (27) to have a monoacylglycerol lipase, and Carter (21) confirmed its location in microsomes. Monoacylglycerol lipases have been reported in microsomes of rat intestinal mucosa (28, 29), in rat adipose tissue (30), and in rat skeletal muscle (31).

Fig. 1, B, C, and D, shows the presence of two peaks of activity in isolated mitochondria, lysosomes, and microsomes. The specific activity in each fraction compared with that of homogenate indicates that the glycerol tridecanoate-hydrolyzing system is mainly located in lysosomes, and that of glycerol monodecanoate in microsomes. The homogeneity and cross-contamination of subcellular fractions in these preparations have been reported elsewhere (32). In Fig. 1C the shift in pH optimum which occurs in the presence of Triton X-100 (30 mg in the incubation mixture) is shown. Triton X 100 at this concentration not only shifted the pH optimum from 5.2 to 4.2, but also inhibited 50 to 90% of the activity. Shifts in pH optimum occur in the presence of bile salts (33) and detergents (34); thus, these compounds show either an activating or an inhibiting effect, depending upon the pH and the concentrations.

Solubilization of Lysosomal Lipase—Lysosomal lipase and esterase are approximately 30% soluble and 70% membrane-bound (5, 6). By the simple procedure of suspending the whole lysosomes in phosphate buffer, 94 to 95% of the lipase activity was solubilized without loss of activity (Table I). An advantage of this technique, seen from Table II, is that solubilization of lipase from the lysosome fraction can be accomplished without appreciable solubilization of contaminating microsomal esterase. Microsomal esterase would interfere with the measurement of lysosomal lipase activity, particularly in its hydrolysis of di- and monoacylglycerol (1, 3, 6). The ratios of activities in soluble lysosomes compared to homogenates, when measured against glycerol tri-, 1,2-di-, and 1-monodecanoate, were 22.8, 9.6, and 0.4, respectively. These decreasing ratios of activity mainly relate to elimination of the highly active microsomal esterase, which enabled us to show that lysosomal lipase hydrolyzes these substrates at different rates. Data supporting this explanation are also presented in Fig. 2. These results suggest the order of hydrolysis of glycerol esters by soluble lysosomal lipase to be: glycerol tridecanoate > glycerol 1,2-didecanoate > glycerol 1-
monodecanoate.

Use of lysosomes from rats without Triton treatment was found

![Fig. 1. Occurrence in rat liver of glycerol acyl ester-hydrolyzing enzymes and effect of pH on the hydrolysis of glycerol tri- and 1-monodecanoate. Incubations were for 10 min at 37° with (A) homogenates (1.4 mg of protein), (B) mitochondria (1.3 mg of protein), (C) whole lysosomes (0.30 mg of protein), and (D) microsomes (1.5 mg of protein) when glycerol tridecanoate (●) was used as substrate, and with (A) homogenates (0.45 mg of protein) (B) mitochondria (0.9 mg of protein), (C) lysosomes (0.15 mg of protein), and (D) microsomes (0.11 mg of protein) when glycerol 1-monodecanoate (▲) was used. Glycerol tridecanoate (●) dispersed in 80 mg of Triton X-100. Specific activity is expressed as millimicromoles of decaenoic acid released per mg of protein per min. Details of assays are as described under "Experimental Procedure." Buffers used were citrate-phosphate buffer (pH 7.2 to 7.3) and 0.1 M Tris-HCl (pH 7.2 to 9.0).](http://www.jbc.org/anchor?section=full&doi=10.1074/jbc.171.4.171)
TABLE I
Distribution of lipolytic activity toward glycerol tridecanoate and p-nitrophenol myristate in membrane and soluble fractions of liver lysosomes from normal and Triton WR 1339-injected rats

The preparations of the lysosome fractions are described under "Experimental Procedure." Specific activity of whole lysosomes of normal liver, expressed as millimicromoles of fatty acid released per mg of protein per min, was 204 against glycerol tridecanoate and 122 against p-nitrophenol myristate; that of Triton-filled liver lysosomes against glycerol tridecanoate was 205. All values shown are from experimental measurement.

| Fraction            | Protein | Total activity |
|---------------------|---------|----------------|
|                     |         | Glycerol tridecanoate | p-Nitrophenol myristate |
| Normal lysosomes    | %       | %               | %                   |
| Whole               | 100     | 100            | 100                 |
| Membrane            | 45      | 6              | 3                   |
| Soluble             | 55      | 94             | 97                  |
| Triton-filled lysosomes | %       | %               | %                   |
| Whole               | 100     | 100            | 100                 |
| Membrane            | 47      | 5              | 5                   |
| Soluble             | 53      | 95             | 95                  |

* Suspension of ammonium sulfate precipitate from Triton-filled lysosomes.

TABLE II
Hydrolysis rates of glycerol esters of decanoic acid by homogenates and soluble lysosomes

Incubations were carried out at pH 5.2 and 8.8 for 10 min at 37° with homogenates (1.4 mg of protein) and soluble lysosomes (0.23 mg of protein) from normal rats. The substrate concentration was 5 mM. Specific activity is expressed as in Table I. The details of assays are described under "Experimental Procedure."

| Glycerol ester       | Homogenates | Soluble lysosomes |
|----------------------|-------------|------------------|
|                      | pH 5.2 | pH 8.8 | pH 5.2 | pH 8.8 |
| Tridecanoate         | 18     | 8      | 410    | 0      |
| 1,2-Didecanoate      | 37     | 100    | 358    | 3      |
| 1-Monodecanoate      | 105    | 430    | 44     | 7      |

to have the following advantages. Relatively high protein contents and yields are obtained in the preparation; Triton is absent; the sucrose concentration is low; and there is negligible release of esterase from contaminating microsomes when lysosomal lipase is solubilized. Therefore, our major experiments were carried out with soluble lysosomes from normal, untreated rats.

Hydroxylapatite Column Chromatography—A peak appeared for each of the eight buffer concentrations used between 0.05 and 0.70 M at pH 6.8, when the soluble fraction of Triton WR 1339-filled lysosomes was chromatographed by stepwise elution (Fig. 3). The first peak, which appeared at 0.05 M buffer concentration, had apparently not been adsorbed to the column. Separate chromatography identified it as Triton WR 1339. The lysosomal lipase activity, as well as the enzyme which hydrolyzes p-nitrophenol esters of fatty acids, appeared in the fourth peak, which was eluted at 0.30 M. Recovery of the total activity applied to the column was never greater than 20%, and the specific activity of Peak 4 was the same as that applied to the column. Since recovery was always low, we studied the possibility of the involvement of cofactors in lipase action, as has been reported by Ory,
Kircher, and Altschul (36) in castor bean lipase and by Daskys, Klein, and Lever (36) in pancreatic lipase. No evidence was found for enzefor involvement in the lysosomal lipolytic system of rat liver. Since acid lipase is quite unstable to organic solvents (8) or to heat treatment (4), it is likely that inactivation of the enzyme by damage to the protein occurs during fractionation. Lipase of rat adipose tissue was reported to be inactivated by acetic acid treatment (37).

Other properties of lipase hindered its purification. Dialysis of soluble lysosomes against water at 0° inactivates lipase completely within 20 hours; acetone fractionation completely destroys the activity; only 5 to 9% of the activity could be recovered after tannic acid precipitation; and hydroxypatite and DEAE-cellulose column chromatography eluted only 15 to 20% of the total activity applied to the columns.

Column fractionation of soluble lysosomes of normal rats was similar to that of the ammonium sulfate-soluble fraction obtained from Triton-filled lysosomes. The p-nitrophenol myristate-hydrolyzing activity fractionated into the same peak with glycerol tridecanoate-splitting activity, indicating that these substrates may be hydrolyzed by similar, if not the same, enzymes. The recovery of activity was also low.

Substrate Specificity and pH Optima—Each substrate used was dispersed in 10% gum arabic solution to give a final concentration of 5 mM, which did not inhibit the activity under the experimental conditions employed. The rate of hydrolysis of glycerol tridecanoate by soluble lysosomes was linear only for the first 10 min of incubation, and activity was directly proportional to the amount of enzyme added to the incubation mixture up to 300 µg of protein. Maximum hydrolysis was obtained at about 5 mM glycerol tridecanoate; the calculated $K_m$ was 1.1 x 10^(-4) M. The rates of hydrolysis of p-nitrophenol derivatives of longer chain fatty acids are given in Table III. Laurate was most rapidly hydrolyzed, followed by myristate. When glycerol esters of decanoic acid were employed, the rate of hydrolysis decreased in the order, glycerol tridecanoate > glycerol 1,2-didecanoate > glycerol 1-monodecanoate, with relative magnitudes of 1:0.9:0.1, respectively. It is not known whether glycerol 1,3-di- and 2-monodecanoate are hydrolyzed at the same rates as those of their corresponding isomers. It is interesting to note that each lipolytic enzyme source seems to have its own characteristic rate of hydrolysis of glycerol esters of homologous fatty acids, regardless of whether the hydrolyses are catalyzed by the same enzyme; e.g., in liver homogenate (24), tri-<di-<monoacylglycerol; in liver and intestinal mucosal microsomes (21, 28), tri-<di-<monoacylglycerol; and in pancreatic juice (38), tri->di->monoacylglycerol. The physiological significance of the fact that microsomes have higher monoacylglycerol-hydrolyzing activity and lysosomes have higher triacylglycerol-hydrolyzing activity is not known.

The pH optima for the hydrolysis of several substrates are shown in Fig. 2 to be similar (pH 5.0 to 5.2). This indicates that the same enzyme catalyzes the hydrolysis of these glycerol esters and the p-nitrophenol derivative. This observation, along with other data presented, strongly suggests that the enzymes described as lysosomal esterase (6) and lysosomal lipase (5) may be the same. Desmuelle and Savary (38) showed that p-nitrophenol esters are suitable substrates for lipase, provided they are employed in an heterogeneous system and are liquid at the temperature of the experiment. They demonstrated that emulsions of p-nitrophenol laurate, which is liquid at 37°, were hydrolyzed to the same extent as trioloin by purified pancreatic lipase.

Effect of Inhibitors and Activators—The most striking effect of the various compounds on lysosomal activity is that of sulfhydryl reagents (Table IV), which inhibit the activity against glycerol tridecanoate in the range of 83 to 100%, and that against p-nitrophenol myristate 80 to 97%. N-Ethylmaleimide partly inhibits (19%) glycerol tridecanoate hydrolysis, but enhances the activity (147%) against p-nitrophenol myristate. Calcium ion seems to have an activation effect, and Mg2+, Na+, and NH4+ have no effect on activity.

There is very little effect by diisopropyl fluorophosphate and protamine sulfate, indicating that the enzyme cannot be classified as diisopropyl fluorophosphate-sensitive esterase or lipoprotein lipase (8). Triton X-100, at the concentrations used here, has a

| Compound          | Final concentration | Activity |
|-------------------|---------------------|----------|
|                   | Glycerol tridecanoate | p-Nitrophenol myristate |
|                   | % of control         | % of control |
| CaCl2             | $1 \times 10^{-4}$ M | 115       | 131          |
| MgCl2             | $1 \times 10^{-4}$ M | 98        | 106          |
| NaCl              | $5 \times 10^{-4}$ M | 96        | 94           |
| NaH2PO4           | $5 \times 10^{-4}$ M | 98        | 105          |
| NaF               | $1 \times 10^{-4}$ M | 80        | 97           |
| KI                 | $1 \times 10^{-4}$ M | 80        | 104          |
| Hg(NO3)2          | $1 \times 10^{-4}$ M | 0         | 4            |
| p-Chloromercuribenzoate | $5 \times 10^{-4}$ M | 3         | 3            |
| N-ethylmaleimide  | $1 \times 10^{-4}$ M | 81        | 147          |
| Diisopropyl fluorophosphate | $1 \times 10^{-4}$ M | 85        | 90           |
| Protamine sulfate | 360 µg/ml            | 85        | 115          |
| Triton X-100      | 64 mg/ml             | 15        | 64           |
|                   | 20 mg/ml             |           |              |
definite inhibitory effect. Lipase may not hydrolyze well those substrates truly in solution, but does hydrolyze the substrate at lipid-aqueous interfaces when it is in an insoluble state (30). The effects of deoxycholate (which is insoluble at pH 5.2), taurocholate, and bovine serum albumin were not studied because of the high blank values obtained with the method employed. However, deoxycholate seems to promote the hydrolysis rate under the assay condition stated in Table V. The data as a whole suggest that sulfhydryl groups are involved in enzymatic hydrolysis, and may be located near the active site of the enzyme. With compounds other than sulfhydryl inhibitors, there is a definite tendency of the lipase activity against p-nitrophenol myristate to be activated more or inhibited less than when glycerol tridecanoate is used as substrate. This may be explained in a manner similar to the interpretation of Singer (40), who found that the extent of inhibition of lipase by each compound tested increased with increasing molecular weight of the substrate. In general, the effects of various compounds on activity with glycerol tridecanoate and p-nitrophenol myristate substrates are more similar than dissimilar.

Hydrolysis Products and Time Course of Glycerol Tri(oleate-1-14C)—Table V gives the results obtained with different substrate-dispersing media and with whole and soluble lysosomes.

Table V
Hydrolysis of glycerol tri(oleate-1-14C) and product formation by whole and soluble lysosomes

|                     | Whole lysosomes | Soluble lysosomes | 10% gum arabic |
|---------------------|-----------------|------------------|---------------|
| Total hydrolysis    | %               | %                | %             |
| Radioactivity       | 7.7             | 13.3             | 8.9           |
| Oleic acid          | 60              | 68               | 40            |
| Glycerol 1,3-dioleate| 6               | 4                | 7.8           |
| Glycerol 1,2-dioleate| 34              | 28               | 52.2          |
| Glycerol monooleate | 0               | 0                | 0             |

Elsbach and Kayden (2) reported that only free fatty acid and 1,2-diacylglycerol accumulation had been observed when

Gum arabic is superior to sonic treatment and deoxycholate as a dispersing medium, even though only 9% hydrolysis is achieved in 1 hour. The pattern of separation and distribution of radioactivity in the hydrolysis products was determined using glycerol tri(oleate-1-14C) as substrate. By thin layer chromatography, most of the radioactivity was recovered in spots corresponding to glycerol 1,2-dioleate and free oleic acid. No accumulation of glycerol monooleate was found under any circumstance. There was always a small accumulation of glycerol 1,3-dioleate. Table V also shows that soluble lysosomes produced more glycerol 1,2-dioleate than free oleic acid in 1 hour, and that whole lysosomes, incubated for 3 hours, produced more free oleic acid than glycerol 1,2-dioleate.

The data in Fig. 4 were obtained from separate experiments from those reported in Table V, but reaction conditions were similar. Fig. 4 shows that during a 3-hour incubation the production of free oleic acid progressively increased as glycerol trilaurate was hydrolyzed. Although the formation of glycerol 1,2- and a small amount of 1,3-dioleate was observed, no accumulation of glycerol monooleate could be demonstrated, indicating the hydrolysis of glycerol dioleate to free oleic acid and glycerol without monooleate accumulation. It is not known whether the small accumulation of glycerol 1,3-dioleate was due to isomerization (41) or to the existence of an enzyme which specifically splits the inner fatty acyl group from triacylglycerol (42).

Fig. 4. Time course of hydrolysis of glycerol tri(oleate-1-14C) by whole lysosomes. Whole lysosomes (2.21 mg of protein) from normal rats were incubated with substrate, prepared by the sonic treatment method, at 37° in a shaking water bath at a speed of 250 rpm. At time intervals indicated, the reaction was stopped, hydrolysis products were separated, and the radioactivity of each compound was measured. Details of the method are described under "Experimental Procedure."
Chylomicron triacylglycerol was hydrolyzed by the lysosomes of rabbit polymorphonuclear leukocytes. Waite and van Deenen (26) and Carter (21) observed the same results when triacylglycerol was hydrolyzed by rat liver homogenates. Higgins and Green (8) demonstrated very little accumulation of mono- and diacylglycerol when chylomicron triacylglycerol was hydrolyzed by rat liver lysosomes. Waite and van Deenen (4) showed that uniformly labeled lecithin-14C hydrolysis by rat liver parenchymal cells. Mellors and Tappel (4) showed that hydrolyzing partial as well as full acyl esters of glycerol by an acid lipase of rat liver. 

The results presented here indicate that lysosomes are capable of hydrolyzing partial as well as full acyl esters of glycerol. The physiological mechanism and significance of lysosomal lipolytic enzymes in intralysosomal lipid digestion and their interaction with the microsomal lipolytic system remain to be elucidated.

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