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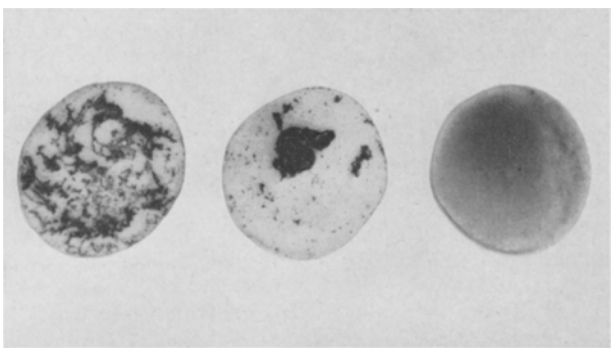
Regional Blood Transfusion Service, Birmingham 15 (England), 11 June 1970.



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

[12] F. OTTENSOOSER and M. SATO, Vox Sang. *8*, 733 (1963).
[13] G. I. PARDOE, G. W. G. BIRD and G. UHLENBRUCK, Z. Immun-Forsch. exp. Ther. *136*, 488 (1968).
[14] A. S. WIENER, Ann. Eugen. Lond. *18*, 1 (1953).
[15] B. E. DODD, Br. J. exp. Path. *33*, 1 (1952).
[16] G. W. G. BIRD, Br. J. exp. Path. *34*, 131 (1953).
[17] E. A. KABAT, *Blood Group Substances. Their Chemistry and Immunochemistry* (Academic Press, New York 1956).
[18] G. W. G. BIRD, Vox Sang. *4*, 66 (1954).
[19] R. R. RACE and R. SANGER, *Blood Groups in Man* (Blackwell, Oxford 1968).
[20] B. E. DODD, P. J. LINCOLN and K. E. BOORMAN, Immunology *12*, 39 (1967).
[21] G. W. G. BIRD, unpublished observations, 1964.

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

[1] C. K. NASPITZ and M. RICHTER, Progr. Allergy *12*, 1 (1968).
[2] L. W. ALLEN, R. H. SVENSON and S. YACHNIN, Proc. natn. Acad. Sci. USA *63*, 334 (1969).
[3] K. HIRSCHHORN, F. BACH, R. L. KOLODNY, I. L. FIRSCHEIN and N. HASHEM, Science *142*, 1185 (1963).
[4] R. L. KOLODNY and K. HIRSCHHORN, Nature *201*, 715 (1964).
[5] P. S. VASSAR and C. F. A. CULLING, Nature *202*, 610 (1964).
[6] D. HUGHES and E. J. FIELD, Int. Arch. Allergy *33*, 45 (1968).

was absorbed out with washed human red cells[7]. A preparation was also fractionated on Sephadex G100 columns. The absorbed or fractionated extracts were titrated for haemagglutinating activity with washed human red cells, and mitogenic activity was assessed by adding samples to human lymphocytes and measuring the uptake of tritiated thymidine after 3 days in culture[8].

Electrophoretic mobility measurements were made on cell suspensions in TC 199. Lymphocytes were used at concentrations of 5×10^6 cells per ml and macrophages at 3×10^6 per ml. Cell suspensions were incubated with PHA at a final dilution of 1/600 or 1/3000 for 1 h at room temperature before measurements were made. Mobility was measured in a Zeiss Cytopherometer at 25 °C on cells not washed after incubation with PHA and controlled against untreated cells in TC 199.

Results showed that absorbtion reduced the titres of the haemagglutinin to 1:2 or zero from an initial 1:10,000 whilst approximately 70% of the mitogenic activity remained. Column fractionation produced 1 fraction with haemagglutinating activity alone, but failed to give any component containing mitogen alone. The Table gives the results of electrophoretic measurements and shows a marked reduction in mobility of lymphocytes after treatment with unabsorbed PHA, and with the haemagglutinating fraction. This reduction of mobility, however, is almost completely abolished when absorbed PHA is used.

Effect of phytohaemagglutinin preparations with and without haemagglutinating activity on electrophoretic mobility of a) lymph node cells and b) peritoneal macrophages

a) *Lymphocytes*[a]

Sample	Migration time (sec)	Change from control (%)	Viability (%)
Control	7.35	0	62
+ PHA 1/600 (native)	9.35	28	47
+ PHA 1/3000 (native)	8.60	17	42
+ PHA 1/600 (haemabsorbed)	7.10	−3	52

b) *Macrophages*[b]

Sample	Migration time (sec)	Change from control (%)
Control	6.70	0
PHA 1/600 (agglutinin, no mitogenic activity)	8.50	27
PHA 1/600 (haemabsorbed, 70% mitogenic activity)	6.85	4

[a] Mean of 2 separate experiments. [b] Mean of 4 separate experiments. Each single result represents the mean of 20 electrophoretic measurements.

The results suggest that the haemagglutinating fraction may be responsible for the electrophoretic change. These findings, whilst confirming the results of earlier experiments with PHA[5,9], showed that the mitogenic component of PHA does not necessarily react on the surface of the cell, at least not to the extent of bound molecules causing an alteration of surface charge. Fractionation of similar commercial preparations of PHA[10] yielded 3 different components 2 of which were mitogenic, whilst a more recent study yielded 2 glycoproteins[2,11] of high and low mitogenic activity with an inverse ratio of haemagglutinating activity. The starting material indeed showed no fewer than 17 components on acrylamide electrophoresis. This biochemical complexity was supported by marked variations in response of lymphocytes stimulated with different preparations of PHA in culture[12]. It is suggested that some agglutinating and mitogenic properties of PHA may occur either on the same molecule[2] or 2 molecules firmly bound to each other. This may explain the absorption of mitogen by lymphocytes[4] and also provide some explanation for the attractive, but possibly misleading, surface hypothesis.

Zusammenfassung. Die elektrophoretische Mobilität von Lymphozyten und Makrophagen wird durch Phytohaemagglutinin um mehr als 20% erniedrigt, wobei das letztere nach Absorbierung des erythrozytagglutinierenden Komponenten diese Eigenschaft verliert.

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20 April 1970.

[7] P. Barkhan and A. Ballas, Nature *200*, 141 (1963).
[8] D. Hughes and E. A. Caspary, Int. Arch. Allergy *37*, 506 (1970).
[9] G. A. Currie, Nature *216*, 694 (1967).
[10] T. Weber, C. T. Nordman and R. Grasbeck, Scand. J. Haemat. *4*, 77 (1967).
[11] M. L. Goldberg, W. Rosenau and G. C. Burke, Proc. natn. Acad. Sci. USA *64*, 281 (1969).
[12] M. Richter and C. K. Naspitz, Int. Arch. Allergy *32*, 288 (1967).

Specifity of the Immunological Inhibition of the Parathyroid Hormone Activity

In the past few years a great progress has been noted in our knowledge about mode of action and the structure of the polypeptide chain of parathyroid hormone[1]. On the other hand, the immunological activity and specifity of this hormone has not been finally determined. The aim of this work was to find out if there is any correlation between the biological and immunological activity of the hormone and the problem of its specifity.

Materials and methods. The sources of parathyroid hormone came from fresh human (from autopsy), bovine or pig glands (from slaughter house). Immunization was performed using rabbits of the Vienna white race. The activity of the hormonal preparations was measured on parathyroidectomized Sprague-Dawley rats.

Two kinds of the active hormonal material were used: native gland homogenate, called in this work extract

[1] J. T. Potts jr., G. D. Aurbach and L. M. Sherwood, Proc. natn. Acad. Sci., USA *54*, 1743 (1965).