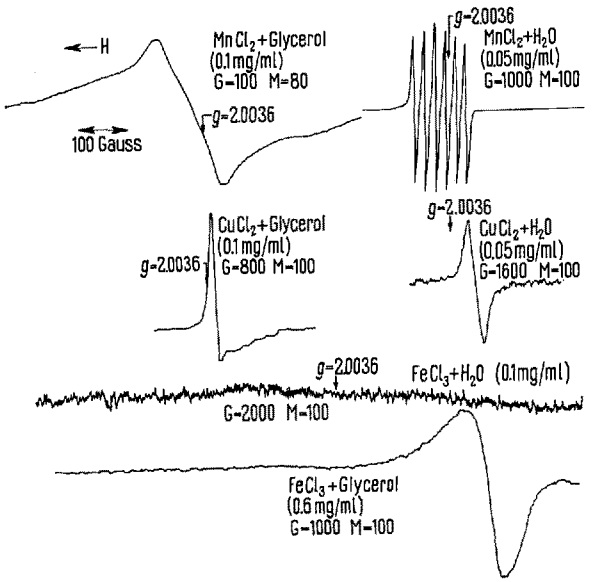
Some Remarks about the Effect of Glycerol on Cells During Freezing and Thawing: Electron-Spin Resonance Investigations Concerning this Effect

Recently, SHERMAN[1] has questioned the importance of intracellular glycerol and has described the influence of intracellular glycerol as detrimental to the maximum survival for certain cells. From this, it was concluded that the site of glycerol action is extracellular rather than intracellular. Other mechanisms proposed previously for glycerol protection of cells during freezing and thawing are: the protection of cells by dehydration, the prevention of mechanical damage to the cells by ice formation[2], and the proposal by LOVELOCK[3] that glycerol acts as a salt buffer. However, none of these proposed mechanisms have been universally accepted. An alternative mechanism for glycerol protection is offered for discussion; this mechanism, however, need not be an exclusive one.

Recently we have shown[4] that glycerol protects catalase against radiation by forming a complex with metal ions present in the enzyme. In these studies it was found that maximum protection could be achieved using relatively small glycerol concentrations, while higher concentrations resulted in a decreased protective effect. It seems likely that a splitting of the coordinative bindings between the metal ion and the remaining macromolecule by glycerol is the reason for the decrease in the protective effect.

These ideas might be applicable to the experiments concerning the freezing and thawing of cells. The bonded metal ions present in the cell are both important and necessary for the biological activity of many of the cellular components, and it is assumed that these ions must be protected against the damaging effect of freezing. The amount of glycerol required for protection by chelation may depend on the biological object as well as the type of metal ion and its coordinative bonding. SHERMAN[1], using mouse eggs, has found that small amounts of glycerol are sufficient to provide maximal protection. Consequently, a high concentration of intracellular glycerol, as in the case of catalase[4] and T1 phages[5], would reduce the maximal survival.



Electron-spin resonance signals of copper, manganese, and iron in water and glycerol, respectively. The curves represent the second derivative of the actual resonance curves.

An influence of copper on the glycerol efficiency has been observed by LOVELOCK[3] and JACOBS[6]. They pointed out that small amounts of copper (cells were washed in solutions containing $10^{-5}\,M$ $CuSO_4$) alter profoundly the glycerol effect; almost no glycerol was observed to permeate the cells. They described the effect as a change in the permeability of the cells by the copper ions. We, however, interpret the effect as the formation of a copper-glycerol complex. The existence of such a complex can be proven by means of electron spin resonance (ESR) signals. We determined the ESR spectra of copper, manganese, and iron in water and glycerol, respectively, using a Varian V 4500 100 kc ESR spectrometer. All observations were made at room temperature at a frequency of 9500 Mc/sec. The ESR signals obtained for the metal ions in the different solutions are shown in the Figure. The arrows indicate the position of the standard DPPH reference signal for which $g = 2.0036$. The normal well-known Mn spectrum (6 lines) is completely changed by glycerol, consisting now of a single broad signal. It should be pointed out that glycerol alone is not paramagnetic. In the case of copper and iron a change in shape and size of the signal is also recognizable. From this, it can be definitely concluded that glycerol forms a complex with the metal ions investigated.

As a result of this complex formation it seems very unlikely that glycerol can penetrate the cell membrane. This complex might occlude the cellular membrane and produce, as a secondary effect, a change in the permeability of the cell. Cells, requiring large amounts of glycerol for protection against freezing, could not be protected if treated with Cu^{++} ions prior to glycerolation. The absence of this protective effect was probably due to an insufficient glycerol concentration available for chelation of the biologically important metal ions in the cell.

Summarizing, we can say it is highly possible that the damaging effect of freezing is exerted on the metal ions and might be avoided by chelating these metal ions with glycerol. The degree of the chelating effect in regard to biological activity depends on the concentration of the chelating substance.

Zusammenfassung. Der Glyzerin-Schutz der Zellen gegen Schäden beim Gefrieren und Auftauen wird mittels einer Komplexbildung zwischen Glyzerin und Metallionen erklärt. Elektron-Spin-Resonanz-Untersuchungen bestätigen die Bildung eines solchen Komplexes.

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