vities[2]. Furthermore AChE activity and O_2 uptake in the presence of methylene blue of erythrocytes from patients developing a positive direct antiglobulin test during cephalothin therapy have so far been found to be within normal limits[10]. Since the in vitro development of the cephalothin-produced positive direct Coombs test has been shown to be influenced by many variables (e.g. the period and temperature of incubation[1], the concentration of the drug in the reaction mixture[1,2], the level of serum gammaglobulins[2] and particularly of γG globulins[11]), the above discrepancy can be explained if we suppose that under particular conditions (e.g. a high protein concentration in the plasma) a positive direct Coombs test develops when the lesion of the membrane is not sufficient to determine a detectable reduction of its metabolic activities. In spite of this discrepancy, therefore, we believe it can be taken as shown that the primary effect of cephalothin on red blood cells is a lesion of the membrane with subsequent adsorption of some serum proteins and the development of a positive direct Coombs test.

Riassunto. L'incubazione in vitro dell'antibiotico cefalotina con sangue intero determina un'anormalità metabolica degli eritrociti e la positività del test di Coombs diretto. Nel presente lavoro sono stati studiati i rapporti tra i due fenomeni e si è osservato che il primo si produce più precocemente del secondo. In base ad una serie di considerazioni, si conclude che l'effetto primo della cefalotina sugli eritrociti è una lesione della membrana, con successivo adsorbimento di alcune proteine dal plasma.

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Agglutinins from Jerusalem Sage (*Phlomis fruticosa*)

Agglutinins active against human blood group A or B red cells and with little or no activity for group O cells have been obtained from various seeds, such as *Crotalaria striata*[1], *Crotalaria usraemonensis*[2], *Calpurnia aurea*[3], *Sophora japonica*[4], *Coronilla varia*[5], *Bandeiraea simplicifolia* (freshly harvested seeds)[2], and *Caragna frutex* var *latifolia*[2]. The two *Crotalaria* agglutinins react more strongly with A (A_1) cells, the *Calpurnia* agglutinin is about as strong with A as with B, and the others are stronger with B cells.

Ottensooser, Leon and Sato[6] reported such agglutinins in the seeds of *Crotalaria mucronata* and *Crotalaria breviflora*. *Crotalaria mucronata* however is another name for *Crotalaria striata*. *Crotalaria breviflora* seeds were reported by Brilliantine, Aranda, Foster and Allen[7] to contain specific anti-A agglutinins. The discrepancy could be due to variation among different strains of the plant or to variations in agglutinin concentration.

We have found in the seeds of *Phlomis fruticosa* (Jerusalem Sage) of the Natural Order Labiatiae, agglutinins which react strongly with A and B cells and fail to agglutinate O cells (Figure). This is the third specific agglutinin to be found in *Labiatiae*, the others being the anti-A of *Hyptis suaveolens*[8] and the anti-(A + N) of *Moluccella laevis*[9]. We here describe the properties of the *Phlomis* agglutinin.

The methods used were as described previously[10]. Screening tests on tiles against various washed red cells suspended in physiological saline solution showed that the *Phlomis* agglutinin strongly agglutinated AB, A and B cells but not O cells. Titre scores by the tube method were: A_1-78, A_2-64, B-57, 0-nil. A_3 cells gave the typical 'weak-mixed field' appearance as is obtained with human group B or O sera. A_x cells were not agglutinated. The group O polyagglutinable cells 'Ba'[11] were strongly agglutinated. AB serum inhibited the agglutinin. There was no crossreaction with O cells suspended in 30% bovine albumin (Armour) or treated with *Vibrio cholerae* neuraminidase (Behringwerke). Papain-treated O cells were seen under the microscope to be very weakly agglutinated.

Absorption with A_1 cells abolished activity for A_1 and B cells; absorption with B cells left some anti-A.

The agglutinin was completely inhibited by group A secretor saliva, which was a more effective inhibitor of the agglutination of B than of A cells. Group B secretor saliva removed all activity for B leaving strong activity for A cells.

Inhibition tests with 2% aqueous solutions of simple sugars showed that L-fucose, D-glucose, lactose, salicin and N-acetylglucosamine did not inhibit the agglutination. N-acetylgalactosamine removed all activity for both A and B cells; D-galactose removed activity for B but not A cells. N-acetylgalactosamine was a greater inhibitor of agglutination of B than of A cells.

The agglutinins did not show the temperature effects (increased anti-B activity at lower temperatures without enhancement of anti-A) described by Ottensooser and Sato[12] for the anti-(A + B) agglutinin of *Crotalaria striata* (*mucronata*). There were individual differences in the strength of agglutination of the various B cells, possibly analogous to those described by Ottensooser, Leon and Sato[6]. The variation paralleled the strength of reaction with human anti-B and could be shown to be inversely proportional to the H antigen content of the various cells

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as measured by the anti-H agglutinins from the seeds of *Cerastium tomentosum, Lotus tetragonolobus, Laburnum alpinum, Cytisus sessilifolius* and *Ulex europaeus*.

The specificity of the *Phlomis fruticosa* agglutinin is anti-(A + B). The results of absorption and inhibition tests support this view and suggest that some specific anti-A might also be present. The possibility that the extract contains free anti-A is only of academic importance: *Phlomis* extract acts as an anti-(A + B) reagent.

An interesting aspect of the inhibition studies is the greater inhibition of agglutination of B cells by A-secretor saliva and by N-acetylgalactosamine, the chief structural determinant of A-specificity. This may be explained by the not unlikely supposition that the binding capacity of the cross-reacting agglutinin for B cells is not as great as for A cells so that its anti-B activity is more easily inhibited.

Anti-(A + B) agglutinins are well known to serologists. Not only are they found in the seeds of various plants, but also in various animal sera as heterophile antibodies produced in response to various stimuli, often microbial[13]. Human O serum contains, in addition to specific anti-A and anti-B, cross-reacting anti-(A + B) antibodies which have been extensively studied. WIENER[14] maintains that these antibodies are anti-C, specific for a factor C common to A and B. DODD[15] and BIRD[16] thought that cross-reacting antibodies had separate receptor sites for A and B. KABAT[17] and others believe that the cross-reaction depends on a structural similarity between A and B. The subject has been reviewed by BIRD[18], RACE and SANGER[19], and, more recently, by DODD, LINCOLN and BOORMAN[20]. DODD has abandoned the concept of two separate receptors for A and B in favour of a single receptor and so, to some extent, has BIRD[21], whose subsequent absorption-elution studies indicated that human group O sera contain, besides specific anti-A and anti-B, a heterogeneous population of cross-reacting antibodies, some better adapted to A than to B and others better adapted to B than to A.

Extracts of *Phlomis fruticosa* or other seeds which contain anti-(A + B) agglutinins could be used instead of O sera in the rapid selection of group O bloods. However, it should be noted that, unlike O sera, these extracts do not agglutinate A_x cells.

Zusammenfassung. Samen des Lippenblütlers *Phlomis fruticosa* (Jerusalem Salbei) enthalten ein wirksames Anti-A- und -B-Agglutinin.

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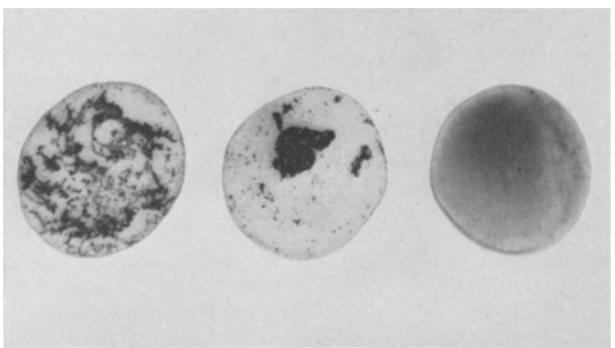
Regional Blood Transfusion Service, Birmingham 15 (England), 11 June 1970.



Strong agglutination of A_1 and B without agglutination of O erythrocytes by *Phlomis fruticosa* extract.

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Effect of Haemagglutinating and Mitogenic Fractions of Phytohaemagglutinin on Electrophoretic Mobility of Lymphocytes and Macrophages

The manifold activities of phytohaemagglutinin (PHA), especially those capable of inducing blastogenesis and mitosis in normal lymphoid cells in culture, have been the subject of many studies in recent years and have been comprehensively reviewed[1]. However, the mode of action of this complex preparation[2] is not fully understood and theories appear to be based either on the similarity to specific stimulation of sensitized cells, indicating an immune mechanism, or on the suggestion that PHA acts by non-specific binding to sites on the cell surface[3,4]. Incubation of lymphocytes with PHA results in reduction of electrophoretic mobility which suggests the presence of a reaction on the cell surface[5].

In the present study the electrophoretic mobility of lymph node cells and of peritoneal macrophages was measured following incubation with PHA before and after absorption of the haemagglutinin.

Lymph nodes and peritoneal macrophages were obtained from adult Hartley strain guinea-pigs of either sex. Peritoneal exudates were extracted following the injection of liquid paraffin[6] giving preparations containing macrophages and 7–20% lymphocytes. The former were 85–95% viable, estimated by dye exclusion. Lymphocytes were extracted from freshly removed lymph nodes by teasing the tissue through 50 and 100 mesh sieves and then washing the cells in TC199. These were 50–60% viable. PHA-M (Difco) was used throughout, and haemagglutinin

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