Liquid nitrogen storage of haemoglobin variants

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The preservation of abnormal haemoglobin solutions for reference purposes is difficult. The majority when stored for a few weeks at −20°C. shows on subsequent electrophoresis a variable degree of blurring of the previously distinct bands. This deterioration is progressive. The most unstable of them is haemoglobin H which rapidly denatures on freezing. When a solution of haemoglobin H is thawed immediately after freezing a precipitate of denatured protein can be seen in the solution.

We have recently been investigating the storage of human red cells in liquid nitrogen for serological purposes (Huntsman, Hurn, Ikin, Lehmann, and Liddell, 1962). We thought that this technique might be useful for the preservation of haemoglobin as well as for that of the red cell agglutinins. We have therefore stored in liquid nitrogen, cells and haemoglobin solutions containing haemoglobins A+S, A+H (+ trace of Bart's), A+$G_{\alpha Norfolk}$, A+$D_{\beta Punjab}$ as well as normal samples containing only haemoglobin A and haemoglobin A_2. All these samples were stored for six months, both in a liquid nitrogen refrigerator at −196°C. and in the conventional deep freeze cabinet at −20°C. The cells were frozen both as packed red cells and as whole blood containing 13% sucrose.

The haemoglobin A+H solution which had been stored at −196°C. showed no precipitation while the subsequent electrophoresis (shown in the figures) was satisfactory. The red cells containing haemoglobin H after similar storage showed no inclusion bodies after

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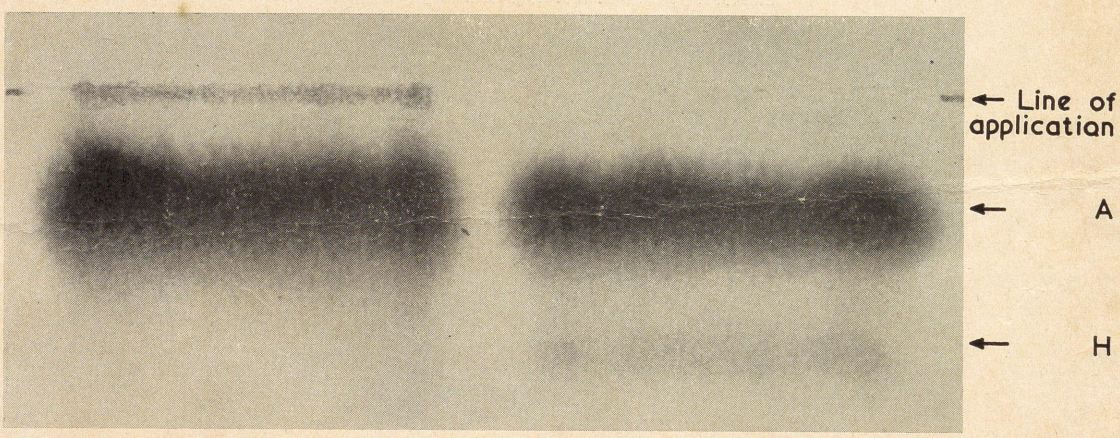

FIG. 1. *Tris buffer electrophoresis (paper, pH 8·9) of two specimens of haemoglobin A+H (+ trace of Bart's).* LEFT *Haemoglobin solution stored for one month in refrigerator. Almost no H is present and denatured haemoglobin is precipitated at the line of application.* RIGHT *Haemoglobin solution stored for one month in liquid nitrogen. There is no denatured haemoglobin at the line of application and haemoglobin H is preserved.*

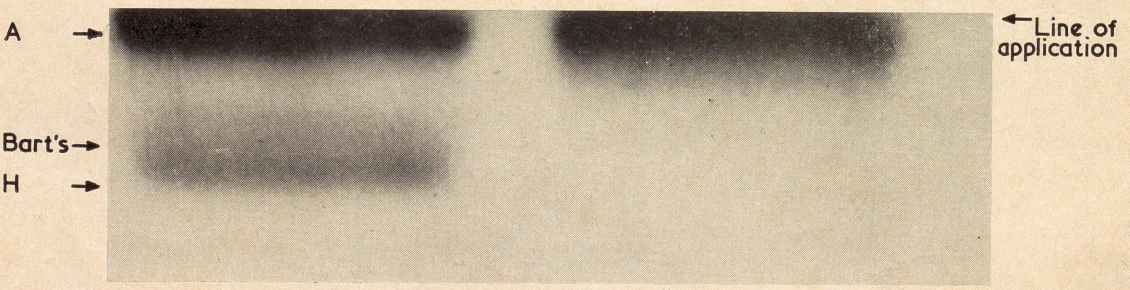

FIG. 2. *Starch gel electrophoresis (pH 7·7) of the same specimens as shown in Figure* 1. LEFT *Stored in liquid nitrogen; the small amount of haemoglobin Bart's is seen moving behind the haemoglobin H band.* RIGHT *Stored in the refrigerator.*

vital staining. These inclusion bodies which arise from the denaturation of haemoglobin H, however, developed after two hours' incubation with cresyl blue as would normally occur with fresh cells. Red cells from a carrier of the sickle-cell trait retained the sickling phenomenon after freezing in liquid nitrogen and subsequent thawing. The stored solutions of haemoglobins A+G, A+D, and A+G respectively had identical electrophoretic properties to fresh solutions. Electrophoresis was also performed on haemoglobin solutions prepared from normal red cells which had been stored at −196°C. for two years. The results were indistinguishable from those obtained with fresh solutions, and in particular the small A_2 component was as clearly visible as with freshly prepared haemolysates. This technique appears of value for the storage of human haemoglobins for references purposes.

REFERENCE

Huntsman, R. G., Hurn, B. A. L., Ikin, E. W., Lehmann, H., and Liddell, J. (1962). *Brit. med. J.*, **2**, 1508.