Separation of Individuals of Any Blood Group into Secretors and Non-Secretors by Use of a Plant Agglutinin (Lectin)

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FOR THE DIAGNOSIS of secretors and non-secretors it is customary to use anti-A or anti-B agglutinating sera, depending on the blood group of the person being tested. In many investigations, no attempt is made to separate the individuals of group O into secretors and non-secretors, although it was shown[9] that the absorbed sera of certain cattle, or the serum of a goat injected with Shiga bacillus,[10] would enable the diagnosis to be made with saliva from individuals of group O. The preparation of a suitable absorbed cow serum is not easy, and the sera of many cattle do not possess the proper agglutinins; furthermore, goat anti-Shiga sera of the proper specificity are not readily available. Grubb[7] has shown that the serum of some eels contain an anti-H, but only a few ml. of blood are obtainable from an eel, and the agglutinin is not found in all eels.

Since Schiff[10] observed that the absorbed cow serum and the goat anti-Shiga serum studied by him could serve for the diagnosis of secretors, not only of group O but of any of the other groups, it is apparent that if such reagents were routinely available the procedure of diagnosing secretors would be appreciably simplified.

The sera found suitable for the separation of group O individuals into secretors and non-secretors were called by Schiff anti-O sera, but it is apparent that in reality they contained agglutinins of the type which Morgan and Watkins[8] propose to call anti-H. When we found,[6] therefore, that an extract of the seeds of *Ulex europeus* contained an anti-H agglutinin* of fair strength, it occurred to us to test the suitability of this reagent for the diagnosis of secretors. The present communication is a report of these experiments.

The anti-H character of the *Ulex* agglutinin is shown in table 1. The extract was made by mixing the seeds, ground to meal in a mill, with ten times their weight of 0.9 per cent saline, in a shaking machine for an hour, centrifuging and filtering. The stock solution was kept in the deep freeze and thawed as needed.

The inhibition of the agglutinin in the *Ulex* extract by saliva from secretors and non-secretors of various groups, compared with the inhibition observed with the usual reagents, is shown in table 2. The anti-A and anti-B reagents

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* One of us has proposed the name *lectin* (from Latin *lego*, to choose or pick out) for these and other antibody-like substances.[2, 5]

TABLE 1.—*Agglutination Reactions of Ulex Extract*

Cells of group	undil.	Dilution of extract, 1:				
		2	4	8	16	32
A_1	1	1	—	—	—	—
A_2	4	4	4	4	2	±
B	1	1	—	—	—	—
O	4	4	4	4	2	1

Equal quantities (0.05 ml.) of the diluted extract and a 1 per cent erythrocyte suspension of the indicated group were mixed, centrifuged, and read with shaking. Degrees of agglutination are indicated on a numerical scale, 4 being the symbol for complete agglutination. The symbol — means no agglutination.

TABLE 2.—*Inhibition Observed with Salivas of Secretors (Se) and Non-secretors (se) of Various Blood Groups*

Blood group	Reagent	Test cells	Dilution of saliva, 1:				
			2	4	8	16	32
A(Se)	anti-A	A	—	—	—	—	—
	Ulex	O	—	±	3	3	3
A(se)	anti-A	A	4	4	4	4	4
	Ulex	O	4	4	4	4	4
B(Se)	anti-B	B	—	—	—	—	1
	Ulex	O	—	—	1	2	3
O(Se)	Eel	O	—	—	—	—	—
	Ulex	O	—	—	—	—	—
O(se)	Eel	O	3	3	3	3	3
	Ulex	O	4	4	4	4	4

Equal quantities (0.05 ml.) of the blood group reagent and the diluted saliva were mixed and allowed to stand at room temperature for 30 minutes, then 0.05 ml. of a 1 per cent suspension of erythrocytes of the indicated group was added. The mixtures were shaken, centrifuged, and read with shaking. The symbol (Se) means secretor, (se) non-secretor. Other symbols as in table 1.

were powerful sera produced by injection of volunteers of groups B and A with mixed A and B substance,[1] diluted 1:30 before use. The eel serum was a serum selected from bleedings of five eels (*Anguilla vulgaris*), used undiluted. The *Ulex* extract was used undiluted.

It will be seen that the salivas from secretors of groups A and B do not inhibit the anti-H or *Ulex* to as high dilutions as they do anti-A and anti-B sera, just as Schiff found with the anti-H sera studied by him. Nevertheless, the inhibition is sufficiently marked to enable secretors of any group to be distinguished from non-secretors merely by the use of the *Ulex* extract alone. In confirmation of this we present in table 3 the results of our tests with the salivas of 22 secretors and 3 non-secretors belonging to groups O, A_1, A_2, B, and AB. In no case was there any disagreement between the diagnosis made in the usual way and that based on the test using *Ulex* extract and group O cells.

We have found it possible to concentrate and purify considerably the anti-A agglutinin from lima beans[4] by ethanol-water fractionation at low temperatures,

TABLE 3.—*Inhibition Reactions Observed with Ulex Extract and Salivas from Secretors (Se) and Non-secretors (se) of Various Groups*

Saliva from individual of group	Test cells	Number giving inhibition	No inhibition
O(Se)	O	13	0
O(se)	O	0	1
A_1(Se)	O	4	0
A_1(se)	O	0	1
A_2(Se)	O	1	0
B(Se)	O	3	0
B(se)	O	0	1
AB(Se)	O	1	0

Equal quantities (0.05 ml.) of the *Ulex* extract and undiluted, boiled, centrifuged saliva were mixed, and allowed to stand 30 minutes at room temperature. Then 0.05 ml. of a 1 per cent suspension of group O cells was added, and the mixture mixed, centrifuged and read with shaking. If no agglutination of the added erythrocytes resulted, the result was recorded as inhibition. If agglutination took place as with the untreated extract, the result was recorded as no inhibition. The symbol (Se) means secretor, (se) non-secretor.

and it seems entirely likely that similar methods, applied to the crude *Ulex* extract, would give a considerably more active preparation. Even without concentration, however, extracts prepared as described above, or by the procedure of Boyd and Reguera,[3] seem quite suitable for the routine diagnosis of secretors. The procedure has the advantage of requiring only one reagent for salivas from individuals of any blood group, and only one type of erythrocytes for testing.

SUMMARY

It has been found that an anti-H agglutinin, prepared by simple saline extraction of seeds of *Ulex europeus*, is suitable for the routine separation of individuals into secretors and non-secretors, merely by testing the ability of the undiluted saliva of the subjects to inhibit the agglutination of group O erythrocytes by the undiluted *Ulex* extract.

SUMMARIO IN INTERLINGUA

Il esseva constatate que un agglutinina anti-H, preparate per simple extraction salin ab semines de *Ulex europeus*, es usabile in le separation routinari de secretores e non-secretores. Le methodo proponite consiste in determinar le capacitate de non-diluite saliva del individuo sub investigation a inhibir le agglutination de erythrocytos del gruppo O per le non-diluite extracto ab *Ulex europeus*.

REFERENCES

[1] BOYD, W. C.: Production of potent blood grouping sera. J. Lab. and Clin. Med. *32:* 1275-1277, 1947.

[2] —: The proteins of immune reactions, in The Proteins, Ed. H. Neurath and K. Bailey, II. New York, Academic Press, (in press).

[3] — AND REGUERA, R. M.: Hemagglutinating substances in various plants. J. Immunol. *62:* 333-339, 1949.

[4] — AND SHAPLEIGH, E.: Antigenic relations of blood group antigens as suggested by tests with lectins. (In preparation.)

[5] — AND —: Specific precipitating power of plant agglutinins (lectins). Science (in press).

[6] CAZAL, P. AND LALAURIE, M.: Recherches sur quelques phyto-agglutinines spécifiques des groupes sanguins A B O. Acta Haematologica *8:* 73–80, 1952.

[7] GRUBB, R.: Some aspects of the complexity of the human A B O blood groups. Acta Path. Microbiol. Scand. suppl. *84:* 1–72, 1949.

[8] MORGAN, W. T. J. AND WATKINS, W. M.: The detection of a product of the blood group O gene and the relationship of the so-called O substance to the agglutinogens A and B. Brit. J. Exp. Path. *29:* 159–173, 1948.

[9] SCHIFF, F.: Der Ausscheidungstypus, ein auf serologischem Wege nachweisbares mendelendes Merkmal. Klin. Wchnschr. *11:* 1426–1429, 1932.

[10] —: Die Diagnose des serologischen Ausscheidungstypus in der Blutgrupe O mittels heterogenetischen Immunserums. Zeitschr. f. Immunitätsforsch. u. exper. Therap. *82:* 302–310, 1934.