HÆMATOLOGY

Anti-A Hæmagglutinins from a Non-Leguminous Plant—*Hyptis suaveolens* Poit

CERTAIN plants, chiefly their seeds, contain agglutinins for the erythrocytes of various species. Whereas most plant agglutinins make no individual distinctions among human erythrocytes, some act selectively on one or other of the following blood-group antigens[1]: A, A_1, B, H and N. Except for separable anti-H and anti-B agglutinins from the seed capsule of certain species of *Euonymus*, of the family Celastraceae, all specific seed agglutinins have hitherto been obtained from Leguminosae[1].

An anti-A agglutinin has now been found in the seeds of *Hyptis suaveolens*, Poit, of the genus Labiatiae. The agglutinin works best when a fresh seed extract is tested on a flat tile which is gently and continuously rocked. Although it is not very avid when tested with erythrocytes suspended in isotonic saline solution, it agglutinates A_1 and A_1B cells strongly, A_2 weakly, and fails to agglutinate A_2B cells. Thus *Hyptis suaveolens* seed extract sharply differentiates A_1 and A_1B erythrocytes from those of the weaker sub-groups of A and AB; however, it is not as satisfactory for this purpose as *Dolichos biflorus* seed extract[2], which is far more avid.

The *Hyptis suaveolens* agglutinin acts as strongly at 37° C. or 4° C. as at room temperature. It does not cross-react with B or O cells after 24 hr. at 4° C. or when erythrocytes are suspended in albumin; albumin, however, potentiates the agglutination of A_2 and A_2B cells. Agglutination is inhibited by A-secretor saliva and by AB serum. A full description will be presented elsewhere.

The 'new' agglutinin is of special interest because it is the first seed anti-A (anti-A_1) agglutinin to be found outside the Leguminosae. The current trend is to confine the search for specific seed agglutinins to leguminous plants; wide examination of other plant families might be profitable.

I am grateful to D. S. Das for collecting the seeds and for technical assistance. The seeds were identified by the Botanical Survey of India, Poona.

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[1] Krüpe, M., "Blutgruppenspezifische pflanzliche Eiweisskörper (Phytagglutinine)" (Stuttgart, 1956).
[2] Bird, G. W. G., *Curr. Sci.*, **20**, 298 (1951); *Nature*, **170**, 674 (1952).

Interaction of Erythrocytes and Endotoxins

CONSIDERABLE diversity of opinion exists as to the sensitivity of hæmagglutination tests. The discrepancy in results may to some extent be attributed to technical differences. The factors influencing the reaction, the elution of endotoxin *in vitro* and the uptake by erythrocytes *in vivo* have been examined using a dehydrated endotoxin derived from *S. typhi* (*TO*–901). This powder consisted of 68·5 per cent ash, 7·8 per cent moisture, 1·6 per cent protein, 2·2 per cent lipids and, based on L-rhamnose hydrate as standard, 2·7 per cent rhamnose. If the method of calculation described by Webster *et al.*[1] is adopted the polysaccharide content is 14·3 per cent and the endotoxin approximately 20 per cent. The powder was dissolved in isotonic saline, heated to 56° C. for 30 min. and stored at 4° C. for at least 24 hr. before use. The solution had a slight buffer action, *p*H 7·2. Erythrocytes from healthy rabbits were washed and measured with the hæmatocrit. A volume of 0·2 ml. packed cells was used in most experiments.

Endotoxin was adsorbed on to the washed and measured erythrocytes at 37° C. for 1 hr. The cells were then washed three times in 6–8 volumes of isotonic saline and accurately made up to a 20 per cent suspension. Agglutination was performed on slides, using 0·05 ml. of sensitized erythrocytes and an equal volume of a diluted standard *TO*-serum. The slide was agitated regularly and the test read after exactly 10 min.

The amount of endotoxin adsorbed on erythrocytes was dependent on both the absolute quantity of endotoxin available and on its concentration. The speed of the process was proportional to the concentration of endotoxin and related to the temperature, being five to six times faster at 37° C. than at 4° C.

Erythrocytes coated with very small amounts of endotoxin were inagglutinable in antiserum, whereas those with greater quantities of endotoxin agglutinated readily. This afforded the basis for the determination of an 'erythrocyte-agglutinating unit', defined as the smallest amount of endotoxin, incubated with 2 ml. of 10 per cent erythrocytes for 1 hr. at 37° C., which rendered the cells agglutinable in standard serum diluted 1 in 10. One erythrocyte-agglutinating unit was found to be equivalent to 0·16 mgm. of the dehydrated powder. The quantity of crude endotoxin adsorbed on erythrocytes in these circumstances was 0·12 mgm., thus removing 75 per cent of endotoxin from the supernatant. Assuming the crude powder contains 20 per cent endotoxin it appears that 0·2 ml. of agglutinable packed cells under our experimental conditions must be coated with a minimum of 24 μgm. of endotoxin.

Erythrocytes, initially sensitized with sub-agglutinable quantities of endotoxin, became agglutinable on subsequent exposure to endotoxin provided that the sum of the two doses constituted at least one erythrocyte-agglutinating unit. Thus the erythrocytes appear to bind the adsorbed endotoxin quite firmly.

Assuming that the endotoxin was not removed or rendered undetectable except by fixation to erythrocytes, the reduction in endotoxin content as measured by determination of the erythrocyte-agglutinating unit on the supernatant, will be a direct, quantitative expression of adsorption. Experiments along these lines indicated that erythrocytes were capable of binding at least 200 times the minimum amount required for agglutination under standard conditions; that is to say 0·2 ml. packed cells could adsorb more than 200 × 24 μgm. or 4·8 mgm. of endotoxin as calculated, corresponding to 2·2 per cent of their own weight.

Erythrocytes of the same batch, coated with amounts of endotoxin ranging from 1 to 200 erythrocyte-agglutinating units, gave final agglutination titres against the standard serum ranging from 1 : 10 to 1 : 1,280. Hence, the titre of a given serum will vary according to the quantity of endotoxin adsorbed on to the cells. This may explain, at least partially, the discrepancy and inconsistency in results obtained by hæmagglutination tests; it could be overcome by rigorous standardization for which accurate measurement of endotoxin is essential.

Elution of endotoxin at our experimental conditions (*p*H 7·2–7·3) was minimal. Erythrocytes coated with 1·5 times minimum agglutinating dose of endotoxin