# The synthesis of higher glycerides via the monoglyceride pathway in hamster adipose tissue

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ABSTRACT The monoglyceride pathway for the synthesis of triglycerides has been investigated employing subcellular fractions and whole cell preparations of white and brown adipose tissue. Conclusive evidence has been obtained for the monoglyceride pathway in these tissues by employing the 2-monoether analogue of 2-monoolein as the substrate. The monoglyceride and $\alpha$-glycerophosphate pathways were primarily found in the microsomal fraction. In these in vitro systems the activity of the monoglyceride pathway compared with the $\alpha$-glycerophosphate pathway was of the same order of magnitude in whole cell preparations and was approximately one-half the activity of the $\alpha$-glycerophosphate pathway when the microsomal fraction was employed.

SUPPLEMENTARY KEY WORDS subcellular location · brown and white adipose tissue

ALTHOUGH recent reviews have suggested that the metabolic pathways associated with the synthesis and degradation of triglycerides (TG) have been well established in adipose tissue (1–3), some questions have arisen regarding the validity of such conclusions. For example, in numerous investigations (4, 5) it has been assumed that adipose tissue did not contain the enzyme glycerokinase (EC 2.7.1.30) and therefore the glycerol which was formed by the hydrolysis of TG could not be reutilized for the synthesis of TG. The presence of this enzyme in adipose tissue has now been established (6). Similarly, it has been categorically emphasized from numerous laboratories that the $\alpha$-glycerophosphate ($\alpha$-GP) pathway is the only mechanism in the adipose tissue for the synthesis of glycerides (7, 8). This pathway was originally described in adipose tissue by Rose and Shapiro (9) and its existence in adipose tissue has been confirmed in numerous laboratories (7, 8). Since the metabolic activity of adipose tissue in regard to the synthesis of TG and to the release of fatty acids has been shown to have certain reciprocal relationships and furthermore, since both of these enzyme systems can be modified by numerous hormones, an understanding of the metabolic reactions of this tissue is necessary in order to interpret these results. Several observations had led us to examine the question of whether or not the $\alpha$-GP pathway was the sole mechanism of TG synthesis in this tissue. The first of these observations was the similarity of metabolic patterns observed between adipose tissue and the intestinal mucosa. This included the synthesis of TG and the presence and subcellular distribution of certain enzymes. For example, it was well documented that the intestinal mucosa contains an enzyme which preferentially hydrolyzes monoglycerides (MG) (10, 11). Several years later a similar enzyme was shown to be present in adipose tissue (12). The original postulate for the mechanism of TG synthesis by the intestinal tissue was that it occurred via the $\alpha$-GP pathway (13), and subsequently it was established that this pathway was not the major reaction sequence involving the synthesis of TG by the intestinal tissue (14–16). Finally, it has been reported that under the influence of certain hormones the ratio of fatty acids to glycerol released in adipose tissue is in excess of the

Abbreviations: 2-MG, 2-monoglyceride; 2-MP, 2-monopalmitin; 2-ME, a 2-monoether analogue of a 2-monoglyceride; 2-MOE, 2-monooleyl ether; DG, diglyceride; TG, triglyceride; FA, fatty acids; $\alpha$-GP, $\alpha$-glycerophosphate; TLC, thin-layer chromatography; Pal-CoA, palmityl CoA.

theoretical value of 3:1 (17, 18). On the basis of the information cited above, experiments were undertaken to investigate the possibility of the existence of the MG pathway for the synthesis of TG in adipose tissue. In addition, several experiments were performed in order to quantitate the significance of this pathway in subcellular systems as well as in the intact adipose cell.

## METHODS AND MATERIALS

CoA and ATP were obtained from Calbiochem, Los Angeles, Calif. The sodium salt of DL-α-glycerophosphate was obtained from Eastern Chemical Corp., Newark, N.J. 2-monopalmitin (2-MP), unlabeled and labeled with $^3H$ in the 9-10 position of the fatty acid ester, was synthesized by the procedure previously described (19). The palmityl CoA was obtained from P-L Biochemicals Inc., Milwaukee, Wisc. Pal-CoA-l-$^{14}C$, glucose-U-$^{14}C$, and palmitic-l-$^{14}C$ acid were obtained from the New England Nuclear Corp., Boston, Mass. The palmitic acid was purified (98%) by TLC. The 2-monoether corresponding to 2-monoolein (2-octadec-9-enyl glyceryl ether, referred to as 2-monooleyl ether [2-MOE]) was prepared with $^3H$ in the 9-10 position of the oleic acid according to the procedure of Wood and Snyder (20). The 1- and 2-octadecyl glyceryl ethers were kindly supplied by Dr. Fritz Paltauf. Collagenase, which was utilized for the preparations for the intact cell, was obtained from the Worthington Biochemical Corp., Freehold, N.J. L-α-Glycerophosphate was determined by the method of Hohorst (21).

### *Enzyme Preparation and Incubation Conditions for the Subcellular Preparations of Adipose Tissue*

The epididymal fat pads were removed from 70–100-g golden hamsters that had been fed ad lib. Homogenization and subcellular fractionation were carried out by the procedure of Steinberg, Vaughan, and Margolis (7) with the exception that the initial homogenization was performed in 0.25 M sucrose rather than in 0.15 M KCl. The intermediate, straw-colored layer which represented the defatted homogenate was removed and was considered in these experiments to be the whole homogenate. From this fraction the mitochondrial and microsomal fractions, as well as the supernatant fluid, were obtained. The mitochondrial and microsomal fractions were washed with an equal volume 0.25 M sucrose prior to their use in the incubation mixture. The final fractions were taken up in 0.15 M KCl prior to addition to the incubation mixture.

For the whole cell preparation of white adipose tissue the method of Rodbell (22) modified by Schotz et al. (23) was employed. Brown adipose tissue was obtained from the subscapular region of the male golden hamster. For the whole cell preparation of brown adipose tissue the procedure of Rodbell, as modified by Fain, Reed, and Saperstein (24), was followed. Similar subcellular fractionation procedures were carried out on intestinal mucosa and liver, employing 0.25 M sucrose, with the exception that in these tissues the homogenization procedure was carried out in a 1:9 w/v ratio, whereas adipose tissue was homogenized in a ratio of 1 part of tissue to 3 parts of 0.25 M sucrose. All fractionation procedures were carried out at 0°C. The protein concentration in each experimental flask was determined by the method of Lowry, Rosebrough, Farr, and Randall (25).

The standard incubation conditions for the various subcellular fractions were as follows: 2 μmoles of the 2-MOE or 2-MP dissolved in benzene was added to a 15-ml centrifuge tube and the solvent was removed under vacuum. 0.1 ml of a solution of 10% Tween 80, which had been previously purified to remove the free fatty acids (26), was then added, and the mixture was vigorously shaken until the substrates were in a clear suspension. 0.5 μmole of Pal-CoA dissolved in 0.5 ml of 0.5 M Tris–maleate buffer, pH 7.0, was added to each incubation vessel. When the α-GP pathway was investigated, 20 μmoles of DL-α-GP was added. In addition, each reaction vessel contained 50 μmoles of $MgCl_2$, 150 μmoles of KCl, 0.6 mmole of Tris–maleate buffer, pH 7.0, and 0.5 ml of the various subcellular fractions. The total incubation volume was 2.0 ml. The assay was shown to be linear during the 30-min incubation. When the fatty acid activation system was substituted for Pal-CoA, the potassium salt of palmitic acid, ATP, and CoA were employed.

### *Incubation Conditions for Whole Cell Preparations*

The 2-MOE or 2-MP in these experiments was suspended by combining the substrate with bovine albumin (Fraction-V) obtained from Armour Pharmaceutical Co., Chicago, Ill. The standard procedure for preparing 10 ml of the albumin–ether complex was as follows: 20 μmoles of 2-MOE, dissolved in benzene, was added to a 15-ml centrifuge tube and the benzene was evaporated under vacuum. The 2-MOE was resuspended in 0.5 ml of diethyl ether, and 10 ml of a 3% solution of albumin in Krebs-Ringer–bicarbonate (pH 7.4), previously equilibrated with 95% $O_2$ and 5% $CO_2$, was added. The buffer contained glucose (30 mM) and half the recommended amount of calcium. The suspension was vigorously mixed and the diethyl ether was removed under vacuum. The resulting 2-MOE–albumin was a perfectly clear solution.

After the isolated cells had been prepared, they were suspended in Krebs-Ringer–bicarbonate–albumin–glucose buffer (pH 7.4; 5 ml/g of original wet weight of

tissue); 2 ml of the cell suspension and 0.5 ml of the labeled 2-MOE solution were then added to a 25-ml plastic beaker. The total incubation volume was 2.5 ml. The incubation vessel contained 2 μmoles of the 2-MOE or 2-MP. When the α-GP pathway was studied in this system, the 2-MOE was omitted and glucose-U-$^{14}$C was employed at a final concentration of 3.0 mM. The incubation tubes with the whole cell preparations each contained 7.6 μmoles of glucose. The incubation conditions were for 1 hr at 37°C under an atmosphere of 95% $O_2$ and 5% $CO_2$. The enzymatic reactions were terminated by the addition of chloroform–methanol 2:1 and the lipids were extracted according to the method of Folch, Lees, and Sloane Stanley (27). The individual lipids were separated by TLC on dichlorofluorescein-impregnated plates as previously described (28). The developing solvent system was chloroform–methanol–acetic acid 98:2:1 contained in lined tanks. The TG analogue containing the diacyl monoether and the corresponding DG analogue were clearly separated. The $R_F$'s were confirmed by using authentic samples of the corresponding ether-ester in the same chromatographic system. When the 2-ME was employed, the DG intermediate formed was exclusively the 1,2-DG analogue. During the study of the α-GP pathway, certain difficulties were encountered in the TLC separation of the substrate Pal-$^{14}$C-CoA and the product phosphatidic acid, since neither compound moved from the origin. In order to distinguish the phosphatidic acid from the unreacted Pal-CoA, the following procedure was utilized. An aliquot of the lipid extract was placed on TLC plates containing Silica Gel H (Brinkmann Instruments, Inc., Westbury, N.Y.), and 25 μg of authentic phosphatidic acid (Pierce Chemical Co., Rockford, Ill.) was added as a carrier for identification purposes. The plates were then placed in lined tanks and developed in a solvent system consisting of diethyl ether–methanol–acetic acid 98:2:0.2 until the solvent reached the top of the plate. In this solvent all neutral glycerides as well as the FA migrate with, or close to, the solvent front. The plates were removed, dried at room temperature, and then developed in chloroform–methanol–30% methylamine 65:25:8. The phosphatidic acid in this system had an $R_F$ of approximately 0.3, while the Pal-$^{14}$C-CoA remained at the origin. The phosphatidic acid area was outlined after it was visualized by spraying the plate with molybdenum reagent (29). The plates were then exposed to ammonia to remove the blue color. The spots corresponding to phosphatidic acid were scraped off and counted in a liquid scintillation counter (28). In addition, the authenticity of synthesized phosphatidic acid was established by two-dimensional chromatography according to the procedure of Rouser, Kritchevsky, Galli, and Heller (30).

## RESULTS

Initial experiments were performed utilizing labeled 2-MP as the acyl acceptor. However, a problem which consistently interfered with the interpretation of the results was the rapid hydrolysis of this substrate. Attempts to inhibit the lipase, such as the addition of fluoride, were unsuccessful. As was previously discussed, a monoglyceride lipase in adipose tissue has been previously reported (12). Since the 2-ME had been utilized in numerous laboratories to study the MG pathway in intestinal mucosa (31, 32) in order to minimize the problem of acyl migration and hydrolysis, this substrate was thought to be ideally suited to study the MG pathway in adipose tissue. Therefore, $^{3}$H-labeled 2-MOE was used in the presence of an acyl donor, Pal-CoA, and the activity of the various subcellular fractions was tested for the ability to synthesize the di- and triglyceride analogues. The results of these studies are given in Table 1 and have been corrected for a control experiment in which Pal-CoA was omitted and in which the endogenous synthesis was less than 10% of the value of the experimental flask. As can be seen, the subcellular component with the highest specific activity is the microsomal fraction. Distribution of radioactivity between the 1,2-di- and triglyceride analogues was approximately 1 to 1. These results clearly demonstrate the incorporation of 2-MOE into TG via a series of reactions similar to that described for the MG pathway. The microsomal fraction obtained from rat epididymal fat pads demonstrated a similar activity; however, this species was only 75% as active as the hamster system. The comparison of the various ethers as precursors of DG and TG is given in Table 2. In these experiments the microsomal preparation was used as the enzyme source. It can be seen that the 2-MOE was a better substrate than the 1- and 2-monoethers which correspond to 1- and 2-monostearin. Therefore,

TABLE I ESTERIFICATION OF 2-MONOOLEYL ETHER BY DIFFERENT SUBCELLULAR FRACTIONS

| | Specific Activity |
|---|---|
| | *pmoles ester bond formed/min/mg protein* |
| Defatted whole homogenate | 300 |
| Mitochondrial fraction | 476 |
| Microsomal fraction | 1,296 |
| Supernatant fraction | 143 |

The flasks contained 2.0 μmoles of $^{3}$H-labeled 2-monooleyl ether and 0.5 μmole of Pal-CoA. Each flask also contained 50 μmoles of $MgCl_2$, 150 μmoles of KCl, 0.1 ml of 10% Tween 80, 1.2 ml of 0.5 M Tris–maleate, pH 7.0, and 0.5 ml of the subcellular fraction suspended in 0.15 M KCl. The final volume was 2.0 ml and the flasks were incubated for 30 min at 20°C. Each value is corrected for a control in which the Pal-CoA was omitted.

TABLE 2 COMPARISON OF THE ESTERIFICATION OF DIFFERENT ETHERS USING PALMITYL-$^{14}$C-CoA

| | Specific Activity |
|---|---|
| | *pmoles glyceride formed/min/mg protein* |
| 1. 1-Monostearyl ether | 210 |
| 2. 2-Monostearyl ether | 1,560 |
| 3. 2-Monooleyl ether | 2,100 |

Flasks 1 and 2 had 1.0 μmole of the respective ether. Flask 3 had 2.0 μmoles of the ether. The conditions of incubation using microsomes from white adipose tissue were exactly the same as in Table 1 with the exception that the incubation was carried out for 1 hr at 20°C.

this substrate was used as the model compound throughout the remainder of this investigation.

In order to obtain some evidence with regard to the relative rates of esterification by the MG and α-GP pathways, experiments were performed in which the 2-MOE was compared with the α-GP as an acyl acceptor for the biosynthesis of higher glycerides. Preliminary experiments in which α-GP was incubated in various subcellular fractions revealed that the highest specific activity was in the microsomal fraction. The results of the comparison are given in Table 3. It should be noted that 20 μmoles of DL-α-GP was added as the acyl acceptor as compared with 2 μmoles of 2-MOE. This was necessary to obtain a significant synthesis of higher glycerides via the α-GP pathway. At the completion of the 30-min incubation 70–90% of the 2-MOE and L-α-GP was still present in the incubation vessel. Although 20 μmoles of DL-α-GP was added, only the L-isomer is an acyl acceptor in this reaction (4, 33). In addition, since the microsomal system was used as the enzyme source for the comparison, and since Tween 80 was also added to the reaction mixture, the product formed was phosphatidic acid. It had been previously demonstrated that in the intestinal mucosa (34) and in adipose tissue (8) the supernatant fraction was required for the synthesis of di- and triglycerides via the α-GP pathway. The supernatant fraction has been shown to contain the enzyme phosphatidic acid phosphohydrolase (EC 3.1.3.4) (8, 34), which is also sensitive to the Tweens used in this experiment (35). Therefore, the accumulation of phosphatidic acid was to be expected. No evidence was obtained for the hydrolytic breakdown of phosphatidic acid by cleavage of either of the acyl moieties since only a small amount of lysophosphatidic acid was found. The results obtained from this experiment clearly established not only the existence of the MG pathway in this tissue but also the quantitation of this pathway compared with the α-GP pathway. The results clearly suggest that the MG pathway may be responsible for significant biosynthesis of TG in white adipose tissue. In order to obtain more information regarding the presence of the MG pathway in various tissues and the comparison of the two pathways in these tissues, 2-MOE and α-GP were utilized as acyl acceptors in white and brown adipose tissues, intestine, and liver. Although it had been reported by Snyder, Piantadosi, and Malone (36) that the 1-ME was acylated to a very limited extent in liver, the α-GP pathway is the major biosynthetic pathway. Intestinal mucosa was used as a control in this experiment since the MG pathway has been established to be the major pathway for the synthesis of TG in this tissue. Therefore, these tissues were compared with the adipose tissue under identical conditions in order to evaluate the MG and α-GP pathways. The results of these experiments in which microsomes from epididymal fat pads of white adipose tissue, subscapular brown fat, liver, and intestine were used are given in Table 4. As can be seen, the results confirm those shown in Table 3; the MG pathway in the white adipose tissue is about 40% as active as the α-GP pathway. When brown adipose tissue was employed as the enzyme source, approximately equal amounts of higher glycerides were synthesized by both pathways. The MG pathway was prevalent in the intestinal mucosa in confirmation of numerous earlier reports. The α-GP pathway is the major pathway of synthesis of glycerides by liver microsomes.

TABLE 3 COMPARISON OF THE ESTERIFICATION OF 2-MONOOLEYL ETHER AND α-GLYCEROPHOSPHATE

| | Specific Activity |
|---|---|
| | *pmoles product formed/min/mg protein* |
| 1. 2-Monooleyl ether | 4,148 |
| 2. α-Glycerophosphate | 10,148 |

Flask 1 had 2.0 μmoles of $^{3}$H-labeled 2-monooleyl ether; flask 2 had 20 μmoles of DL-α-glycerophosphate. Each flask had 0.5 μmole of Pal-CoA. Flask 2 had palmityl-$^{14}$C-CoA. The other conditions were exactly the same as in Table 1. Incubation was carried out at 20°C for 30 min.

TABLE 4 A COMPARISON OF ESTERIFICATION OF 2-MONOOLEYL ETHER AND α-GLYCEROPHOSPHATE BY MICROSOMES OF VARIOUS TISSUES

| | Specific Activity | | | |
|---|---|---|---|---|
| | Adipose Tissue | | | |
| | White | Brown | Intestine | Liver |
| | *pmoles higher product formed/mg protein/min* | | | |
| $^{3}$H-2-Monooleyl ether | 1,560 | 2,200 | 14,400 | 166 |
| α-Glycerophosphate | 4,440 | 2,880 | 4,000 | 3,380 |

The ether flasks had 2.0 μmoles $^{3}$H-labeled 2-monooleyl ether and 0.5 μmole of unlabeled Pal-CoA; the α-glycerophosphate flask had 20 μmoles of DL-α-GP and 0.5 μmole Pal-$^{14}$C-CoA. The other conditions were exactly the same as those described in Table 1.

One of the disadvantages of using the various subcellular fractions to study these enzymatic reactions is the question of their importance in intact cell preparations. Whole cell preparations were obtained from both white and brown adipose tissue. The resulting whole cells were incubated in the presence of 2-MOE and uniformly labeled glucose to evaluate the MG and $\alpha$-GP pathways and the results are given in Table 5. Since $\alpha$-GP could not be utilized directly as a precursor for higher glycerides in this system, 7.6 $\mu$moles of glucose-U-$^{14}$C was employed as an $\alpha$-GP precursor. The calculations of Table 5 are based on the assumption that during the process of glycolysis twice this amount of $\alpha$-GP could be synthesized from the added glucose. No correction was made for the glycogen content of the adipose tissue. In addition, in order to establish that the radioactivity incorporated into higher glycerides in white adipose tissue was incorporated primarily into the glycerol portion of the molecule, the glycerides were saponified by the method described by Rodbell (22), and aliquots of the resulting water-soluble glycerol and hexane-soluble fatty acids were counted. In the four experiments in which the saponification procedure was performed, an average of 68,500 cpm were saponified. 64,345 cpm were recovered in the water-soluble fraction and 340 cpm were present in the hexane phase. The former phase would contain the glyceride-glycerol and the latter the fatty acid fraction. Although the glyceride-glycerol fraction is somewhat higher than previously reported (22), our incubations were for a much shorter time period. This was necessary in order to insure the linearity of the assay. When the 2-MOE, 2-MP, and $\alpha$-GP were compared with the whole cell preparation (Table 5), labeled higher glycerides were found in both white and brown adipose tissue. In contrast to the microsomal system, the major compound synthesized via the $\alpha$-GP pathway when the whole cell preparation was used was triglyceride, with small amounts of phosphatidic acid and diglyceride. Although considerable hydrolysis was evident when 2-MP was employed in the whole cell preparations, the synthesis of higher glycerides was similar to that when 2-MOE was used. From the results of these experiments it can be concluded that the MG pathway is operable in whole cell preparations as well as the microsomal system.

TABLE 5 COMPARISON OF ESTERIFICATION OF 2-MONOOLEYL ETHER AND $\alpha$-GLYCEROPHOSPHATE IN WHITE AND BROWN ADIPOSE TISSUE USING WHOLE CELL PREPARATIONS

| | Adipose Tissue | |
|---|---|---|
| | White | Brown |
| | *pmoles higher glyceride formed/mg wet wt* | |
| $^{3}$H-labeled 2-monooleyl ether | 490 | 344 |
| Glucose-U-$^{14}$C* | 365 | 167 |
| $^{3}$H-labeled 2-monopalmitin | 607 | ... |

The ether flasks had 2.0 $\mu$moles $^{3}$H-labeled 2-monooleyl ether in 0.5 ml Krebs-Ringer–bicarbonate–3% albumin–3.0 mM glucose buffer. For the 2-monopalmitin flask, 2.0 $\mu$moles of the 2-monoglyceride was substituted for the 2-monoether. The $\alpha$-glycerophosphate flask had glucose-U-$^{14}$C in 0.5 ml of the same buffer. 2 ml of the isolated intact cells in the Krebs buffer was added and incubated for 1 hr at 37°C in a 95% $O_2$–5% $CO_2$ atmosphere. The final concentration of glucose was calculated to be 7.6 $\mu$moles in each flask.

* Calculated using the theoretical amount of 2 moles of $\alpha$-glycerophosphate from each mole of glucose.

## DISCUSSION

Previous investigations of the occurrence of the MG pathway in adipose tissue had suggested that this pathway was not present (7, 8). The results of the present investigations clearly demonstrate the MG pathway in white and brown adipose tissue. It should be emphasized that the ability to demonstrate the pathway in adipose tissue arises primarily from the incorporation of 2-MOE substrates rather than the 2-MG. It has clearly been established that the ethers can serve as substrates in the intestinal mucosa and that in adipose tissue the ethers circumvent the problem of the loss of 2-MG substrate by hydrolysis. However, as can be seen from Table 5, a similar synthesis of higher glycerides was obtained when 2-MOE and 2-MP were employed as substrates, indicating that these substrates were utilized by similar enzyme systems.

As can be seen from Table 1, which shows the distribution of the enzyme system, the microsomal fraction appears to be the major site for the synthesis of higher glycerides via the MG and $\alpha$-GP pathways, in confirmation of previous reports (37). It has been suggested that the mitochondrial fraction is the major site of TG biosynthesis in this tissue (8). When microsomes obtained from adipose tissue were employed as the enzyme source for the investigations on the $\alpha$-GP pathway, phosphatidic acid was the major compound synthesized. Based on the results obtained for this pathway in the intestinal mucosa and the reported stimulatory effect of the supernatant fraction in adipose tissue, this observation was not unexpected. If one examines the data presented in Tables 1 through 4 from a quantitative standpoint, it becomes apparent, at least with subcellular fractions, that the MG pathway may indeed account for a significant amount of TG turnover and synthesis in both white and brown adipose tissue under the reported conditions. Further, the data in Table 4 demonstrate that the activity of the MG pathway in adipose tissue is intermediate between the intestinal mucosa, in which the pathway is prevalent, and the liver, in which this reaction sequence

is questionable from a quantitative standpoint. If the calculations of the white adipose tissue whole cell preparation are converted to pmoles of higher glyceride synthesis/mg protein per min for the MG and α-GP pathways, the values obtained are 464 and 347, respectively. These values are of a similar order of magnitude as those obtained for the whole homogenate system and are reassuring as to the validity of the experiments carried out with various subcellular preparations. In addition, the rates of esterification of α-GP reported here for adipose tissue are of the same order of magnitude as those previously reported (8, 21, 23).

We realize that these in vitro studies cannot be quantitatively extrapolated to the in vivo situation, but they suggest that the monoglyceride pathway may indeed play a significant role in triglyceride synthesis in adipose tissue.

The physiological importance of the MG pathway in adipose tissue has rather wide ramifications since the metabolism of this tissue can be affected by numerous hormones. Whether the MG pathway is stimulated under various conditions that have been shown to increase TG biosynthesis, such as the action of insulin on adipose tissue or, conversely, the release of FA under the influence of epinephrine, awaits future investigations. The formation of MG in adipose tissue stimulated by epinephrine has been reported by Wadström (17).

Although various ratios have been reported of fatty acid to glycerol release by adipose tissue, the presence of the MG pathway may explain those results which have been reported under conditions in which this ratio was in excess of the theoretical value 3 to 1. One might postulate that this pathway could function in the rapid recovery phase for the synthesis of TG following the release of FA by the action of the hormone-sensitive lipase shown to be present in this tissue (12). In addition, this pathway may explain the results of Angel (38) in which it was reported that adipose tissue contains a DG fraction which was rapidly turning over. The presence of the MG pathway in brown adipose tissue may be of significance in the process of thermogenesis. It is theoretically possible that TG are hydrolyzed to MG which, in turn, are resynthesized into TG. The net effect of the breakdown and the resynthesis of TG would be a $-\Delta H$. Finally, the relationship and quantitation of the MG and α-GP pathways may be of importance in certain genetically related obese conditions. Investigations are now in progress to test some of these postulates.

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