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USE OF FORMALINIZED HUMAN CORD BLOOD ERYTHROCYTES IN THE HAEMAGGLUTINATION INHIBITION TEST FOR RUBELLA

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Summary. — The use of formalinized human cord blood erythrocytes in the haemagglutination inhibition test for rubella is proposed. These cells retain their sensitivity to rubella virus haemagglutinin for several months and give serum titres comparable with those obtained with fresh cord blood erythrocytes.

Key words: *rubella virus*; *haemagglutination inhibition test*; *human cord blood erythrocytes*

Introduction

Since first introduced by Stewart *et al.* in 1967, the haemagglutination inhibition (HI) test has been successfully employed in the diagnosis of rubella and immunological screening. The technique has greatly enhanced with respect to the methods used to remove nonspecific inhibitors (Feldman, 1968; Liebhaber, 1970*b*; Haukenes and Aasen, 1971), the diluent employed (Liebhaber, 1970*a*; Campbell *et al.*, 1975) and the type of red cells required (Negro Ponzi *et al.*, 1978).

Trypsinized human 0 Rh — red cells (Quirin *et al.*, 1972) and cord red cells (Negro Ponzi and Pugliese, 1977; Negro Ponzi *et al.*, 1978) belonging to the same group have proved particularly suitable. Fresh cells, however, can only be kept for short periods. In some cases, too, the titre decreases with time, so that the agglutinin must be titrated before the antibody assay (Negro Ponzi *et al.*, 1978). The fact that human cord blood erythrocytes are not always easy to obtain is another point that must be taken into consideration. Attention has therefore been directed to their treatment with formaldehyde for longer preservation since this method has been successfully used for one day-old chick (Ito and Iwasa, 1976) and sheep red cells used in HI tests for rubella (Gupta and Harley, 1970).

Materials and Methods

Reagents. HEPES (N-2-hydroxyethylpiperazine-N'2-ethanesulphonic acid), bovine serum albumin (in powder form, Cohn V fraction) and heparin (in powder form, sodium salt, extracted from pig intestine mucosa, activity 163 U/mg.) were obtained from the Sigma Chemical Co., St. Louis, Missouri.

Red cells. 0 Rh — cord blood erythrocytes supplied by Avis, Turin, were washed by centrifugation in Alsever solution at 2000 rev/min for 10 min and then twice in DGV (dextrose-gelatine--veronal) buffer (pH 7.2) prepared according to Stewart *et al.* (1967), prior to suspension in the same buffer at 10%.

Red cell formalinization. The technique of Butler (1963) was slightly modified. Equal volumes of red cells resuspended in DGV buffer at a concentration of 8% and the same buffer containing 3% formaldehyde (Merck) were mixed and lightly shaken for 18 hr at 37 °C. The cells were then washed twice in GDV buffer and four times in distilled water by centrifugation at 2000 rev/min for 10 min. Lastly, they were resuspended at a concentration of 10% in DGV buffer supplemented with 1 : 10000 sodium azide and stored at 4 °C.

HA and HI with formalinized cord blood red cells. Freeze-dried rubella agglutinin was obtained from Behringwerke (Marburg-Lahn). Both the HA and HI were carried out by a micromethod in plates with 96 V-bottomed wells as described (Negro Ponzi *et al.*, 1975). In both reactions HSAG buffer (pH 6.5) was employed according to Liebhaber (1970*a*, *b*).

Sera obtained from male and female adults were kept either at 4 °C for not more than 8 days or divided into 0.5 ml portions and frozen at −30 °C. Prior to titration, they were adsorbed with formalinized red cells to rule out the possibility of nonspecific agglutination. To 0.05 ml of serum was added an equal volume of a 1 : 1 mixture of 5000 U/ml heparin and 1 M $MnCl_2$ and 0.05 ml of HSAG. The mixture was kept at room temperature for 30 min and shaken occasionally. Next, each portion was mixed with 0.1 ml of a 50% suspension of formalinized cord blood red cells. After 2 hr adsorption at 4 °C and several resuspensions, the supernatant obtained after centrifugation at 2000 rev/min for 10 min was used for the titrations.

Results and Discussion

Treatment with formaldehyde was used as a means of preserving cord blood red cells for long periods. Treatment at 37 °C for 18 hr in the presence of a final formaldehyde concentration of 1.5% gave the best results: no sings of haemolysis after repeated washings in distilled water and a cell loss of not more than 10%. The incubation temperature was an equally important parameter. The results were, in fact, disappointing when the same formaldehyde concentrations were used at room temperature for 24 hr and at 4 °C for a week.

The rubella agglutinin titres obtained in HSAG buffer with 0.3% fresh cord blood red cells and those treated with 1.5% formaldehyde for 18 hr at 37 °C were as follows: 1024 and 1024 with fresh cells kept for 0 and 15 days, respectively as compared with 1024, 1024, 768, 768, 768 and 768 with formalinized cells kept for 0, 15, 30, 45, 60 and 90 days, respectively. Normal cells began to show signs of haemolysis after 15 days.

In a subsequent set of tests we determined the best concentration of formalinized red cells to be used in HA reactions, i e. that at which the differences between agglutination and its absence were most evident. The HA titres obtained with red cell concentrations of 0.2, 0.3, 0.4, 0.5, 0.6, 0.8 and 1% were 1024, 768, 512, 384, 256, 192 and 128, respectively. The lowest concentrations were associated with the highest sensitivity but the clearest results were obtained with concentrations of 0.4 and 0.5%. Formalinized cells precipitate less readily than fresh cells and at low concentrations the distinction between agglutination and its absence is less marked (van Weemen and Kacaki, 1976).

Lastly, we caried out HI tests on 100 adult sera with both fresh and formalinized red cells, stored for over 3 months. Normal cells were used at a concentration of 0.3% in the light of an earlier finding (Negro Ponzi *et al.*, 1975)

that this value is particularly suitable, while formalinized cells were used at 0.5% to ensure clear readings; this value has also been recommended for formalinized sheep red cells by van Weemen and Kacaki (1976). Fig. 1 shows that formalinized red cells gave much the same results as fresh cells.

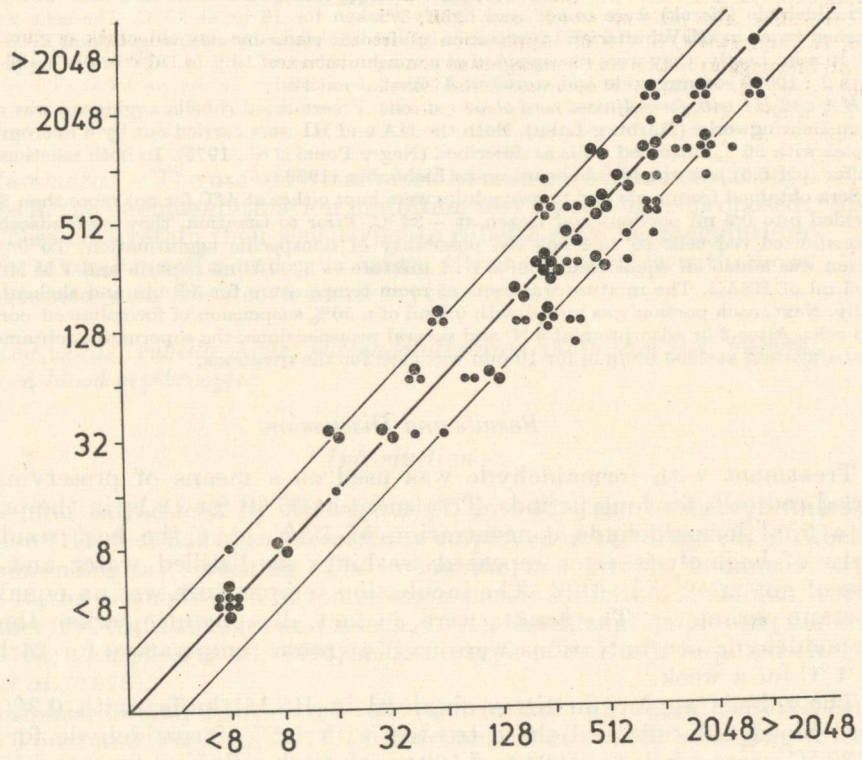

Fig. 1.
Comparison of HI titres of sera determined with normal (ordinate) and formalinized (abscissa) cord blood red cells

Our findings indicate that formalinized cord blood cells may be employed in HA and HI for rubella, retaining high sensitivity to agglutinin for a long time. Chick and sheep red cells preserved in this way have been successfully employed in the HI test for rubella: their sensitivity can even be maintained for over one year (Gupta and Harley, 1970; van Weemen and Kacaki, 1976; Ito and Iwasa, 1976).

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