Preliminary Communications

Storage of Red Cells for Blood-grouping after Freezing in Liquid Nitrogen

Use of Sucrose to Protect Against Haemolysis

An increasing number of laboratories find it necessary to keep a panel of erythrocytes of known antigenic structure for blood-grouping work. Long-term storage of such cells has been made possible by freezing after the addition of glycerol (Chaplin and Mollison, 1953). However, care must be taken in the gradual adding and withdrawal of the glycerol, as otherwise considerable haemolysis may result. In particular, recovery of the cells from the glycerol is a rather tedious procedure.

An alternative is very rapid freezing in liquid nitrogen, storage at a suitably low temperature, and equally rapid thawing. Valuable work has been done by Meryman and Kafig (1955a, 1955b) along these lines, concerned with preserving blood for transfusion purposes. The present note describes a simple method suitable for keeping the small quantities needed for serological work.

PROCEDURE

Preparation.—Blood is anticoagulated with E.D.T.A. in the proportion of 1 mg. per ml. Shortly before freezing, one volume of 40% (w/v) sucrose solution is added to two volumes of blood. A very fine-pointed Pasteur pipette is then filled with the blood–sucrose mixture.

Freezing.—Not more than four or five test-tubes of about 15-ml. capacity are filled one-third full of liquid nitrogen. (This substance is quite easily handled, being poured from the storage vessel into the tubes with the aid of a small funnel. It will boil vigorously for a few seconds and then more quietly.) Blood is dropped slowly into the tubes from the pipette, each drop freezing apparently instantaneously as it enters the nitrogen. When a sufficient quantity (say 20 drops) has been added in each tube, the frozen blood and the small quantity of nitrogen now remaining is decanted into small storage tubes. (The glass tubes can safely be held at their upper ends with bare hands.) The storage tubes, preferably already labelled, are tightly plugged with cotton-wool and placed in the storage cabinet at once, the remaining nitrogen boiling off at storage temperature.

Thawing.—The selected tubes are removed from the storage cabinet and, without delay, the cotton-wool plugs are removed and the frozen blood is tipped into about 5 ml. of saline at 45° C. The tube containing the saline is at once thumb-stoppered and inverted several times, the blood thawing within one or two seconds. The cells may then be centrifuged and resuspended in saline or test serum as required for use.

PRE-TREATMENT OF BLOOD

Various anticoagulants were tried and E.D.T.A. was found to give the best recovery of intact cells (20–30% haemolysis when thawed immediately after freezing). Acid citrate–dextrose showed an improvement on this figure, with 10–20% haemolysis, but this is due to the presence of dextrose. Meryman and Kafig (1956a), using a rather complex device to decrease droplet size, have claimed losses of only 2–3% after the addition of 7% dextrose to the blood. By the present method, the best result, using dextrose, was about 10% haemolysis when one volume of 40% (w/v) dextrose was added to four of E.D.T.A. blood (giving a final concentration of 8% dextrose). Adding to two volumes of blood one of 40% (w/v) sucrose proved a considerable improvement, giving a loss of 4–6% when used as described above. The sugar concentration has a definite optimal level, recovery falling off quite sharply if the concentration be changed either way.

STORAGE

The ordinary laboratory deep-freeze at −10 to −20° C. is not cold enough for this purpose. We have, however, stored the frozen blood in CO_2 snow for a few weeks. The amount of haemolysis in the thawed blood appeared to increase from the original 5% to about 10% after the first day or two of storage, but then remained at this level for the rest of the short test period. It is possible that the CO_2 vapour may be the cause of this immediate deterioration.

CO_2 snow is obviously impracticable, as it needs such frequent replenishment and, regrettably, refrigerators working at a similar temperature are at present very expensive. Further work is necessary to ascertain the optimum (or maximum effective) temperature which will preserve the cells; it may well be that −40° C. is sufficient, and refrigerators working to this temperature are much less costly.

A great saving in storage space and time taken in freezing can be effected by freezing the blood in larger quantities (several hundred drops) in 25-ml. universal containers, replenishing the nitrogen from time to time. When thawed blood is required, a bunch of frozen droplets is picked out of the container with a pair of long forceps (kept at storage temperature) and dropped into warm saline as before.

GROUPING PROPERTIES

Cells representing A, B, C, D, E, $\bar{c}$, M, N, K, and Fy^a groups showed unimpaired reactions after freezing and thawing by the above method.

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Members of the Association of British Pharmaceutical Industry, in response to an appeal put out by the "Save the Children Fund," have donated essential supplies for victims of the recent earthquake in Agadir. Among the medical supplies flown out were antibiotics, sulphonamides, various types of iron and vitamin tablets, and numerous other pharmaceutical products, including sera.