A SIMPLIFIED METHOD FOR THE SEROLOGICAL DIAGNOSIS OF GLANDULAR FEVER

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It is well known that the simple sheep cell agglutination test of Paul and Bunnell as an aid to the diagnosis of glandular fever is subject to considerable limitations. To obtain reliable serological evidence of infection it is necessary to resort to the absorption test, which is, however, somewhat laborious. The purpose of this note is to draw attention to the fact that virtually the same information as is given by the absorption test can be obtained by the use of a simpler method based on the principle of agglutination inhibition. The rationale of the latter test depends on the observation that the supernatant fluids obtained by centrifuging the autoclaved tissue suspensions as usually prepared for the absorption test possess antigenic properties in tests *in vitro* corresponding to those of the tissues from which they are derived.

Methods

Antigens.—Twenty per cent. suspensions in saline of finely ground guinea-pig kidney and of well-washed ox r.b.c. are prepared and autoclaved for 15 min. at 15 lb. pressure. When cool the suspensions are centrifuged, the supernatants pipetted off and phenol to a final concentration of 0.5% is added. The fluids obtained are of course in effect saline extracts of the respective tissues. When kept in the refrigerator at 4° C. they appear to retain their antigenic activity unimpaired for long periods.

Test.—The serum is inactivated and three rows of serial doubling dilutions from 1/2 upwards are prepared in 3 × $\frac{1}{4}$ in. tubes using a dropping pipette with a unit volume of 5 drops approximately equivalent to 0.2 ml. It is convenient to use 10 tubes in the first two rows and seven in the third. A unit volume of the diluted serum is not discarded from the last tubes in each row, as these tubes are set aside for further dilutions should this later prove necessary. A unit volume of saline is added to the first row and a unit volume of the guinea-pig kidney and ox cell extracts to the second and third rows respectively. The measurement of these volumes need not be very exact; a sufficiently accurate unit volume is given by 7 drops of the extracts from the same pipette as is used for the serum

and saline. The tubes are shaken and set aside at room temperature for not less than one hour. A unit volume of 1% washed sheep cells is then added to all tubes, which are shaken and immediately centrifuged for five to 10 min. at 1,000 r.p.m. (In practice only the last six tubes in the first two rows and the six in the third row are used for this purpose.) On removal from the centrifuge the results are read by holding the tubes between finger and thumb, flicking the base, and observing the behaviour of the sediment. Titres are recorded as the initial dilution of serum in the last tube in which distinct cell aggregates can easily be seen with the naked eye, no attempt being made to read to fine limits. (It is necessary to use the centrifuge method for the development of agglutination, as prolonged incubation with guinea-pig kidney extract results in discoloration and ultimately lysis of the sheep cells.)

The demonstration that the guinea-pig kidney extract is an effective Forssman antigen naturally depends largely on the use of artificially prepared sera. An anti-Forssman serum obtained by the intravenous inoculation of a rabbit with finely ground guinea-pig kidney suspension showed a sheep cell agglutinin titre of 1:256 and a lysin titre of 1:2,048. After treatment of the serum in the way described above with guinea-pig kidney extract these titres were reduced to 1:8 and 1:4 respectively. Absorption with kidney tissue annulled both reactions entirely. Mixtures of serum and extract gave complement fixation up to serum dilution 1:128. When rabbit v. sheep cell sera are treated with the extract the agglutination of sheep cells subsequently added is not inhibited because of the presence of the isophile antibody, but such sera at relatively high dilutions, e.g., 1:128, readily fix complement with the extract. On the other hand, neither agglutination inhibition nor complement fixation is observed with horse v. sheep cell serum due respectively to the presence of isophile agglutinin in the serum and of Forssman antigen in the tissues of the horse. From these experiments it is concluded that the saline extract of guinea-pig kidney is an

effective and specific antigen for the detection of Forssman antibody.

The reduction of agglutinin titre resulting from the treatment with ox cell extract of the serum of 95 patients suffering from glandular fever is shown in Table I, which gives for each titre observed with untreated serum the corresponding titres with extract-treated serum.

TABLE I

Initial Titre 1 in	Titre of Extract-treated Serum 1							Total
	2	4	8	16	32	64	128	
64	2	6	4					12
128		4	4	2				10
256	1	5	5	8	1			20
512	1	2	4	8	3			18
1,024			2	4	6			12
2,048			4	2	7			13
4,096				4	2			6
8,192				1		1	2	4
Total	4	17	23	29	19	1	2	95

It will be noticed that the degree of titre reduction is not constant but varies directly with the titre of the untreated serum. The figures suggest that a reduction by three tubes is the minimum acceptable as significant, but if the original titre exceeds 1 in 512 a reduction by five tubes or more is to be expected. Comparison with results of the absorption test is somewhat difficult on account of the variety of techniques recommended for the latter. A number of parallel tests have shown that the titre reduction obtained with the inhibition test approximates to that which results when the (undiluted) serum is absorbed with an equal volume of 20% ox cell suspension.

It was thought that further simplification of the test might result from observing lysis instead of agglutination, for while this would require the addition of complement it would obviate the necessity for centrifuging the tubes. This modification, however, proved impracticable because it was found that lysis titres were always much lower than agglutinin titres, sometimes not exceeding normal values. Indeed, it appears that in glandular fever the relationship between the sheep cell agglutinins and lysins is almost the reverse of that which obtains in artificially prepared sera.

The antigenic properties of saline extracts of ox cells can also be used as the basis of a complement-fixation test, and indeed the present work was begun in this way.

The method was essentially that used in this laboratory for the Wassermann reaction, merely substituting the (undiluted) ox cell extract for the

Wassermann antigen. (It should be noted, however, that 0.5% phenol has some anticomplementary effect which must be allowed for in determining the complement dose.) Briefly, the results showed 28 positive reactions among 1,400 "control" sera, but in only six of these was the titre as high as 1 in 8. This titre was not exceeded by 13 out of 92 sera from glandular fever patients, but in 79 the titres ranged from 1 in 16 to 1 in 1,024.

The method is of course less convenient than the inhibition test for routine use when as a rule single specimens are being dealt with. It was, however, pursued for some time because it was thought possible that complement-fixing antibodies might occur in the absence of agglutinins, but no evidence of this was obtained. It may be added that the Wassermann reaction was carried out on 84 of the glandular fever sera with two positive results, the nature of which remained in doubt owing to the lack of clinical details. Price (1954) found only one false positive Wassermann reaction in 92 cases of glandular fever, and it appears therefore that the frequency of such reactions has generally been overstated.

Comment

The purpose of this paper is mainly to draw attention to a simple technical modification which facilitates the application of the principles of the absorption test, the value of which is well established, in the diagnosis of glandular fever. While this work was in progress, however, both Mason (1951) and Leyton (1952) reported favourably on the use of an ox cell lysis test. This method has the attraction of perhaps even greater simplicity, and both workers found that diagnostic ox cell lysis titres are sometimes observed before, or even without, the development of sheep cell agglutinins in significant amount. Further, according to Leyton, absorption of glandular fever serum with sheep cells does not remove the ox cell lysis, and it therefore appears that the two antibodies are independent entities. Whether either is the same as the complement-fixing antibody referred to above which reacts with saline extracts of ox cells has not been determined. Other workers have shown that the serum of glandular fever patients will agglutinate to high titre human red blood cells of group O which have been sensitized by treatment with Newcastle disease virus (Burnet and Anderson, 1946) or with phage lysates of strains of haemolytic streptococci and *Staph. aureus* (Fraser, 1954). It is evident that much work remains to be done to elucidate the nature

of the antibody response which occurs in glandular fever.

A point of some interest is the demonstration of the water solubility of both the Forssman and the glandular fever antigens, at least to a degree which permits the use of simple saline extracts as antigens in test-tube reactions. Early work on the Forssman antigen led to the conclusion that it was of lipid nature, largely because of its solubility in alcohol. Later investigation has of course shown it to be a lipo-polysaccharide complex, closely related to the Group A substance of human red blood cells, and it is of interest that the latter can also be readily obtained by saline extraction of certain tissues of "secreters." Both saline and alcoholic extracts of guinea-pig kidney can therefore act as Forssman antigens, but a curious discrepancy has been observed between normal human sera and rabbit anti-Forssman serum as regards their behaviour with these extracts in complement-fixation tests. The rabbit serum, which was Wassermann negative, reacted with both to an equal degree, but human sera reacted only with the saline extract, positive results with alcoholic extraction being confined to sera which were Wassermann positive.

With regard to the glandular fever antigen in the ox cell, simple alcoholic extraction does not yield active preparations, but this may mean only that the concentration of antigen in the extract is too small to be effective when the solution is diluted to the extent required for tests *in vitro*.

And indeed Schwarzweiss and Tomcsik (1948) obtained highly active fractions by a complex process involving successive extractions with hot alcohol. The saline extracts described above give a weak biuret and a positive Molisch reaction. It seems probable that the antigen concerned is analogous to the Forssman antigen and Group A substance, and is another addition to the number of polysaccharide haptens which are of increasing importance as serologically determinant groups.

Summary

The Forssman antigen in guinea-pig kidney and the glandular fever antigen in the ox red cell have both been found to be sufficiently soluble in water for simple saline extracts of these tissues to act as effective reagents in tests *in vitro* for the detection of the corresponding antibodies. These observations enable a simple agglutination inhibition test, which is described, to be substituted for the more laborious absorption technique in the serological diagnosis of glandular fever.

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REFERENCES

- Burnet, F. M., and Anderson, S. G. (1946). *Brit. J. exp. Path.*, **27**, 236.
- Fraser, K. B. (1954). *J. Path. Bact.*, **67**, 301.
- Leyton, G. B. (1952). *Journal of Clinical Pathology*, **5**, 324.
- Mason, J. K. (1951). *J. Hyg. (Lond.)*, **49**, 471.
- Price, I. N. O. (1954). *Brit. med. J.*, **1**, 637.
- Schwarzweiss, H., and Tomcsik, J. (1948). *Proc. Soc. exp. Biol. (N.Y.)*, **69**, 558.