Curves $E$ and $F$ (Fig. 1) show that added succinyl-CoA inhibited in the presence but not in the absence of added NADH. Inhibition by succinyl-CoA is abolished at infinite [CoASH], and also by increasing the $E_h$ of the NAD system or by replacing NAD with a comparable concentration of APyAD. The requirement for a partially reduced enzyme complex and the reversal by infinite [CoASH] suggest that the inhibitory effects of succinyl-CoA are associated with succinylation of lipoyl residues in the enzyme.

A possible common mechanism for the inhibitory effects of NADH and succinyl-CoA is that accumulation of reduced and succinylated lipoyl residues in the enzyme complex results in a decrease of oxidized lipoyl residues, which then behave as a rate-limiting cofactor in the overall oxoglutarate dehydrogenation. Product inhibition of oxoglutarate dehydrogenase provides a mechanism whereby the enzyme activity could be related to the state of mitochondrial reduction and, via succinyl-CoA synthetase (EC 6.2.1.4), to the phosphorylation state of mitochondrial nucleotides. The behaviour of oxoglutarate dehydrogenase resembles that of pig-heart pyruvate dehydrogenase, which is inhibited by its products acetyl-CoA and NADH (Garland & Randle, 1964, and unpublished work).

As shown in Fig. 1, CoASH can be assayed by measuring the reduction of APyAD (curve C). Under the conditions described for assay of CoASH in tissue extracts, the extinction change is proportional to the CoASH added and is completed within 1 min. of adding the enzyme. Any continued slow increase in extinction due to hydrolysis of succinyl-CoA and recycling of CoA can be corrected graphically (Bergmeyer, 1963). Added acetyl-CoA, butyryl-CoA or 3-hydroxybutyryl-CoA (20 μm-moles) does not interfere.

The content of CoASH in liver from fed rats was 47 ± 3·3 (5) μm-moles of CoASH/g. wet wt. of tissue (mean ± s.e.m.; number of observations in parentheses). In liver from rats starved for 36 hr. the content was 76 ± 6·0 (5), and from alloxan-diabetic rats 77 ± 8·9 (6). The percentage of total acid-soluble CoA (see Tubbs & Garland, 1964) accounted for as CoASH was 39 ± 2·7 (5), 50 ± 1·9 (5) and 45 ± 2·1 (6) in liver from normal, starved and alloxan-diabetic rats respectively.

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**The Structure of Cardiolipin**

**By M. G. Macfarlane**

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From the time of Thudichum the pathways of identification of natural phospholipids have been enlivened by controversy and strewn with 'red herrings'. To this, cardiolipin is no exception. It is common ground that it is a polyglycerophosphatide (Pangborn, 1942) with a molar ratio of fatty acids to phosphorus of 2:1, which on mild alkaline saponification gives a single water-soluble polyglycerophosphate without liberation of phosphoric acid. In the last few years structure (I) (Table 1), proposed by Pangborn (1947), has generally lost ground in favour of structure (II); but recently
Rose (1964) suggested structure (III), and de Haas & van Deenen (1963) consider that the choice between structures (I) and (II) is still open. It is perhaps timely to consider various structures for the molecule (Table 1) by the null hypothesis, i.e. a postulated structure is ‘in’ until it is ‘out’ by experimental results. The nature of the fatty acids is immaterial for this.

Structure (I) is a hexa-acyl αβ-polyglycerophosphatidate with no free hydroxyl group. Macfarlane & Gray (1957, 1958) proposed structure (II), a diphasphatidylglycerol in which the phosphatidyl groups were arbitrarily assigned to the αβ-positions, leaving a median β-hydroxyl group free. Rose (1964) proposed structure (III), triphosphatidylglycerol, postulating that the β-phosphatidy1 group would be very labile to acid hydrolysis, giving compound (II) and phosphatidic acid. Structure (IV), now set down for consideration, is the αβ-isomer of structure (II), and structure (V) is a variant of structure (II) in which there is a primary or secondary hydroxyl group free in a terminal glycerol.

Evidence on chain structure of deacylated glycerol.
(i) Oxidation of deacylated cardiolipin with periodate yielded 1 mol. of formaldehyde/mol. of phosphorus (Macfarlane, 1958). This excludes structure (I) and any chain longer than in structures (II) or (III), in which the number of vicinal hydroxyl groups gives a ratio less than one.

(ii) Benson & Strickland (1960) synthesized αβ-bis(glycerylphosphoryl)glycerol (GPGPG) by treatment of synthetic glyceridophosphatidic acid (PGP, formed by phosphorylation of 1,3-dichloropropan-2-ol) with glycidol. This compound co-chromatographed with the deacylation product from Chlorella polyglycerophosphatide, which in turn co-chromatographed with the corresponding product from sheep-heart mitochondria (Strickland & Benson, 1960). The synthetic GPGPG also co-electrochromatographed with the Chlorella ester, and the electrophoretic mobility was consistent with the presence of two phosphoric diester groups in the molecule. Brief hydrolysis of the synthetic and the Chlorella ester at 100° in 0.1 N-hydrochloric acid gave the same initial products, α-glycerophosphate and glycerylphosphorylglycerol (GPG), the latter decreasing in time with increase in glycerophosphate. These facts virtually exclude structure (III) (as well as structure I), whose deacylation product contains 3 phosphoric diester groups/mol. and is therefore unlikely to co-chromatograph with GPGPG, and which should give different initial products on hydrolysis. As Benson & Strickland’s (1960) data do not specifically distinguish between the deacylation products of compounds (II) and (IV), which are presumably very similar in behaviour, both these structures remain ‘in’.

Evidence from acid hydrolysis of cardiolipin. On hydrolysis of cardiolipin in 90%, (v/v) acetic acid Macfarlane & Wheeldon (1959) obtained 76% of the fatty acids as diglycerides, with only 5% of free acids, and 80% of the phosphorus as water-soluble esters of which not more than 10% was glycerophosphate and the rest was glycerodiphosphate. This excludes structures (I) and (V) but, as Rose (1964) points out, does not exclude structure (III), as the residual ether-soluble phosphorus was not identified. However, Coulon-Morelec, Faure & Maréchal (1960) found that on hydrolysis of cardiolipin in acetic acid the first products were diglycerides and phosphatidylcyclo-glycerophosphate. On hydrolysis of cardiolipin as the free acid in water at 18° (Coulon-Morelec, Faure & Maréchal, 1962) the first products were a triacyl-lysocardiophosphatidylcyclo-glycerophosphate; phosphatidic acid and glycerophosphate were not detected. These facts exclude structure (III) (whose first product is postulated to be phosphatidic acid) and are consistent with either structure (II) or structure (IV).

Synthesis of an acetyl-cardiolipin. Coulon-Morelec (cited by Faure & Coulon-Morelec, 1963) synthesized an acetyl derivative of cardiolipin by the action of excess of acetic acid and dicyclohexylcarbodi-imide in pyridine; it contained 0.48 mol. of acetyl group/mol. of phosphorus. This is the first direct evidence that cardiolipin contains a free hydroxyl group, but it does not distinguish between structures (II) and (IV).

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**Table 1. Structures for cardiolipin**

(GP). Glycerylphosphoryl; phosphatidyl and lysophosphatidyl, di- and mono-acylglycerylphosphoryl respectively.

<table>
<thead>
<tr>
<th>Structure</th>
<th>Designation</th>
<th>Deacylation product</th>
<th>Lipid product of acid hydrolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>(I)</td>
<td>Polyglycerophospholipid</td>
<td>GPGPGPG</td>
<td>2 Diglyceride + 2 fatty acid</td>
</tr>
<tr>
<td>(II)</td>
<td>αβ-Diphasphatidylglycerol</td>
<td>αβ-Di(GP)G</td>
<td>2 Diglyceride</td>
</tr>
<tr>
<td>(III)</td>
<td>Triphosphatidylglycerol</td>
<td>Tri(GP)G</td>
<td>Phosphatidic acid + 2 diglyceride</td>
</tr>
<tr>
<td>(IV)</td>
<td>αβ-Diphasphatidylglycerol</td>
<td>αβ-Di(GP)G</td>
<td>2 Diglyceride</td>
</tr>
<tr>
<td>(V)</td>
<td>α-Phosphatidyl-β-acyl-γ-lysophosphatidylglycerol</td>
<td>αγ-Di(GP)G</td>
<td>1 Diglyceride + 1 monoglyceride + 1 fatty acid</td>
</tr>
</tbody>
</table>
Synthesis of an acyl derivative of diphasatidylglycerol. A derivative of compound (II), αγ-bis-(γ-oleoyl-β-palmitoyl-L-α-glycerylphosphoryl)-β-myristoylglycerol (VI), was synthesized by de Haas & van Deenen (1963); this is clearly not cardiolipin as the ratio of fatty acids to phosphorus is different. They compared the action of phospholipase A on cardiolipin and on compound (VI), examining the products chromatographically and by gas-liquid chromatography. Cardiolipin after 6 hr. incubation gave two products: after 18 hr. the original cardiolipin and the faster product (presumably an intermediate triacyl-lysocardioplin) had disappeared and half the original fatty acids had been released. The enzyme did not release any of the ‘β-located’ myristic acid from compound (VI), and the rate of hydrolysis was much less. After 18 hr. much of the material was unhydrolysed and two products were present, apparently with the same $R_P$ values as those from cardiolipin. This result is puzzling, for if no myristic acid was liberated the expected products of compound (VI) would be different from those of compound (II), i.e. a tetra-acyl intermediate and a triacyl final product.

An early attempt to determine the structure of cardiolipin by oxidation of the hydroxyl group and identification of the oxidation product was inconclusive (Macfarlane & Gray, 1957). At present it appears that cardiolipin can be either compound (II) or compound (IV). The route of biosynthesis is not yet known and perhaps the possibility that it proceeds through compound (IV) to compound (II) should be considered.


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Gluconic Acid in the Human Lens

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This paper reports the presence of free gluconic acid in the human lens. The formation of xylonic acid from xylose by the intact calf lens in vitro has already been shown (van Heyningen, 1958), and also the presence of an enzyme, in dialysed extracts of bovine and rat lens, which will reduce NAD$^+$ in the presence of a high concentration of glucose or xylose.

Lens extracts. Human cataractous lenses were removed at operation; a few normal lenses were obtained post mortem; both were stored frozen until required. Animal lenses were used soon after death. Trichloroacetic acid was used to precipitate lens proteins. It was removed from the protein-free extracts by shaking with methylidioctylamine in chloroform (Hughes & Williamson, 1951). The extracts (pH 7) were then concentrated in vacuo.

Distribution and preparation of acid. The lens extracts were found, by means of paper electrophoresis in pyridine (2·5 %, v/v)-acetic acid (0·25 %, v/v), pH 6 (Runeckeles & Krotkov, 1959), to contain a negatively charged substance revealed by AgNO$_3$-NaOH (Trevelyan, Procter & Harrison, 1950). The concentration of the acid was judged by the strength of the spot on an electrophoresis paper, compared with that given by known amounts of gluconic acid. In three clear (post-mortem) lenses from non-diabetics its concentration was 0·1 μmole/g. of lens or less. In two clear (post-mortem) lenses from diabetics it was at concentrations of 2·5 μmoles/g. of lens and 0·4 μmole/g. of lens. About 100 cataractous lenses were examined from non-diabetic and diabetic patients (Pirie & van Heyningen, 1964). The amount of the acid in these lenses was not assessed, but it was present in nearly all, and at a considerably higher concentration (probably up to 0·5 μmole/g. of lens) in those from diabetics.