The effect of heat on the A, B, and H antigenic strength of human red cells

J. R. BOOTH, W. WAGSTAFF, AND D. VOAK

From the Regional Blood Transfusion Centre, Sheffield

SYNOPSIS The reaction of A cells with anti-A, B cells with anti-B, O cells with *Ulex europaeus* anti-H is reduced by heat (56°C) treatment. This is demonstrated using an automated haemagglutination technique with which minor differences in reactions can be detected.

This report presents the results of experiments to determine the effect of heat on the A, B, and H activity of human red cells.

A study of the literature revealed only the work of Hulse (1951) who reported that certain antigens are heat labile and others, including the A, B, and H antigens, are heat stable. The technique used did not include titres to detect any changes, but only one-tube tests, which in the case of A, B, and H antigens gave 'complete' agglutination before and after heat treatment. This present study of the phenomenon was carried out using an automated haemagglutination titration technique.

The use of the Technicon AutoAnalyzer for ABO typing was described by Sturgeon, Cedergren, and McQuiston (1963) and for Kidd and Duffy typing by Allen, Rosenfield, and Adebahr (1963). The technique used in this experiment is based on the one described by Marsh (1967) and Marsh, Nichols, and Jenkins (1968) for antibody detection.

Reagents

A₁, A₂, B, and O cells from clotted samples, taken less than 24 hours before use, were suspended in a protein-enriched medium in a ratio of packed cells to 20% bovine albumin to saline of 1:1:3. Also used were: isotonic saline, 1% Triton X-100 in distilled water, methyl cellulose, 3 g/l isotonic saline, bromelain 0.75 g/l, human

anti-A and anti-B, and *Ulex europaeus* extract prepared as described by Voak, Lodge, and Reed (1969).

Method

Each cell sample was washed four times in isotonic saline, divided into two equal aliquots, and one of these diluted to a 50% suspension in saline in a thin-walled conical flask. This diluted subsample was heated at 56°C for eight minutes in a water bath, diluted with a large volume of saline at room temperature immediately on removal from the water bath, and washed a further four times. These washes were performed with a ratio of at least 20 volumes of saline to 1 volume of packed cells, to ensure minimal residual haemolysis. Treated and untreated aliquots were then adjusted to the same measured packed cell volume, in the range $20\% \pm 2\%$, using bovine albumin and saline as diluents in the proportion already stated.

The reagents, apart from the cells, were held for use in flasks standing in melting ice to prevent deterioration; when not in use the cell suspensions were stored at 4°C. Water from a reservoir of melting ice was pumped through the jacketed mixing coils, and the pulse suppressor controlling the saline diluent cooled by melting ice, thus minimizing lysis of the cells during the reaction. A methyl cellulose and bromelain

Received for publication 23 July 1969.

mixture in the ratio of 5:3 was used for the O cell - anti-H reactions, the bromelin being replaced by saline in the A cell-anti-A and B cell-anti-B reactions.

The reagents were pumped through the system as shown in Fig. 1, the base line being set at 0.01 optical density (OD) units with all reagents running except the cell suspensions.

Appropriate dilutions of antiserum or *Ulex europaeus* extract were made, after preliminary titrations in the system to find the most suitable ranges, and pumped sequentially through the system via pump line (no. 11, Fig. 1) changing the dilutions manually and allowing the corresponding paired, heated and unheated, cells to be sampled in turn during the flow of each dilution. Readings for the negative reactions of each set of cells were obtained by replacing antisera with saline. No significant change in these readings was found when checked later

during testing, thus providing a control of cell stability over the duration of the experiment. Separation of cells in the sampler by two saline washes was found sufficient to allow recovery of the base line between peaks, at a sample rate of 60/hour with a 1:2 ratio between the sample and the wash, each cell suspension being thoroughly mixed immediately before sampling.

Results

The results in Fig. 2 show a considerable reduction in the activity of A₁, B, and H antigens after heat treatment, compared with those before, and a smaller reduction with the A₂ antigen. As can be seen from the graphs, increasing dilution of the antibody results in fewer agglutinated cells being available for decantation, lysis of the larger proportion of free cells thus giving a higher optical density.

With this system, optical density measurements of more than 0.6 OD units on Technicon apparatus have been found to be associated with less reliable results. Hence the attempt was made to adjust the reactions to give readings below this level, which was accordingly chosen as the base line for the charts of results.

Discussion

A technique measuring free cells or a representative sample of free cells is ideal for comparative antigen-antibody studies, and the automated technique measures the latter. Sampling the cells from the sample module results in an increase of optical density on a straight base line set without cells at 0.01 OD units conversely, running the cells continuously and sampling the antisera would result in a decrease of optical density on an irregular base line set

Fig. 1 The manifold.

Fig. 2 Effect of heat (56°C) in A, B, and H red cell antigen strength.

at about 0.8 OD units, this point usually being chosen as a compromise between sensitivity and base line regularity. Cell sampling, with its superior base line and recording on the more sensitive part of the optical density scale, is preferable to serum sampling. With accurately prepared cell suspensions of a strength giving a reading of less than 0.6 OD units the sensitivity is such that minor differences in reaction can be detected. The method allows high dilutions of antisera to be used, especially the *Ulex europaeus* extract which is used with a rouleaux-inducing agent (methyl cellulose) and an enzyme (bromelin) in the reaction mixture. Enhancement of *Ulex europaeus* has been reported by Voak *et al* (1969) with bovine albumin, human group AB serum, and papain.

No conclusions as to the cause of the reduction of activity can be made, but Kościelak (1963) suggests that the state of aggregation in solution of compounds with A activity, extracted from human red cells, affects their activity. Molecular

disorientation on the cell surface by heat treatment could contribute to the reduction of activity.

The authors gratefully acknowledge the encouragement of Dr C. C. Bowley in carrying out this study.

References

- Allen, F. H., Rosenfield, R. E., and Adebahr, M. E. (1963). Kidd and Duffy blood typings without Coombs serum. Adaptation of the auto-analyzer hemagglutination system. *Vox. Sang. (Basel)*, **8**, 698-706.
- Hulse, E. V. (1951). The influence of heat upon the Rh, Lewis, and H agglutinogens. *J. Lab. clin. Med.*, **38**, 402-406.
- Kościelak, J. (1963). Blood group A specific glycolipids from human erythrocytes. *Biochim. Biophys. Acta (Amst.)*, **78**, 313-328.
- Marsh, W. L. (1967). In *European Technicon Symposium, Brighton*.
- Marsh, W. L., Nichols, M., and Jenkins, W. J. (1968). Automated detection of blood group antibodies. *J. med. Lab. Technol.*, **25**, 335-342.
- Sturgeon, Ph., Cedergren B., and McQuiston, D. (1963). Automation of routine blood typing procedures. *Vox. Sang. (Basel)*, **8**, 438-451.
- Voak, D., Lodge, T. W., and Reed, J. V. (1969). The enhancement of *Ulex europaeus* anti-H activity by human serum. *Vox. Sang. (Basel)*, **17**, 134-138.