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THE INFLUENCE OF HORMONES ON LIPID METABOLISM IN RELATION TO ARTERIOSCLEROSIS

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THE INFLUENCE OF HORMONES ON LIPID METABOLISM IN RELATION TO ARTERIOSCLEROSIS*

Conference Co-Chairman and Consultin
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INTRODUCTION

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Even a slight familiarity with recent literature on the pathogenesis of atherosclerosis conveys the strong impression that there is a widely held belief that cholesterol and dietary fat are the important factors in the development of this disease. However, what are the basic roles of the cellular and hormonal factors that regulate lipid metabolism and their relation to the pathogenesis of atherosclerosis? With advances in knowledge of the confluent roles of enzymes and hormones in biological processes, these factors can no longer be considered in terms of peripheral or secondary actions bearing on the balance of forces that result in the deposition of lipid in arterial tissue. An even more neglected area of investigation, which may hold the key to arteriosclerosis, is the "ground substance." Since several hormones influence the cellular structure and function of this tissue, there is reason for speculation and study of the role of this tissue in the intrinsic biochemical lesions of the arterial wall. Recent studies of the physicochemical state of lipids in blood have changed some concepts about lipid metabolism; not less significantly, the activity of cellular elements of the ground substance was found to be involved in lipid metabolism and the lipid-transporting mechanism.

The objective of this publication is the provision of information about biological processes germane to the development of arteriosclerosis, since it is time for the presentation, objective evaluation, and integration of our knowledge and ideas of the physiological control and biochemical mechanisms of lipid metabolism. The contributors to this monograph have brought into focus fundamental data that will assist all who are interested in arriving at a better understanding of the processes that may lead to development of atheromatous lesions in the arterial wall.

Part I. Metabolism of Lipids: Biosynthesis, Absorption, Transport

BIOGENESIS OF PHOSPHATIDES AND TRIGLYCERIDES*

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Introduction

Atheromatous deposits in the aorta and other blood vessels contain considerable quantities of both phosphatide and triglyceride. Buck and Rossiter[1] summarized the evidence indicating that certain of the phosphatides are major constituents of these intimal deposits in man. The amount of phosphatide was found to increase with increasing severity of the atherosclerotic process.

Weinhouse and Hirsch[2] showed that phosphatides and triglycerides also entered into the atheromatous deposits of rabbits maintained on a diet rich in cholesterol. For this reason, and for the reason that phosphatides may be involved in the absorption, transport and, possibly, the oxidation of triglycerides (see Deuel,[3] Beveridge,[4] and Dawson[5] for reviews), it is appropriate to consider here the general problem of the biological formation of phosphatides and triglycerides.

Isotope experiments indicate that glycerophosphatides may be formed *in vivo* from many different precursors, such as inorganic phosphorus labeled with P^{32}; fatty acids labeled with deuterium, C^{14}, I^{131}, or elaidic acid; acetate labeled with deuterium or C^{14}; choline, ethanolamine, or serine labeled with C^{14} or N^{15}; and glycerol labeled with C^{14}. Similar experiments carried out *in vitro* with tissue slices indicate that for most tissues the glycerophosphatides are formed *in situ* from the appropriate precursors (see Deuel,[6] Dawson,[5] and Rossiter[7] for references)

Chernick *et al.*[8] showed that inorganic P^{32} was incorporated into the phosphatides of slices of rat artery. These experiments demonstrated that blood vessels, like most other tissues, can form phosphatides *in situ* from smaller molecules. More recently, Zilversmit and his colleagues[9, 10] have shown that the major portion of the phosphatides in the aorta of rabbits with atheromatosis caused by a high-cholesterol diet was synthesized *in situ* and was not transported to the aorta from the plasma lipids.

Glycerophosphatides

Chiefly owing to the work of Kennedy,[11, 12] much is known of the metabolic pathways whereby glycerophosphatides, notably lecithin, are formed in liver tissue. Similar metabolic pathways are operative in brain[7, 13] and probably in many other tissues.

Formation of phosphatidic acid. As the result of early *in vivo* experiments[14, 15]

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† These studies were performed during the tenure of a Lederle Medical Faculty Award from Lederle Laboratories Division, American Cyanamid Corp., Pearl River, N. Y.

TABLE 1

EFFECT OF COENZYME A ON THE INCORPORATION OF LABELED α-GLYCEROPHOSPHATE INTO THE LIPID OF RAT BRAIN*

Preparation	Precursor	Specific activity (counts/min./μg. P)	
		Control	CoA†
Water homog. (gas, N_2)	α-GP^{32}	13.4	35.5
Mitochondria (gas, O_2)	α-GP^{32}	25.4	89.2
Water homog. (gas, N_2)	α-GP-C^{14}	4.1	10.3

* Conditions of incubation as described previously.[24]
† Final concentration, 5×10^{-5} M.

and later *in vitro* experiments with rat liver mitochondria[16] and cell-free enzyme preparations from rat liver,[17] α-glycerophosphate (α-GP) was implicated as a precursor of tissue glycerophosphatides. The α-GP could arise from the glycolysis intermediate, dihydroxyacetone phosphate, by the action of L-α-GP dehydrogenase,[18-20] or by the transfer of phosphate from adenosine triphosphate (ATP) to glycerol.[21] The enzyme that catalyzes this reaction, glycerokinase, was isolated and partially purified by Bublitz and Kennedy.[22]

When inorganic P^{32} was added to metabolizing cell-free preparations from liver and brain tissue, radioactivity was incorporated into the lipids, but most of this radioactivity was recovered from the phosphatidic acid fraction.[16, 23, 24] Similarly, when α-GP^{32} was used as a precursor, Kennedy[16] and Kornberg and Pricer[17] found that the radioactivity appeared in phosphatidic acid. This incorporation was stimulated by the addition of coenzyme A (CoA), long-chain fatty acids, and ATP.[25]

TABLE 1, taken in part from the results of McMurray *et al.*,[24] shows that the radioactivity of α-GP^{32} and α-GP-C^{14} was incorporated into the lipid of glycolyzing homogenates or phosphorylating mitochondria from rat brain. Examination of the hydrolysis products of the labeled lipid by the method of Dawson[23] showed that for both α-GP^{32} and α-GP-C^{14} most of the radioactivity was in the phosphatidic acid, with negligible activity in the glycerophosphatides. The incorporation was greatly stimulated by the addition of CoA. Moreover, a supply of metabolic energy was required for the labeling of the phosphatidic acid, since the incorporation was abolished either by the addition of inhibitors that prevented the production of ATP (iodoacetate for glycolyzing homogenates and 2:4-dinitrophenol for phosphorylating mitochondria) or by the omission of factors (such as oxidizable substrate and adenine nucleotides) necessary for the continued formation of ATP.

The above experiments are explained by the important contribution of Kornberg and Pricer[26] that guinea pig liver contained an enzyme system capable of activating long-chain fatty acids to form thioesters of CoA, together with the production of adenosine 5′-monophosphate (AMP) and inorganic pyrophosphate (P-P):

$$\text{Fatty acid} + \text{ATP} + \text{CoA} \rightleftharpoons \text{acyl-CoA} + \text{AMP} + \text{P-P} \qquad (1)$$

Presumably, the mechanism of the reaction is similar to that of the acetic thiokinase reaction, which has been the subject of several recent studies.[27, 28]

Kornberg and Pricer[29] also demonstrated the presence of a second liver enzyme system that carried out the esterification of L-α-GP to form L-α-phosphatidic acid:

$$\text{L-}\alpha\text{-GP} + 2 \text{ acyl-CoA} \rightleftharpoons \text{L-}\alpha\text{-phosphatidic acid} + 2 \text{ CoA} \qquad (2)$$

Jedeikin and Weinhouse[30] and Stansly[31] have provided further evidence for such a reaction. The details of the esterification must still be clarified. It is not certain whether the α′- or the β-hydroxyl group of L-α-GP is esterified first.

Glycerol was inactive[29] in an enzyme system similar to that catalyzing REACTION 2 and glycerol-C^{14} was not incorporated into brain lipids in experiments similar to those described in TABLE 1. It thus seems that phosphatidic acid is formed by the esterification of α-GP, but that diglyceride cannot be formed by a similar esterification of glycerol.

Dephosphorylation of phosphatidic acid. The evidence summarized above indicates that in many cell-free preparations the radioactivity of α-GP^{32} is incorporated into phosphatidic acid, but not into the glycerophosphatides. This finding raises the question of the role of phosphatidic acid in glycerophosphatide synthesis. Since it has been shown by Kennedy[12] that D-α,β-diglycerides are intermediates in the formation of both glycerophosphatides and triglycerides (see below), considerable interest has arisen concerning possible sources of D-α,β-diglycerides. Smith *et al.*[32] showed that D-α,β-diglyceride can be formed by the enzymatic dephosphorylation of phosphatidic acid. The enzyme, phosphatidic acid phosphatase, causes the liberation of inorganic P (P_i) from phosphatidic acid:

$$\text{L-}\alpha\text{-phosphatidic acid} \rightleftharpoons \text{D-}\alpha,\beta\text{-diglyceride} + P_i \qquad (3)$$

The enzyme is inhibited by divalent cations such as Mg^{++}. This type of inhibition probably accounts for the accumulation of phosphatidic acid[16, 17, 23, 24, 33, 34] or a "phosphatidic acidlike" compound[25, 36] in many tissue preparations, although only small amounts of phosphatidic acid may be present in the same tissues from freshly killed animals.[37-39]

TABLE 2 shows the hydrolysis of a number of phosphatidic acids by chicken liver and rat brain preparations. It is seen that in general the chicken liver was much more active than the rat brain. With both tissues L-α-dioleyl-GP* was less well hydrolyzed than phosphatidic acids from natural sources. Two samples of natural phosphatidic acid were prepared from lecithin by the action of the phospholipase C of carrot chromoplasts, as outlined by Kates.[40] One sample of lecithin was obtained from ox spinal cord† by the method of Pangborn.[41] The other was a commercial preparation of egg lecithin purified by passage over an alumina column as described by Hanahan *et al.*[42]

* Kindly provided by E. Baer, Banting and Best Department of Medical Research, University of Toronto, Toronto, Canada.

† Extract of spinal cord kindly provided by K. K. Carroll, Department of Medical Research, University of Western Ontario, London, Canada.

TABLE 2

DEPHOSPHORYLATION OF PHOSPHATIDIC ACID*

Tissue	Substrate	Homogenate	Mitochondria plus microsomes
Chicken liver	L-α-Dioleyl-GP	0	0.5†
	Phosphatidic acid (from egg lecithin)	3.1	2.2
Rat brain	L-α-Dioleyl-GP	0	0.05
	Phosphatidic acid (from spinal cord lecithin)	1.08	—
	Phosphatidic acid (from egg lecithin)	0.87	0.86
	Phosphatidic acid (from egg lecithin) $MgCl_2$ (8 mM)	0.22	0.24

* Incubated at 37° C. in Tris buffer (0.05 M) *p*H 7.4 for 1 hr. Each vessel contained phosphatidic acid, 4 μmoles; 100 mg. homogenate or mitochondria plus microsomes from 300 mg. tissue. Total volume, 3 ml.

† In μmoles, P liberated.

$MgCl_2$, in a concentration (8 mM) similar to that used in the experiments reported in TABLE 1, was inhibitory to the rat brain phosphatidic acid phosphatase.

Formation of lecithin from phosphorylcholine. Early *in vivo* experiments with phosphorylcholine (PCh) gave little support to the supposition that this substance might be an intermediate in the biosynthesis of lecithin.[43] However, interest in PCh was renewed when Kornberg and Pricer[25] showed that PCh labeled with both C^{14} and P^{32} was incorporated, as a unit, into the lipid of a rat liver preparation. Subsequently, Rodbell and Hanahan[44] and McMurray *et al.*[24] showed that P^{32}Ch was incorporated into the lecithin of liver and brain preparations. The earlier claim[45] that choline-C^{14} was incorporated more rapidly than PCh-C^{14} into the lecithin of rat liver mitochondria has not been substantiated.[11]

A great stimulus to the study of the biosynthesis of the glycerophosphatides was the important finding of Kennedy and Weiss[46, 47] that cytidine 5′-triphosphate (CTP) was necessary for the incorporation of P^{32}Ch into lecithin. The requirement was specific for CTP, none of a number of other nucleoside 5′-triphosphates being active. Kennedy and Weiss[47] showed that lecithin was formed according to the following reactions:

$$\text{CTP} + \text{PCh} \rightleftharpoons \text{CMP-PCh} + \text{P-P} \qquad (4)$$

$$\text{CMP-PCh} + \text{D-}\alpha,\beta\text{-diglyceride} \rightleftharpoons \text{L-}\alpha\text{-lecithin} + \text{CMP} \qquad (5)$$

The formation of cytidine diphosphate choline (CMP-PCh) in REACTION 4 represents a novel metabolic role for cytidine nucleotides. PCh is transferred from the intermediate CMP-PCh to a D-α,β-diglyceride acceptor, with the formation of cytidine 5′-monophosphate (CMP). The CMP-PCh was isolated, characterized, and synthesized.[48] The enzyme catalyzing REACTION 4, which was subsequently studied by Borkenhagen and Kennedy,[49] was called PCh-cytidyl transferase and that catalyzing REACTION 5 was called PCh-

TABLE 3

EFFECT OF CYTIDINE TRIPHOSPHATE ON THE INCORPORATION OF LABELED PHOSPHORYLCHOLINE INTO THE LIPID OF RAT BRAIN*

Preparation	Precursor	Specific activity (counts/min./ μg. P)	
		Control	CTP
Water homog. (gas, N_2)	$P^{32}Ch$	3.4	49.8†
Mitochondria (gas, O_2)	$P^{32}Ch$	1.7	31.7‡
Water homog. (gas, N_2)	PCh-C^{14}	1.1	9.3†

* Conditions of incubation as described previously.[24]
† Final concentration of CTP, 7×10^{-4} M.
‡ Final concentration of CTP, 3.5×10^{-4} M.

TABLE 4

EFFECT OF D-α,β-DIGLYCERIDE ON THE INCORPORATION OF LABELED CYTIDINE DIPHOSPHATE CHOLINE INTO THE LIPID OF RAT BRAIN*

Preparation	Precursor	Specific activity (counts/min./ μg. P)	
		Control	D-α,β-Diglyceride
Water homog. (gas, N_2)	CMP-$P^{32}Ch$	35.0	54.8†
Mitochondria (gas, O_2)	CMP-$P^{32}Ch$	48.6	81.0†
Water homog. (gas, N_2)	CMP-PCh-C^{14}	28.0	44.1‡
Water homog. (gas, N_2)	CMP-PCh-C^{14}	25.0	36.8†

* Conditions of incubation as described previously.[50]
† D-α,β-Diglyceride from egg lecithin (1 μmole).
‡ D-α,β-Diglyceride from spinal cord lecithin (1 μmole).

glyceride transferase. Similar enzymes are also present in brain[13, 50] and seminal vesicle.[51]

TABLE 3 shows that the radioactivity of P^{32}- and C^{14}-labeled PCh was incorporated into the phosphatide of glycolyzing homogenates and phosphorylating mitochondria from rat brain. Choline-C^{14} was not incorporated. For both $P^{32}Ch$ and PCh-C^{14}, examination of the labeled lipid by the method of Dawson[23] indicated that the greater part of the radioactivity was in the lecithin. It would seem that the labeling occurred by way of REACTIONS 4 and 5, since activity was dependent upon the presence of CTP. Again, the provision of metabolic energy was found to be necessary for incorporation.

Further evidence that in brain preparations lecithin is formed by REACTION 5 is presented in TABLE 4. In these experiments the radioactivity of P^{32}- and C^{14}-labeled CMP-PCh, synthesized by the method of Kennedy,[48] was incorporated into the phosphatide of rat brain preparations. The incorporation was stimulated by the addition of emulsified D-α,β-diglyceride preparations. The diglycerides were prepared enzymatically, either from egg lecithin or from spinal cord lecithin, by the method of Hanahan and Vercamer,[52] using the lecithinase D of *Clostridium perfringens*.* Examination of the labeled

* Supplied by Lederle Laboratories Division, American Cyanamid Co., Pearl River, N. Y.

lipid again showed that lecithin was the only glycerophosphatide containing any significant radioactivity.

Of a number of synthetic α,β-diglycerides* tested, D-diolein was the only one that approached the activity of the natural diglycerides. D-Dimyristin, D-dipalmitin and L-diolein were without effect. This lack of activity of the saturated synthetic D-α,β-diglycerides may be due to their greater insolubility, or to the fact that saturated diglycerides are poor acceptors of PCh for reasons of chemical specificity rather than for physical reasons. The inactivity of L-diolein is not unexpected since the D-configuration is necessary in order for the diglyceride to give rise to glycerophosphatides having the natural L-α-configuration.[53]

There is good evidence that PCh, the precursor of CMP-PCh, can arise in most tissues. Wittenberg and Kornberg[54] described and partially purified an enzyme, which they called choline phosphokinase, that catalyzed the transfer of phosphate from ATP to choline. The enzyme, prepared from yeast, was said to be present in a number of mammalian tissues. Berry *et al.*[55] showed that a similar enzyme was present in extracts of acetone-dried powders from brain and peripheral nerve.

Evidence for the participation of phosphatidic acid in the biosynthesis of lecithin. The low concentration of phosphatidic acid in fresh tissues[38, 39] and in liver slices[56] has led to the suggestion that phosphatidic acid may not be an intermediate in the formation of glycerophosphatides. However, Rodbell and Hanahan[44] reported that the addition of phosphatidic acid increased the incorporation of P^{32}Ch into the lecithin of guinea pig liver mitochondria. Subsequently, Smith *et al.*[32] showed that the addition of phosphatidic acid to a rat liver preparation caused an increase in the incorporation of CMP-PCh-C^{14} into lecithin, provided the phosphatidic acid was first incubated in an Mg^{++}-free medjum to allow dephosphorylation to diglyceride.

Experiments in our laboratory with homogenates of rat brain and rat liver mitochondria have provided further evidence for the view that phosphatidic acid is an intermediate in the biosynthesis of glycerophosphatides. TABLE 5 shows the effect of added phosphatidic acid on the incorporation of the radioactivity of CMP-PCh-C^{14} into the lipids of a rat brain homogenate. Before the addition of the CMP-PCh-C^{14}, the phosphatidic acid was preincubated with the homogenate in an Mg^{++}-free medium to allow dephosphorylation to diglyceride according to REACTION 3. It is noted that the presence of Mg^{++} (8 mM) in the preincubation medium greatly decreased the stimulation of the incorporation brought about by the addition of the phosphatidic acid.

In experiments with cell-free preparations from both brain (TABLE 1) and liver,[16, 17] phosphatidic acid is labeled from α-GP^{32}, but none of the radioactivity is incorporated into the glycerophosphatides because, as shown above, the radioactivity is lost as inorganic P^{32} when the phosphatidic acid is converted into diglyceride. TABLE 1 shows that C^{14}-labeled α-GP also was incorporated into the lipid of a rat brain homogenate. Examination of the labeled lipid

* Supplied by E. Baer, Banting and Best Department of Medical Research, University of Toronto.

TABLE 5

EFFECT OF PHOSPHATIDIC ACID ON THE INCORPORATION OF CYTIDINE DIPHOSPHATE CHOLINE-C^{14} INTO LIPIDS OF RAT BRAIN HOMOGENATE*

Conditions†	Specific activity‡	Increase (percentages)
No additions	15.1	—
Phosphatidic acid (from egg lecithin)	32.6	116
Phosphatidic acid (from egg lecithin) + $MgCl_2$ (8 mM)	26.0	48
Phosphatidic acid (from spinal cord lecithin)	54.5	260

* Water homogenate of rat brain (100 mg.) preincubated in 0.05 M Tris buffer pH 7.4 for 60 min. at 37° C. in the presence or absence of phosphatidic acid (4 μmoles). At end of 1 hr. $MgCl_2$ (final concentration, 8 mM), Tween 20 (0.25 mg./ml.), and CMP-PCh-C^{14} (0.5 μmole) were added and incubation continued for another 60 min.

† Refers to the conditions of preincubation.

‡ Counts/min./μg. P of sample to which no phosphatidic acid has been added.

TABLE 6

EFFECT OF CYTIDINE DIPHOSPHATE CHOLINE ON THE INCORPORATION OF α-GLYCEROPHOSPHATE-C^{14} INTO THE PHOSPHOLIPIDS OF RAT LIVER MITOCHONDRIA*

Expt. No.		Specific activity of phospholipid (counts/min./μg. P)	Radioactivity (counts/min.)	
			Phosphatidic acid	Lecithin
1	Control	51.4	187	91
	CMP-PCh†	84.2	124	319
2	Control	42.7	44	166
	CMP-PCh†	74.5	33	489

* Conditions of incubation as described previously.[24]

† At 0.5 μmole CMP-PCh per vessel.

again revealed that most of the radioactivity was in phosphatidic acid, and that negligible amounts were in the lecithin. Presumably in this brain system there was sufficient $MgCl_2$ in the medium to prevent the dephosphorylation of the newly formed phosphatidic acid, now labeled in the glycerol portion of the molecule. However, this was not so with preparations from rat and chicken liver. TABLE 6 shows that in rat liver mitochondria α-GP-C^{14} was incorporated into both the phosphatidic acid and the lecithin. Even when Mg^{++} was present in the medium there was sufficient dephosphorylation of phosphatidic acid to enable lecithin labeling to proceed.

TABLE 6 also shows that when unlabeled CMP-PCh was added to rat liver mitochondria capable of incorporating the radioactivity of α-GP-C^{14} into lecithin, the specific activity of the total phospholipid was increased. It is of interest to note that the addition of the CMP-PCh caused a slight decrease in the total number of counts recovered in the phosphatidic acid fraction and a considerable increase in the counts recovered in the lecithin. In this experiment the phosphatidic acid is, no doubt, labeled by way of REACTION 2, and the lecithin is labeled by way of REACTIONS 3 and 5. The addition of an

TABLE 7

INCORPORATION OF RADIOACTIVITY OF LABELED CYTIDINE DIPHOSPHATE CHOLINE AND CYTIDINE DIPHOSPHATE ETHANOLAMINE INTO THE LIPID OF RAT BRAIN HOMOGENATE*

	Specific activity (counts/min./μg. P)	
	CMP-P^{32}-Ch	CMP-P-ethanolamine-C^{14}
Lecithin†	77.3	0
Phosphatidyl ethanolamine	2.7	29
Phosphatidyl serine	1.0	0
Sphingomyelin‡	6.5	—
Phosphatidic acid	0	0
Inositol lipid	0	0

* Conditions of incubation as described previously.[50]
† Hydrolysis products of labeled lipid separated by the method of Dawson.[23]
‡ Lipid stable to the alkaline hydrolysis procedure of Schmidt *et al.*[57]

excess of CMP-PCh has aided REACTION 5 from left to right, with a consequent increase in the labeling of lecithin.

Other glycerophosphatides. It seems probable that the reaction sequence described above for the biosynthesis of lecithin is general, and that similar reactions are involved in the formation of at least some of the other glycerophosphatides. For instance, Kennedy and Weiss[47] showed that an enzyme preparation from chicken liver catalyzed the incorporation of the radioactivity of CMP-P-ethanolamine into phosphatidyl ethanolamine, indicating that this phosphatide is formed by a process involving the cytidine nucleotides.

The data reported in TABLE 7 strengthen this conclusion. The radioactivity of CMP-P-ethanolamine-C^{14} was found to be incorporated into the phosphatidyl ethanolamine of rat brain homogenates. No other fraction examined contained radioactivity.

Suitable precursors occur in most tissues for the formation of other glycerophosphatides. Ethanolamine can be phosphorylated in brain[58] and yeast.[54] Also, Ichihara and Greenberg[59] have described a pathway whereby phosphoserine may be formed from 3-phosphoglyceric acid, a glycolysis intermediate.

Inositol Lipids

In recent years considerable interest has been shown in the inositol lipids. TABLE 8, taken in part from the data of McMurray *et al.*,[24] shows that the incorporation of inorganic P^{32} into the inositol lipid of glycolyzing homogenates and phosphorylating mitochondria from rat brain was stimulated by the addition of CTP. In addition, TABLE 8 shows that the incorporation of inositol-C^{14}, obtained from rye seedlings grown in an atmosphere of $C^{14}O_2$, also was dependent upon the presence of CTP. Agranoff *et al.*[60] have shown that cytidine nucleotides are necessary for the incorporation of *myo*inositol-2-H^3 into the inositol lipids of cell-free preparations of guinea pig kidney.

These findings do not necessarily indicate that inositol lipids are formed by a reaction sequence similar to that outlined above for lecithin. In fact, they may indicate the contrary. However, the results do underline the importance

TABLE 8

EFFECT OF CTP ON THE INCORPORATION OF LABELED PRECURSORS INTO THE INOSITOL LIPID* OF RAT BRAIN†

Preparation	Precursor	Specific activity (counts/min./μg. P)	
		Control	CTP‡
Water homog. (gas, N_2)	Inorg. P^{32}	1440	2430
Mitochondria (gas, O_2)	Inorg. P^{32}	530	1860
Water homog. (gas, N_2)	Inositol-C^{14}	0.4	8.7
		0.7	9.2

* Hydrolysis products of labeled lipid separated by the method of Dawson.[23]
† Conditions of incubation as described previously.[24]
‡ Final concentration, 7×10^{-4} M.

of cytidine nucleotides in the biogenesis of inositol lipids of both brain and other tissues.

Sphingomyelin

Weinhouse and Hirsch[61] reported that the phospholipid obtained from the atheromatous deposits of human aortas was largely ether-insoluble. Buck and Rossiter[1] showed that a considerable quantity of this phospholipid was stable to the alkaline hydrolysis procedure of Schmidt *et al.*[57] It is thus possible that this lipid is sphingomyelin or a similar diaminophosphatide.

Sribney and Kennedy[62] described the presence in chicken liver of an enzyme, PCh-ceramide transferase, that catalyzed the formation of sphingomyelin by the transfer of PCh from CMP-PCh to *N*-acylsphingosine (ceramide):

$$\text{CMP-PCh} + \textit{N}\text{-acylsphingosine} \rightleftharpoons \text{sphingomyelin} + \text{CMP} \qquad (6)$$

REACTION 6 is analogous to REACTION 5 catalyzed by PCh-glyceride transferase. *N*-acylsphingosine rather than D-α,β-diglyceride is the acceptor for the PCh. The formation of CMP-PCh by REACTION 4 has been described above. In addition, Brady and Koval[63] and Zabin[64] have reported that cell-free preparations from rat brain can form sphingosine and ceramide, respectively.

According to Sribney and Kennedy[65] the substance that accepts PCh in REACTION 6 is an "active ceramide." The PCh-ceramide transferase is highly specific, both for CMP-PCh and "active ceramide." Ceramides with short-chain fatty acids in amide linkage are much more active than those with long-chain fatty acids, presumably because the short-chain compounds are more soluble. Sribney and Kennedy[65] summarized the evidence for the conclusion that the sphingosine moiety of the "active ceramide" is threo-1,3-dihydroxy-2-amino-4-*trans*-octadecene, rather than the corresponding erythro compound, which is a constituent of some naturally occurring sphingolipids.[66]

TABLE 7 shows that in a rat brain homogenate a fraction designated as sphingomyelin received some of the radioactivity from CMP-P^{32}Ch. This fraction was stable to the alkaline hydrolysis procedure of Schmidt *et al.*[57]

and so contained the sphingomyelin, which presumably was labeled by a mechanism similar to that of REACTION 6. The presence in this fraction of a phosphosphingolipid that does not contain choline, as has been suggested by work from a number of laboratories,[67-70] would require the revision upward of the specific activity of sphingomyelin.

Triglyceride

Because of the current interest in the effect of dietary triglycerides on cholesterol metabolism, particularly in relation to the development of atheroma, some mention will be made of the biosynthesis of triglycerides. The data of McArthur,[71] incidentally, indicate that the intima of atheromatous aortas contains considerable quantities of triglyceride.

Surprisingly, little is known of the biosynthesis of triglycerides. Borgström[72] showed that it was possible to form triglyceride from fatty acid and diglyceride by reversal of the action of pancreatic lipase. A further suggestion that this method of triglyceride formation may be of importance was made by Jedeikin and Weinhouse,[30] who reported that palmitate-1-C^{14} was incorporated into the triglyceride of slices and cell-free preparations of a number of tissues, without the requirement of a coupled energy source.

That the formation of triglyceride may occur by a pathway different from a reversal of hydrolysis was suggested by the work of Tietz and Shapiro.[73] These workers showed that the incorporation of palmitate-1-C^{14} into tryglyceride of a rat liver homogenate was dependent upon the presence of ATP in the medium. Subsequently Stein and Shapiro[74] reported that this incorporation was greatly accelerated by the addition of α-GP (not free glycerol) and CoA. In addition, they demonstrated that the radioactivity of α-GP-C^{14} was incorporated into triglyceride.

Recently Weiss and Kennedy[75] showed that mitochondria from chicken liver contained an enzyme system capable of forming triglyceride from D-α,β-diglyceride and palmitoyl-CoA, according to the following general equation:

$$\text{Acyl-CoA} + \text{D-}\alpha,\beta\text{-diglyceride} \rightarrow \text{triglyceride} + \text{CoA} \qquad (7)$$

For the formation of triglyceride the acyl-CoA is formed according to REACTION 1, as described by Kornberg and Pricer,[26] and the D-α,β-diglyceride is formed according to REACTIONS 2[29] and 3.[32]

In the experiments reported in TABLE 6 it was shown that the radioactivity of α-GP-C^{14} was incorporated into both the phosphatidic acid and the lecithin of rat liver mitochondria. TABLE 9 shows that in such experiments there was even more radioactivity in the acetone-soluble lipid. That this radioactivity was not due to the presence of phosphatide remaining after the acetone precipitation is shown in the second experiment reported in TABLE 9 in which CMP-PCh-C^{14} was the source of the radioactivity. In this instance, although there was good labeling of the phosphatide, only negligible radioactivity was observed in the acetone-soluble lipid, indicating no gross contamination of this fraction with lecithin.

It thus appears that D-α,β-diglycerides are intermediates in the formation

TABLE 9

INCORPORATION OF C^{14}-LABELED α-GLYCEROPHOSPHATE AND CYTIDINE DIPHOSPHATE CHOLINE INTO THE LIPID OF RAT LIVER MITOCHONDRIA*

Precursor	Acetone-insoluble lipid (phospholipid)	Acetone-soluble lipid (chiefly triglyceride)
α-GP-C^{14}	689† 753	2210† 1340
CMP-PCh-C^{14}	2320 1690	24 40

* Conditions of incubation as described previously.[24, 50]

† Counts per min. per sample. Subsequent examination of the fraction by silicic acid chromatography revealed that much of the activity is present in a phosphorus-containing lipid that is readily eluted by methanol. Mild alkaline hydrolysis and chromatographic separation of this material yielded a spot corresponding to GP.[23] A small but significant portion of the activity remained in the triglyceride fraction. This activity was reduced by the presence of CMP-PCh in the reaction mixture.

of both lecithin and triglyceride. This does not mean that both lecithin and triglyceride are derived from a common metabolic pool of diglyceride. The diglycerides destined to become triglycerides may differ considerably from those destined to become glycerophosphatides, particularly in the nature of their constituent fatty acids. Hanahan[76] has pointed out that each of a series of lecithins from different sources has its characteristic arrangement of fatty acids. Also, there is good evidence that each of the fatty acids of the lecithin molecule is formed by a characteristic metabolic process.[77]

It has been reported that the *in vitro* incorporation of certain precursors into the fatty acids of phospholipid is quite sensitive to metabolic inhibitors[30] and reduced oxygen tensions,[30, 78] whereas the incorporation of the same precursors into triglyceride is less sensitive. This is, perhaps, not surprising. In the formation of both glycerophosphatide and triglyceride, fatty acid must be activated as in REACTION 1 but, in the formation of glycerophosphatide, choline also must be activated as in REACTION 4. For this, an additional source of ATP is necessary for the maintenance of adequate concentrations of CTP. Presumably the CTP is regenerated by the rephosphorylation of the CMP formed in REACTION 5 by enzymes similar to those described by Herbert and Potter.[79]

Summary

Tissue slice experiments have demonstrated that in most tissues, including blood vessels, phosphatides can be formed *in situ* from suitable precursors.

Evidence is given for the conclusion that in cell-free enzyme systems D-α,β-diglyceride, derived from L-α-phosphatidic acid, is an intermediate in the formation of lecithin. Cytidine triphosphate is necessary for the enzymatic transfer of phosphorylcholine to the D-α,β-diglyceride by way of the intermediate, cytidine diphosphate choline.

Cytidine nucleotides are also necessary for the formation of phosphatidyl ethanolamine and some of the inositol lipids.

Sphingomyelin is formed by the enzymatic transfer of phosphorylcholine from cytidine diphosphate choline to *N*-acylsphingosine (ceramide).

Evidence also is given for the conclusion that triglyceride is formed by the enzymatic esterification of D-α,β-diglyceride with a long-chain thioester of coenzyme A.

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