The 'Free' Lipids of *Brucella abortus* Bang, II. The Positional Distribution of the Phospholipid Fatty Acids

Phospholipids of higher organisms are known to contain considerable amounts of polyenoic fatty acids which are preferentially located at their 2-positions while the 1-positions are preferentially occupied by saturated fatty acids[1]. Since phospholipids are essential constituents of biological membranes, research has recently focused much attention on chemical structure of membrane lipids[2]. The occurrence of polyenoic acids seems to be of great importance for the function of membrane phospholipids. Decreasing levels of polyenoic acids of membrane phospholipids result in increasing fragility of membranes[3]. Studies of monolayer phospholipids show a marked effect of unsaturation of the component fatty acids on their physical-chemical properties[4]. The specific positional distribution of saturated and unsaturated fatty acids is controlled by specific acyl-transferases as was recently demonstrated[5]. This positional distribution can be assumed to play an important role in the function of phospholipids as membrane constituents. In addition, cholesterol is believed to have a stabilizing effect on the lipid core of membranes[4].

Bacterial total lipids contain neither polyenoic fatty acids[6] nor sterols[7]. Since bacterial membranes are no less stable than membranes of higher organisms, the knowledge of the positional distribution of fatty acids of bacterial phospholipids is of interest. So far investigation of this type has been done only on phosphatidylethanolamine of some (mostly Gram-negative) bacterial species and lecithin of *Agrobacterium* tumefaciens[8]. In most cases cyclopropane fatty acids occupied preferentially the 2-positions.

We isolated further phospholipids from *Brucella abortus* Bang (strain Scherle II) which were grown on solid media and harvested after approximately 70 h. The 'free' lipids turned out to contain much larger amounts of C_{19} cyclopropane acids than has been reported on most other bacterial species, but no detectable amounts of C_{17} cyclopropane acids. The lipids of *B. abortus* display an exceptional case in that they contain lecithin[9] as their major component, while other bacteria do not contain any lecithin or only trace amounts – with the only exception to date being *Agrobacterium*[10]. We determined the positions of the component fatty acids by virtue of the positional specificity of phospholipase A from snake venom. The results are summarized in the Table; the usual saturated and unsaturated fatty acids are not mentioned: they exhibit no markedly specific positioning. The hydroxy acids were recognized by their gas-chromatographic properties after acetylation[11].

Although we did not prepare phospholipids from isolated bacterial membranes, most of the phospholipids studied are probably membrane derivatives. It is not known whether the marked positional distribution pattern (Table) is essential for the membrane function of bacterial phospholipids. Thus, the results stimulate the investigation of the physical-chemical properties of phospholipids containing cyclopropane and/or hydroxy fatty acids. Recently, it was suggested[12] that mitochondrial protein has special sites for hydrophobic bonding of apolar moieties of phospholipids. If we apply this suggestion to bacterial membranes, we have to postulate that the qualities of bacterial membrane proteins differ markedly from those of higher organisms.

Zusammenfassung. Aus *Brucella abortus* Bang (Stamm Scherle II) wurden 6 Phosphatide in reiner Form isoliert. Bei allen fand sich die 2-Stellung bevorzugt von einer C_{19}-Cyclopropansäure besetzt, während in 1-Stellung verschiedene Hydroxysäuren angereichert waren.

O. W. THIELE and D. BUSSE

Physiologisch-Chemisches Institut der Universität, Göttingen (Germany), 14 September 1967.

Positional distribution of the principal phospholipid fatty acids of *Brucella abortus* Bang (Strain Scherle II)

		% of principal fatty acids	
		C_{19} cyclopropane	hydroxy[a]
Lecithin	1-position	33.8	45.2
	2-position	84.0	6.0
Phosphatidyl-*N*-dimethyl ethanolamine	1-position	2.0	21.4
	2-position	42.8	12.6
Phosphatidyl-*N*-methyl-ethanolamine	1-position	3.0	29.6
	2-position	69.2	8.0
Phosphatidyl-ethanolamine	1-position	2.8	60.6
	2-position	51.4	14.2
Phosphatidyl-glycerol	1-position	2.2	44.6
	2-position	58.2	2.2
Cardiolipin	1-position	14.8	37.2
	2-position	52.0	5.6

[a] Saturated and unsaturated.

[1] L. L. M. VAN DEENEN and G. H. DE HAAS, A. Rev. Biochem. *35*, 173 (1966).
[2] D. J. HANAHAN and G. A. THOMPSON, A. Rev. Biochem. *32*, 215 (1963); R. O. BRADY and E. G. TRAMS, A. Rev. Biochem. *33*, 75 (1964); A. A. BENSON, A. Rev. Plant Physiol. *15*, 1 (1964).
[3] B. L. WALKER and F. A. KUMMEROW, Proc. Soc. exp. Biol. Med. *115*, 1099 (1964); M. SUGANO and O. W. PORTMAN, Archs Biochem. *108*, 302 (1965).
[4] L. L. M. VAN DEENEN, U. M. T. HOUTSMULLER, G. H. DE HAAS and E. MULDER, J. Pharm. Pharmac. *14*, 429 (1962).
[5] W. E. M. LANDS and P. HART, J. Am. Oil Chem. Soc. *43*, 290 (1966).
[6] W. M. O'LEARY, Bact. Rev. *26*, 421 (1962).
[7] J. ASSELINEAU, *Les lipides bactériens* (Hermann, Paris 1962), p. 287.
[8] J. G. HILDEBRAND and J. H. LAW, Biochem. *3*, 1304 (1964); L. M. G. VAN GOLDE and L. L. M. VAN DEENEN, Chem. phys. Lipids *1*, 157 (1966).
[9] W. WOBER, O. W. THIELE and B. URBASCHEK, Biochim. biophys. Acta *84*, 376 (1964).
[10] M. KATES, Adv. Lipid Res. *2*, 17 (1964).
[11] O. W. THIELE, Biochim. biophys. Acta *84*, 482 (1964).
[12] G. G. DE PURY and F. D. COLLINS, Chem. phys. Lipids *1*, 1 (1966).