carcinomas provides the opportunity for the selection of a dominant cell type, which characterizes the invasive growth.

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Medical Memoranda

Blood Groups and Enzymes of Human Red Cells After Two Years' Storage in Liquid Nitrogen

The possible advantages of liquid nitrogen for preserving red cells for grouping purposes were reported in a previous paper (Huntsman, Hurn, Ikin, Lehmann, and Liddell, 1962). The present communication describes the results found after the storage period had been extended from one to two years. The methods employed were identical to those previously reported.

RESULTS

1. *Recovery of Intact Cells.*—The average recovery in eight samples was 92.8% (range 90 to 96%), compared with the average recovery after one year of 94.1% (range 93 to 96%). It was noted with all specimens that haemolysis in the thawed cell suspensions continued during the next twelve hours while the specimens were held at 4° C. This feature was more pronounced with the two-year specimens.

2. *Grouping Properties.*—Preliminary "run-through of all groups" of the same seven samples showed no deterioration when thawed after two years' storage. Titrations of all the antigens tested previously showed no significant alterations.

3. *Enzyme Estimations.*—The glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase activities were well maintained, and there was in fact some increase of activity, as was also seen after one year. This could be due to the release of an enzyme activator in the stroma. The deterioration in the glutamic-oxaloacetic transaminase and the aldolase continued, dropping to 80% and 50% of the original levels respectively (see Chart). This is the typical enzyme pattern seen on storage at 4° C., as distinct from *in vivo* ageing of the red cells (Rapoport, 1961). Its progress during the present experiment confirms our observation after the first year of storage, that complete

stability of biological systems has not been obtained even at the very low temperature of -196° C.

COMMENT

The recovery of red cells stored for two years in liquid nitrogen averages 92%. The grouping properties of these cells are satisfactory, but the thawed cell suspensions show an increased tendency to progressive haemolysis. If this lysis after thawing increases with more prolonged storage, this may prove to be the factor which ultimately limits the effective storage life. The red-cell enzymes showed no fall in original activity of two dehydrogenases, but there was further deterioration in the second year for glutamic-oxaloacetic transaminase and for aldolase.

Enzyme activity of red cells during two years' storage in liquid nitrogen. Results expressed as a percentage of the level before freezing. (1) Aldolase. (2) Glutamic-oxaloacetic transaminase. (3) Glucose-6-phosphate dehydrogenase. (4) 6-Phosphogluconate dehydrogenase.

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