

Lovelock J E. Het [*sic*] mechanism of the protective action of glycerol against haemolysis by freezing and thawing. *Biochim. Biophys. Acta* 11:28-36, 1953. [National Institute for Medical Research, Mill Hill, London, England]

The protective action of glycerol against freezing damage to living cells is explained and shown by experiment to be a consequence of its capacity to buffer against harmful increases of salt concentration when saline solutions are frozen. Practical suggestions are made for improving the technique of cryopreservation. [The SCI® indicates that this paper has been cited in over 320 publications since 1955.]

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I am astonished by the interest shown in this paper. It is true that it was one of two papers that established the principal cause of freezing damage to living cells and overturned the previously held belief that cell death was caused by rupture or mechanical damage by ice crystals.¹ The fact that my proofreading was so bad that I missed the incorrect spelling of the first word of the title, a three letter word at that, should have ensured its oblivion.

In 1950,² my colleagues at the National Institute for Medical Research in London discovered that spermatozoa could be frozen in glycerol without loss of motility. At that time, I was working ineffectually on the intractable problem of the common cold. I was delighted to be moved to the Department of Experimental Biology and directed to find out what happened when living cells were frozen and how glycerol prevented the damage.

It was the tradition at our institute never to read the literature before beginning a re-

search project and this no doubt saved me from the discouragement of discovering that everything was already known about damage to living cells by freezing. Secure in this innocence, the first thought to come was that as freezing takes place, ice separates as a pure substance and any solutes will be concentrated in the liquor between the crystals. In other words, freezing pickled cells in brine is a bad thing to happen to them. That this was a fact was soon demonstrated experimentally and reported.³ It was not difficult then to show that any neutral solute, like glycerol, would act as a salt buffer and prevent the salinity rising to harmful levels during freezing.

Theories are best judged by the accuracy of their predictions and it was pleasing later to be able to predict that the neutral solute dimethyl sulfoxide would be as good or better a protective agent than glycerol. Strangely, the paper reporting this prediction and its experimental confirmation, although published in *Nature*,⁴ seems to have attracted less interest. The National Institute for Medical Research under the direction of Sir Charles Harington was a delightful and productive workplace, and I was soon collaborating with my biologist colleagues in the more challenging problem of freezing and reanimating whole animals. We succeeded with hamsters,⁵ but no one has yet preserved a larger animal and the method is almost certainly unavailable for humans.

Subsequent research has uncovered other mechanisms of damage during freezing, and cryobiology has developed powerful practical techniques for preserving cells and tissue in the frozen but still viable state.

I spent only five years in freezing experiments and in 1957 moved on through instrument science to atmospheric research and geophysiology, my present interest. Memories are short and sometimes I am asked if I am a relative of that early cryobiologist.

For a recent review, see reference 6.

1. Smith A U. Prevention of haemolysis during freezing and thawing of red blood-cells. *Lancet* 2:910-11, 1950. (Cited 110 times since 1955.)
2. Polge C, Smith A U & Parkes A S. Revival of spermatozoa after vitrification and dehydration at low temperatures. *Nature* 164:666, 1949. (Cited 380 times since 1955.)
3. Lovelock J E. The haemolysis of human red blood-cells by freezing and thawing. *Biochim. Biophys. Acta* 10:414-26, 1953. (Cited 295 times since 1955.)
4. Lovelock J E & Bishop M W H. Prevention of freezing damage to living cells by dimethyl sulphoxide. *Nature* 183:1394-5, 1959. (Cited 290 times.)
5. Smith A U, Lovelock J E & Parkes A U. Resuscitation of hamsters after supercooling or partial crystallization at body temperatures below 0°C. *Nature* 173:1136, 1954. (Cited 40 times since 1955.)
6. Mazar P. Freezing of living cells. Mechanisms and implications. *Amer. J. Physiol.* 247:C125-C142, 1984.