The effect of high sugar intake on the esterification of dihydroxyacetone phosphate by rat liver microsomes

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Abstract Rat liver microsomes were used as an enzyme source to study dietary-induced changes in the rate of dihydroxyacetone phosphate esterification. Rats were fed (*1*) 75% glucose or fructose diets for various time intervals, or (*2*) fed a fructose diet for six days and then a chow diet. Both the glucose and fructose diets produced a 2–3-fold increase in total and neutral glycerolipid formation from dihydroxyacetone phosphate measured in the presence of ATP, palmitate, CoA, and NADH. The increased rate of dihydroxyacetone phosphate esterification and a simultaneous rise in serum triglyceride level in rats fed fructose was rapidly reversed when chow was substituted for the fructose. The results indicate that an increased rate of dihydroxyacetone phosphate esterification may contribute to the acceleration of endogenous glycerolipid biosynthesis noted under these dietary conditions.

Supplementary key words carbohydrate diets · enzyme regulation

It is generally accepted that the major pathway of hepatic glycerolipid biosynthesis proceeds via *sn*-glycerol-3-phosphate as outlined by Kennedy (1) and Kornberg and Pricer (2). More recently, Hajra and Agranoff (3–4) have suggested that DHAP also may be an important precursor in the formation of hepatic glycerolipids. Hill and Lands (5), Okuyama and Lands (6), and Rognstad, Clark, and Katz (7) studied the relative importance of these two pathways and concluded that the DHAP pathway is quantatively unimportant. However, Manning and Brindley (8), Bowley, Manning, and Brindley (9) and Pollock, Hajra, and Agranoff (10), using different experimental techniques, suggested that DHAP is a significant precursor of hepatic glycerolipid. Therefore, the quantitative importance of the DHAP pathway in the regulation of hepatic glycerolipid metabolism is not clear, although Rao, Sorrels, and Reiser (11) have shown that phosphatidate formation from DHAP is influenced by diet.

High fructose diets elevate serum triglycerides in man (12–13) and experimental animals (14–15). Several studies suggest that the elevated serum glyceride levels result from increased hepatic glycerolipid formation (16–19). This has been attributed to increased activity of several reactions in microsomal neutral lipid synthesis from *sn*-glycerol-3-phosphate (20–21). However, no measurements of the alternative DHAP pathway were made under these dietary conditions. Therefore, the present studies were undertaken to determine the effects of high sugar diets on DHAP esterification by rat liver microsomes.

MATERIALS AND METHODS

Lipid standards for thin-layer chromatography were purchased from Applied Science Labs, Inc., State College, Pa. Fatty acid-poor albumin (Fraction V) and dithiothreitol were obtained from Pentex, Kankakee, Ill., and Calbiochem, San Diego, Cal., respectively. NADH, CoA, ATP, and the dimethyl ketal of DHAP were purchased from Sigma Chemical Co., St. Louis, Mo. The free carbonyl compound was generated from the ketal derivative by hydrolysis with Dowex 50-X4 (H^+). [1-^{14}C]Palmitate was purchased from New England Nuclear, Boston, Mass.

Male Sprague-Dawley rats (250–300g) were obtained from Zivic Miller, Inc., placed in individual cages and fed diets containing 75% fructose, 75% glucose, or Purina laboratory chow. High sugar diets were prepared as described previously (20). Control rats were pair fed Purina laboratory chow. Daily caloric intake and weight gain were similar in each

Abbreviations: DHAP, dihydroxyacetone phosphate; MCSM, microsomes; NL, neutral lipid; TLC, thin-layer chromatography.

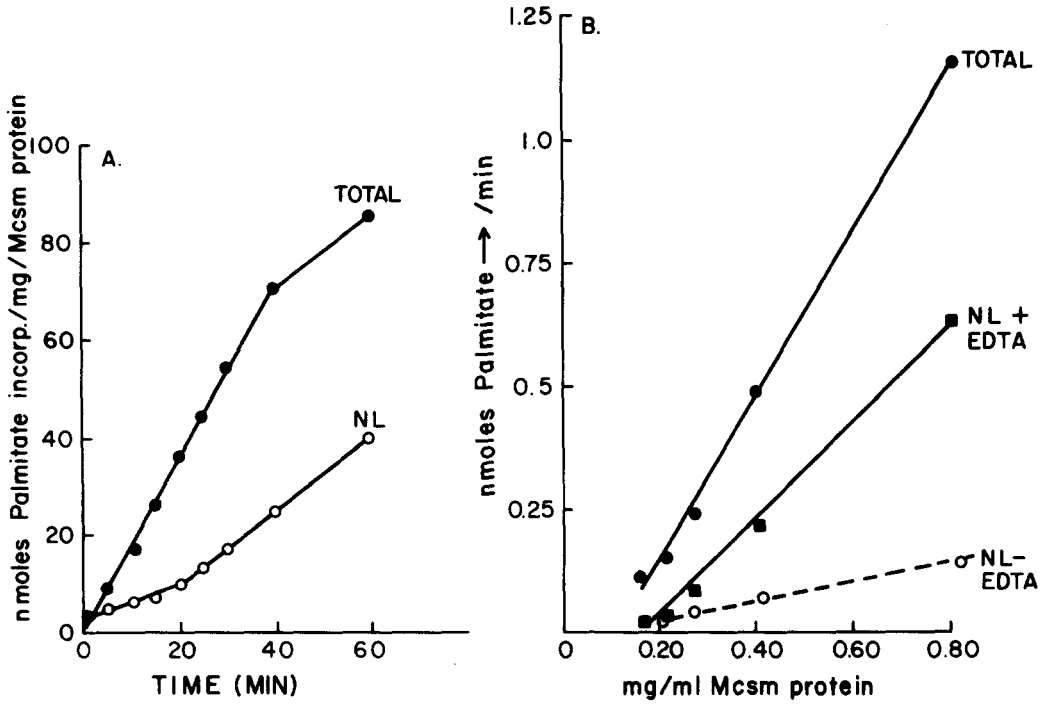

Fig. 1. The effect of increasing incubation time (A) and microsomal protein (B) on the incorporation of [1-^{14}C]palmitate into total and neural lipid (NL). EDTA (±) refers to whether or not microsomes were prepared in sucrose–Tris buffer containing 2.5 mM EDTA.

experiment. Rats were killed by exsanguination from the dorsal aorta, and the livers were rapidly removed and homogenized in 4 volumes of ice-cold 0.225 M sucrose containing 0.05 M Tris-HCl, pH 7.5, and 2.5 mM EDTA. Microsomes were isolated as described by Sedgwick and Hubscher (22). Isolated microsomes were suspended in the sucrose-Tris buffer and resedimented at 105,000 *g* to remove particle-free supernatant. Aliquots of washed microsomes were diluted with the sucrose–Tris buffer containing 2.5 mM EDTA (3–4 mg protein/ml), quickly frozen in dry ice and acetone, and then stored at −30°C. Frozen microsomes were used within one week and were thawed only once before being used. DHAP acyltransferase activity was not significantly altered by freezing and storage at 0°C for one week. Microsomal protein was determined as described by Lowry et al. (23).

The standard incubation mixture that was optimum for DHAP esterification contained 1.5 mM DHAP, 1.5 mM NADH, 25 mM Tris-HCl, pH 7.5, 1.5 mM $MgCl_2$, 40 μM CoA, 2.8 mM ATP, 0.3 mM dithiothreitol, 0.7 mM [1-^{14}C]palmitate (0.1 μCi), 1.25 mg fatty acid-poor albumin (Fraction V Pentex), and 0.3 mg microsomal protein in a total volume of 0.5 ml. Reactions were started by the addition of microsomal protein and stopped after a 30 or 60 min incubation at 37°C by the addition of 10 ml of chloroform–methanol 2:1 (v/v) containing 1% 1N HCl. Radioactive lipid products were extracted as described by Folch, Lees and Sloane Stanley (24) and identified by chromatography on TLC plates coated with 0.25 mm silica gel G containing 0.1 M borate with a solvent system containing chloroform–methanol–3.5 M NH_4OH 65:35:8 (v/v) or hexane–ether–glacial acetic acid 146:50:4 (25).

RESULTS AND DISCUSSION

Under the standard incubation conditions of DHAP esterification, both total lipid (phosphatidate + neutral lipid) and neutral lipid (diglyceride + triglyceride) formation were optimum. The synthesis of neutral and total lipids was markedly enhanced by the addition of CoA, palmitate, DHAP, ATP, $MgCl_2$, and NADH, since less than 20% of the optimum activity was present in the absence of these substrates and cofactors. Maximum incorporation of palmitate into total lipids occurred with approximately 5 μM CoA, 0.75 μM palmitate and 2 mM DHAP. The rate of palmitate incorporation was unchanged by 5–40 μM CoA, but it decreased at palmitate concentrations above 0.75 μM and DHAP concentrations above 2 mM. Glycerolipid formation was proportional to incubation time for 60 min and

to microsomal protein concentration of 0.2–0.8 mg/ml as shown in **Fig. 1**. The optimum conditions of phosphatidate formation from DHAP reported by Rao et al. (11) are similar to the conditions described here. However, these investigators did not describe the formation of neutral lipids under their incubation conditions. This is most likely explained by their use of microsomes prepared in the absence of EDTA and the presence of KF in their incubation mixture. The latter inhibits phosphatidate phosphohydrolase (26).

When rat liver microsomes are prepared in buffer containing 2.5 mM EDTA, neutral lipid formation is 3–4-fold greater than that noted for microsomes prepared in the absence of EDTA as shown in Fig. 1B. The increased glyceride synthesis is a result of a Mg^{2+}-stimulated phosphatidate phosphohydrolase that is inhibited by a variety of other divalent cations (27). Similar results with respect to EDTA-treated microsomes and Mg^{2+}-stimulated phosphatidate phosphohydrolase activity have been observed in intestinal (28) and adipose tissues (29). EDTA presumably stimulates neutral lipid formation by removing endogenous metal cations that decrease phosphatidate phosphohydrolase activity. Under the incubation conditions described here, neutral lipid comprises 25–50% of the total glycerolipid formed from DHAP.

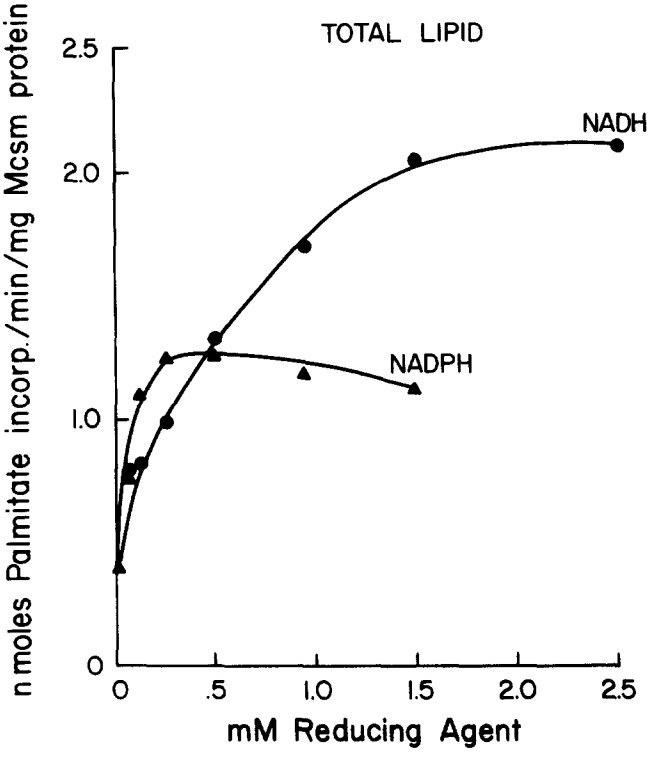

Fig. 2. The effect of increasing concentrations of NADH and NADPH on DHAP esterification by rat liver microsomes.

TABLE 1. Effect of additions on the formation of glycerolipids from dihydroxyacetone phosphate (DHAP)

Additions	Palmitate Incorporated into Total Lipid (nmoles/min/mg MCSM protein ± SEM)
None	0.30 ± 0.01
DHAP	0.67 ± 0.03
DHAP, NADPH	1.39 ± 0.15
DHAP, NADH	2.21 ± 0.20
DHAP, NADH, NADPH	4.89 ± 0.40

DHAP esterification was measured as described in Methods. When indicated, each incubation mixture contained a final concentration of 1.5 mM NADH, 0.25 mM NADPH and 1.5 mM DHAP. These results represent the means of experiments performed on several different occasions.

LaBelle and Hajra reported that NADH could not replace NADPH in the reduction of acyl- or alkyl-DHAP by Ehrlich ascites tumor (30) or rat liver microsomes (31). However, Chae, Piantadosi, and Snyder (32) and Wykle, Piantadosi, and Snyder (33), using mouse preputial gland tumor and Ehrlich ascites tumor microsomes, respectively, have shown that alkyl-DHAP could be reduced by NADH as well as NADPH under appropriate incubation conditions. The results presented in **Fig. 2** support the latter observations, since both reducing agents were effective cofactors in the formation of glycerolipids from DHAP by rat liver microsomes. However, at the optimum concentration of NADH (1.5 mM) and NADPH (0.25 mM), NADH was about 2-fold more effective in forming glycerolipids from DHAP than NADPH. Rao, Sorrels, and Reiser (11) also noted that NADH was more effective than NADPH as a reducing agent in the esterification of DHAP with rat liver microsomes. The difference in optimum concentrations for these reducing agents might indicate that more than one enzyme mediates the reaction. **Table 1** presents data supporting this concept. The formation of glycerolipids from DHAP in the presence of optimum concentrations of NADPH + NADH was greater than the sum of glycerolipid formation with each individual agent alone.

LaBelle and Hajra (31) suggested that rat liver microsomal glycerophosphate dehydrogenase might utilize NADH to reduce DHAP to *sn*-glycerol-3-phosphate before the esterification occurs. Therefore we studied DHAP esterification in the presence of labeled *sn*-glycerol-3-phosphate and compared the rate of isotope incorporation with a similar incubation in which the DHAP was omitted. If significant amounts of DHAP were reduced to *sn*-glycerol-3-phosphate, the amount of nonlabeled *sn*-glycerol-3-phosphate would be correspondingly larger and isotope incorporation into glycerolipids reduced in the presence of DHAP. The results of

TABLE 2. Effect of dihydroxyacetone phosphate (DHAP) and NADH addition on the esterification of *sn*-glycerol-3-phosphate

Additions	nmoles *sn*-[1-3-^{14}C]glycerol-3-P incorporated/mg MCSM protein/ 30 min ± SEM	
	PA[a]	NL[a]
None	28.88 ± 4.79[b]	7.00 ± 1.28[b]
NADH	29.68 ± 1.08	6.96 ± 1.69
DHAP	29.55 ± 2.54	6.49 ± 2.25
NADH, DHAP	26.14 ± 2.99	3.63 ± 0.87

[a] PA refers to phosphatidate and NL to neutral lipids (the sum of diglyceride and triglyceride).

[b] Mean ± SEM of assays in which *sn*-glycerol-3-phosphate esterification was measured in duplicate on three different occasions under incubation conditions that were similar to the usual DHAP esterification reaction mixture. The incubation mixtures contained 200 nmoles of *sn*-[1,3-^{14}C]glycerol-3-phosphate (0.1 μCi), 0.7 mM NH_4palmitate, 1.25 mg fatty acid-poor albumin, 1.5 mM $MgCl_2$, 40 μM CoA, 2.8 mM ATP, 0.3 mM dithiothreitol, 25 mM Tris-HCl pH 7.5, and 0.3 mg microsomal protein in a total volume of 0.5 ml. NADH and DHAP were added to the indicated assays to give final concentrations of 1.3 and 1.5 mM, respectively.

these studies are shown in **Table 2**. The incubation conditions were similar to those described for the standard DHAP esterification reaction except for the indicated omissions and the addition of 200 nmoles of *sn*-[1,3-^{14}C]glycerol-3-phosphate. In the presence of DHAP and NADH, the amount of labeled phosphatidate was not significantly altered, although neutral lipid radioactivity was reduced by 50%. Even when only 10 nmoles of labeled *sn*-glycerol-3-phosphate were added to the incubation mixture there was no significant reduction of phosphatidate radioactivity. This was true whether animals were fed chow, glucose, or fructose diets. These findings suggest that DHAP is probably not significantly converted to *sn*-glycerol-3-phosphate under these incubation conditions but does form phosphatidate, resulting in a reduced phosphatidate specific activity and correspondingly lower neutral glyceride radioactivity. Furthermore, the results indicate that the intermediates of the DHAP and *sn*-glycerol-3-phosphate pathways apparently do not equilibrate until phosphatidate is formed.

Table 3 shows the effect of feeding 75% glucose and fructose diets for various time intervals on the relative rate of glycerolipid synthesis. Previous studies have shown an increase in phosphatidate phosphohydrolase activity under these conditions (20), which is compatible with the increases in neutral lipid formation shown here. In the absence of added DHAP, there was no significant increase in the incorporation of labeled palmitate into neutral lipids in animals fed high sugar diets compared to those fed chow. The increase observed in total lipid formation represents increased conversion of DHAP to

TABLE 3. Effect of feeding 75% glucose and fructose diets for various time periods on the relative rate of total and neutral lipid formation from DHAP

Diet	Days	Relative Rate[a] of Lipid Formation	
		Total Lipid	Neutral Lipid
Chow		1.00	1.00
75% Glu	3	1.69	1.77
75% Glu	5	1.96	2.73
75% Glu	10	2.22	3.27
75% Fru	3	1.89	2.50
75% Fru	15	2.79	3.90

[a] Relative rate refers to the rate of palmitate incorporation into the indicated lipid in the experimental groups divided by the rate in rats fed chow. Each value represents the mean of five animals.

phosphatidate, indicating enhanced DHAP acyltransferase activity in rats fed high glucose or fructose diets.

Table 4 demonstrates that fructose-induced increases in serum triglyceride concentrations and DHAP esterification are promptly reversed when rats are returned to a chow diet. A significant reduction in the effects of fructose intake occurred within one day of chow consumption.

DHAP and *sn*-glycerol-3-phosphate acyltransferase (21) as well as phosphatidate phosphohydrolase (20) activity are significantly increased by high sugar intake, suggesting that these enzymes participate in the accompanying acceleration of hepatic glycerolipid formation. The time course of dietary-related changes in serum triglyceride concentrations, hepatic triglyceride synthesis, and the increases in microsomal enzyme activity reported here and in earlier studies (16, 20) suggest a possible causal relationship. However, other factors, including alterations in substrate levels under various dietary conditions, may also influence hepatic glycerolipid metabolism (18).

The relative importance of the DHAP pathway

TABLE 4. Effect of chow intake for various time periods on fructose induced changes in serum triglyceride concentrations and dihydroxyacetone phosphate esterification

Diet	Days	Palmitate Incorporated (nmoles/min/mg MCSM protein)		Serum TG (mg/100 ml)
		Total ± SEM	NL ± SEM	
Chow		1.70 ± 0.43	0.65 ± 0.06	88.8 ± 20.3
75% Fru	11	4.43 ± 0.27[b]	1.47 ± 0.14[b]	340.0 ± 66.5[b]
Chow[a]	1	2.59 ± 0.29	1.00 ± 0.01	123.6 ± 52.9
Chow[a]	2	2.32 ± 0.10	0.86 ± 0.08	116.4 ± 39.3
Chow[a]	4	2.51 ± 0.18	1.06 ± 0.15	94.8 ± 16.4
Chow[a]	6	1.95 ± 0.09	0.87 ± 0.09	94.0 ± 25.6

[a] Each group of animals (5) was fed a 75% fructose diet for 11 days prior to receiving a chow diet for the indicated number of days.

[b] Difference from chow groups significant ($P < 0.01$).

in hepatic glycerolipid metabolism is still unresolved. Pollock et al. (10) and Manning and Brindley (8) suggest that the DHAP pathway may play a major role in hepatic glycerolipid metabolism. However, other investigators (5–7) have concluded that DHAP esterification is a minor route of hepatic glycerolipid formation. In these studies the maximum in vitro rate of DHAP esterification in normal fed animals was 1.0–2.2 nmoles/min/mg of microsomal protein, which is significantly less than the maximum in vitro rate of *sn*-glycerol-3-phosphate esterification (5.0–7.0 nmoles/min/mg of MCSM protein) obtained under similar incubation conditions (27). Pollock et al. (10) also noted that the acyltransferase activity was considerably lower (6-fold) with respect to DHAP than *sn*-glycerol-3-phosphate in rat liver homogenates. These results along with the observation that the concentration of *sn*-glycerol-3-phosphate is 8-fold higher than DHAP in liver (34) suggest that under optimum conditions DHAP esterification plays a minor role in hepatic glycerolipid metabolism. Nevertheless, DHAP esterification as shown here and previously (11) is influenced by diet, suggesting that this pathway may participate in modulating hepatic glycerolipid biosynthesis.

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REFERENCES

1. Kennedy, E. P. 1953. Synthesis of phosphatides in isolated mitochondria. *J. Biol. Chem.* **201:** 399–412.
2. Kornberg, A., and W. E. Pricer, Jr. 1953. Enzymatic esterification of α-glycerophosphate by long chain fatty acids. *J. Biol. Chem.* **204:** 345–357.
3. Hajra, A. K., and B. W. Agranoff. 1966. Acyl dihydroxyacetone phosphate. *J. Biol. Chem.* **242:** 1074–1075.
4. Hajra, A. K. 1968. Reduction of palmitoyl dihydroxyacetone phosphate by mitochondria. *J. Biol. Chem.* **243:** 3542–3543.
5. Hill, E. E., and W. E. M. Lands. 1970. Formation of acyl and alkenyl glycerol derivatives in *Clostridium butyricum*. *Biochim. Biophys. Acta.* **202:** 209–211.
6. Okuyama, H., and W. E. M. Lands. 1970. A test for the dihydroxyacetone phosphate pathway. *Biochim. Biophys. Acta.* **218:** 376–377.
7. Rognstad, R., D. G. Clark, and J. Katz. 1974. Pathways of glyceride glycerol synthesis. *Biochem. J.* **140:** 249–251.
8. Manning, R., and D. N. Brindley. 1972. Tritium isotope effects in the measurement of glycerol phosphate and dihydroxyacetone phosphate pathways of glycerolipid biosynthesis in rat liver. *Biochem. J.* **130:** 1003–1012.
9. Bowley, M., R. Manning, and D. N. Brindley. 1973. The tritium isotope effect of *sn*-glycerol-3-phosphate oxidase and the effects of clofenapate and *N*-(2-benzoyloxyethyl) norfenfluramine on the esterification of glycerol phosphate and dihydroxyacetone phosphate by liver mitochondria. *Biochem. J.* **136:** 421–427.
10. Pollock, R. J., A. K. Hajra, and B. W. Agranoff. 1975. The relative utilization of the acyl dihydroxyacetone phosphate and glycerol phosphate pathways for synthesis of glycerolipids in various tumors and normal tissues. *Biochim. Biophys. Acta.* **380:** 421–435.
11. Rao, G. A., M. F. Sorrels, and R. Reiser. 1971. Dietary regulation of phosphatidic acid synthesis from dihydroxyacetone phosphate and fatty acid by rat liver microsomes. *Lipids.* **6:** 88–92.
12. Kaufmann, N. A., R. Poznanski, S. H. Blondheim, and Y. Stein. 1966. Effect of fructose, glucose, sucrose, and starch on serum lipids in carbohydrate induced hypertriglyceridemia and in normal subjects. *Israel J. Med. Sci.* **2:** 715–726.
13. Nestel, P. J., K. F. Carroll, and N. Havenstein. 1970. Plasma triglyceride response to carbohydrates, fats, and caloric intake. *Metabolism.* **19:** 1–18.
14. Laube, H., H. U. Klör, R. D. Fussgänger and E. F. Pfeiffer. 1973. Effect of starch, sucrose, glucose and fructose on lipid metabolism in rats. *Nutr. Metab.* **15:** 273–280.
15. Hill, P. 1970. Effect of fructose on rat lipids. *Lipids.* **5:** 621–627.
16. Waddell, M., and H. J. Fallon. 1973. The effect of high-carbohydrate diets in liver triglyceride formation in the rat. *J. Clin. Invest.* **52:** 2725–2731.
17. Zakim, P., R. S. Parkidi, R. H. Herman, and H. E. Sauberlich. 1967. Mechanism for the differential effects of high carbohydrate diets on lipogenesis in rat liver. *Biochim. Biophys. Acta.* **144:** 242–251.
18. Cahlin, E., J. Jonsson, B. Person, H. Stakenberg, P. Bjorntorp, A. Gustafson, and T. Schersten. 1973. Sucrose feeding in man. Effects on substrate incorporation into hepatic triglycerides and phosphoglycerides in vitro and on removal of intravenous fat in patients with hyperlipoproteinemia. *Scand. J. Clin. Lab. Invest.* **32:** 21–33.
19. Quarfordt, S. H., A. Frank, D. M. Shames, M. Berman, and D. Steinberg. 1970. Very low density lipoprotein triglyceride transport in type IV hyperlipoproteinemia and the effects of carbohydrate rich diets. *J. Clin. Invest.* **49:** 2281–2297.
20. Lamb, R. G., and H. J. Fallon. 1974. An enzymatic explanation for dietary induced alterations in hepatic glycerolipid metabolism. *Biochim. Biophys. Acta.* **348:** 179–188.
21. Fallon, H. J., and E. L. Kemp. 1968. Effects of diet on hepatic triglyceride synthesis. *J. Clin. Invest.* **47:** 712–719.
22. Sedgwick, B., and G. Hubscher. 1965. Metabolism of phospholipids. IX. Phosphatidate phosphohydrolase in rat liver. *Biochim. Biophys. Acta.* **106:** 63–77.
23. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
24. Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A

simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226:** 497–509.
25. Lamb, R. G., and H. J. Fallon. 1970. The formation of monoacylglycerol phosphate from *sn*-glycerol-3-phosphate by a rat liver particulate preparation. *J. Biol. Chem.* **245:** 3075–3083.
26. Vavrecka, M., M. P. Mitchell, and G. Hubscher. 1969. The effect of starvation on the incorporation of palmitate into glycerides and phospholipids of rat liver homogenates. *Biochem. J.* **115:** 139–145.
27. Lamb, R. G., and H. J. Fallon. 1974. Glycerolipid formation from *sn*-glycerol-3-phosphate by rat liver cell fractions—the role of phosphatidate phosphohydrolase. *Biochim. Biophys. Acta.* **348:** 166–178.
28. Lamb, R. G., T. G. Maynard, and H. J. Fallon. 1973. Inhibition of intestinal glycerolipid formation by chlorophenoxyisobutyrate and betabenzalbutyrate. *Federation Proc.* **32:** 690.
29. Jamdar, S. C., and H. J. Fallon. 1973. Glycerolipid synthesis in rat adipose tissue. II. Properties and distribution of phosphatidate phosphatase. *J. Lipid Res.* **14:** 517–524.
30. LaBelle, E., and A. Hajra. 1972. Enzymatic reduction of alkyl and acyl derivatives of dihydroxyacetone phosphate by reduced pyridine nucleotides. *J. Biol. Chem.* **247:** 5825–5834.
31. LaBelle, E. F., and A. K. Hajra. 1972. Purification and kinetic properties of acyl and alkyl dihydroxyacetone phosphate oxidoreductase. *J. Biol. Chem.* **247:** 5835–5841.
32. Chae, K., C. Piantadosi, and F. Snyder. 1973. Reductase, phosphatase, and kinase activities in the metabolism of alkyldihydroxyacetone phosphate and alkyldihydroxyacetone. *J. Biol. Chem.* **248:** 6718–6723.
33. Wykle, R. L., C. Piantadosi, and F. Snyder. 1972. The role of acyldihydroxyacetone phosphate, reduced nicotinamide adenine dinucleotide, and reduced nicotinamide adenine dinucleotide phosphate in the biosynthesis of *O*-alkyl glycerolipids by microsomal enzymes of Ehrlich ascites tumor. *J. Biol. Chem.* **247:** 2944–2948.
34. Greenbaum, A. L., K. A. Gumaa, and P. McLean. 1971. The distribution of hepatic metabolites and the control of the pathways of carbohydrate metabolism in animals of different dietary and hormonal status. *Arch. Biochem. Biophys.* **143:** 617–663.