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LETTER TO THE EDITORS

CHANGES IN THE CHEMICAL AND THE BARRIER PROPERTIES OF THE MEMBRANE LIPIDS OF E. COLI BY VARIATION OF THE TEMPERATURE OF GROWTH

Analytical studies on the structural lipids of biological interfaces have shown that for a given membrane[1]) the lipid composition is very constant and characteristic. Normally only limited variations can be brought about by dietary means[2]). This suggests that the physical properties of these membrane lipids, determined by the characteristic chemical composition, are very essential for the proper function of the membrane at the conditions under which the membrane has to operate. Micro-organisms offer the possibility that the physical conditions of the growth media can be changed drastically. As a consequence of for example changes in temperature[3]) and *p*H[4]) interesting changes in the composition of the structural lipids have been observed.

In this paper we deal with differences in lipid composition of *Escherichia coli* grown at different temperatures and the consequent variations in the barrier properties of these structural lipids as apparent from studies on simple model systems. In all experiments the wild type of E. coli K_{12} was used. The bacteria were cultured in a glucose minimal medium.

The cells were harvested at the end of exponential growth, the lipids were extracted by the method of Bligh and Dyer[5]) and separated in neutral lipids and phospholipids by chromatography on silicagel. Thin layer chromatograms demonstrated that the main phospholipid was phosphatidyl ethanolamine, taking in about 90% of the total phospholipids, whereas also small amounts of phosphatidyl glycerol and cardiolipin were present. Variations in the temperature of growth did not demonstrate any apparent change in the distribution over the various phospholipid classes. However, there was a marked increase in saturated and a decrease in unsaturated fatty acids when the temperature of growth was elevated, as is shown in Table 1. The force-area curves of the monolayers of the total phospholipid, as obtained from the bacteria grown at the three different temperatures, are given in Fig. 1. It appeared that at all pressures the mean surface area per phospholipid molecule was lower when the temperature of growth was higher. This is in agreement with the observations of Demel *et al.*[7]), which demonstrated that the packing of the phospholipid molecules in monolayers is primarily de-

TABLE 1

Fatty acid composition of phospholipids from E. coli grown at different temperatures in glucose minimal media

Fatty acid	Temperature of growth		
	20°	30°	40°
14:0	1,7	2,3	2,7
16:0	21,0	26,5	36,7
16:1	12,8	6,7	7,3
17:0	8,4	20.0	23,0
18:1	49,0	35,5	15,2
19:0	7,0	9,0	15,1
Total straight chain saturated acids	22,7 →	28,8 →	39,4
Total monounsaturated plus cyclopropane acids	77,2 ←	71,2 ←	60,6

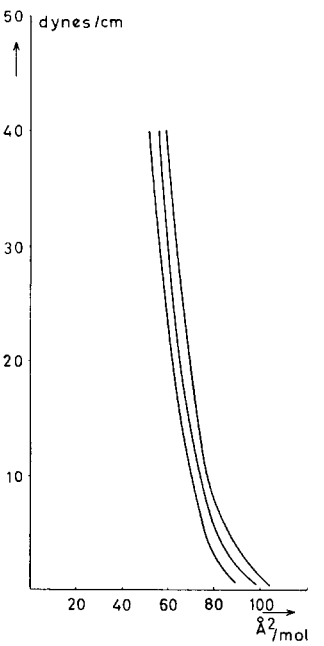

Fig. 1. Force-area curves of phospholipids isolated from E. coli cultures grown at 20, 30 and 40°C respectively.

termined by the nature of the fatty acid chains and that the introduction of double bounds increases this area.

When dry phospholipids are brought into contact with an excess of an aqueous phase, this normally causes the spontaneous formation of osmotic sensitive liposomes[8]).

Addition of 50 m M KCl to thin films of the bacterial phospholipids did not result in a spontaneous swelling and dispersion of the lipids in the water phase. This may be due to the fact that phosphatidyl ethanolamine is the main phospholipid in these lipids.

Papahadjopoulos *et al.*[8,9]) noticed that phosphatidyl ethanolamine in the chromatographic pure form did not give completely closed structures in KCl solutions. In agreement with their observations the addition of 20 mole% of egg yolk lecithin to the bacterial lipids completely changed the behaviour.

Now we noticed spontaneous formations of phospholipid liquid crystals in 50 m M KCl with an identical osmotic behaviour as those prepared of natural and synthetic lecithins containing 4 mole% phosphatidic acid[10,11]).

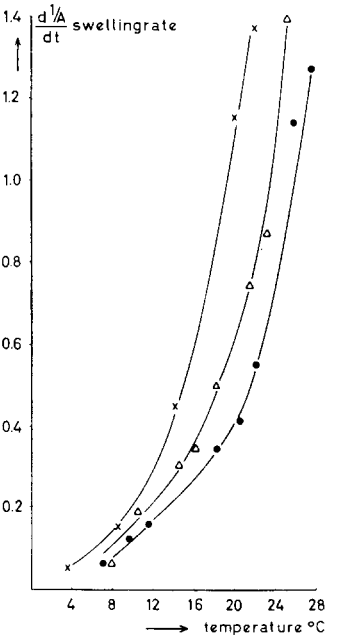

Fig. 2. Initial swelling rates in isotonic glycerol of liposomes prepared from E. coli phospholipids mixed with 20 mole % egg lecithin.

× phospholipids from E. coli grown at 20°C
△ phospholipids from E. coli grown at 30°C
● phospholipids from E. coli grown at 40°C

These structures were considered to be consistent with liposomes composed of fully closed concentric lipid lamellae separated by aqueous layers.

The permeability of the liposomes for glycerol was determined by optical measurements of the initial swelling rate of the liposomes in an isotonic solution of this non-electrolyte[11]). In Fig. 2 these initial swelling rates are given as a function of temperatures. It is clearly shown that the permeability of the liposomes decreases markedly when the phospholipids are used of bacteria grown at a higher temperature. This is consistent with what could be expected from the result on the packing in monolayer and with our conclusions on the relation between lipid bilayer permeability and unsaturation of the chains made from studies on liposomes of synthetic lecithins.

From the present observations on the chemical and physico-chemical properties of the E. coli lipids it can tentatively be concluded that when the temperature of growth is enhanced the bacteria compensate the increase in permeability as a consequence of the change in this parameter by reducing the degree of unsaturation of the phospholipid paraffin chains.

The analytical data in Table 1 are in agreement with the observations of Marr and Ingraham[3]). Careful comparison of their data with ours demonstrate that they found lower values for the cyclopropane acids. This may be explained by difference in strain or by the fact that they used a chemostat and consequently harvested younger cell than we did. Analysis on the lipids of bacteria harvested in the stationary phase even demonstrated considerable higher values in cyclopropane fatty acids. However, the permeability of the liposomes prepared with these lipids did not differ from those obtained with the lipids from bacteria grown at the same temperature and harvested in the logarithmic phase. Therefore we suggest that the bacteria are stabilizing their unsaturated fatty acids by conversing them to the corresponding cyclopropane chains and that this does not change the physicochemical properties of the membrane.This is also in agreement with observations of Demel[6]) who showed that the packing of phospholipids with cyclopropane fatty acids in monolayers is identical to those of these lipids with monounsaturated acids.

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ERRATUM

F. C. REMAN, R. A. DEMEL, J. DE GIER, L. L. M. VAN DEENEN, H. EIBL and O. WESTPHAL: Studies on the lysis of red cells and bimolecular lipid leaflets by synthetic lysolecithins, lecithins and structural analogs. Chem. Phys. Lipids 3 (1969) 221–233

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