THE EFFECT OF LOW TEMPERATURES ON PERMEABILITY IN STREPTOMYCES HYDROGENANS*)

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While studying the active transport of amino acids in the microorganism Streptomyces hydrogenans we observed that the permeability of these cells drastically rises upon cooling down the suspension to about 0°C. This behaviour contrasts with that of many animal cells, in which the passive permeability, in accordance with the laws of diffusion, usually decreases with lowering the temperature. On the other hand it agrees well with the views of MEYNELL (1958) and STRANGE and DARK (1962) who attribute the lethal cold shock observed with some coliform bacteria to an irreversible depletion of cellular constituents, due to an intensively increased permeability.

Kinetic investigations of the above mentioned cold effect suggest a profound alteration of the membrane structure, probably due to a reversible temporary phase transition within the lipid layer associated with a widening of the membrane pores. The findings recall experiments of THOMPSON (1964) on artificial membranes, the electrical conductivity of which drastically changes at the critical temperatures of 30° and 23°C. Since these mem-

*) Part of the results were reported on at the autumn congress of the "Deutsche Gesellschaft für Physiologische Chemie" in Cologne, October 10, 1964.

branes were structured according to the DAVSON-DANIELLI model in which a lipid bilayer determines the permeability, such permeability changes suggest that some lipid components have undergone phase transitions, which might affect the permeability of the membrane.

Methods : Cells of the logarithmic growth phase of Strept.hydrogenans from aerated cultures in modified nutrient broth at 30°C were washed twice in bidistilled water and once in buffer, then suspended and standardized to constant cell density. The buffers (pH 7,1) were 0,05 molar and contained 1% of glucose additionally. For determination of the cellular potassium, tris-HCl-buffer was used, phosphate buffer (SÖRENSEN) for transport experiments with α-aminoisobutyric acid (AIB) or thiourea. In these experiments cells were incubated at 29°C with ^{14}C-AIB (0,85 mM, 130 µC/mM) or ^{14}C-thiourea (12,8 mM, 16,5 µC/mM). The reaction was stopped by rapid filtration of 2 ml of the suspension by means of vacuum through a membrane filter (group 4, "Membranfiltergesellschaft", Göttingen), and washing with the same quantity of buffer. The cooled suspensions were filtered through precooled filters. The radioactivity of filter or filtrate were determined with a GEIGER-MÜLLER counter. The symbol u'_c designates the radioactivity per mg of cells (dried over $CaCl_2$), whereas u'_{rel} is the radioactivity per mg of cells, divided by the radioactivity per ml of suspension (a'_f). Potassium and sodium were determined by flamephotometer.

Results : At 30°C the efflux of the actively accumulated AIB is relatively small (Fig.1). It drastically rises, however, if the temperature is suddenly lowered to 0°C. This effect is hardly due to a metabolic block, since inhibitors like antimycine A, azide or dinitrophenol do not increase the normal efflux appreciably. Nor can it be attributed to an irreversible injury of the cell wall, because immediately after restoring the temperature to 30°C the AIB is reaccumulated at normal rate, reaching the same steady state distribution as the control (Fig.1). Even 2,5 hours treatment at -4°C had no significant effect on the subsequent reaccumulation.

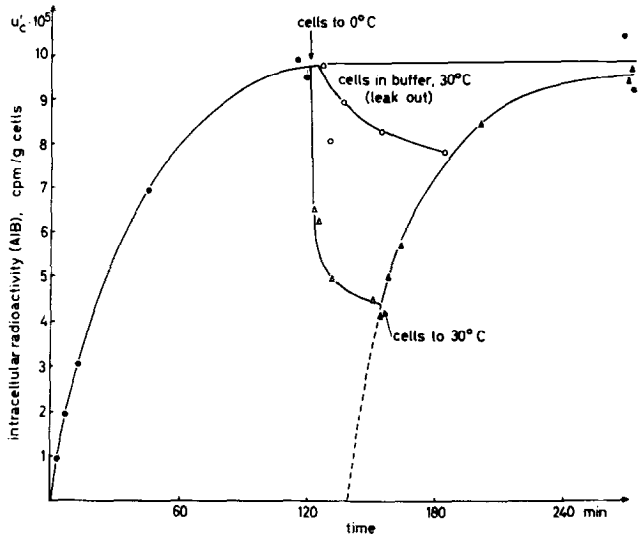

Fig.1: Uptake of ^{14}C-AIB at 30^{0}C and leakage in AIB-free buffer. Efflux at 0^{0}C in AIB containing medium and reaccumulation after reheating up to 30^{0}C.

As shown on Fig.2 the extent of the permeability change observed after 60 minutes of cooling seems to be an S-shaped function of the cooling temperature. The temperature of the turning point is remarkably uniform in different experiments.

The alteration of the permeability of the membrane resulting from the above temperature effect suggests a considerable widening of the membrane pores. The question, whether the temperature shock affects all cells of the suspension equally, or whether only part of the cells are, owing to a particularly labile membrane structure, cold-sensitive, cannot be answered yet. The last mentioned interpretation, however, seems less likely in view of the strictly controlled conditions of growth.

If the observed increased permeability is due to a widening of the membrane pores, it should affect also the entrance of other substances into these cells (STRANGE and POSTGATE, 1964). This

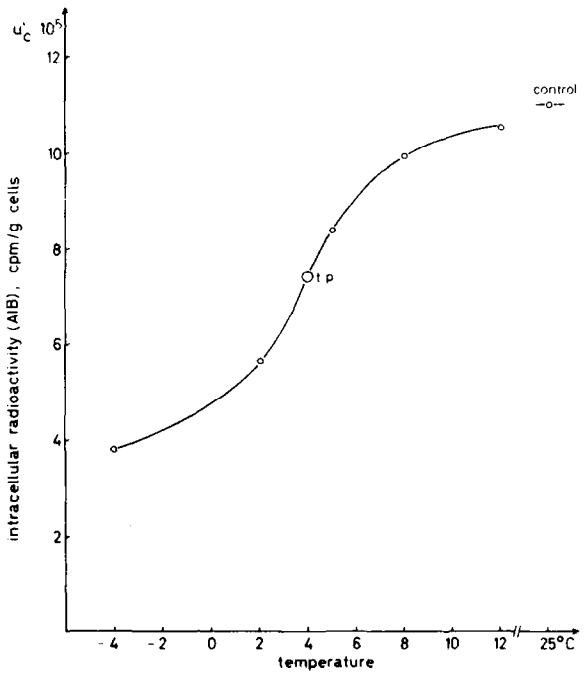

Fig.2: Efflux of AIB as a function of the cooling temperature. The cells have been preloaded with ^{14}C-AIB at 30°. After cooling to various temperatures for 60 minutes the cellular radioactivity was determined.

could, indeed, be shown with thiourea, which at 30°C enters the cells very slowly, but does so much more rapidly at 0°C (Fig.3). The two stage kinetics is very similar to that of the AIB efflux. There is some indirect evidence that also ATP, to which the cell is normally impermeable, may get into these at low temperature. As will be reported elsewhere, the onset of active transport by these cells is normally associated with an increase of O_2 uptake by 13 - 15%. No such increase, however, occurs, if the cells are preincubated for one hour at 0°C with 5×10^{-3} M/l ATP prior to the active transport test. Anticipating that ATP donates the energy for these transport processes, one may follow from the last mentioned observation that a considerable quantity of ATP has penetrated the cellular membrane.

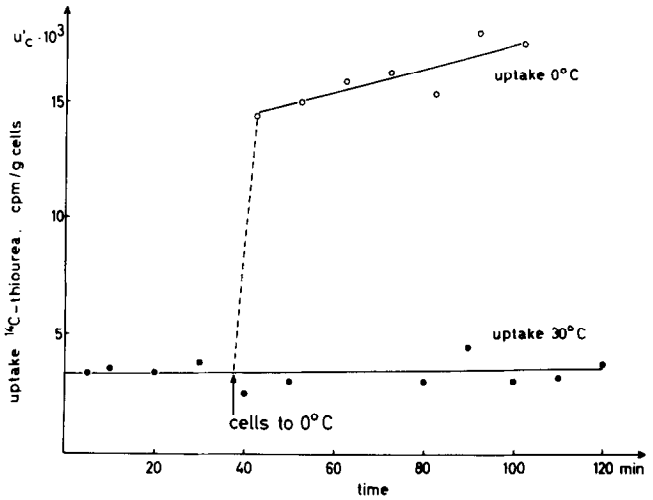

Fig.3: Influx of ^{14}C-thiourea at 30°C. After 38 minutes part of the solution was cooled to 0°C and further incubated at this temperature.

Discussion : The increase in permeability in Streptomyces hydrogenans upon lowering the temperature has been tentatively explained in terms of a reversible alteration of the membrane structure. This alteration seems to reflect a phase transition, i.e. a "precipitation" of some of the membrane lipids. Such an interpretation is supported by the similarity between the function of the permeability change versus the temperature and a melting diagram. It gets further support by the observation with cells grown at low temperature (RING, 1965). In such cells the temperature critical for the permeability change is shifted towards lower values whereas the opposite is true with cells grown at higher temperatures. It is known that adaptation of bacterial cells to lower temperatures shifts the composition of fatty acids in favour to the unsaturated acids which will lower the melting point of the lipids (INGRAHAM, 1962). We therefore believe that in Streptomyces hydrogenans the critical temperature, at which

the permeability increases, depends on the composition of the lipids with respect to saturated and unsaturated fatty acids. During adaptation to the cold this composition seems to be adjusted towards lowering the melting point of the lipids, thus making the cell more resistant to cold. A decrease of the temperature to a critical zone would then cause a phase transition of the membrane lipids in the sense of a "crystallization", which, consequently, should lead to alterations of the physical properties of the membranes.

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References :

Ingrahm,J.L., in: Recent Progress in Microbiology, Congress Montreal 1962, ed.N.E.Gibbons (University of Toronto Press, 1963) p. 201

Meynell,G.G., J.Gen.Microbiol., 19, 380 (1958)

Ring,K., Biochim.Biophys.Acta, 94, 597 (1965)

Strange,R.E. and F.A.Dark, J.Gen.Microbiol., 29, 719 (1962)

Strange,R.E. and J.R.Postgate, J.Gen.Microbiol., 36, 393 (1964)

Thompson,Th.E., in: Cellular Membranes in Development, ed. M.Locke (New York, Academic Press, 1964) p. 83