LIPID METABOLISM IN THE FRUIT OF *PERSEA AMERICANA* MILL.

III. Lipogenesis and Complex Lipid Synthesis

Yoshio KIKUTA

(Department of Botany, Faculty of Agriculture
Hokkaido University, Sapporo, Japan)

Received January 9, 1969

INTRODUCTION

Interesting features of the chemical constituent of mesocarp of the fruit of *Persea americana* are an increase in lipid content (as high as 30 per cent of the flesh tissue) and a corresponding dissimilation of carbohydrates (8). The formation of lipids depends on the breakdown of carbohydrate to acetate followed by synthesis of fatty acids from the acetate. The fatty acids may then from complex lipids by combining with glycerol which is also provided from carbohydrates. Contrary to much information concerning the fatty acid synthesizing system of avocado preparations, information is lacking about lipogenesis in the fruit of avocado particularly in relation to the sources of glycerol, acetyl CoA and NADPH, and to relate this, synthesis of triglyceride, glycolipids and some important phospholipids.

The biosynthesis of triglyceride in plants was described by BARRON and STUMPF (2) in 1962. Microsomes isolated from avocado mesocarp appear to synthesize glyceride via a pathway similar to the system in animal tissue (9). The route, called the KENNEDY and KORNBERG pathway (13), proceeds from α-glycerophosphate to L-α-phosphatidic acid to D-α, β-diglyceride and finally to triglycerides. Whether avocado mesocarp mitochondria can also synthesize glycerides is not certain. Since glyceride synthesis is effected by microsomes in plants (2, 6), it is interesting that the addition of the supernatant fraction (after the centrifugation at $10{,}000 \times g$) to the mitochondrial fraction had little effect on the incorporation of glycerol to lipids. Glycerol kinase, but not

Abbreviations: ACP, Acyl carrier protein; ATP, Adenosine-5′-triphosphate; CoA, Coenzyme A; CTP, Cytidine-5′-triphosphate; NADH, Reduced nicotinamide-adenine dinucleotide; NADPH, Reduced nicotinamide-adenine dinucleotide phosphate; Tris, Tris (hydroxymethyl) aminomethane; glucose-1, glucose-6, glucose-U and acetate-1, the corresponding carbon atom, the hydrogen and the phosphate attached thereto.

glycerophosphate phosphatase, appeared to be associated with microsomes from avocado mesocarp. A time course study of the triglyceride synthesis indicated that glycerophosphate appeared first, next were phospholipids, followed by monoglycerides, diglyceride and triglycerides in that order. The origin of the monoglycerides remained a question, although they concluded the monoglycerides would arise from lysophosphatidic acid by the hydrolytic removal of the inorganic phosphate (2). The direct acylation of glycerol was not studied. BRADBEER and STUMPF (3) found that peanut mitochondria incorporated P^{32} from ATP^{32} predominantly into phosphatidic acid together with a small incorporation into phosphatidyl ethanolamine. This incorporation did not involve glycerophosphate as an intermediate but resulted from the phosphorylation of 1, 2-diglyceride by ATP. This diglyceride phosphokinase also phosphorylated 1-monoglyceride to yield lysophosphatidic acid. The significance of the enzyme, originally reported in microsomes of guinea-pig brain (11) and in cytoplasmic particulate fraction of *E. coli* (24), in triglyceride and phospholipid synthesis in plants, is uncertain.

The role of phosphatidic acid as an intermediate in the biosynthesis of phospholipids and triglycerides has now been well established (9, 13, 17, 27, 28, 31). There are two main pathways by which phosphatidic acid is metabolized in living organisms: 1) The formation of CDP-diglycerides by interaction with CTP (1), and 2) the hydrolytic removal of inorganic phosphate with the formation of diglycerides (30, 31), a common precursor of triglycerides and phospholipids. Alpha-glycerophosphate which has been shown to dominate in the role of fatty acid esterification is catalyzed by microsomes and mitochondria of rat liver, and functions as a regulatory factor in fatty acid esterification (29). Interestingly, BRANDES and SHAPIRO (4) reported that phosphatidic acid phosphatase was inhibited by palmitoyl CoA in a concentration as low as 5×10^{-5} *M*. This inhibition is competitive with respect to phosphatidic acid. The incorporation of glycerophosphate into lipids has been reexamined by CHENIAE (6) with microsomes extracted from spinach leaves. Kinetic studies on the products indicated that phosphatidic acid was a precursor for triglyceride. The occurrence of monoglyceride resulted from another phospholipid, probably lysophosphatidic acid.

Little is known concerning the mode of biosynthesis of glycerides in plants even though a number of oil bearing plants, commercially and dietetically important, store triglyceride almost exclusively. In this paper, some evidence concerning avocado fruit lipogenesis will be discussed, and to relate this, the synthesis of complex lipids in the fruit of avocado.

MATERIALS AND METHODS

Plant materials

The developing fruits of avocado, *Persea americana* MILL. (*P. gratissima* GAERTH.), variety HASS, were used in the studies on the lipogenesis and complex lipid synthesis. The tissue slices and subcellular particles were prepared by the technique described in the previous papers of this series (15, 16). The fruits were grown on the Riverside campus of the University of California, and after harvest were stored at 5°C for at least 24 hours before being used for experiments.

Isolation of lipids

The lipids after the incubation in a respiratory flask at 25°C following vacuum infiltration of glucose-C^{14} or acetate-1-C^{14} were extracted from the tissue slices of avocado mesocarp and separated by silicic acid column chromatography (10). The fatty acid composition was determined by gas liquid column chromatography. Specific radioactivity of the isolated fatty acid was determined by gas liquid chromatographic analysis followed by C^{14} counting of the corresponding effluent or 0.01 *N* NaOH titration after saponification of the lipids followed by C^{14} counting.

Chemicals

All chemicals were obtained from commercial sources. Acetate-1-C^{14} (7 mC/mM), glucose-1-C^{14} (5 mC/mM), glucose-6-C^{14} (1 mC/mM) and glucose-U-C^{14} (73 mC/mM) were purchased from Calbiochem Co., California. Coenzyme A and ATP were obtained from Biochemicals Inc., Wisconsin, U. S. A., and NAD and NADP from C. F. Boehringer & Soehne GmbH, Mannheim, Germany.

Estimation of catabolic pathway participation

A batch of 100 tissue slices from the mesocarp of a single chilled fruit were infiltrated with a specifically labelled glucose-1-C^{14} and/or glucose-6-C^{14} of equal specific radioactivity (37,000 cpm/μmole glucose) in 5 m*l* of 0.05 *M* phosphate buffer, pH 7.0 for 15 minutes under vacuum (7 to 10 mm Hg). The resulted slices were rinsed once with non-radioactive buffer, blotted dry with filter paper, and placed on a stainless wire net covered with cheese cloth in a 125 m*l* respiratory flask. The tips of the cloth were dipped in 20 m*l* of non-radioactive phosphate buffer solution so that the slices were kept moist during the incubation period.

The side arm of the respiratory flask was used for trapping respiratory

carbon dioxide in 5 per cent NaOH. In the present procedure, 33,300 cpm of glucose were infiltrated into one gram of tissue slices. Respiratory carbon dioxide, lipids and water soluble acids were measured in cumulative sequence.

After incubation, the slices were transferred to chilled 10 per cent trichloroacetic acid and then frozen. The frozen samples were crushed in a mortar and the crude lipids were extracted with chloroform-methanol (2 : 1, by volume). The slurry was centrifuged in a crinical centrifuge and then the precipitate was again homogenized to extract residual lipids with additional chloroform-methanol mixture. The aqueous and chloroform fractions were washed with chloroform or distilled water, respectively.

The residues were extracted with cold 5 per cent trichloroacetic acid and resulting debris was hydrolyzed overnight in *N* NaOH at 50°C. Only the chloroform fraction was subjected to further analysis.

An aliquot of the chloroform fractions was plancheted for radioactive counting. The remainder of the fractions was saponified and separated into aqueous fraction and lipid fraction.

Estimation of fatty acyl-C^{14}/glyceryl-C^{14} ratio

Complex lipids were dried and re-extracted with diethyl ether and added 1 m*l* of 5 per cent ethanolic KOH. After standing for 2 hours at room temperature, the mixture was added 3 m*l* of *N* HCl to acidify and to separate aqueous glycerol moiety and fatty acyl moiety. The C^{14} ratio of fatty acyl/glyceryl is a measure of complex lipid synthesis *de novo*. The theoretical value was assumed hexadecanoic acid for minimal value and octadecanoic acid for maximal value, while hexose, glycerol, ethanolamine, inositol and choline were also included in glyceryl fraction.

Preparation of cytoplasmic particles

The peeled mesocarp tissue (100 grams) from a single fruit stored in crushed ice was grated into a glass container and dispersed in 200 m*l* of 0.4 *M* sucrose containing 4 m*M* glutathione, and 20 m*M* Tris-HCl buffer, pH 8.0. The brei was squeezed in a 4-layered cheese cloth bag with a hand press. The extract was centrifuged at $1{,}000 \times g$ for 10 minutes. The supernatant was again centrifuged at $10{,}000 \times g$ for 30 minutes. After the centrifugation at $10{,}000 \times g$, the pellet was suspended with 0.4 *M* sucrose-tris buffer, pH 7.0 in a Potter-homogenizer tube with a Teflon pestle, and the suspension was again centrifuged at $10{,}000 \times g$ for 30 minutes. The collected pellet was homogenized and taken up in 0.4 *M* sucrose containing 20 m*M* Tris-HCl buffer, pH 7.0. All the processes were carried out in a room at 4°C, with equipment that had been a cold room for at least 12 hours before the preparation.

Enzyme assays

NADPH dependent dehydrogenase activity was measured by the absorbancy change followed at 340 mμ with continuous recording the progress of the reaction in the DU type of Beckman spectrophotometer with speedmax recorder and a one cm wide cell. One unit of enzyme activity is defined as a change of O. D. in 0.01 unit at 340 mμ per minute per one cm path. The assay reaction mixtures contained 10 μmoles of substrate (glucose-6-phosphate, malate or isocitrate), 2 μmoles of pyridine nucleotide ($NADP^+$ or NAD^+), 10 μmoles of $MnSO_4$ and 0.1 m*l* of homogenate as enzyme source. All reaction mixtures were in a total volume of 3 m*l* 0.2 *M* Tris-HCl buffer, pH 7.5 containing 0.005 *M* reduced glutathione. The reaction was initiated stepwise addition of Mn^{++}, substrate and pyridine nucleotide. The data were taken from a change at the initial velocity of 15 seconds.

NADPH: dehydrogenase dependent fatty acid synthetase activity was measured by coupling the fatty acid synthesizing system with NADPH generating system. The reaction mixtures contained 10 μmoles of ATP, 50 mμmoles of Co A, 70 mμmoles of $NADP^+$ and 0.5 μmoles of substrate (glucose-6-phosphate, malate and/or isocitrate) as hydrogen donor, and 30 μmoles of HCO_3^-, and 10 μmoles of $MnSO_4$. Total volume of reaction mixture was adjusted to 1 m*l* with 0.4 *M* sucrose-tris buffer, pH 7.0, and 0.2 to 0.4 m*l* of cytoplasmic particulate fraction (2–4 mg nitrogen) in 0.4 *M* sucrose-tris buffer, pH 7.0 containing 0.5 m*M* reduced glutathione. The reactions were initiated in 15 m*l* centrifuge tubes at 28°C by the addition of 100 mμmoles of acetate-1-C^{14} and were stopped by the addition of 4 m*l* chloroform-methanol-hydrochloric acid (200 : 100 : 1, by volume) and lipids were isolated.

Protein determination

The protein concentration of cytoplasmic particles was determined by micro-Kjeldahl digestion followed by Nesslerization. The samples were first precipitated with 10 per cent trichloroacetic acid, washed with chloroform-methanol mixture, and then digested with concentrated H_2SO_4.

Radioactivity determination

All radioactivity determinations were made using a Tracer Lab. Compumatic II scaler with a gas flow G. M. tube of thin end window type. For counting lipids, a thin layer (less than 0.1 mg/cm^2) of the sample was mounted to avoid self absorption of C^{14}. For $BaC^{14}O_3$, either infinite thickness of the sample (more than 20 mg/cm^2) or a calibration made from the self-absorption curve was necessary to determine the radioactivity of $BaC^{14}O_3$ precipitate.

RESULTS

Vacuum infiltrated glucose could be metabolized via catabolic pathways and then converted to lipids in Hass avocado mesocarp tissue slices. Figure 1 shows that the time course studies on the accumulation of lipids following the dissimilation of uniformly labelled glucose by the tissue slices during six hours' incubation. Approximately 50% of total labelled carbons was finally

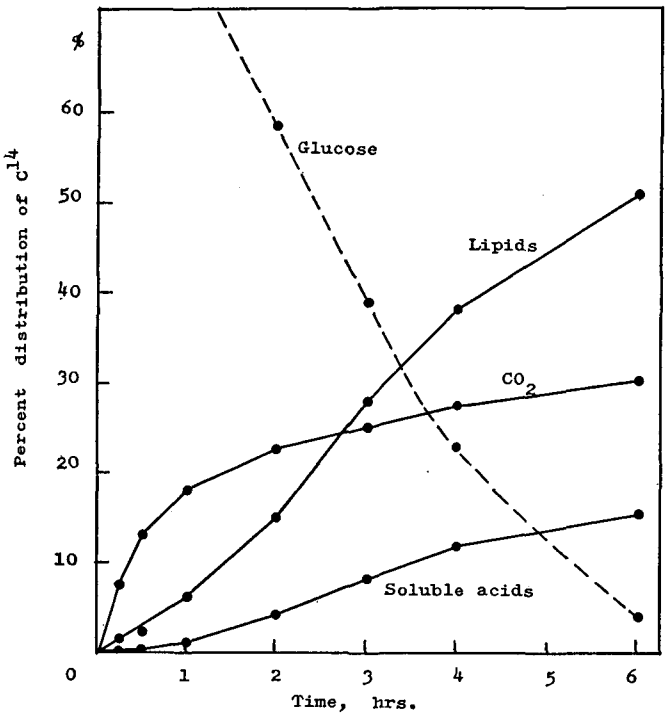

Figure 1. Percentage incorporation of uniformly labelled glucose into respiratory carbon dioxide, lipids and soluble acids by tissue slices of Hass avocado fruit.

found to accumulate in lipids and 30% of that was in the respiratory carbon dioxide after 6 hours' incubation. The remainder was in alcohol soluble and insoluble fractions. The lipid fraction was further separated into the glyceryl and fatty acyl moieties of the comlex lipids by saponification. It is then indicated in figure 2 that approximately 4% of total labelled carbons was in the glyceryl moiety and 46% the fatty acyl moiety. Furthermore, the fatty acyl-C^{14} to glyceryl-C^{14} ratio was initially low, but later, increased upto 11.3 where glucose-U-C^{14} was used up. This value of 11.3 meets the complete labelling

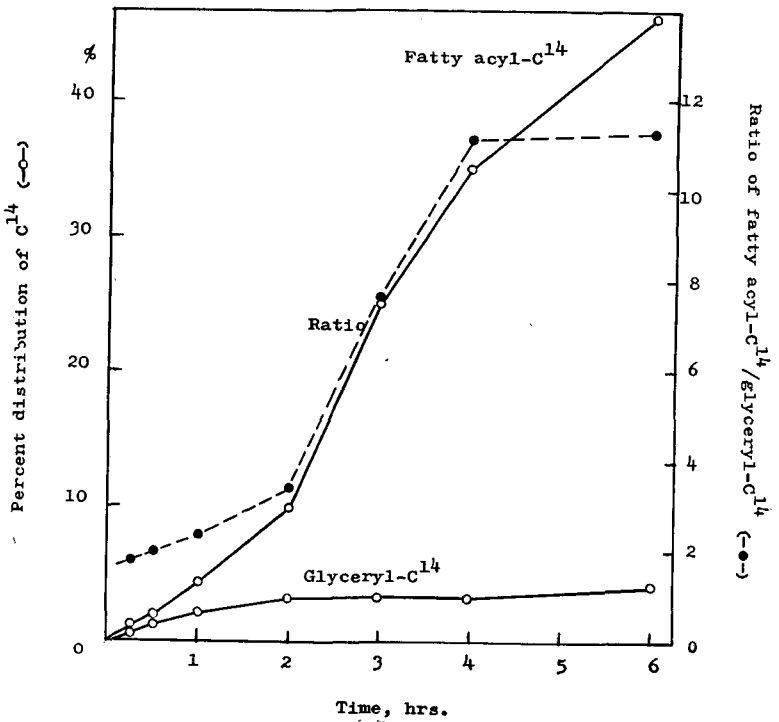

Figure 2. Changes in distribution of label in lipids synthesized from glucose-U-C^{14} (solid line) and in the fatty acyl/glyceryl ratio (broken line).

TABLE 1. Distribution of label in lipids synthesized from uniformly labelled glucose by tissue slices of Hass avocado fruit after four hours' incubation

Lipid	Distribution %	Fatty acyl/Glyceryl Ratio	
		Observed	Theoretical
Triglyceride	45.6	15.9	16.0–18.0
Diglyceride	14.1	11.7	10.7–12.0
Monoglyceride	12.1	5.8	5.3– 6.0
Glycolipid	12.1	8.2	2.2– 4.0
Phosphatidic acid	4.3	3.1	10.7–12.0
Phosphatidyl glycerol	1.9	1.5	5.3– 6.0
Phosphatidyl ethanolamine	1.8	6.7	9.4– 7.2
Phosphatidyl inositol	0.7	1.9	3.5– 4.2
Phosphatidyl choline	7.4	10.0	4.6– 4.5

of diglyceride or phosphatidic acid appearing in tissue slices, whereas the ratio fails to show its maximum expectation of 17 even after the six hours' incubation. In Table 1, the ratios for neutral lipids are close to the theoretical values but those for the phospholipids indicate the multiplicity of their metabolism.

The relative participation of the catabolic pathways of glucose was examined in such a way that specifically labelled glucose-C^{14} was used as a substrate and the rate of release of carbon number 1 (C–1) and carbon number 6 (C–6) of glucose in the respiratory carbon dioxide and of incorporation of label into lipids and organic acids in tricarboxylic acid cycle members were determined with cumulative time. The lipid fraction was again separated into the glyceryl moiety and fatty acyl moiety of the complex lipids by saponification. The results are shown in figure 3. The evolution of $C^{14}O_2$ from

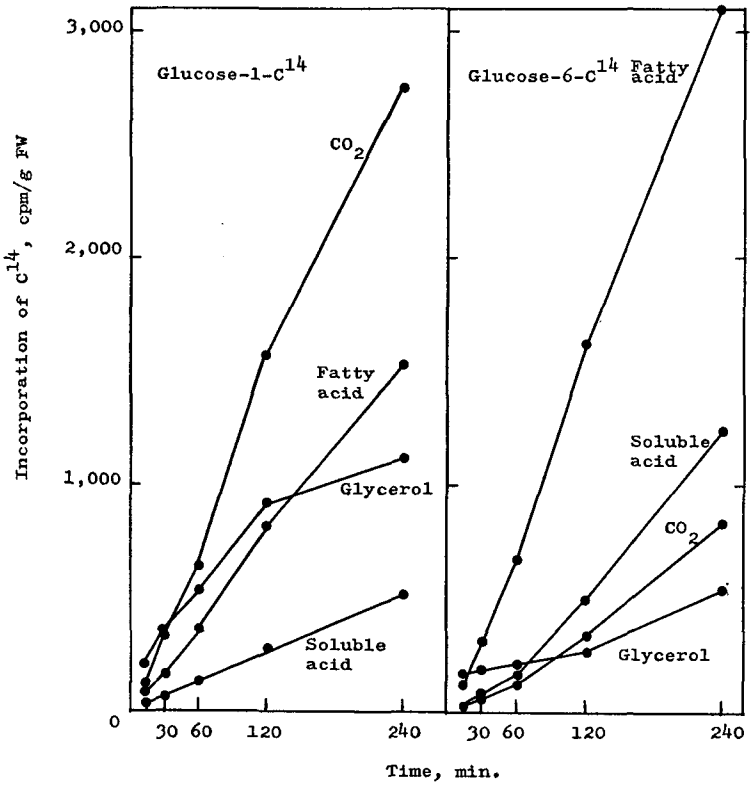

Figure 3. Incorporation of specifically labelled glucose-1-C^{14} and glucose-6-C^{14} of equal specific radioactivity by tissue slices of Hass avocado fruit.
Left: Glucose-1-C^{14}, and Right: Glucose-6-C^{14}.

glucose-1-C^{14} and glucose-6-C^{14} is clearly shown to indicate the participation of the oxidative pentose phosphate pathway in the tissue slices. Although the use of the $C^{14}O_2$ from C–6 to $C^{14}O_2$ from C–1 ratio may be quite misleading as a measure of the participation of pathways, the change in this ratio are only an indication of qualitative changes in the pathway participation. On the other hand, the label was accumulated in the lipid fraction both from C–1 and C–6 of glucose, whereas C–1 of glucose was preferentially incorporated into glyceryl moiety and C–6 was incorporated into fatty acyl moiety during two hours' period. Later, the label from C–6 of glucose was found to accumulate in the lipid fraction in which both glyceryl and fatty acyl moieties were labelled. This is due to the rapid elimination of C–1 from glucose released as carbon dioxide by the direct oxidation of glucose-6-phosphate and yet, the C–6/C–1 ratio could not approximate a measure of the oxidative pentose phosphate pathway participation in such tissue that the lipid synthesis was a major interest in the tissue metabolism.

TABLE 2. Relative enzyme activity levels in avocado mesocarp

Assay reaction mixtures: 10 μmoles of substrate (glucose-6-phosphate, malate or isocitrate), 2 μmoles of pyridine nucleotide (NAD^+ or $NADP^+$), 10 μmoles of $MnSO_4$ and 0.1 ml of homogenate as enzyme source. All reaction mixtures were in a total volume of 3 ml 0.2 *M* Tris-HCl buffer, pH 7.5, containing 0.005 *M* reduced glutathione. O. D. change at 340 mμ was measured in a Beckman spectrophotometer with speedmax recorder and a one cm wide cell. One unit of enzyme activity is defined as a change of O. D. in 0.01 unit at 340 mμ per minute per one cm path. Total activity is the activity in 100 g fresh mesocarp tissue.

	Total activity	Requirement
Experiment I: Particulate fraction		
Malic enzyme	65	$NADP^+$, Mn^{++}
NADPH: glucose-6-phosphate dehydrogenase	140	$NADP^+$, Mn^{++}
NADPH: isocitrate dehydrogenase	220	$NADP^+$, Mn^{++}
NADH : isocitrate dehydrogenase	130	NAD^+, Mn^{++}
Experiment II: Supernatant		
Malic enzyme	1,425	$NADP^+$, Mn^{++}
NADPH: glucose-6-phosphate dehydrogenase	3,337	$NADP^+$, ?
NADPH: isocitrate dehydrogenase	3,600	$NADP^+$, ?
NADH: isocitrate dehydrogenase	0	
Experiment III: NADPH: isocitrate dehydrogenase		
Supernatant after 10,000×*g*	4,300	$NADP^+$, ?
Supernatant after wash	218	$NADP^+$, Mn^{++}
Particulate fraction after wash	84	$NADP^+$, Mn^{++}

Because of the importance of the relative participation of the oxidative pentose phosphate pathway alongside the glycolytic degradation of glucose followed by *de novo* synthesis of lipids in avocado frurit, the relative dehydrogenase activity levels which may supply reducing power supporting lipid synthesis were investigated. Table 2 summarized the levels of three NADPH dependent dehydrogenase activities in the homogenate from the mesocarp tissue of avocado fruit. The reaction was initiated by the stepwise additions of 10 μmoles of substrate (glucose-6-phosphate, malate or isocitrate), 2 μmoles of $NADP^+$ and 10 μmoles of $MnSO_4$, pH 7.0. The change of optical density at 340 mμ was measured with a Beckman spectrophotometer. Most of the NADPH: dehydrogenase activity was found in the supernatant fraction. Although the assays were done with crude extracts, it is significant that the high level of NADPH: glucose-6-phosphate dehydrogenase in the extract may reflect the participation of the oxidative pentose phosphate pathway of glucose catabolism by the mesocarp tissue. In plants, and probably in any organism, the rate of NADPH utilization may be a primary factor in the control of the

TABLE 3. Relative participation of enzyme activity levels supporting acetate assimilation by the particulate system isolated from mesocarp of avocado fruit

Reaction mixtures: 10 μmoles ATP, 70 mμmoles $NADP^+$, 50 mμmoles CoA, 10 μmoles Mn^{++}, 30 μmoles HCO_3^- and 0.5 μmoles substrate (isocitrate, malate or glucose-6-phosphate, as hydrogen donor) with 0.4 ml of avocado particulate preparation (approx. one mg protein per 0.1 ml) in total volume of 1.0 ml 0.4 *M* sucrose-0.02 *M* Tris-HCl buffer, pH 7.0 containing 0.005 *M* reduced glutathione. The reactions were initiated with the addition of 0.1 ml of 200 mμmoles (200.000 cpm) acetate-1-C^{14}, in a 15 ml centrifuge tube. The incubation duration was 2-hour at 28°C. The data show cpm acetate incorporated per mg particle protein.

Substrate	Acetate incorporation	
	Lipids cpm	Water soluble acids cpm
Experiment I		
Isocitrate	2,054	3,489
Malate	1,608	4,114
Glucose-6-phosphate	1,582	4,560
None	—	6,300
Experiment II		
Isocitrate	2,249	4,876
Malate	1,716	5,529
Glucose-6-phosphate	1,578	5,036
None	701	7,400

oxidative pentose phosphate pathway participation. It is also noteworthy that NADPH: isocitrate dehydrogenase showed the highest activity among the examined system. Even though malic enzyme was the least active system in the mesocarp of avocado fruit, malate and isocitrate could also provide NADPH for lipid synthesis by the particulate system isolated from the fruit (Table 3).

The label of glucose-6-C^{14} was metabolized by ways of the oxidative pentose phosphate pathway and of the glycolytic pathway without loss of label, and there could eventually be an equilibrium between the two trioses which showed steady state incorporation of the label into the lipid fraction of the tissue slices. Figure 4 shows the incorporation of the label from glucose-6-C^{14} into the fatty acyl moiety of the classes of lipids separated by silicic acid column chromatography. The pooled fractions were saponified by 5% KOH in methanol. In figure 5, the ratio of fatty acyl-C^{14} to glyceryl-C^{14} of the

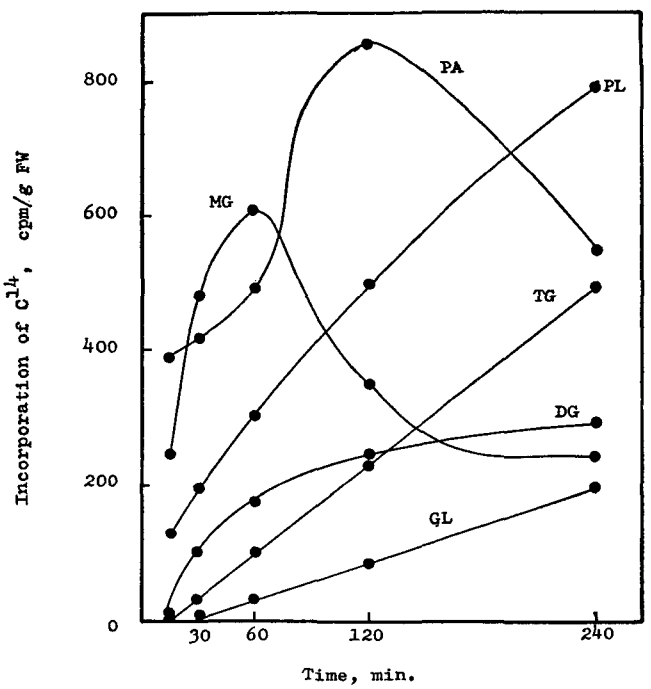

Figure 4. Incorporation of glucose-6-C^{14} into the fatty acids of classes of lipids by tissue slices of avocado fruit[1].

1) The following symbols are used in figures: NL, Neutral lipid; TG, Triglyceride; DG, Diglyceride; MG, Monoglyceride; GL, Glycolipid; FA, Free fatty acid; PL, Phospholipid; PA, Phosphatidic acid; PG, Phosphatidyl glycerol; PE, Phosphatidyl ethanolamine; PI, Phosphatidyl inositol; and PC, Phosphatidyl choline.

classes of lipids has been plotted versus incubation time. This value is an indication of complex lipid synthesis *de novo*. It is clear from the figures 4, 5 and 6 that the phosphatidic acid was labelled rapidly followed by the diglyceride and finally triglyceride fractions. It is interesting that the labelling behavior of monoglycerides gave high fatty acyl/glyceryl ratios up to the theoretical value of 8 and was labelled faster than the phosphatidic acid fraction.

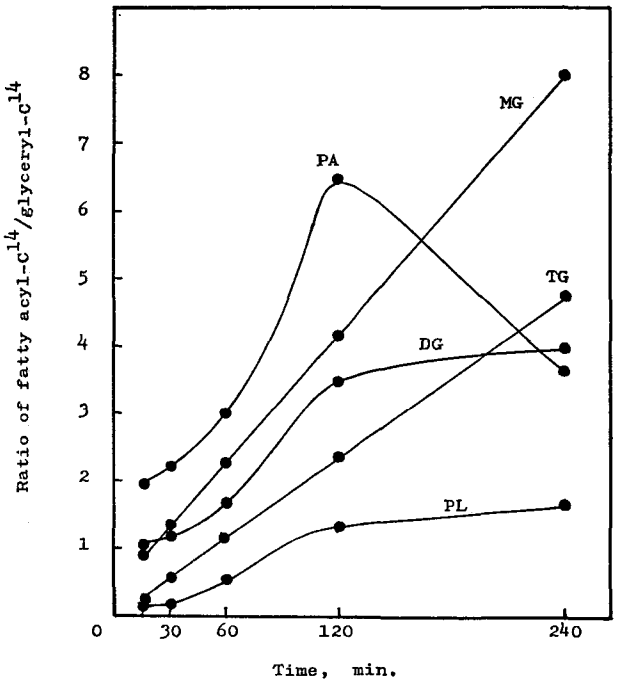

Figure 5. Changes in ratios of fatty acyl-C^{14}/glyceryl-C^{14} in classes of lipids synthesized from glucose-6-C^{14} by tissue slices of the mesocarp of avocado fruit.

This is an indication that the hydrolysis of the phosphate from lysophosphatidic acid was occurring in the tissue slices. Specific radioactivity of the fatty acyl moiety in the classes of lipids shown in figure 6 indicates that all fractions of phospholipid are diluted in time by non-labelled fatty acids. The following possibilities may be considered in explaining the observation of figures 4, 5 and 6. 1) There is a metabolically labile lysophospholipid. 2) Direct acylation of lysophospholipid occurred. 3) Fatty acyl moiety was exchanged with the other phospholipid which had not been labelled, *vice versa*. 4) Phospholipid synthesis *de novo* occurred. The multiplicity in phospholipid formation might

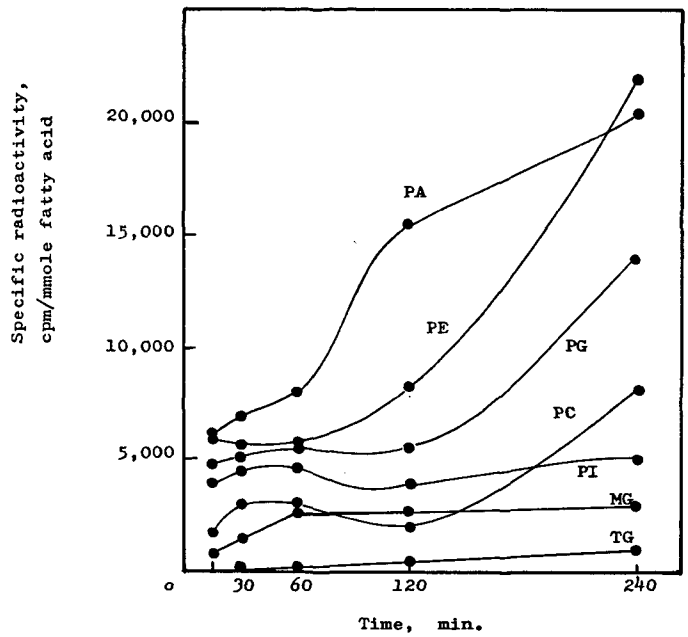

Figure 6. Specific radioactivity of fatty acids in classes of lipids synthesized from glucose-6-C^{14} by tissue slices of Hass avocado fruit.

be due to the selective incorporation of a different fatty acid into the specific position of each kind of phospholipids.

Since acetate was a primary precursor of long-chain fatty acid in the synthesized complex lipids by the tissue slices (15), the time course studies on the fatty acid synthesis from acetate-1-C^{14} were conducted over a four-hour period. The crude lipids were extracted from the tissue slices with acidic methanol chloroform mixture. The complex lipids were separated by silicic acid column chromatography. The radioactive peaks of the classes of complex lipids, *i. e.*, triglyceride, diglyceride, monoglyceride, phosphatidic acid, lysophosphatidic acid, phosphatidyl glycerol, phosphatidyl ethanolamine and phosphatidyl choline, were examined their fatty acid composition and the distribution of the label. Table 4 shows the percentage distribution of labelled fatty acid in the classes of complex lipids and their normal fatty acid composition. The specific radioactivity was also determined in Table 5. From the data presented, palmitoleic, linoleic, linolenic and arachidic acids were least labelled fatty acid among those examined. On the other hand, palmitic and oleic acids in monoglyceride, diglyceride and phosphatidic acid were quickly labelled and

TABLE 4. Changes of percentage distribution of labelled fatty acid in the classes of lipids synthesized from acetate-1-C^{14} by tissue slices from avocado mesocorp

Lipid	% Distribution of labelled fatty acid in lipid						
Time, min.	16:0	16:1	18:0	18:1	18:2	18:3	20:0*
	%	%	%	%	%	%	%
Triglyceride							
30	14.7	4.8	31.9	42.3	3.2		3.1
60	19.0	4.7	23.9	47.3	3.0		3.0
120	21.6	4.7	16.0	53.7	2.2		1.8
240	21.2	4.4	5.4	65.4	1.9		1.7
Diglyceride							
30	19.4	3.2	17.9	56.5	1.4	1.2	0.4
60	4.23	4.2	29.8	16.9	3.8	2.0	1.0
120	14.9	3.0	28.5	40.8	4.6	3.0	5.2
240	11.4	2.0	14.7	65.4	2.3	1.7	2.5
Monoglyceride							
30	39.6	5.1	23.9	6.3	2.3	2.7	20.1
60	46.0	7.2	28.7	5.5	4.0	4.0	4.6
120	10.3	5.4	52.2	17.8	3.9	4.2	6.2
240	11.1	8.5	26.0	23.9	9.2	9.1	12.2
Phosphatidic acid							
30	35.5	6.3	28.8	22.0	3.6	0.3	3.5
60	24.2	5.3	13.8	46.0	2.6	1.6	6.5
120							
240	12.7	0.8	16.5	67.0	1.9	2.0	0.1
Phosphatidyl glycerol and Lysophosphatidic acid**							
30	41.5	4.6	12.3	11.5	1.8	3.1	25.2
60	33.7	4.0	16.8	34.2	3.2	3.3	4.8
120	9.7	7.4	21.8	32.0	11.6	7.8	9.7
240	10.1	13.7	15.0	26.7	13.6	9.2	11.7
Phosphatidyl ethanolamine							
30	25.7	5.0	31.8	24.0	3.6	1.8	8.1
60	19.1	4.5	25.0	30.4	11.2	4.7	5.1
120	12.9	4.4	23.6	35.2	11.5	5.8	6.6
240	7.9	4.3	23.8	39.5	11.8	7.0	5.7
Phosphatidyl choline							
30	3.9	1.4	14.7	73.5	4.3	2.0	0.2
60	3.3	2.4	12.3	70.0	4.8	4.1	3.1
120	2.2	1.3	12.7	73.1	5.6	2.9	2.2
240	2.2	3.0	11.7	67.9	9.5	3.7	2.0

Lipid	% Normal fatty acid composition of lipids						
	16:0	16:1	18:0	18:1	18:2	18:3	20:0*
	%	%	%	%	%	%	%
Triglyceride	17.7	7.8	0.2	61.2	12.0		1.1
Diglyceride	12.6	6.2	0.4	67.4	11.2	0.8	1.8
Monoglyceride	65.2	7.0	2.4	7.5	5.4	6.3	6.2
Phosphatidic acid	18.8	5.6	5.6	52.5	8.0	9.0	0.5
Phosphatidyl glycerol and Lysophosphadic acid**	23.4***	1.9	0.6	39.7	14.2	17.0	3.2
Phosphatidyl ethanolamine	16.8	3.7	1.0	30.5	24.4	22.8	0.8
Phosphatidyl choline	8.2	2.8	0.6	50.9	5.1	32.0	0.4

* Including several unknown fatty acids.

** Compounds not positively identified.

*** Including trans-3-hexadecenoic acid.

later diluted with endogenous substrate or equilibrated with non-radioactive fatty acids. Stearic acid was synthesized almost as rapidly as palmitic and oleic acids after vacuum infiltration of acetate, and this was due to the anaerobiosis prevailling during vacuum infiltration period (16). The phospholipids showed the highest specific radioactivity as indicated in figure 6, and triglyceride was the least labelled lipid and later equilibrated with diglyceride in the tissue slices.

DISCUSSION

Inasmuch as carbohydrate is a major source of acetyl CoA for lipogenesis, it is of considerable interest to examine the overall mechanism of lipid synthesis from glucose. The process of glycolysis and pyruvate decarboxylation results in formation of 2 moles of CO_2 and 2 moles of acetyl CoA from each mole of glucose. Thus four of every six carbon atoms of glucose are available for fatty acid synthesis. Fatty acid arising from malonyl CoA pathway yields

TABLE 5. Changes of specific radioactivity of fatty acid in the classes of lipids synthesized from acetate-1-C^{14} by tissue slices of the mesocarp of developing avocado fruit

Lipid	Specific radioactivity, cpm/μg fatty acid						
Time, min.	16:0	16:1	18:0	18:1	18:2	18:3	20:0
Triglyceride							
30	86	38	1048	75	3		106
60	99	57	7546	95	33		88
120	276	121	9810	180	35		136
240	250	152	10421	254	32		308
Diglyceride							
30	868	462	9555	593	49		98
60	1106	438	41641	680	734		
120	358	459	10700	166	359		1055
240	208	86	9229	253	47	542	475
Monoglyceride							
30	528	540	15688	472	144		
60	1026	1494	16734	1083	1093		
120	172	384	6210	255	1612	210	
240	158	407	1861	198	444	336	
Phosphatidic acid							
30	1669	1989	16038	234	106		
60	1055	729	9550	445	320		
120							
240	435	368	8825	670	366		
Posphatidyl glycerol and Lysophosphatidic acid*							
30	2334	1385	26597	465	436	145	
60	763	369	9550	301	285		830
120	179	360	2875	194	378	211	890
240	345	1232	3780	270	920	143	1812
Phosphatidyl ethanolamine							
30	1962	1972	13875	596	60	60	
60	1237	1153	14156	706	685	120	
120	3483	16160	150375	4928	3316	211	
240	406	958	25050	1142	388	184	
Phosphatidyl choline							
30	770	468	36030	2524	708	86	
60	333	850	75180	1631	3700	655	
120	740	1667	30475	3648	3000	866	
240	621	3407	38645	2506	3508	271	

* Compounds not positively identified.

primarily palmitic acid. The process may be represented as follows:

$$4C_6H_{12}O_6 + O_2 \longrightarrow C_{16}H_{32}O_2 + 8CO_2 + 8H_2O$$

The theoretical energetic efficiency of this process is high. It is noted that 4 moles of glucose approximate 2,744,000 cal., whereas 1 mole of palmitate approximates 2,400,000 cal. when these are completely oxidized, where 66.7% of glucose carbon atoms is stored in palmitic acid, and the process has 87.5% of the theoretical energy efficiency of this conversion of glucose to palmitic acid. Although the metabolic efficiency cannot be stated precisely, it may be formed an estimate of biological energetics that 152 moles of $ATP_{equiv.}$[1] produced from every four moles of glucose and 130 moles of $ATP_{equiv.}$ by that of each mole of palmitic acid are the resulted picture of the possible evaluation, when the above substrates are biologically oxidized. In such case of the maximum metabolic efficiency under the proper conditions will be 85.5%.

Suppose the metabolic process were the conversion of glucose to acetyl CoA via glycolytic pathway followed by the fatty acid synthesis, every four moles of glucose could provide 8 moles of acetyl CoA, 8 moles of CO_2, 8 moles of ATP and 16 moles of NADH. While the requirements for the synthesis of palmitic acid are 8 moles of acetyl CoA, 7 moles of HCO_3^-, 7 moles of ATP and 14 moles of NADPH. Thus 7 moles of $ATP_{equiv.}$ are obtained when extra 2 moles of NADH are completely oxidized by this system. Note that the transformation of NADH to NADPH in a given biological system is remained uncertain, although NADH is equivalent to NADPH on the basis of biological energetics, in that the transformation of NADH to NADPH by mitochondria requires ATP. Fatty acid synthesis by mitochondrial system, *i.e.*, non-biotin system which does not form malonyl CoA during condensation reactions, 50% of total NADPH required may be replaced by NADH, could be facilitated.

Contrary to the mitochondrial system, the fatty acid synthesis, especially palmitic acid synthesis in avocado, seems to be the property of soluble enzyme system and to take an avidin sensitive pathway. Thus NADPH generating system appears to be important to elucidate the metabolic process of lipogenesis in the soluble fraction of avocado mesocarp tissue. The participation of three NADPH generating systems is predicted from the experiment *in vitro*. They are NADPH: glucose-6-phosphate dehydrogenase, NADPH: isocitrate dehydrogenase and malic enzyme or NADPH: malate dehydrogenase. All of them facilitate NADPH for the lipid synthesis of avocado shown in Table 3. It

1) $ATP_{equiv.}$ means the heat content change for the formation of ATP, hence NADH and NADPH give 3ATP, respectively, FADH gives 2ATP when these are oxidized.

is evident that the metabolism of organic acid in the tissue also regulates lipogenesis in plants. In addition, citrate-1, 5-C^{14} and succinate-1, 4-C^{14} did not give the label incorporation into lipid fraction of the tissue, and acetate-1-C^{14} and acetate-2-C^{14} incorporated into lipids and tricarboxylic acid which, the latter, was further metabolized to indicate the presence of complete cyclization of the tricarboxylic acid cycle operation in the avocado fruit (16).

In plants, presumably in any organism, the rate of NADPH utilization is a primary factor for controlling the oxidative pentose phosphate pathway participation (25). The release of $C^{14}O_2$ from glucose-1-C^{14} ($G_{1(CO_2)}$) and glucose-6-C^{14} ($G_{6(CO_2)}$) is clearly shown in figure 3 to indicate the presence of the oxidative pentose phosphate pathway in the tissue slices. Since the label of C-1 from glucose was considerably incorporated in the lipids of avocado fruit, the use of $G_{6(CO_2)}/G_{1(CO_2)}$ ratio may be misleading as a measure of the participation of this pathway. For the estimation of the relative participation of catabolic pathways by glucose, the following assumptions are required: 1) Emden-Meyerhof-Parnas (glycolytic) pathway and Warburg-Dickens (oxidative pentose phosphate) pathway account for the primary breakdown of glucose and the operations of other minor pathways are dismissed, 2) the evolution of $C^{14}O_2$ from C-1 of glucose is a rapid process, 3) the pentose formed in direct oxidation of glucose is not metabolized extensively in further catabolic process during the experimental period, and 4) trioses formed in the glycolytic process are equivalent to each other and they are promptly incorporated into tricarboxylic acid and fatty acid which are trapped in chloroform-methanol extract. The calculation was based on the above assumptions. The total amount of glucose engaged in catabolic function (G_t) is the sum of the respiratory $C^{14}O_2$ from C-1 of glucose ($G_{1(CO_2)}$), C-1 in lipids ($G_{1(lipid)}$), and C-1 in tricarboxylic acid ($G_{1(TCA)}$) fractions.

$$G_t = G_{1(CO_2)} + G_{1(lipid)} + G_{1(TCA)}$$

Then, the relative participation of pentose phosphate pathway (G_p) and glycolytic pathway (G_g) are:

$$G_p = \frac{G_{1(CO_2)} - G_{6(CO_2)}}{G_t}, \text{ and } G_g = 1 - G_p$$

It is evident that the equation above gives a minimum value for G_p and maximum value for G_g, since any recovery of $C^{14}O_2$ from C-6 via recombination of trioses by oxidative decarboxylation of glucose will tend to reduce the value for G_p, and trioses formed by the action of aldolase and triose phosphate isomerase are not actually equivalent to each other in respect to

TABLE 6. Metabolism of specifically labelled glucose by tissue slices of Hass avocado fruit

Time, min.	15	30	60	120	240
C-6/C-1 Ratio*					
CO_2	0.133	0.166	0.176	0.210	0.297
Lipids**	1.025	0.960	0.905	1.100	1.423
Glyceryl	0.852	0.515	0.389	0.295	0.488
Fatty acyl	1.417	1.955	1.825	2.020	2.110
Souble acids***	1.335	1.590	1.345	1.845	2.410
G_p****	0.250	0.320	0.326	0.386	0.381
G_{NADPH}	0.475	0.570	0.508	0.515	0.409

* C-6/C-1 ratio: C^{14} from glucose-6-$1C^{14}$/C^{14} from glucose-1-C^{14}.

** Lipids were saponified and separated into glyceryl moiety and fatty acyl moiety.

*** Soluble acids represent aqueous methanol soluble fraction. The fractions were dried and extracted with acetone to eliminate residual glucose.

**** G_p: Relative participation of the oxidative pentose phosphate pathway. G_{NADPH}: Relative participation of the oxidative pentose phosphate pathway supporting the fatty acid synthesis.

further metabolic reaction such as complex lipid synthesis, *i.e.*, dihydroxyacetone phosphate was quickly eliminated from the glycolytic sequence before isomerization occurred. Table 6 shows the evaluation of the relative participation of the oxidative pentose phosphate pathway during four hours' incubation of the tissue slices. Then, the relative participation of this pathway supporting lipid synthesis as a donor of NADPH could be estimated by the following assumption and calculation. 1) One mole of $C^{14}O_2$ produced by the way of 6-phosphogluconate decarboxylation ($G_{1(CO_2)}-G_{6(CO_2)}$) could provide two moles of NADPH which could be equilibrated for the assimilation of one mole of acetyl CoA into fatty acid. 2) Total fatty acid synthesized during the experimental period could be the total amount of C^{14} found in fatty acyl moiety from C-1 and C-6 of glucose ($G_{1(Acyl)}+G_{6(Acyl)}$). The relative participation of the oxidative pentose phosphate pathway supporting lipid synthesis (G_{NADPH}) is thus defined as,

$$G_{NADPH}=\frac{G_{1(CO_2)}-G_{6(CO_2)}}{G_{1(Acyl)}+G_{6(Acyl)}}.$$

Table 6 indicated that approximately 50% of required NADPH could be

provided by the oxidative pentose phosphate pathway. Obviously this is a minimum value for G_{NADPH}, since any recovery of C–6 in carbon dioxide and in lipids *via* pentose phosphate tends to reduce the relative participation value for lipid synthesis.

Although there is no direct evidence from the data, there is little incorporation of label in organic acid that might not produce a detectable amount of $C^{14}O_2$ from the organic acid *via* the tricarboxylic acid cycle operation, because C–6 of glucose is equivalent to C–2 of acetate, from which the production of CO_2 is at a slower rate than that from C–1 of acetate by the avocado tissue (16). If any recyclization of the pentose phosphate pathway occurred, the C–6/C–1 ratio of carbon dioxide production tends to increase. From the re-evaluation of data, the oxidative pentose phosphate pathway might support some 70–75% of NADPH required for lipid synthesis. Even though there is a discrepancy from the assumption, the lipid synthesis from C–6 is greater than that from C–1 of glucose after two hours' incubation which indicates that the triose formed by way of this pathway was incorporated into fatty acid.

The trioses formed by the action of aldolase and triose phosphate isomerase are not equivalent, since these trioses did not incorporate in equivalent manner into lipids as shown by the distribution of label in glyceryl and fatty acyl moieties, yet the utilization of C–1 in the glyceryl moiety is greater than that of C–6 and the opposite is true in the fatty acyl moiety of complex lipids. The reason for the lack of equivalence of trioses is unknown. In general, it is believed that there is a metabolic pool of dihydroxyacetone phosphate in the tissue and the turnover of triose isomerase is somewhat high. These observation customarily make it possible to assume the equivalence of dihydroxyacetone phosphate and phosphoglyceraldehyde in their labels (5).

The C–1 of glucose was preferentially incorporated into the glyceryl moiety in lipids synthesized by the mesocarp tissue. Since glycerophosphate plays a dominant role in fatty acid esterification (29), the possibility of the coupled reaction between glycerophosphate dehydrogenase and phosphoglyceraldehyde dehydrogenase may be suggested for at least initiation of the esterification process, although the amount of glycerophosphate required for the complex lipid synthesis is quite small but probably critical. Later, the bulk of NADH might be transferred from phosphoglyceraldehyde dehydrogenase to NADH: malate dehydrogenase where NADH might be converted to NADPH by the catalytic action of malic enzyme. Thus it is apparent from the fatty acyl/glyceryl ratios that the C–1 of glucose incorporated mainly into glyceryl moiety of lipids and carbon dioxide, and the dihydroxyacetone phosphate pool may

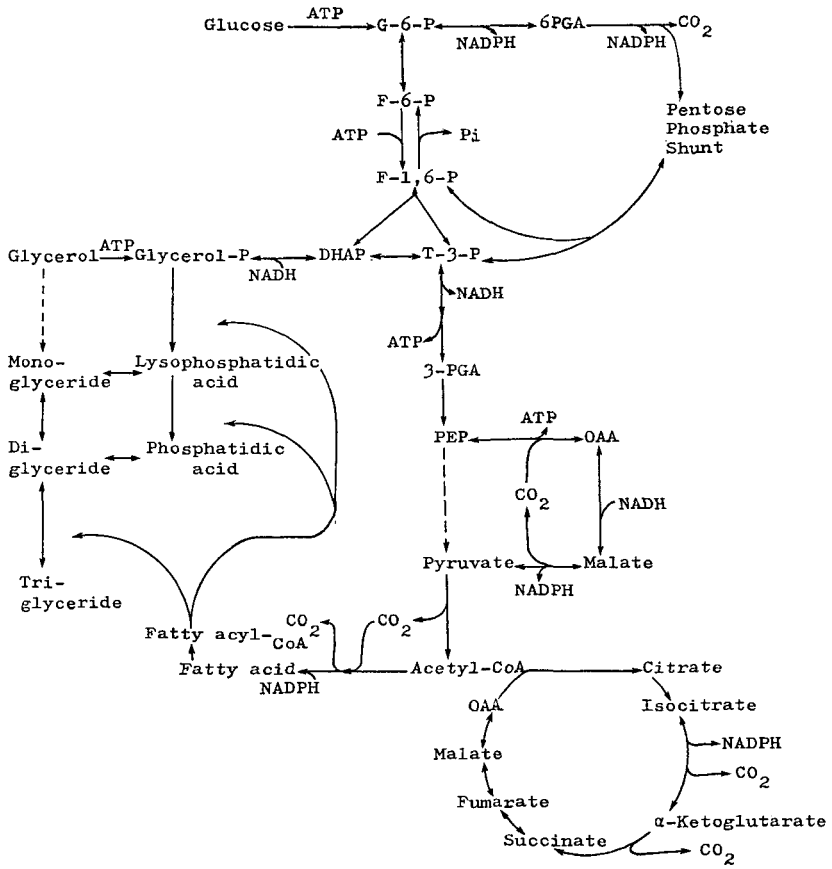

Figure 7. Scheme for the conversion of carbohydrate to complex lipids by the tissue slices of mesocarp of the developing Hass avocado fruit.

be small in the developing avocado fruit. Figure 7 shows the overall scheme for the possible conversion process of carbohydrate to complex lipids in the developing fruit of avocado, in which approximate 50% of glucose metabolized was found to accumulate in lipids whose synthesis might be regulated by the availability of glycerophosphate, into which a fatty acyl CoA was incorporated. In this way, phosphatidic acid formed from glucose by the tissue slices has been shown to act as a precursor for triglycerides and phospholipids in plants (2, 3, 6).

The evidence for the existance of phosphatidic acid in plants was the presence of large amounts of its phospholipid in cabbage leaves (7). But it

was found later that this was mainly an artifact caused by activation of phospholipase *D* during isolation procedures (10, 20). Recently some evidence was presented suggesting the presence of phosphatidic acid and its role in plants (6).

Phosphatidic acid can also arise from mono- and diglycerides by the phosphorylation with ATP (3, 22). This ATP: diglyceride kinase, which is responsible for the above reaction and is likely associated with the particulate fraction of the cell (3), may cause the difference in chromatographic elution patterns in lipids synthesized by the particles and the tissue slices prepared from the mesocarp of avocado fruit (15), and yet, another key reaction is catalyzed by phosphatidic acid phosphatase which has been shown to be inhibited by fatty acyl CoA (4) but this is not clear in plant system.

It is convenient to discuss the synthesis of phospholipids and triglyceride together because their synthesis is closely linked through the common intermediate, D-α, β-diglyceride and L-α, β-phosphatidic acid.

Intensive studies with animal tissues worked out by KENNEDY (13) suggested the diglyceride pathway for the formation of phospholipids and triglyceride, but phosphatidyl inositol formed from phosphatidic acid by interaction with CTP followed by inositol incorporation (1). Judging from the results presented, the quantitative KENNEDY's original scheme is not clear enough to explain the following observations. 1) The fatty acid composition of each complex lipid was different from that of diglyceride, which seemed to be a common intermediate. 2) The fatty acyl/glyceryl ratio in triglyceride and phospholipid was not same, when glucose-U-C^{14} was metabolized. 3) The highest specific radioactivity of fatty acid synthesized from acetate-1-C^{14} and glucose-6-C^{14} was found in phosphatidyl ethanolamine and phosphatidyl choline. The oleic acid synthesis in avocado was a predominant mode of lipid accumulation during fruit development, and the fruit stored triglyceride exclusively. Since the concentration of palmitic and unsaturated fatty acid in triglycerides and monoglycerides indicated the precursor-product relationship, it is suggested that the triglyceride was synthesized from monoglyceride with the incorporation of unsaturated fatty acids. While the fatty acid in diglyceride, phosphatidic acid, and other phospholipids was highly unsaturated, it is difficult to suggest the monoglyceride pathway. Studies with intestinal mucosa (14, 26) showed the monoglyceride pathway for triglyceride synthesis in relation to the lipid transport through the membrane. LANDS (18) studied the preferential incorporation of unsaturated fatty acid into β-position of complex lipids. Table 7 shows an approximation of the fatty acid concentration on the precursor-product relationship in the classes of complex lipids found in avocado under the assumption that palmitic acid is first incorporated in monoglyceride followed

TABLE 7. An estimate of fatty acid concentration on the precursor-product relationships among complex lipids

Number of fatty acyl	Palmitic acid			Unsaturated acid		
	mono-	di-	tri-	mono-	di-	tri-
	%	%	%	%	%	%
Triglyceride	53.1	26.5	17.7	19.6	59.8	73.2
Diglyceride	25.2	12.6	8.4	56.8	78.4	85.5
Monoglyceride	65.2	32.6	21.7	19.2	59.6	76.2
Phosphatidic acid	3.96	18.8	12.6	39.0	69.5	79.8
Lysophosphatidic acid	23.4	11.7	7.5	70.9	85.5	90.3
Phosphatidyl ethanolamine	33.6	16.8	11.3	55.4	77.7	85.1
Phosphatidyl choline	16.4	8.2	5.4	76.0	88.0	92.0

Underlined figures are the observed concentration of fatty acid. The others are the estimated concentration after the incorporation of unsaturated fatty acids (oleic, linoleic and linolenic acids) into mono- and diacyl glyceride to form phospholipids and triglyceride.

by the incorporation of unsaturated faty acids. From these data, it is possible to predict the two distinguishable diglyceride pathways in the avocado. The one related to triglyceride is formed from monoglyceride, and the other from lysophosphatidic acid which is in turn a precursor of phosphatidic acid and diglyceride, and of, more probably, phosphatidyl ethanolamine and phosphatidyl choline.

Palmitic acid synthesized from acetate-1-C^{14} by the avocado tisssue slices was first incorporated into phosphatidic acid, later into monoglyceride and diglyceride, while oleic acid was incorporated into monoglyceride and diglyceride, and later, into phosphatidic acid as well as triglyceride. Since stearic acid was also incorporated into lysophosphatidic acid first, then monoglyceride and phosphatidic acid were equally labelled, and finally the label was appeared in diglyceride and triglyceride, there are two pathways of lysophosphatidic acid metabolism, *i.e.*, via phosphatidic acid to diglyceride and via monoglyceride to triglyceride. Although stearic acid synthesized from acetate-1-C^{14} was a resulted product prevailled by the vacuum infiltration of acetate into tissue slices, and the fatty acid composition of phospholipids was highly unsaturated by discriminating saturated fatty acids, the pathway of phospholipid synthesis seems to be more complicated that the fatty acyl moiety had the highest specific radioactivity throughout the experimental period, and the fatty acyl/glyceryl ratio could not approximate a complete synthesis *de novo* of these biologically important phospholipids. The metabolism of diglyceride is of

strict interest in biosynthesis of phospholipids and glycolipids in plants (23).

From the kinetic evidence, it may be concluded that the synthesis of triglyceride from monoglyceride and diglyceride by the esterification of unsaturated fatty acids, mainly oleic acid, is the major route in the accumulation of reserve lipid in the mesocarp of avocado fruit, and it seems likely that palmitic acid is first incorporated into lysophosphatidic acid as well as monoglyceride which, the former, is more able to be a precursor of phospholipids and the latter is to be triglyceride, and that the phosphatase action on lysophosphatidic acid and phosphatidic acid and the phosphorylation of monoglycerid and diglyceride serve as a key reaction in complex lipid synthesis depending upon the stage of developing of the avocado fruit.

SUMMARY

Studies on the lipid metabolism relative to the lipogenesis from glucose and complex lipid synthesis in the fruit of avocado, *Persea americana* Mill., were undertaken to elucidate the pathways of glucose degradation followed by the synthesis of phospholipids and triglyceride, and to discuss the biological efficiency of the conversion of carbohydrate to reserve lipids.

In the mesocarp tissue slices of developing avocado fruit which predominated the accumulation of lipids, approximately 50 per cent of total glucose applied was converted to lipids, where 83.9 per cent of the labelled lipids was the neutral lipids.

The relative participation of the oxidative pentose phosphate pathway was determined. Approximately 30 per cent of the glucose metabolized by catabolic pathways was directly oxidized to release carbon dioxide and to provide some 50 per cent of the total reducing power responsible for the fatty acid synthesis. Whereas carbon number 1 of glucose was incorporated into the glyceryl moiety, carbon number 6 was incorporated into the fatty acyl moiety of complex lipids in the tissue slices of the developing avocado fruit. Whilst the NADPH produced by malic enzyme and soluble isocitrate dehydrogenase could also participate the fatty acid synthesis by the isolated system from the mesocarp.

The conclusions of the lipid synthesis that there may be two pathways of lysophosphatidic acid, *via* phosphatidic acid to diglyceride and phospholipid, and *via* monoglyceride to triglyceride, by the esterification of unsaturated fatty acids were based on the analysis of the tracer experiments with glucose-C^{14} and acetate-1-C^{14} by the tissue slices from the mesocarp of avocado fruit.

ACKNOWLEDGMENTS

The author wishes to express the deepest appreciation to Dr. L. C. ERICKSON for making this study possible and to Dr. Takashi TAGAWA for his kind advice in the preparation of manuscripts. He extends his appreciation to Dr. John B. MUDD and Mr. G. G. PORTER for valuable advice and discussion relative to the biochemical studies of lipids in plants, and to the technical assistance.

The data presented here were taken in part from a dissertation submitted by the author in partial satisfication of the requirements of a graduate program in Biochemistry, the University of California, Riverside, for the degree of Doctor of Philosophy.

Literature Cited

1. AGRANOFF, B. W., R. M. BRADLEY and R. O. BRADY. 1958. The enzymatic synthesis of inositol phosphatide. Jour. Biol. Chem. 233: 1077–1083.
2. BARRON, E. J. and P. K. STUMPF. 1962. Fat metabolism in higher plants. XIX. The biosynthesis of triglycerides by avocado mesocarp enzymes. Biochim. Biophys. Acta 60: 329–337.
3. BRADBEER, Clive and P. K. STUMPF. 1960. Fat metabolism in higher plants. XIII. Phosphatidic acid synthesis and diglyceride phosphokinase activity in mitochondria from peanut cotyledons. Jour. Lipid Research 1: 214–220.
4. BRANDES, R. and B. SHAPIRO. 1967. Inhibition of phosphatidic acid phosphatase by palmitoyl CoA. Biochim. Biophys. Acta 137: 202–204.
5. BUTT, V. S. and H. BEEVERS. 1961. The regulation of pathways of glucose catabolism in maize roots. Biochem. Jour. 80: 21–27.
6. CHENIAE, G. M. 1965. Phosphatidic acid and glyceride synthesis by particles from spinach leaves. Plant physiol. 40: 235–243.
7. CHIBNALL, A. C. and H. J. CHANNON. 1927. The ether soluble substances of cabbage leaf cytoplasm. II. Calcium salts of glyceride phosphoric acid. Biochem. Jour. 20: 233–246.
8. DAVENPORT, J. B. and S. C. ELLIS. 1959. Chemical changes during growth and storage of the avocado fruit. Aust. Jour. Biol. Sci. 12: 445–454.
9. DAWSON, R. C. M. 1966. The metabolism of animal phospholipids and their turnover in cell membranes. Essay in Biochemistry 2: 69–115.
10. HIRSCH, J. and E. H. AHRENS, Jr. 1958. The separation of complex lipide mixtures by the use of silicic acid chromatography. Jour. Biol. Chem. 233: 311–320.
11. HOKIN, L. E. and M. R. HOKIN. 1964. The incorporation of P^{32} from triphosphate polyphosphoinositides, r-P^{32} adenosine and phosphatidic acid in erythrocyte membrane. Biochim. Biophys. Acta 84: 563–575.
12. JOHNSTON, J. M. and G. A. RAO. 1965. Triglyceride biosynthesis in the intestinal mucosa. Biochim. Biophys. Acta 106: 1–9.
13. KENNEDY, E. P. 1957. Biosynthesis of phospholipids. Federation Proc. 16: 847–852.

14. KERN Jr, F. and B. BORGSTROEM. 1965. Quantitative study of the pathway of triglyceride synthesis by hamster intestinal mucosa. Biochim. Biophys. Acta 98: 520–531.
15. KIKUTA, Y. 1968. Lipid metabolism in the fruit of *Persea americana* Mill. I. Studies on the chemical composition of lipids and their changes during fruit development and storage. Jour. Fac. Agri. Hokkaido University 55: 469–495.
16. KIKUTA, Y. 1968. Lipid metabolism in the fruit of *Persea americana* Mill. II. Metabolism of acetate in relation to lipid synthesis. Jour. Fac. Agri. Hokkaido University 56: 117–144.
17. KORNBERG, A. and W. E. PRICER Jr. 1953. Enzymatic esterification of α-glycerophosphate by long chain fatty acids. Jour. Biol. Chem. 204: 345–357.
18. LANDS W. E. M. 1965. Effects of double bond configuration on lecithin synthesis. Jour. Amer. Oil Chem. Soc. 42: 465–467.
19. MCCAMAN, R. E., M. SMITH, and K. COOK. 1965. Intermediary metabolism of phospholipids in brain tissue. II. Phosphatidic acid phosphatase. Jour. Biol. Chem. 240: 3513–3517.
20. MCCARTY, R. E. and A. T. JAGENDORF. 1965. Chloroplast damage due to enzymatic hydrolysis of endogenous lipids. Plant Physiol. 40: 725–735.
21. MATTSON, F. H. and R. A. VOLPENHEIN. 1963. The specific distribution of unsaturated fatty acids in the triglycerides of plants. Jour. Lipid Research 4: 392–396.
22. MAZELIS, M. and P. K. STUMPF. 1955. Fat metabolism in higher plants. VI. Incorporation of P^{32} into peanut mitochondrial phospholipids. Plant Physiol. 30: 237–243.
23. NEUFELD, E. P. and C. W. HALL. 1964. Formation of galactolipids by chloroplasts. Biochim. Biophys. Acta 70: 417–422.
24. PIERINGER, R.A. and R. S. KUNNES. 1965. The biosynthesis of phosphatidic acid and lysophosphatidic acid by glyceride phosphokinase pathway in *Escherichia coli*. Jour. Biol. Chem. 240: 2833–2838.
25. RAGLAND, T. E. and D. P. HACKETT. 1965. Radioactive tracer studies of the metabolic fates of intracellularly generated NADH and NADPH in higher plant tissues. Plant Physiol. 40: 1191–1197.
26. RAO, A. G. and J. M. JOHNSTON. 1966. Purification and properties of triglyceride synthetase from the intestinal mucosa. Biochim. Biophys. Acta 125: 465–473.
27. STEIN, Y. and B. SHAPIRO. 1957. Glyceride synthesis by rat liver mitochondria. Biochim. Biophys. Acta 26: 286–293.
28. STEIN, Y. and B. SHAPIRO. 1958. Glyceride synthesis by microsome fractions of rat liver. Biochim. Biophys. Acta 30: 271–277.
29. TZUR, R., E. TAL, and B. SHAPIRO. 1964. α-glycerophosphate as regulatory factor in fatty acid esterification. Biochim. Biophys. Acta 84: 18–23.
30. WEISS, S. B., S. W. SMITH, and P. E. KENNEDY. 1956. Net synthesis of lecithin in an isolated enzyme system. Nature 178: 594–595.
31. WEISS, S. B., E. P. KENNEDY, and J. Y. KIYASU. 1960. The enzymatic synthesis of triglycerides. Jour. Biol. Chem. 235: 40–44.