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Phospholipids and their metabolism

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Phospholipids are characterized by the presence of non-polar hydrophobic side chains and polar hydrophilic head groups. These chemical groupings make them particularly suitable compounds to serve as major constituents for biological interfaces. The structure of hydrophobic paraffin chains varies from phospholipid to phospholipid and greatly influences their physicochemical behaviour. There is now a vast literature concerning the chemistry of phospholipids in relation to biological membranes (see van Deenen, 1971). However, while studies of artificial phospholipid membrane systems have been pursued with vigour, the role of phospholipids in other systems, more related to clinical problems, has also attracted attention. For example, the solubilizing power of bile for cholesterol depends on the combined phospholipid and bile salt composition of the bile, and lithogenic bile is characterized by an altered ratio of cholesterol, phospholipid, and bile salts; this is discussed by Dr Dowling later in this Symposium. The separate pathways for the synthesis of biliary lecithins and liver structural lecithins have been studied in detail. Phospholipids are also involved in a later stage of fat absorption, ie, the assembly of chylomicrons during the exit of dietary triglyceride from the enterocyte. The various classes of plasma lipoproteins all carry phospholipids and are characterized by varying proportions of phospholipids, cholesterol, and triglycerides; this too is discussed elsewhere in this Symposium (Dr B. Lewis).

An important new aspect of phospholipid metabolism is the production of dipalmitoyl lecithin in the lung as an essential constituent of surfactant. Dipalmitoyl lecithin is synthesized from the 35th week of gestation onwards. Babies delivered before this date have inadequate surfactant production and a high proportion of them develop the respiratory distress syndrome. The measurement of the lecithin-sphingomyelin ratio in amniotic fluid gives an indication of the maturity of the foetus and is proving useful in deciding when to induce delivery (Gluck, 1971).

The glycerides are both structurally and biosynthetically the parent compounds of the glycerophospholipids. In fig. 1, the structural relationships between glycerol, diglyceride, and phosphatidic acid are shown. Both diglyceride and phosphatidic acid are important intermediates in phospholipid biosynthesis. Phosphatidic acid is chemically the parent of the glycerophosphatides, and the relationship between this compound and lecithin is shown in figure 2. Here choline is esterified via its hydroxyl group onto phosphatidic acid to give phosphatidyl choline. Some of the major phosphatides are illustrated in figure 3. Phosphatidyl choline and phosphatidyl ethanolamine are related biosynthetically and are referred to as the neutral glycerophosphatides; the other glycerophosphatides are acidic, having a net excess of negatively charged groups.

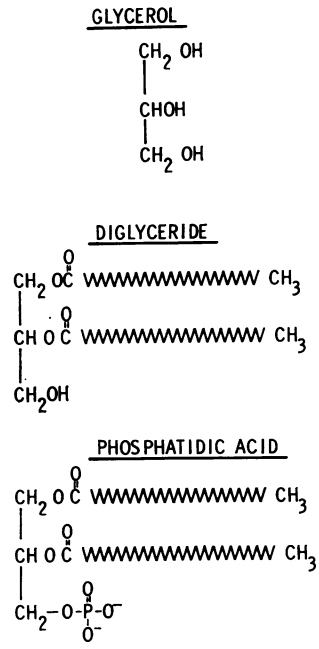

Fig. 1 *Structural relationships of glycerol, diglyceride, and phosphatidic acid.*

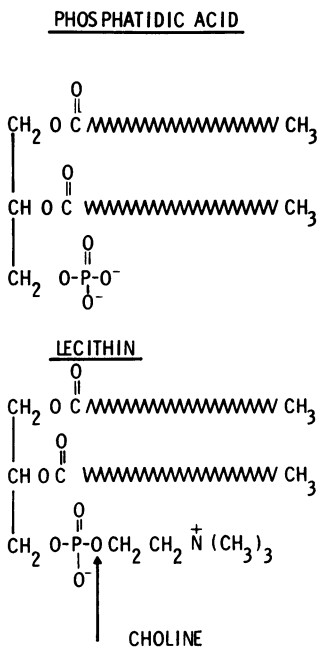

Fig. 2 *Structure of phosphatidic acid and lecithin.*

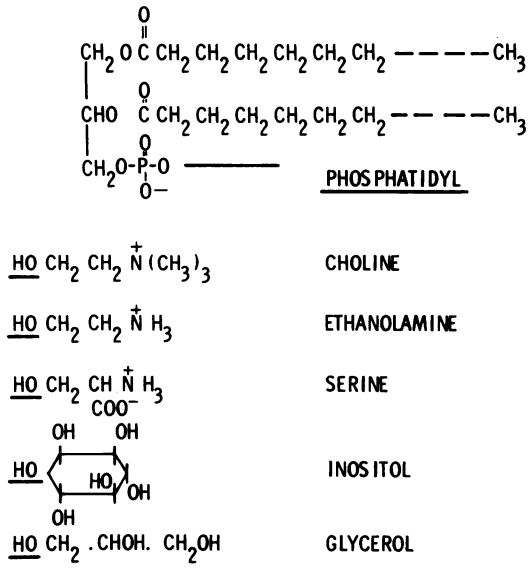

Fig. 3 *Some important mammalian glycerophosphatides.*

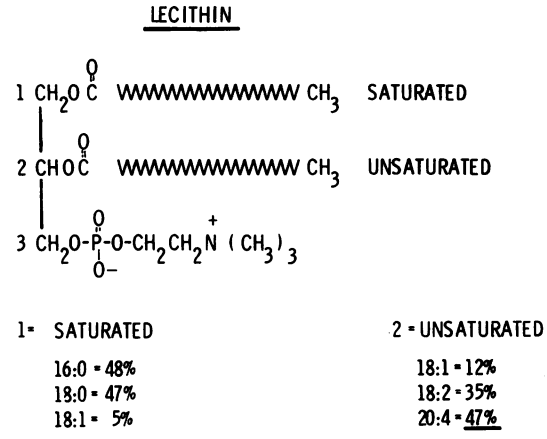

Fig. 4 *A typical distribution of fatty acids between the 1- and 2-positions of lecithin. By convention fatty acids are represented by the number of carbon atoms followed by a colon and then the number of double bonds. Thus 16:0 = palmitic acid; 18:0 = stearic acid; 18:1 = oleic acid; 18:2 = linoleic acid; 20:4 = arachidonic acid.*

Lecithin appears to be quantitatively the most important glycerophosphatide both in membrane systems and in lipid-transporting mechanisms, and much of the biosynthetic work has been concerned with its synthesis. This work has been reviewed in detail several times recently (see bibliography) and only an outline of the major findings will be given here. The distribution of fatty acids between the 1- and 2-positions of the glycerol backbone of rat liver lecithins is shown in figure 4. The data for rat liver lecithins are similar to those from other tissues and to data from other mammalian species that have been investigated. There are two important characteristics of the fatty acid esterification pattern that have concerned investigators in the field: (1) the tendency for the fatty acids in the 1-position to be predominantly saturated, and for those in the 2-position to be unsaturated or polyunsaturated, and (2) the much higher proportion of polyunsaturated longer chain fatty acids, eg, arachidonic acid, 20:4, in the 2-position of lecithin (fig. 4) than in diglycerides or triglycerides.

Investigation of the positional distribution of the fatty acids in various lipids has drawn attention to the range of molecular species of each individual lipid present in each tissue. A representative series of the molecular species of rat liver lecithins is shown in figure 5. Each of these species has its individual turnover rate and their proportions vary from tissue to tissue. The mechanisms by which this specificity of esterification is established are best discussed in

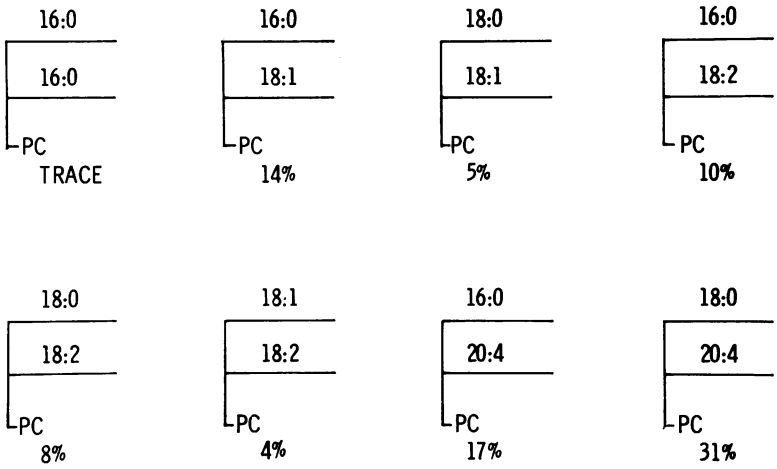

Fig. 5 *Some molecular species of rat liver lecithins.*

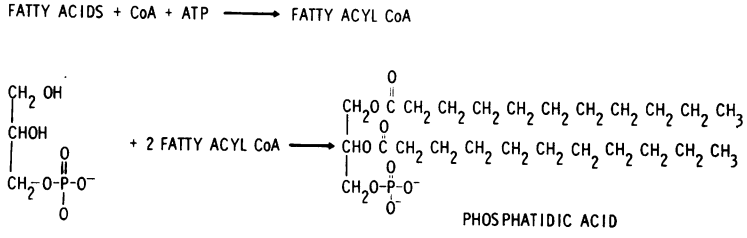

Fig. 6 *The first two reactions in the synthesis of glycerophospholipids by the Kennedy pathway.*

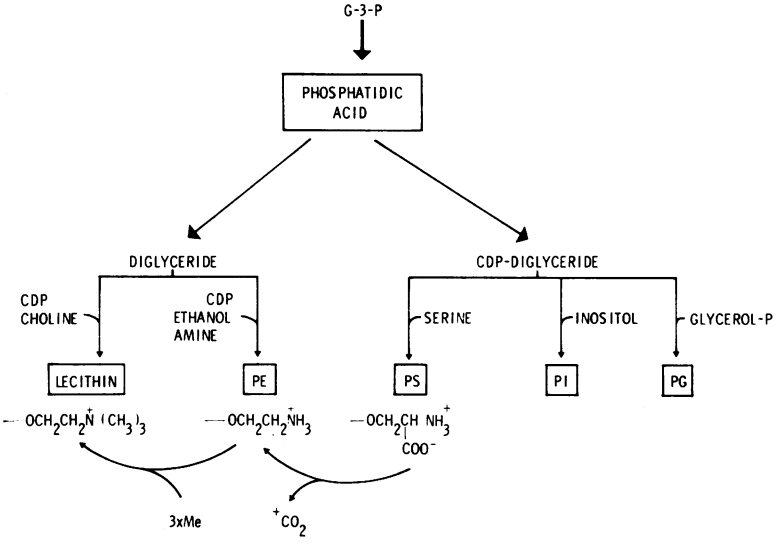

Fig. 7 *The major pathways of glycerophospholipid biosynthesis. PE, phosphatidyl ethanolamine; PS, phosphatidyl serine; PI, phosphatidyl inositol; PG, phosphatidyl glycerol; and CDP, cytidine diphosphate.*

relation to the known pathways for glycerophosphatide biosynthesis.

The first reaction leading to synthesis of phospholipid molecules *de novo* is the acylation of glycerol 3-phosphate (fig. 6), giving rise to phosphatidic acid. After phosphatidic acid is formed, there are separate pathways for the synthesis of neutral and acidic glycerophosphatides. The neutral glycerophosphatides, phosphatidyl choline and phosphatidyl ethanolamine, are synthesized from diglycerides formed by the action of phosphatidate phosphatase (EC 3.1.3.4) on phosphatidic acid (fig. 7). However, the acidic glycerophosphatides are synthesized from cytidine diphosphate-diglyceride (CDP-diglyceride); this intermediate, half lipid, half nucleotide, can be regarded as a nucleotide-activated diglyceride. Thus synthesis of the neutral phosphatides is accomplished by activating the bases, choline and ethanolamine, with cytidine triphosphate, while the acidic phosphatides are synthesized from an activated diglyceride without the further activation of the polar head group (fig. 7). The pathway of lecithin biosynthesis from glycerol 3-phosphate via diglyceride and CDP-choline was the first pathway of phospholipid biosynthesis to be established in detail and is referred to as the 'Kennedy pathway'.

Two other reactions are indicated in figure 7. The first is the tri-methylation of the ethanolamine residue of phosphatidyl ethanolamine with S-adenosyl methionine to give phosphatidyl choline. The other is the decarboxylation of phosphatidyl serine to give phosphatidyl ethanolamine. The former of these two reactions (the methylation pathway) appears to be quantitatively important in several mammalian tissues.

However, this outline of the biosynthetic pathways leading to the glycerophosphatides gives no indication of the mechanisms involved in establishing the 1-saturated, 2-unsaturated distribution of fatty acid esterification in lecithin and other phosphatides. In the 1950s, Lands noticed that the fatty acids of lecithin turn over faster in relation to the glycerol backbone than the fatty acids of triglycerides. Lands postulated that there were tissue enzymes that could remove fatty acids independently from the 1- and 2-positions of lecithin, and other enzymes that could re-esterify new ones back in their place. This was soon demonstrated experimentally (fig. 8). Lands demonstrated that the two isomeric lysolecithins could be re-acylated by enzyme systems present in the microsomal fraction of the cell. The enzyme system acylating a free hydroxyl at the 1-position was shown to be specific for saturated acyl CoAs, while the enzyme acylating a free hydroxyl at the 2-position was specific for unsaturated and poly-

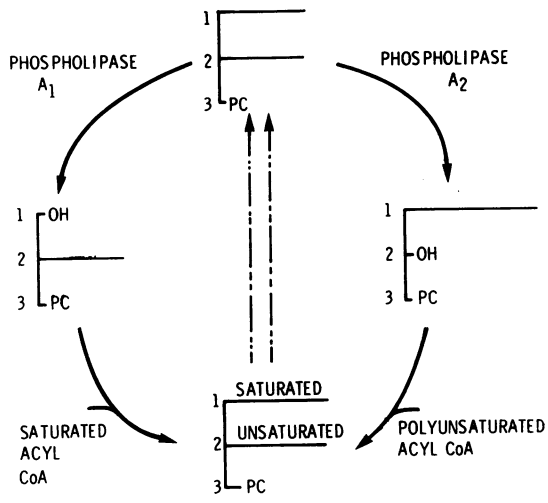

Fig. 8 *The Lands deacylation-reacylation cycle.*

unsaturated fatty acids. Van Deenen (1971) and his colleagues then demonstrated the presence of tissue phospholipases (A_1 and A_2), and this completed the Lands/van Deenen cycle. This cycle will obviously take a lecithin molecule synthesized *de novo* by the Kennedy pathway and remould it to form a lecithin with a 1-saturated, 2-unsaturated distribution.

This mechanism of deacylation and reacylation was considered for some time to account entirely for the positional specificity of fatty acid esterification in phosphatides. Subsequently the specificities of other steps in phospholipid biosynthesis were investigated in more detail (fig. 9), and one of the new pieces of information that had to be explained was that phosphatidic acid and diglyceride also have a 1-saturated, 2-unsaturated distribution. The other important observation was that there is a much higher proportion of arachidonic acid (20:4) in lecithin than in di- and triglycerides and phosphatidic acid (fig. 9).

This extra arachidonic acid in lecithin could be introduced by three separate mechanisms: (1) selection of arachidonyl diglycerides specifically for lecithin biosynthesis at the cholinephosphotransferase (EC 2.7.8.2) step (ie, CDP-choline + diglyceride); (2) synthesis of arachidonyl lecithins by methylation of arachidonyl-phosphatidyl ethanolamines; and (3) introduction of arachidonic acid by the Lands deacylation-reacylation cycle. Experimental work has shown that the cholinephosphotransferase step does not select arachidonyl diglycerides specifically, and now the general weight

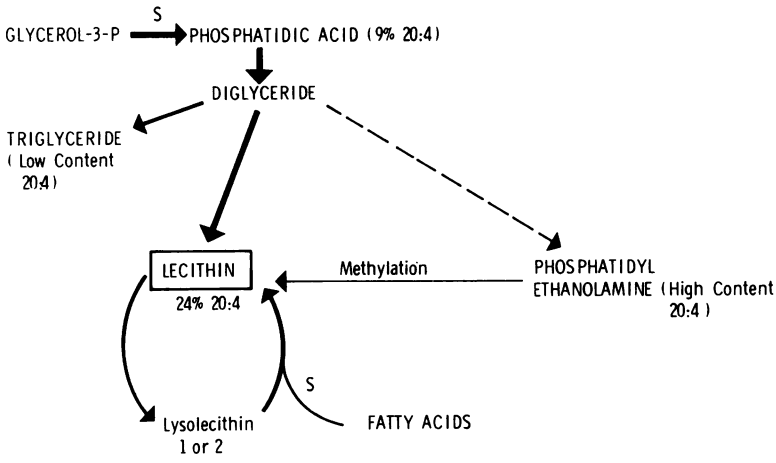

Fig. 9 *Specificity of esterification in the synthesis of lecithin. S = reactions shown to have specificity in relation to fatty acid composition.*

of evidence favours the third explanation, ie, that it is the deacylation-reacylation cycle that is responsible for the high proportion of arachidonic acid in lecithin.

The pathways that have been discussed so far indicate that there are three mechanisms for introducing fatty acids into lecithin: (1) the acylation of glycerol 3-P; (2) methylation of phosphatidyl ethanolamine; and (3) fatty acid exchange reactions. There are also three mechanisms for introducing a choline residue on to the glycerol backbone: (1) cholinephosphotransferase, (2) synthesis of choline by methylation of phosphatidylethanolamine, and (3) another pathway not mentioned so far, a lecithin-free choline exchange. It was originally thought that, although these parts of the lecithin molecule could originate in several different ways, the glycerol backbone was always derived from glycerol 3-phosphate. Recent work has shown that there are two other possible sources, namely, the triose phosphates of the glycolytic pathway (fig. 10). Both dihydroxyacetone phosphate and glyceraldehyde 3-phosphate can be acylated first and reduced afterwards. The reduction products are isomeric lysophosphatidic acids. The product from dihydroxyacetone phosphate contains a saturated fatty acid

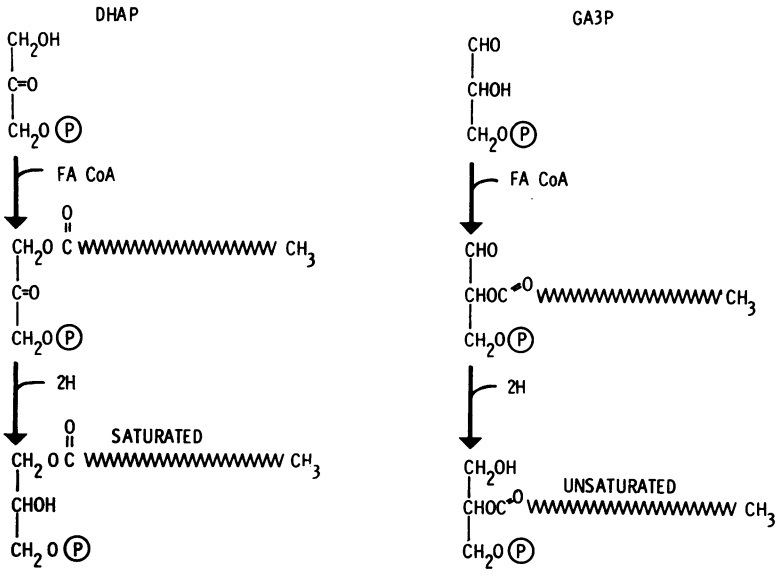

Fig. 10 *Acylation of triose phosphates with their subsequent reduction to form lyso-phosphatidic acids.*

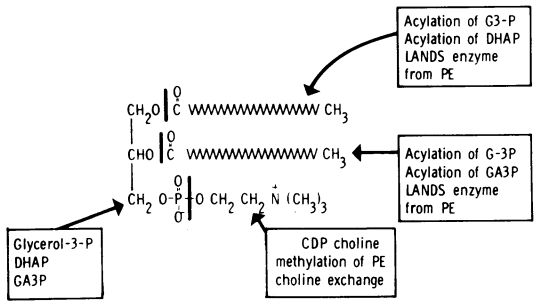

Fig. 11 *Various pathways giving rise to different functional groups of the lecithin molecule. G3-P, glycerol 3-phosphate; DHAP, dihydroxyacetone phosphate; PE, phosphatidyl ethanolamine; GA3P, glyceraldehyde 3-phosphate; CDP, cytidine diphosphate.*

and that from glyceraldehyde 3-phosphate contains an unsaturated fatty acid. These lysophosphatidic acids can be converted to phosphatidic acid in the same manner as lysolecithins are reacylated in the Lands cycle. These phosphatidic acids can enter the pathways of phosphatide and triglyceride synthesis (fig. 7).

Figure 11 summarizes the possible sources of the individual parts of the lecithin molecule, and demonstrates the complexity involved in the biosynthesis and turnover of just one phospholipid. It is important to realize that, although these pathways have in part been worked out using isotope data, their complexity makes it difficult to establish the quantitative importance of each pathway using isotopic methods alone. These separate pathways ensure that lecithin molecules can be made, and remodelled even after synthesis, in response to different metabolic requirements. Increased synthesis *de novo* might be required for an increase in lipid-transporting activity, while remodelling might be a consequence of or a response to a change in membrane permeability.

Full details of the experimental studies giving rise to this outline of phospholipid metabolism and the important contributions of the major groups working in this field are well described in the reviews listed below.

Reviews

van Deenen, L. L. M. (1971). Chemistry of phospholipids in relation to biological membranes. *Pure appl. Chem.*, **25**, 25-56.

Gluck, L. (1971). Biochemical development of the lung: clinical aspects of surfactant development. R.D.S. and the intrauterine assessment of lung maturity. *Clin. Obstet. Gynec.*, **14**, 710-721.

Hill, E. E., and Lands, W. E. M. (1970). Phospholipid metabolism. In *Lipid Metabolism*, edited by S. J. Wakil, ch. VI, pp. 185-272. Academic Press, New York.

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