THE SEROLOGICAL DIAGNOSIS OF GLANDULAR FEVER (INFECTIOUS MONONUCLEOSIS): A NEW TECHNIQUE

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Sheep-cell agglutination tests: the disagreement concerning the normal range of titres

The sheep-cell agglutination test for glandular fever described by Paul & Bunnell (1932) has become widely used, and it is now generally agreed that an increased sheep-cell agglutination titre is strong evidence of glandular fever provided that serum sickness can be excluded; but on the other hand, there is no general agreement as to the proportion of cases of glandular fever in which the test is positive (Himsworth, 1941). In a large measure this difference of opinion may be due to the lack of any clinical criteria by which the disease may be recognized with certainty; in part it no doubt arises because some authors, but not all, are content with a single test taken more or less at random during the illness; but in part also, and perhaps considerably more than is generally realized, it may result from the unwitting use by different workers of different standards in the agglutination tests. Probably few of those who perform such tests have themselves determined the normal range of titres and so decided where to draw the line between negative and positive reactions. Usually the published results of some other worker are used for this purpose, which is only justifiable on the assumption that the same tests performed by different workers would give the same titres. That this is unlikely to be true unless the same technique is used is clear from the work of Stuart, Burgess, Lawson & Wellman (1934), who showed that there is general disagreement in the literature concerning the sheep-cell agglutination titres of normal people, and that the titre depends to a large extent on such factors as the temperature at which the test is performed, the concentration of sheep cells employed, and whether the titre

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is expressed in terms of the final concentration of serum after adding the sheep cells and any other ingredients or of the initial concentration before such addition. Therefore no statement of sheep-cell agglutination titre is of value without information concerning the technique. It seems probable that the importance of such factors has not always been realized, and that technical modifications have sometimes been made without any adjustment of the titre value assumed to represent the upper limit of normal; but even when no intentional modifications of technique have been made (as is presumably the general rule) it is uncertain how closely the results of the same tests performed by different workers would agree. I have determined the sheep-cell agglutination titre of 100 apparently healthy people using the technique described by Stuart, Burgess, Lawson & Wellman (1934), and in Table 1 my results are

Table 1. *Frequency distribution of sheep-cell agglutination titres of people not suffering from glandular fever or serum sickness: comparison of results obtained by different workers using the same technique*

	No. of tests	Titre (final concentrations; 0·5 % cells; 37° C.)							
		1 : <10	1 : 10	1 : 20	1 : 40	1 : 80	1 : 160	1 : 320	1 : 640
Stuart, Burgess, Lawson & Wellman (1934)	300	3·00	23·00	46·33	20·66	6·00	0 66	0·33	0
Author	100	90	9	1	0	0	0	0	0

compared with theirs: the difference is considerable. Using my own normal standards I have found the sheep-cell agglutination test for glandular fever a satisfactory test, but if I had used the values given by Stuart, Burgess, Lawson & Wellman (1934) I should have obtained a smaller proportion of positive reactions.

I am unable to account satisfactorily for the difference between my results and those of Stuart, Burgess, Lawson & Wellman (1934). It is true that my sera were all from apparently healthy people, whereas some of theirs were from patients with various diseases other than glandular fever or serum sickness, but Bunnell (1933) compared the titres of normal people and of patients with such diseases and found no significant difference. It is also true that instead of standardizing my sheep-cell suspensions in the way that Stuart, Burgess, Lawson & Wellman recommend I determined the haematocrit of a concentrated suspension and hence calculated how much dilution was necessary to give a 1 % suspension, which seems to me a more accurate method than any involving the measurements of volumes of packed cells; but it is exceedingly unlikely that this alone could be responsible for the difference in the results. Other possible factors include: (*a*) *A difference in the method of reading the tests.* I disregarded slight degrees of agglutination not definitely visible with the naked eye: Stuart, Burgess, Lawson & Wellman do not state their criterion of agglutination. (*b*) *A difference in temperature.* I read my results in a warm room at 37° C. having found that if the tubes were taken out of the incubator to be read the titre sometimes increased appreciably in a few minutes: Stuart, Burgess, Lawson & Wellman incubated their tests at 37·5° C. but do not state what precautions were taken to prevent cooling while the results were being read. However, temperature alone cannot account for the difference between the results, because my tests were read at room temperature as well as at 37° C. and even the latter values (Table 2) are lower than those of Stuart, Burgess, Lawson & Wellman. (*c*) *A qualitative*

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difference in the sheep cells. Are sheep erythrocytes identical the whole world over? (*d*) *A real difference in the titres* of people in different communities may exist—but I am loathe to accept this until it is established beyond doubt.

It appears, therefore, that at present only he who determines the range of normal variation for himself can be certain of the most satisfactory results in the diagnosis of glandular fever by means of sheep-cell agglutination tests; but it is unreasonable to expect so much of everyone who uses the test, and there is need for a more precise standardization of technique. Although the common practice of using any normal serum selected at random as a control in such tests is obviously of little value, a standard sheep-cell agglutinating serum which would enable different workers to compare their results might be very useful. Even at present, however, the sheep-cell agglutination test seems to compare quite favourably with most other diagnostic tests, and indeed to be more satisfactory than many tests which have a rational instead of a purely empirical foundation; nevertheless, it is not perfect, and two attempts to improve upon it are described below.

Horse-cell agglutination tests

Agglutination tests on a certain glandular fever serum using sheep, horse, guinea-pig, ox, rabbit, pig, dog, cat and fowl erythrocytes gave the following results: the horse-cell titre was 1 : 10,240, the sheep-cell and guinea-pig-cell titres were each 1 : 1280, and the titres with all the other kinds of cells were lower. The ox-cell haemolytic titre was greatly increased (1 : 1280), although the agglutination titre was normal (negative in a dilution 1 : 20): a curious phenomenon first observed by Bailey & Raffel (1935). Treatment of the serum with sheep cells did not completely remove the agglutinins for horse cells, but treatment with horse cells or ox cells did completely absorb the agglutinins for sheep cells; neither horse cells, ox cells nor sheep cells completely absorbed the agglutinins for guinea-pig cells. In another sample of serum from the same patient 3 months later the horse-cell agglutination titre was 1 : 160, the sheep-cell titre 1 : 10, and the guinea-pig cell titre 1 : 20. These observations suggested the possibility that horse cells might provide a more sensitive test for the glandular fever antibody than sheep cells.

The patient on whom the above observations were made was of interest because by chance the characteristic cytological changes in the blood were observed 2 days before the appearance of the first symptoms of glandular fever. He was a medical student, and while examining his own blood he noticed an excess of lymphocytes. I performed a blood count which revealed 9750 white cells per cu. mm.; polymorphonuclear neutrophils 28·5%, eosinphils 1·0%, large lymphocytes 44·5%, small lymphocytes 18·5%, monocytes 7·5%. About one-fifth of the large lymphocytes were atypical cells, of the type characteristic of glandular fever. The patient stated that he felt quite well at the time but a complete physical examination was not made. Two days later he developed a sore throat and he had a typical attack of the anginose form of glandular fever.

Increased horse-cell agglutination titres in glandular fever have been reported previously by Beer (1936)—who, however, found that the agglutina-

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tion titre for guinea-pig erythrocytes was not increased in glandular fever—and a little earlier by Stuart, Griffin, Wheeler & Battey (1936); but the possible superiority of horse cells over sheep cells for use in diagnostic agglutination tests seemed to me worthy of more consideration than it had yet received. Accordingly, I determined the horse-cell and sheep-cell agglutination titres of twenty-seven samples of serum from patients with glandular fever and 100 samples of serum from apparently healthy people (Table 2). All the glandular fever patients presented clinical features entirely compatible with the diagnosis, and all gave a definitely positive sheep-cell agglutination test at some time during the illness; but it was obviously desirable to include some sera containing insufficient glandular fever antibody to give a positive reaction by the ordinary sheep-cell test, and such sera were obtained from convalescent patients. The normal sera were the same that were used for the tests reported

Table 2. *Comparison of sheep-cell and horse-cell agglutination tests*

A. Frequency distribution of titres of 100 healthy people

	Titre (final concentrations; 0·5% cells; room temperature)							
	1 : <10	1 : 10	1 : 20	1 : 40	1 : 80	1 : 160	1 : 320	1 : 640
Sheep cells	68	19	9	4	0	0	0	0
Horse cells	40	25	22	9	2	1	1	0

B. Frequency distribution of titres of twenty-seven specimens of serum from eighteen cases of glandular fever

	Titre (final concentrations; 0·5% cells; room temperature)							
	1 : <40	1 : 40–1 : 80	1 : 160–1 : 320	1 : 640–1 : 1280	1 : 2560–1 : 5120	1:10,240–1:20,480	1:40,960–1:81,920	1 : 81,920
Sheep cells	6	5	6	7	2	1	0	0
Horse cells	2	2	7	8	3	4	1	0

The dotted lines separate results regarded as normal or negative (to the left) from those regarded as abnormal or positive.

in Table 1, and the technique was also the same except that these tests were performed at room temperature (approximately 20° C.) instead of at 37° C. Comparison of the sheep-cell titres given in Table 2 with those in Table 1 therefore illustrates the effect of temperature on these tests; the difference is quite in accordance with the results of Stuart, Burgess, Lawson & Wellman (1934). In order to compare the diagnostic values of the two kinds of cells, I decided in each case to regard as positive all tests in which the titre exceeded that of 99% of apparently healthy people. Very occasional normal people (up to 1%) will give positive reactions according to this criterion, and I have in fact encountered two such false positives in doing about 500 sheep-cell agglutination tests, one of them being serum 9211 A of Table 5; nevertheless, a compromise between the antagonistic demands of specificity and sensitivity is unavoidable in this as in most serological tests, because any attempt to eliminate the false positives (and so increase the specificity of the test) by

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raising the minimum titre regarded as positive will considerably decrease the proportion of positive results in cases of glandular fever. On the whole the standard given above seems to me the most suitable for use with sheep-cell agglutination tests, and accordingly sheep-cell titres exceeding 1 : 80 and horse-cell titres exceeding 1 : 320 were regarded as positive, the boundary between negative and positive being marked by the dotted line in Table 2. It is true that among the 100 normals no sheep-cell titres of 1 : 80 were encountered, but on the other hand there were four sera with titres of 1 : 40, and I did not feel confident that the percentage of normal sera with a titre of 1 : 80 was really less than 1. Larger series of normals would of course be necessary to establish the limits accurately, but probably the values given above represent approximately the same standard for the two kinds of cells. Table 2 shows that although the horse-cell titres are definitely higher than the sheep-cell titres in glandular fever, the normal values are also higher, and with each kind of cells eleven of the twenty-seven tests on glandular fever sera must be regarded as negative: there is therefore no reason to advocate the substitution of horse cells for sheep cells in agglutination tests for glandular fever.

Absorption tests: a new technique

Bailey & Raffel (1935), Stuart, Fulton, Ash & Gregory (1936), Stuart, Welch, Cunningham & Burgess (1936) and Davidsohn (1937) have claimed that the heterophile sheep-cell agglutinins present in normal human sera, those found in serum sickness, and those found in glandular fever are different and may be distinguished by determining whether or not they are absorbed by ox cells (either fresh or autoclaved) and by an emulsion of guinea-pig kidney (Table 3). It is claimed that by means of such absorption tests it may be

Table 3. *Differentiation of sheep-cell heterophile antibodies in human sera by means of absorption tests (according to Bailey & Raffel* (1935) *and others)*

	Treated with	
Variety of heterophile antibody:	Ox cells	Guinea-pig kidney
'Normal'	Not absorbed	Absorbed
Serum sickness	Absorbed	Absorbed
Glandular fever	Absorbed	Not absorbed

possible to detect the presence of glandular fever antibody even when the ordinary sheep-cell agglutination test is negative, and to avoid mistaking the increased sheep-cell agglutination in serum sickness for glandular fever: of which advantages the former is in my opinion undoubtedly the greater. It seemed to me, however, that before such absorption tests could confidently be recommended for use in the diagnosis of glandular fever a series of at least 100 normal sera ought to be tested in order to discover whether antibodies having the characteristics of glandular fever antibody (i.e. absorbed by ox cells but not by guinea-pig kidney) were ever present normally. This I attempted to do, but I soon found that the methods hitherto described, though apparently

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suitable for distinguishing glandular fever from serum sickness, were not very suitable for tests on normal sera, in which the initial titre was often so low that it was difficult to decide whether or not absorption of the antibody occurred; yet sera with initial titres within the normal range were just those on which it seemed to me that absorption tests might prove most valuable. Eventually a technique was evolved which overcomes this difficulty and is at the same time less laborious than previous methods because it is only intended to demonstrate the presence or absence of glandular fever antibody; this technique is described in detail in the appendix. The serum is first treated with an emulsion of autoclaved guinea-pig kidney, then tested for sheep-cell agglutinins. If there is no agglutination—which is true of about 95% of sera other than glandular fever sera—the test is negative and nothing more need be done. If there is agglutination, the titre is determined and the serum is further treated with autoclaved ox erythrocytes. Four degrees of absorption by the ox cells are represented by the symbols + +, +, ± and −, and only if the degree of absorption is + + or + is the antibody regarded as glandular fever antibody. In order to increase the titres and so make the test suitable for use when the titre is within the normal range, a more dilute suspension of sheep cells than usual is employed; but with such a suspension agglutination is retarded although the final titre is increased, and if the results were read in the ordinary way the test could not be completed in one day. This disadvantage is overcome by centrifuging the tubes, when the result may be read almost immediately (Levine & Mabee, 1923, p. 428). At first I thought it necessary to allow an hour or two for the absorption by ox cells to take place, as is usual in absorption tests, but I have found that if the tubes are centrifuged there is no need for any such delay. No matter how soon after the addition of the ox cells the tubes are centrifuged, the glandular fever antibody is always found to have been absorbed completely; which is rather surprising. Neither the guinea-pig kidney emulsion nor the suspension of autoclaved ox cells need be prepared fresh for each test: both are relatively stable and can be stored for many months without deterioration. Using this technique 300 sera from apparently healthy people, thirty-one sera from glandular fever patients, and seven sera from patients who had received injections of horse serum (scarlet fever anti-toxin) have been tested.

Results in healthy people

In these tests, as in the sheep-cell and horse-cell agglutination tests recorded above, the normal sera were the residues of samples used in determining the blood groups of transfusion donors of various ages between seventeen and sixty-five and of both sexes, females predominating. In 285 of the 300 absorption tests no agglutination was observed after treatment of the serum with guinea-pig kidney emulsion. These sera were not tested before absorption, but it is certain that if they had been many would have shown agglutination; for even in the tests recorded in Table 2, in which the final

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concentration of sheep cells was 0·5%, 32% of normal sera were found to have titres of at least 1 : 10, and the more dilute suspension employed in the absorption test gives higher values (see Table 5). Actual tests on thirty-three unabsorbed normal sera using the agglutination technique employed in the absorption test gave the result shown in Table 4: in all except one of these sera the titre was at least 1 : 10, so that the failure of that concentration to produce agglutination in 95% of the absorption tests supports the view that the sheep-cell agglutinins present in the majority of normal sera are absorbed by guinea-pig kidney (Table 3).

Table 4. *Frequency distribution of agglutination titres of thirty-three normal sera determined by the technique used in the absorption test but omitting the absorption*

	Titre (final concentrations; 0·1% cells; room temperature)						
	1 : <10	1 : 10	1 : 20	1 : 40	1 : 80	1 : 160	1 : >160
Frequency	1	6	13	8	4	1	0

Table 5. *Results of investigations on fifteen normal sera containing sheep-cell agglutinins not completely removed by treatment with guinea-pig kidney emulsion*

	Absorption test		Sheep-cell agglutination titres	
Ref. no.	Ox-cell absorption	Titre (after treatment with guinea-pig kidney)	0·1% cells (as used in absorption test)	0·5% cells = (as in the tests of Table 2)
9211 A	±	1 : 160	1 : 640	1 : 160
1169 A	–	1 : 80	1 : 160	1 : 20
4678	–	1 : 80	1 : 80	1 : 40
5150	±	1 : 20	1 : 80	1 : 20
3747	–	1 : 20	1 : 160	1 : 20
4677	–	1 : 20	1 : 160	1 : 40
4622	–	1 : 10	1 : 80	1 : 20
5244	–	1 : 10	1 : 80	1 : 10
5264	–	1 : 10	1 : 160	1 : 20
5181	–	1 : 10	1 : 40	1 : 10
4658	+ +	1 : 40	1 : 40	1 : <10
5125	+ +	1 : 40	1 : 40	1 : <20
1264 A	+ +	1 : 40	1 : 40	1 : 10
4673	+	1 : 20	1 : 40	1 : 20
4689	+	1 : 20	1 : 40	1 : 20

There were, however, among the 300 normal sera fifteen in which sheep-cell agglutinins were demonstrated even after treatment with guinea-pig kidney emulsion: the individual results are given in Table 5, where it will be seen that these sera may be divided into two groups according to their behaviour with ox cells. *Group I* consists of ten sera in which ox-cell absorption was negative (– or ±) and which therefore appear to contain an antibody different from any shown in Table 3. An inquiry was made into the medical history of the people concerned in the hope of discovering some clue to the origin of the antibody, but without success. It is indeed possible that the antibody present in these sera was not essentially different from that present in most normal sera, but for some reason it was less readily absorbed by guinea-

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pig kidney. This possibility is favoured by the often considerable difference between the titres before and after absorption with guinea-pig kidney: the absorption has removed much of the antibody though not all. No mere technical error can explain the incompleteness of the absorption, however, for most of the tests were repeated with the same results. Whatever may be the truth about these sera, their practical importance is that they show the necessity of using ox cells in addition to guinea-pig kidney emulsion in the absorption test. According to Table 3 any sheep-cell antibody not absorbed by guinea-pig kidney should be glandular fever antibody, but clearly it is not safe to rely upon this in practice. *Group II* consists of five sera in which ox-cell absorption was positive (+ or + +), but with these sera, unlike those of Group I, absorption by guinea-pig kidney was at most very slight, there being little or no difference between the titres before and after absorption. According to Table 3, therefore, the antibody appears to be identical with that of glandular fever; nor have I found any way of distinguishing the two. Nevertheless, despite inquiry into their medical histories, there is no evidence at all that any of the people concerned had ever suffered from glandular fever. It remains possible that they had had unrecognized attacks: a mild attack of glandular fever might easily escape diagnosis, and it is by no means inconceivable that 1·67% of apparently healthy people had at some time been afflicted in this way. On the other hand, in my experience (which admittedly is in this respect very limited) the glandular fever antibody usually disappears from the blood within a few months of the illness, and it is not easy to believe that the five people in question had all had unrecognized attacks of glandular fever during the few months preceding the test. Further, in one case (4689) I have been able to test another sample of serum taken 9 months after the first and it gave exactly the same result: the titre had not diminished. These facts are opposed to the otherwise attractive hypothesis that the antibody in question was a residual glandular fever antibody from a previous infection. However that may be, the practical importance of these sera is that they show that the mere presence in a patient's serum of a sheep-cell agglutinin which is absorbed by ox cells but not by guinea-pig kidney does not provide proof that the patient is suffering from glandular fever.

Results in glandular fever patients

In Table 6 the results of absorption tests on thirty-one samples of serum from twenty-two cases of glandular fever compared with the results of sheep-cell agglutination tests on the same samples. All the glandular fever patients presented clinical features entirely compatible with the diagnosis, and all at some time during the illness had a definitely positive sheep-cell agglutination test. In order to compare the diagnostic values of the two tests I decided to use essentially the same criterion that was used in comparing the sheep-cell and horse-cell tests, and in each case to regard as positive all tests in which the titre exceeded that of 99% of apparently healthy people; provided that in the

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case of the absorption test the antibody was absorbed (+ or + +) by ox cells. As recorded above, in five of the 300 absorption tests on normal people ox-cell absorption was positive, and reference to Table 5 will show that in three (=1·0%) of these the titre was 1 : 40, and that no higher titres were encountered. According to my criterion, therefore, the absorption test could be regarded as positive if ox-cell absorption was + or + + and the titre at least 1 · 80. Sheep-cell agglutination titres of 1 : 160 or more were regarded as positive, the same technique being used as for the tests recorded in Table 2. Using these standards it soon became apparent that with the glandular fever sera the absorption test was giving considerably the higher proportion of positive tests, and that I could afford to increase the minimum absorption test titre regarded as positive to 1 : 160 and thus meet any criticism on the grounds that the standard for the absorption test was perhaps less stringent than that for the agglutination test. This has been done in Table 6, where the results of

Table 6. *Comparison of sheep-cell agglutination test and absorption test. Frequency distribution of titres of thirty-one samples of serum from twenty-two cases of glandular fever*

	Titre						
	1 : <10	1 : 10–1 . 20	1 : 40–1 : 80	1 : 160–1 : 320	1 : 640–1 · 1280	1 : 2560–1 : 5120	1 : >5120
Agglutination test	0	6	8	8	6	2	1
Absorption test	0	2	4	14	6	3	1
		1	0	0	0	0	0

The upper of the two rows of absorption test results includes all tests in which the antibody was absorbed (+ or + +) by ox cells.

The dotted line separates results regarded as normal or negative (to the left) from those regarded as abnormal or positive.

the two tests are compared: even with this more stringent standard the absorption test gives twenty-four positive reactions compared with seventeen for the agglutination test. But this is not all: in six of the seven negative absorption tests ox-cell absorption was + or + + and the titre at least 1 : 20, and as such results occurred in less than 2% of the series of normal people they may be regarded as suggestive of glandular fever though not definitely positive. This leaves only one completely negative absorption test, whereas at least the six agglutination titres below 1 : 40 must be regarded as negative, for Table 2 shows that such titres occur so commonly among normal people that no significance can be attached to them. Therefore I feel justified in claiming that the absorption test described in the appendix is a more sensitive test for glandular fever than the ordinary sheep-cell agglutination test.

Results in patients who had received injections of horse serum

The sheep-cell agglutinin found in serum sickness is reported to be absorbed by guinea-pig kidney (Table 3), so the absorption test ought to give negative

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results. I have not tested any patients actually suffering from serum sickness, but sera from seven scarlet fever patients who had each received intramuscular injections of 30 c.c. anti-streptococcal (horse) serum 8–13 days before all gave completely negative absorption tests, six of them showing no agglutination after treatment with guinea-pig kidney and one having a titre of 1 : 80 but with negative (−) ox-cell absorption. Sheep-cell agglutination tests on the unabsorbed sera, however, showed that the two highest titres were 1 : 160 (final concentrations, 0·5% cells, room temperature), a value which only just exceeds that of 99% of normal people (Table 2); but according to Davidsohn (1938, p. 2629) *concentrations of serum sickness antibody sufficient to raise the* titre considerably above the normal range are rare in the absence of clinical signs of the disease, so the completely negative absorption tests with the sera just mentioned suggest that false positive absorption test results due to serum sickness antibody are unlikely in people not at the time suffering from serum sickness. Positive absorption tests in patients who may be suffering from serum sickness should at present be regarded as of doubtful significance, because it is possible that the serum of such patients may contain too much serum sickness antibody to be absorbed completely by the quantity of guinea-pig kidney used in the test, and the residue, being absorbed by ox cells, would be mistaken for glandular fever antibody. But even if further investigation reveals that this does actually occur, the scope of the test will not be greatly restricted; for the possibility of serum sickness does not often complicate the diagnosis of glandular fever.

SUMMARY

1. The agglutination titres for sheep and for horse erythrocytes of 100 normal sera and twenty-seven glandular fever sera have been determined.

2. The results of the sheep-cell agglutination tests on normal sera differed considerably from those of Stuart, Burgess, Lawson & Wellman (1934) whose technique was used. The reason for this difference was not determined. It was thought to have some bearing upon the interpretation of sheep-cell agglutination tests in the diagnosis of glandular fever.

3. Although in the glandular fever sera the horse-cell titre was usually higher than the sheep-cell titre, the normal range for horse cells was also higher, and in the diagnosis of glandular fever there did not appear to be any advantage in using horse cells instead of sheep cells.

4. The value of absorption tests in the diagnosis of glandular fever is discussed and a new technique described which has practical advantages over other methods although it does not embody any important new principles.

5. Of 300 normal sera examined by this technique, five contained small amounts of an antibody indistinguishable from glandular fever antibody and ten others contained a sheep-cell agglutinin apparently, though not certainly, different from any yet recognized in human serum.

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6. A comparison of the results of the two tests on thirty-one samples of glandular fever serum showed the absorption test to be a more sensitive diagnostic test for glandular fever than the ordinary sheep-cell agglutination test.

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APPENDIX

Technique of the absorption test for glandular fever

A. *Preparation of reagents.*

(1) *Guinea-pig kidney emulsion.* The kidneys may be used fresh or they may be kept frozen until required. One pair of kidneys should yield at least 10 c.c. emulsion—sufficient for a dozen or more tests. Strip off the capsules and peri-renal fat and cut the kidneys into small pieces. Wash in running water until the fluid comes away clear and colourless, then transfer the tissue to a fine wire sieve such as is used for straining coffee. Rub the tissue through the sieve with a pestle into normal saline solution: this operation will be easier if

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the sieve be partially immersed in the saline. Reject any fat and connective tissue which adheres to the sieve. Autoclave the sieved kidney at 15 lb. per sq. in. for 20–30 min. When cool, rub the tissue through the sieve twice more, the second time lining the sieve with two thicknesses of butter-muslin. More saline may be used in order to wash as much as possible of the tissue through the sieve: the volume is quite unimportant at this stage. Centrifuge the suspension of kidney tissue; replace the supernatant fluid with fresh saline, stirring with a glass rod to break up any lumps and centrifuge again; then once more renew the saline and centrifuge. It does not matter if the supernatant fluid is still opalescent: pour it off and replace it by a small quantity (not more than four times the volume of the deposit) of a 0·5% solution of phenol in normal saline adding this in fractions and stirring to break up any lumps. Measure the volume of the resulting suspension after filling a Wintrobe haematocrit tube with some of it; centrifuge the Wintrobe tube until no further packing occurs; read the percentage of solid matter; calculate the total volume of solid matter in the suspension; and add sufficient phenol-saline to make the total volume six times that of the solid matter. The emulsion is now ready for use; it will keep for at least 6 months in the refrigerator.

(2) *Ox-cell suspension.* Wash the fresh ox cells in saline until the supernatant fluid is clear and colourless. Pour off the supernatant fluid from the last wash until the total volume is three to four times that of the packed cells. Re-suspend the cells and autoclave the suspension at 15 lb. per sq. in. for 20–30 min. When cool, shake well and strain through two thicknesses of butter muslin. Determine the haematocrit of the suspension, measure its volume, and add sufficient saline to make the total volume five times that of the solid matter. Add an equal volume of 1% solution of phenol in normal saline, thus making the final concentration of cells 10% and of phenol 0·5%. This stock suspension will keep for at least 6 months in the refrigerator. It should be diluted ten times with normal saline before use.

(3) *Sheep-cell suspension.* The sheep cells should have been kept at 4° C. for not less than 1 and not more than 7 days before use. After washing them three times with normal saline (when the supernatant fluid should be clear and colourless) a suspension containing 0·2 vol. % of packed cells is prepared either by measuring 1 vol. of packed cells and diluting to 500 vol. with normal saline or more accurately, by the haematocrit method used in standardizing the ox-cell suspension.

(4) *Standardized glandular fever antibody.* This is intended for use as a control, but experience has shown that it is not essential and that no serious misgiving need be felt if it is lacking. Obtain a sample of serum from a definite case of glandular fever and heat it to 56° C. for 15–30 min. Determine the agglutination titre of the unabsorbed serum using the dilute sheep-cell suspension (final concentration of cells 0·1%), reading the result by the centrifuge method (see 'Procedure (5)' below), and expressing the titre in terms of final concentration. The titre should be at least 1 : 320: the higher the better.

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Dilute the remainder of the serum with saline until its concentration is 32 times the titre value: e.g. if the titre is 1 : 320 (final concentration) dilute the serum 1 : 10. The glandular fever antibody appears to be very stable and if sterile precautions are observed throughout the diluted serum will keep for many months at 4° C., and one case of glandular fever will provide enough material for many tests.

B. *Procedure.*

(1) Heat 1·0 c.c. of the patient's serum in a water bath at 56° C. for 15–30 min.

(2) Prepare a negative control tube containing 1·0 c.c. saline, and also a positive control tube containing 0·3 c.c. of standardized glandular fever antibody and 0·7 c.c. of saline, if the former is available.

The exact size of the tubes used is immaterial provided that they will contain 2 c.c., will fit into a centrifuge, and have a bore not more than about 1 cm. diam.

(3) Add 0·1 c.c. of guinea-pig kidney emulsion to the tube containing the serum and also to the control tubes. Mix by inversion and allow the tubes to stand for 40 min. then add another 0·1 c.c. kidney emulsion to each tube. Repeat yet again after another 40 min. Then 0·3 c.c. kidney emulsion has been added to 1 c.c. of serum, and since the former contains one-sixth its volume of solid matter 0·25 c.c. of fluid will have been added; thus diluting the serum 1 : $1\frac{1}{4}$.

(4) Allow the tubes to stand for 40 min. after the last addition of kidney emulsion, then centrifuge them at moderate speed for a few minutes. Measure 0·3 c.c. of supernatant fluid from the serum tube into the first, 0·25 c.c. into the second, and 0·5 c.c. into the third of three tubes labelled 1–3. Add 0·25 c.c. saline to tube 2 and 1·5 c.c. saline to tube 3. Now tube 1 contains a serum dilution 1 : $1\frac{1}{4}$; tube 2, 1 : $2\frac{1}{2}$; and tube 3, 1 : 5.

(5) *Preliminary test.* Measure 0·5 c.c. of 0·2% sheep-cell suspension into each of three tubes labelled A–C. Transfer 0·5 c.c. of the contents of tube 3 to A and 0·5 c.c. of the supernatant fluid from the negative and positive control tubes to B and C respectively. Centrifuge tubes A–C at a speed between 2000 and 2500 r.p.m. for 5–10 min. Tap each tube until the deposit is suspended, leave them for 15 min. at room temperature, then tap again and examine for agglutination. Definite agglutination should be observed in the positive but not in the negative control tube, and unless there is definite agglutination in the tube containing the serum (tube A) the whole test is negative and nothing more need be done. As about 95% of people not suffering from glandular fever will give a negative result at this stage, the preliminary test is well worth while. If there is definite agglutination in tube A, proceed as follows:

(6) *Determination of titre.* Prepare two rows of tubes, the first consisting of six tubes each containing 1·0 c.c. saline, the second consisting of nine tubes of which the first three are those already numbered 1–3 and containing serum

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dilutions, followed by six empty tubes. Transfer 1 c.c. of the 1 : 5 serum dilution from tube 3 of row II to the first tube of row I. Since this tube already contained 1 c.c. saline the dilution is now 1 : 10. Transfer 0·5 c.c. from it to tube 4 of row II and 1 c.c. to tube 2 of row I, which will now contain 2 c.c. of serum dilution 1 : 20. After mixing transfer 0·5 c.c. from the latter tube to tube 5 of row II and 1 c.c. to tube 3 of row I. Continue in a similar manner to the end of the rows, saving the 1 c.c. from the last tube lest it should be necessary to put up further dilutions. Now each tube of row II contains eight times the concentration of serum in the tube of the same number in row I. Prepare an ox-cell control tube by measuring 0·3 c.c. of the standardized glandular fever antibody into an extra tube at the end of row II. Add 0·3 c.c. of ox-cell suspension (1%) to this control tube and also to tube 1 of row II; add 0·5 c.c. of ox-cell suspension to tubes 2–6 of row II; and add 0·5 c.c. of sheep-cell suspension to each tube of row I. Centrifuge all these tubes at a speed between 2000 and 2500 r.p.m. for 5–10 min. and read the sheep-cell agglutination titre after allowing the tubes to stand for 15 min. as in the preliminary test. Sometimes it will be necessary to extend the series of dilutions. The titre should be expressed in terms of final concentration (e.g. tube 1 of row I = 1 : 20 not 1 : 10). Do not shake the tubes of row II.

(7) *Test whether the antibody is absorbed by ox cells.* Having selected the last tube in row I which shows agglutination, take the tube of the same number ($=n$) from row II and also the tubes next on either side of this one (tubes $n+1$ and $n-1$). Transfer 0·5 c.c. of the supernatant fluids from each of these tubes and from the ox-cell control tube to four other tubes appropriately labelled, and add to each 0·5 c.c. of sheep-cell suspension. Mix, centrifuge and examine for agglutination after 15 min. as before. There should not be any agglutination in the ox-cell control tube, thus demonstrating that the ox-cell suspension employed does in fact absorb glandular fever antibody. The other three tubes all contain a greater concentration of serum than tube n of row I, so that unless some of the antibody has been absorbed by the ox cells they will show agglutination. If they do, the result is recorded as 'ox-cell absorption negative (−)'; but if, on the other hand, so much antibody has been absorbed that none of them shows agglutination the result is 'ox-cell absorption + +'. Intermediate results are denoted by ±, when only tube $n+1$ is free from agglutination; and +, when both $n+1$ and n are so free.

(8) *Interpretation of results*:

(*a*) Ox-cell absorption ± or − : the whole test must be regarded as negative, whatever the titre may be.

(*b*) Ox-cell absorption + or + + : suggestive of glandular fever whatever the titre, positive if the titre exceeds 1 : 40.

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