

irradiation on the synthesis of bacteriochlorophyll. Fifty-ml. Esbach dishes covered with a glass plate were used as culture chambers. The other experimental conditions are described elsewhere<sup>7</sup>.

If dark-grown cultures are transferred to conditions of anaerobic light-growth, synthesis of chlorophyll starts only after irradiation with far-red or blue or green (Fig. 1). Under the experimental conditions we have observed a relation between light-intensity and the amount of bacteriochlorophyll synthesized in 48 h (Fig. 2). These two facts suggest that light controls the synthesis of bacteriochlorophyll through photosynthesis. In contrast to the observations of Wolken<sup>8</sup> concerning the synthesis of chlorophyll in *Euglena*, light which is absorbed by protobacteriochlorophyll is not utilized for synthesis of bacteriochlorophyll. Further, we tested the effect of pre-illumination with low light intensities of different wave-lengths; but we could not detect any influence of other pigment systems different from bacteriochlorophyll and carotenoids.

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### Similar Effects of Various Neutral Solutes on the Survival of *Aerobacter aerogenes* and of Red Blood Cells after Freezing and Thawing

*Aerobacter aerogenes* from continuous culture is killed by slow freezing in buffer or by freeze-drying. Rapid freezing in liquid nitrogen results in 50 per cent survival if the organisms are suspended in distilled water, and almost complete survival if they are suspended in 10 per cent glycerol. Ten per cent solutions of some other polyhydroxy-compounds, sugars or of polyethylene glycol protect equally well<sup>1</sup>. The work recorded here extends the investigation to other, mainly non-hydroxylic, compounds. Like the sugars, these had already been tested on human red blood cells subjected to relatively slow freezing (various workers, references below) and it seemed possible that the response of *A. aerogenes* would not be very different from that of red cells.

Table 1. EFFECT OF AQUEOUS SOLUTIONS ON FREEZING DAMAGE TO *Aerobacter aerogenes* FROM CONTINUOUS CULTURE COMPARED WITH THEIR EFFECT ON FREEZING DAMAGE TO RED BLOOD CELLS

Solute	Conc. (%)	Survival (%)	Protection	Protection (red cells)
Dimethyl sulphoxide	10	97	Complete	Complete (ref. 5)
Dimethyl acetamide	10	95, 96.5	Complete	Complete (ref. 6)
Dimethyl formamide	10	93, 92.5	Good	Complete (ref. 6)
N-Methyl-2-pyrrolidone	2.5	92, 87	Good	Good (ref. 7)
Acetamide	10	93, 90	Good	Good (ref. 8)
Formamide	10	80, 80	Fair	Slight (ref. 8)
2-Pyrrolidone	2.5	51, 52	None	None (ref. 6)
Tetrahydrofuran	4	47, 40	None	None (ref. 6)
Acetone	10	36, 37	None	None (ref. 6)
Pyrrolidone N-oxide	2.5	44, 26	None	Partial (ref. 9)
Urea	10	2, 1.3	None (aug.)	None (ref. 6)
Butyrolactone	2	1, 0.5	None (aug.)	None (ref. 6)
Acetonitrile	10	0, 2	None (aug.)	None (ref. 6)
Polyethylene glycol	5	94	Good	Good (ref. 10)
Polyvinyl pyrrolidone	10	89, 96	Good	Good (ref. 11)
Human albumin	10	98, 97, 96	Complete	Good (ref. 12)
Ovalbumin	10	97, 96, 95	Complete	Not tested

(aug.), augmentative.

The experimental procedure for freezing in liquid nitrogen and measuring viability has been described<sup>1</sup>. Apart from the higher rate of freezing, the main difference between the present system and that normally used in experiments on red cells is that distilled water and not saline had to be used, because *A. aerogenes* is very sensitive to salt on freezing. The solutes were at 10 per cent strength (v/v for liquids, w/v for solids) except when this concentration was found to be toxic at room temperature; in such cases the highest tolerated concentration was used.

The results are shown in Table 1; each test includes controls using distilled water which gave viabilities very near to 50 per cent, except for acetone when the controls were a little low. The unfrozen populations were uniformly 95–99 per cent viable. The effect of each solute on the haemolysis of red cells by freezing and thawing is also given, with a reference; the concentrations used are assumed to be optimal, generally between 10 and 20 per cent for the good protectors. *A. aerogenes* was protected from freezing damage by very much the same compounds as those which protect red cells despite differences in the technique used to determine response of these systems. Another way in which *A. aerogenes* resembles red cells is its sensitivity to cold shock<sup>2</sup>, which is, however, observed only with bacterial cells in the logarithmic phase of growth and not with those from continuous culture; with red cells special conditions are also required<sup>3</sup>.

It is of particular interest that *A. aerogenes* is protected by the same non-penetrating polymeric solutes which protect red cells, and these results may help in resolving the present controversy regarding the role of intracellular salts in the lethal effects of low temperatures. It should also be noted that the best low-molecular weight protectors are, under other conditions, particularly lethal to gram-positive bacteria such as micrococci, and it is believed that this is connected with the strong solvent action that such compounds can exert on the cell wall, when the solution is sufficiently concentrated<sup>4</sup>.

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## CYTOLOGY

### Multinuclearity of Osteoclasts

THE origin and function of the multinuclear osteoclast cell are of continuing interest among contemporary investigators, and have a long history among investigations on amphibian regeneration<sup>1,2</sup>.

The reported origin of this cell is varied<sup>3</sup>, with reticulocytes, osteoblasts, and osteocytes receiving complete or partial support from Bloom *et al.*<sup>4</sup> and Tonna<sup>5</sup>, whereas a haematogenic origin from mononuclear leucocytes is recommended by Fishman and Hay<sup>1</sup> and others.

The phagocytic activity of osteoclasts<sup>3,6</sup> lends itself well to this cell, being considered with other macrophages as a