

AD No. 27035  
ASTIA FILE COPY

OFFICE OF NAVAL RESEARCH

Contract Nonr 19201

Annual Progress Report

THE ELECTRONIC CONFIGURATION OF ANTIBODIES

William C. Boyd, Burnett M. Pitt, Elizabeth Shapleigh

January 1, 1953 to December 31, 1953

BOSTON UNIVERSITY SCHOOL OF MEDICINE

80 East Concord Street

Boston 18, Massachusetts

## THE ELECTRONIC CONFIGURATION OF ANTIBODIES

### Summary of Results

#### A. Since start of project:

Since this project began, about 1000 varieties of seeds have been examined for specific agglutinating activity for human erythrocytes of blood groups O, A, and B, including in many cases erythrocytes of type O (Rh positive) and O (Rh negative). A number of plants yielding agglutinins of various specificities have been discovered.

In collaboration with Professor A. F. Blakeslee of Smith College, a large number of plants resulting from crosses performed by Professor Blakeslee and a number of plants grown from seeds which had been exposed to varying doses of x-rays or thermal neutrons have been tested. Other seeds of known specificities have been sent Professor Blakeslee for further crosses and irradiation treatment.

#### B. During current report period:

(1) A technique of water-ethanol fractionation at low temperatures has been developed which yields colorless protein fractions of high activity. In a series of over ten large-scale experiments, starting each time from about 500 grams of seeds, it has proven possible to remove inactive impurities by preliminary adjustment of the pH of the extracts to approximately 4.5. Further impurities are removed by precipitating the active proteins by dialysis or by diluting to low ionic strengths and collecting the precipitate in the Sharples Supercentrifuge. By thorough extraction of this precipitate with limited volumes of 0.15 molal sodium chloride solution, it is possible to extract the active proteins and leave behind large amounts of inactive material. The dissolved material is now adjusted to 10 per cent ethanol in the cold (0°C.),

and a precipitate, which is largely inactive, is removed by centrifugation in the refrigerated centrifuge. By now raising the ethanol concentration to 20 per cent, the active protein is precipitated, leaving behind considerable inactive material.

Precipitin tests indicate that this purified material is about 10 per cent specifically precipitable with blood group A substance. Titrations and protein determinations by the quantitative biuret method (Lovin, R. and Brauer, R.W. - Journal of Laboratory and Clinical Medicine - 38, pp. 474-480) indicate that this represents approximately a forty-fold concentration over the starting material.

(2) We have discovered that the active material is insoluble in 1.70 molal sodium chloride solutions, whereas nearly all the inactive material remaining after the above steps is soluble at this ionic strength. This discovery opens up the possibility of obtaining the material in nearly 100 per cent pure form, and this possibility is being actively investigated. If the active material resembles the Concanavalin A isolated by Sumner (J. Biol. Chem. 64, 256, 1925) from the Jack bean, Canavalia ensiformis, as now seems likely, it should be possible to obtain it in crystalline form. The advantages of having a material with antibody activity in pure crystalline form is obvious.

(3) We have discovered that these plant agglutinins, for which I have suggested the name lectins (Science, in press; The Proteins, Ed. H. Neurath and K. Bailey, Academic Press, in press) are also specific precipitins for the blood group substances (Science, in press; W. C. Boyd and E. Shapleigh, in preparation). This discovery provides a means of quantitative assay of the degree of purity of the various purified preparations, and also suggests the possibility of making calorimetric and other thermodynamic studies of the

reaction between the lectins and their antigens.

(4) We have been carrying out, in collaboration with Professor P. W. Bridgman of Harvard University, studies of the effect of high pressures (of the order of 5000 atmospheres) on these plant agglutinins. This will supplement a similar study made of the effect of pressure on agglutinins of human origin in 1946 (Boyd, W. C. - J. Exp. Med. 83, 401-407, 1946).

(5) We have developed the following practical applications of the lectins:

(a) We have demonstrated that extracts of several varieties of Lima beans (Sieva, Baby Potato Lima) are specific for blood group factor A, and may be used as routine reagents for this purpose. Large scale tests using such reagents have been made by Dr. Harold Kenton of the Deaconess Hospital and Dr. Saint-Paul of the Institute Pasteur, Paris, France. These Lima bean extracts are about as satisfactory for routine blood grouping as are the best normal human sera of blood group B, but like these latter they react more weakly with  $A_2$  and other weak subgroups of (Dunsford and Hutchinson - Vox Sanguinis 3, 6, 1953) and are inferior to the potent sera which are sometimes obtained by injection of human volunteers of blood group B with hog A substance (Boyd, W. C. - J. Lab. and Clin. Med. 32, 1275-1277, 1947). However, they are perfectly satisfactory for large scale screening tests, and are much easier to prepare than the human reagents.

(b) We have found that extracts of Dolichos biflorus (Bird, G.W.C. Ind. J. Med. Research 40, 289, 1952) are entirely specific for subgroups  $A_1$  and  $A_1B$ . This reagent, in connection with an extract of Cytisus (Renkonen, K. O. - Am. Med. Exp. Biol. Fenn. 26, 66, 1948), which agglutinates bloods of groups C,  $A_2$ , and  $A_2B$ , make a perfect combination for the routine diagnosis of subgroups  $A_1$  and  $A_2$ . In particular, the

Dolichos extract is much more powerful than the human group B serum absorbed with A<sub>2</sub> cells which is generally recommended.

(c) We have found that an extract of Ulex is extremely useful for the differentiation of secretors and non-secretors. The saliva of secretors of any blood group, O, A, B, or AB inhibits the agglutinating action of Ulex cyropaeus on human cells, whereas the salivas of non-secretors has no inhibiting effect. This means that a single reagent, an extract of Ulex, will replace for purposes of diagnosis of secretors and non-secretors the anti-A, anti-B, and anti-H ("anti-O") which are otherwise required. The Ulex extract is especially useful for detecting secretors of group O, as the reagents formerly used for this purpose, eel serum and the serum of goats immunized with Shiga bacilli, are not easy to obtain.

(6) Our studies on the reaction of antibody and antigen by light scattering methods, carried out in collaboration with Professor Paul Doty of Harvard University, have continued. They have involved the study of practical methods of separating antibody from the compound. By treating the compound formed by rabbit anti-arsanilic antibody and the trivalent hapten prepared by coupling diazotized arsanilic acid with resorcinol with borate buffer at pH 8, we have been able to recover about 40 per cent of the antibody in usable form.

We have also experimented with the absorption of the antibody on a column of protein coupled with arsanilic acid and subsequently treated with heat and formaldehyde to render it insoluble, and with acyl chloride resins condensed with arsanilic acid. This method gives promise, but has not as yet been reduced to a procedure which will yield amounts of purified antibody sufficient for our purposes.

The light scattering studies, carried out in Professor Doty's

laboratory by Mr. Samuel Epstein, have shown that the purified antibody combines with one molecule of divalent hapten, or sometimes two molecules of antibody combine with one molecule of hapten, giving particles with a weight of about 320,000.

By studies of the equilibrium between antibody and haptens at different temperatures, Mr. Epstein has been able to calculate the free energy change of the reaction of this antibody with one haptenic group. A value of 7.6 Kcal. was obtained (*Science* 118, 570, 1953).

(7) Use has been made of a calculating machine purchased from Navy funds in calculations of gene frequencies for three papers which are now in press (Boyd, W. C. and Boyd, L. G. - *Am. J. Phys. Anthropology*, in press; Boyd, W. C. - *Am. J. Human Genetics*, in press).

#### Plans for Future

##### A. Immediate:

Our immediate plans call for:

(1) Development of methods of further purification of the lectins, leading, it is hoped, to their preparation in crystalline form.

(2) Study of the change in enthalpy (heat of reaction) of the lectins and the reactive sites on the erythrocytes by the methods developed by Filitti-Wurmser and associates (*Arch. Sci. Physiol.* 1, 151-164, 1947; *Biochem. Biophys. Acta* 4, 238-243, 1950; *J. Chimie Phys.* 47, 419-431, 1950; 49, 550-556, 1952; 50, 236-239, 1953; 50, 240-242, 1953; 50, 317-320, 1953; *Biochem. Biophys. Acta* 12, 92-97, 1953; *Arch. Sci. Physiol.* 7, 233-240, 1953).

(3) Direct measurement of the heat of reaction of the lectins and specific blood group substances in a calorimeter.

(4) Continuation of the light-scattering experiments in collaboration with Professor Doty.

(5) More vigorous prosecution of the studies of the effects on specificity of chemical modification.

B. Long range:

Our long range plans call for:

(1) A study of the amino acid composition of the purified lectins, including studies of the order of the amino acids in the polypeptide chain, with a view to possible correlation with the specific activity displayed.

(2) Continued study of the effects of chemical modification, especially coupling of haptens containing charged groups, on the specificity of the purified lectins.

(3) Attempts to make an extract of Sophora japonica, (Krupe, M. J. Imm. 107, 450, 1950) which agglutinates erythrocytes of groups A and B, more nearly specific for B, by (a) chemical modification, (b) treatment with group A substance to inhibit the anti-A activity, (c) partial digestion with enzymes in the hope of removing the anti-A activity and leaving the anti-B.

(4) Further thermodynamic studies, leading to measurement of the equilibrium constants and calculation of the free energy change and entropy change of the reaction.

(5) Further study of the equilibria by light-scattering methods.

Reports and Publications

A. Reports submitted to the Office of Naval Research during the period covered by this report:

Progress Report, January-June, 1953.

B. Scientific papers based on this work:

(1) In press:

- (a) Specific precipitating activity of plant agglutinins (lectins).  
W. C. Boyd and Elizabeth Shapleigh. Science, in press.
- (b) The blood groups in Pakistan. W. C. Boyd and L. G. Boyd.  
Am. J. Phys. Anthropol., in press.
- (c) Maximum likelihood method for the estimation of gene  
frequencies from MNS data. W. C. Boyd, Am. J. Human Genetics,  
in press.
- (d) Simplified maximum likelihood method for Rh data. W. C. Boyd,  
Am. J. Human Genetics, in press.

(2) In preparation:

- (a) Antigenic structure of blood group antigens revealed by  
reaction with plant agglutinins (lectins). W. C. Boyd  
and Elizabeth Shapleigh.
- (b) Practical use of group-specific plant agglutinins (lectins).  
W. C. Boyd and Elizabeth Shapleigh.
- (c) Effects of high pressures on group specific plant agglutinins  
(lectins). W. C. Boyd and Elizabeth Shapleigh.