adequately compete with the few carboxyl groups which appear on the surface as the reaction proceeds. Consequently ether has no activating effect. In contrast when mixed micelles of phosphatidyl ethanolamine and fatty acid are prepared artificially the negative charge of such particles is much higher than that of the phospholipid alone. This indicates that, in this case, carboxyl groups are present on the surface and, in agreement with this, it is found that the enzymatic hydrolysis of the substrate is inhibited by the presence of the fatty acid.

To summarize, it is suggested that a phospholipase *A*-calcium complex links with anion sites (phosphate) on the surface of the lecithin micelle. The carboxyl groups of the fatty acids liberated in the reaction appear to a limited extent in the micelle–water interface, and inhibit the reaction by competing with the anion sites for enzyme. Saturation of the system with ether removes carboxyl groups from the surface and the reaction can proceed. With phosphatidyl ethanolamine micelles, there are many more free anion sites on the surface. The few carboxyl groups appearing on the surface as the reaction proceeds only have a very limited competing effect and consequently ether does not activate.

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[1] Bangham, A. D., and Dawson, R. M. C., *Biochem. J.*, **72**, 486 (1959).
[2] Dawson, R. M. C., and Bangham, A. D., *Biochem. J.*, **81**, 29, P (1961).
[3] Thompson, W., and Dawson, R. M. C., *Proc. Intern. Symp. Enzyme Activity of Nervous System, Göteborg*, 34 (1962).
[4] Magee, W. L., and Thompson, R. H. S., *Biochem. J.*, **77**, 526 (1960).
[5] Dawson, R. M. C., Hemington, N., and Davenport, J. B., *Biochem. J.*, **84**, 497 (1962).

Positional Specificity of Phosphatide Acyl Hydrolase (Phospholipase *A*)

Snake venom phospholipase *A* was shown by de Haas and van Deenen to release the fatty acid constituent present in the β-ester position of mixed-acid phospholipids obtained by a synthesis *de novo*[1–3]. This result confirmed those of Tattrie[4] and Hanahan *et al.*[5], who worked on fatty acid distribution in egg lecithin, using phospholipase *A*, phospholipase *D* and pancreatic lipase. Hence, the positional specificity of phospholipase *A* appeared to be settled. Recently Bennett and Tattrie[6], however, reported, because of reacylation investigations of snake-venom-formed lysolecithin, that this enzyme may not invariably hydrolyse the β-fatty acid ester bonds. This assumption supports the interpretation previously made by Marinetti *et al.*[7], indicating a preference of the enzyme for liberating long-chain poly-unsaturated fatty acids, irrespective of their position in the phospholipid molecule. For this reason further investigations into the mode of action of phospholipase *A* were made.

The synthetic lecithins and cephalins investigated so far by us contained either two dissimilar saturated fatty acid constituents or one saturated and one mono-unsaturated fatty acid of equal chain-length in different positions. In view of the conflicting opinions, a further check on the specific action of phospholipase *A* required examination of phospholipids containing, in different positions, a poly-unsaturated fatty acid with a chain-length dominating that of the saturated fatty acid constituent. The synthesis of mixed-acid lecithins and cephalins, involving the preparation of defined γ,β-diacylglycerol iodohydrins, has already been published[8,9]. In the present investigation (γ-linolenoyl-β-palmitoyl)-L-α-phosphatidyl ethanolamine and (γ-palmitoyl-β-linolenoyl)-L-α-phosphatidyl ethanolamine were prepared by a coupling of the corresponding mixed-acid γ,β-diacylglycerol iodohydrins with silver benzyl-2-ethyl-phthalimido phosphate, as previously described[10]. Experimental results of the synthesis of phospholipids with polyenoic acyl chains will be described elsewhere. Emulsions of 10 mg of both structural isomeric phospholipids, prepared in 1 ml. of borate buffer, *p*H 7, calcium chloride $2{\cdot}5 \times 10^{-3}$ M, with a few drops of ether, were incubated together with 2 mg of snake venom (*Crotalus adamantheus*) for 6 h at room temperature. The entire reaction mixture was lyophilized and adequate samples dissolved in chloroform were subjected to chromatography on silica-impregnated paper according to Marinetti[11]. The paper chromatogram demonstrated a complete breakdown of both substrates. The spots of the liberated fatty acid and formed lysophospholipid were treated directly with methanol/hydrochloric acid, and the fatty acid methylesters thus obtained were analysed by gas-liquid chromatography.

Table 1. Action of Phospholipase *A* on Two Synthetic Mixed-Acid L-α-Phosphatidyl Ethanolamines, containing Linolenic Acid in Different Molecular Positions

Substrates	Fatty acid composition after hydrolysis by phospholipase *A*: Liberated fatty acid	Lysophospholipid
(γ-Palmitoyl-β-linolenoyl)-L-α-phosphatidyl ethanolamine	98·5 per cent linolenic acid	99 per cent palmitic acid
(γ-Linolenoyl-β-palmitoyl)-L-α-phosphatidyl ethanolamine	97 per cent palmitic acid	97·5 per cent linolenic acid

The results obtained (Table 1) showed that during the enzymatic hydrolysis of both synthetic phospholipids, the β-fatty acid is liberated, thus confirming our previous conclusions. It should be noted that the results presented here have not been corrected for a non-enzymatical 'at random' degradation, which was shown to occur to a very small extent under the conditions used. The experiments did not reveal a preferential enzymatic release of the long-chain poly-unsaturated fatty acid constituent, and hence do not support the reports[6,7] attributing a lack of positional specificity to snake-venom phospholipase *A*. In addition, it should be pointed out that experiments on the phosphatide-(mono)acyl hydrolase from bee venom and pancreas demonstrated that these enzymes also exhibit a β-specificity for synthetic phospholipids.

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[1] Haas, G. H. de, and Deenen, L. L. M. van, in *The Enzymes of Lipid Metabolism*, edit. by Desnuelle, P., 53 (Pergamon Press, 1961).
[2] Haas, G. H. de, Mulder, I., and Deenen, L. L. M. van, *Biochem. Biophys. Res. Comm.*, **3**, 287 (1960).
[3] Haas, G. H. de, and Deenen, L. L. M. van, *Biochim. Biophys. Acta*, **48**, 215 (1961).
[4] Tattrie, N. H., *J. Lipid Res.*, **1**, 60 (1959).
[5] Hanahan, D. J., Brockerhoff, H., and Barron, E. J., *J. Biol. Chem.*, **235**, 1917 (1960).
[6] Bennett, J. R., and Tattrie, N. H., *Canad. J. Biochem. Physiol.*, **39**, 1357 (1961).
[7] Marinetti, G. V., Erbland, J., and Stotz, E., *Biochim. Biophys. Acta*, **38**, 534 (1960).
[8] Haas, G. H. de, and Deenen, L. L. M. van, *Rec. Trav. Chim.*, **80**, 952 (1961).
[9] Haas, G. H. de, and Deenen, L. L. M. van, *Proc. Kon. Akademie (Amsterdam)*, C, **64**, 592 (1961).
[10] Daemen, F., Haas, G. H. de, and Deenen, L. L. M. van, *Rec. Trav. Chim.*, **81**, 216 (1962).
[11] Marinetti, G. V., Erbland, J., and Kochen, J., *Fed. Proc.*, **16**, 837 (1957).