Effect of Hot Formamide on Gram-Positive and Gram-Negative Cell Walls

Hot formamide extraction[1] was shown to separate the streptococcal cell wall into soluble carbohydrate and insoluble mucopeptide[2,3]. The mucopeptide residue was found under the electron microscope as fine streptococcus-shaped membranes susceptible to the lytic action of lysozyme.

In our experiments the attempt was made to obtain pure mucopeptide membranes from some Gram-positive and Gram-negative bacteria by hot formamide extraction.

Gram-positive bacteria *B. megaterium* M and *B. subtilis* CCM 1997 were used. 18-h-old cells were treated in the following way: (a) mechanical disintegration with Ballotini No. XIV in a device described by NOVOTNY[4], then extraction of purified cell walls by formamide at 160°C for 20 min or at 205°C for 3 min. (b) Extraction of whole washed cells with formamide at 205°C for 5 min. The initially turbid suspension cleared within 2 min, and after 4 min it became yellow to orange. This material was purified by repeated washing with distilled water, centrifugation for 15 min at 12,000 *g*, and prepared for electron microscopic observation (Figures). Another part was fixed either with 1% osmic acid or with 1% uranyl acetate, or with 2% potassium permanganate for 1 h at 4°C, embedded in Vestopal W and sectioned with the Porter-Blum ultramicrotome. The samples were examined under the electron microscope Tesla BS 242, SEM III (Werk für Fernsehelektronik, Berlin) or the Elmiskop Typ I (Siemens u. Halske)[5].

Results. Both procedures were successful. Very fine rod-shaped membranes were obtained with both the strains used (Figure 1). In most cases very fine fibrils were found at the edges of the membranes. In comparison with this, membranes obtained after the procedure described by PARK and HANCOCK[6] were thicker and not so pure. Formamide membranes were lysed by lysozyme (200 µg/ml) within 8 min, (examined by measurement of

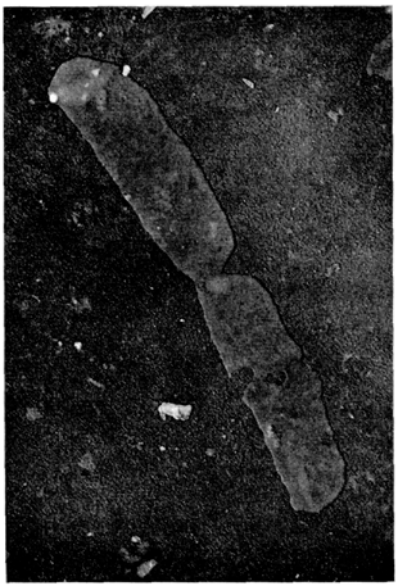


Fig. 1. Fine rod-shaped membranes obtained after treating the cells of *B. subtilis* with formamide at 205°C for 5 min. Shadowed by gold palladium, enlarged × 12,000.

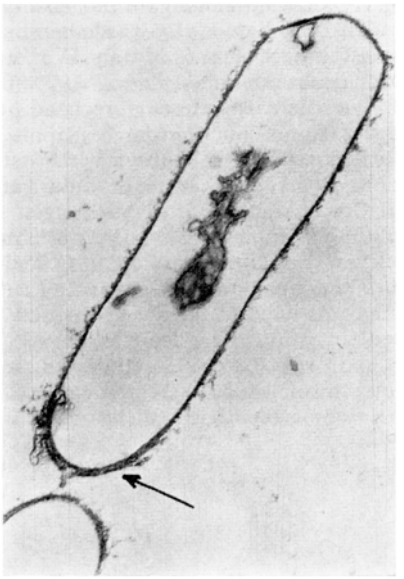


Fig. 2. Ultra-thin section of *B. megaterium* after formamide extraction of whole cells (205°C for 5 min). Contrasted with uranyl acetate. The cell wall is rather thin, only at one pole (arrow) a remnant of the outer layer can be seen, enlarged × 24,000.

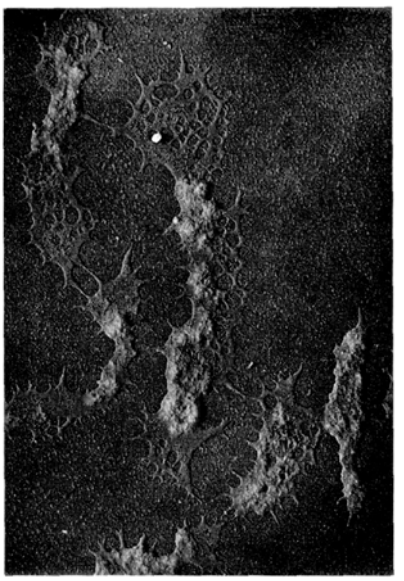


Fig. 3. Cell walls of *Proteus vulgaris* after the action of formamide at 170°C for 20 min. Shadowed by gold-palladium, enlarged × 12,000.

[1] A. T. FULLER, Br. J. exp. Path. *19*, 130 (1938).
[2] R. M. KRAUSE and M. MCCARTY, J. exp. Med. *114*, 127 (1961).
[3] R. M. KRAUSE and M. MCCARTY, J. exp. Med. *115*, 49 (1962).
[4] P. NOVOTNÝ, Nature *202*, 364 (1964).
[5] The author wishes to thank Miss E. FRITSCHE from the Institute of Microbiology and Experimental Therapy in Jena for the painstaking preparation of ultra-thin sections, and to Mr. WACHSMUTH from the same Institute for electron microscopic recordings.
[6] J. T. PARK and R. HANCOCK, J. gen. Microbiol. *22*, 249 (1960).

turbidity and by phase contrast microscopy). The thickness of the cell walls of *B. megaterium* when measured in ultra-thin sections was about 310 Å. After formamide extraction it dropped to 110 Å. At the poles of some membranes, remnants of the surface layer were found, this being evidence for the superficial localization of the removed layer (teichoic acid in the case of *B. megaterium*, Figure 2). Considering some literary data[2,3] and the lysozyme sensitivity of the fine membranes, it may be assumed that they represent the mucopeptide basal layer of *B. megaterium* and of *B. subtilis*, respectively. Detailed results, together with a conception of the ultrastructural arrangement of the Gram-positive cell wall, will be published elsewhere[7].

Gram-negative bacteria, 18-h-old cells of *Proteus vulgaris* P_2 (CCM 1799), were treated with formamide at 170°C for 20 min or longer. In no case were regular rod-shaped membranes observed. On the contrary, irregular, partly solubilized material with remnants of another substance was found (Figure 3). This unsuccessful attempt to isolate the mucopeptide layer could be explained by stronger covalent bonds between the mucopeptide and a substance of the overlying layer (peptides or lipopolysaccharides)[7–10].

Zusammenfassung. Die obere Schicht der Zellwände von *B. megaterium* M und *B. subtilis* wurde mittels Extraktion mit heissem Formamid beseitigt. Zurückbleibende stäbchenförmige, etwa 110 Å dicke Membranen entsprechen offenbar der rigiden Mucopeptid-Basalmembran der Bakterienzellwände.

M. V. NERMUT

Institute of Virology, Czechoslovak Academy of Sciences, Bratislava (Czechoslovakia), April 23, 1965.

[7] M. V. NERMUT, in press.
[8] J. MANDELSTAM, Biochem. J. *84*, 294 (1962).
[9] H. H. MARTIN and H. FRANK, Z. Naturf. *17b*, 190 (1962).
[10] R. PLAPP and O. KANDLER, Archiv Mikrobiol. *50*, 171 (1965).

Metabolism of Phospholipids in Scorbutic Guinea-Pigs

The role played by *l*-ascorbic acid in the metabolism of phospholipids is as yet quite unexplored. There are a number of works concerned with the catalysing effect of vitamin C in phospholipid oxidation (ELLIOTT and LIBET[1], WILLIAMS[2], RADSMA and VAN GRONINGEN[3]); but there is only a very small amount of work on the metabolism of phospholipids in organisms subjected to C-avitaminosis.

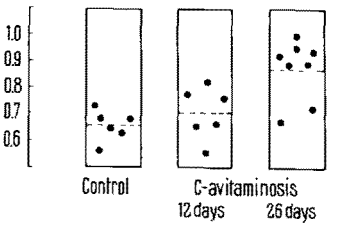
50 male guinea-pigs, weighing 200 g on the average, were divided into 4 groups and fed *ad libitum* on a slightly modified Lunde scorbutogenic diet. The control group obtained daily 5 mg *l*-ascorbic acid perorally. The experimental animals were killed on the 9th, 18th and 25th day respectively of the scorbutogenic regime, and subsequently the concentration of phospholipids was determined according to the method of STEWART and HENDRY[4] in their blood serum and liver. In further experiments, on a group of control animals, guinea-pigs fed for 12 and 26 days on scorbutogenic diet, $Na_2HP^{32}O_4$ was administered intraperitoneally in doses of 0.16 μC for 1 g of their weight; 24 h later the animals were killed, and phospholipids were isolated from their liver according to the method of DAVIDSON et al.[5]. In the fraction obtained, the phosphorus content was determined by means of phosphomolybdenate and also the radioactivity was determined. The results were expressed in the form of relative specific activity given by the relationship of the specific activity of phospholipids to the specific activity of acid-soluble phosphorus.

The changes which occurred in the concentration of phospholipids in the course of C-avitaminosis are shown in the Table. At the beginning a very significant increase can be seen in the blood serum, returning to normal not earlier than in the terminal phase of scurvy. In the liver, a provable decrease takes place until the 9th day of the avitaminosis; up to the 18th day the level increases, and continues to increase until the terminal phases of C-avitaminosis.

On studying the incorporation of P^{32} into liver phospholipids, it was found that this process undergoes no change during a 12-day administration of scorbutogenic diet; on the other hand, under conditions of an already developed C-avitaminosis (in a phase when the animals refuse food) a provable acceleration of the process ($P < 0.01$) takes place (Figure).

Concentration of lipid phosphorus (mg %) in blood serum and liver of guinea-pigs during the development of C-avitaminosis

		Blood serum	Liver
Control		2.8 ± 0.2	134.2 ± 2.8
C-avitaminosis	9	7.0 ± 0.2	109.2 ± 1.7
days	18	5.8 ± 0.4	148.5 ± 8.5
	25	3.5 ± 0.4	204.3 ± 4.6



Relative specific activity of liver phospholipids in normal and scorbutic guinea-pigs.

[1] K. A. ELLIOTT and B. LIBET, J. biol. Chem. *152*, 617 (1944).
[2] J. N. WILLIAMS, Proc. Soc. exp. Biol. Med. *77*, 315 (1951).
[3] W. RADSMA and H. E. M. VAN GRONINGEN, Acta physiol. pharmac. néerl. *8*, 15 (1959).
[4] C. P. STEWART and E. B. HENDRY, Biochem. J. *29*, 1683 (1935).
[5] J. N. DAVIDSON, S. C. FRAZER, and W. C. HUTCHINSON, Biochem. J. *49*, 311 (1951).