A VALUATION OF THE "AGGLUTINABILITY-FACTOR" IN DREYER'S SYSTEM.

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ONE of the main postulates, upon which Dreyer's system for agglutination-tests is based, is that no two suspensions of any organism can be taken as identical in agglutinability. This fact is now too well proven to need further demonstration, though it is still disregarded in many a current method.

There are two ways of meeting the difficulty, and both have been adopted at different periods in the manufacture of sterilized cultures for the Widal test (see Dreyer, 1909).

(1) All suspensions are brought to one and the same degree of agglutinability by suitable additions of culture-filtrate, to reduce the sensitiveness, or of salt-solution, to increase it.

(2) Each suspension is diluted with salt-solution to a suitable constant density, and its sensitiveness is carefully estimated by comparison with an arbitrarily chosen "standard" suspension, or with any other suspension already standardised against the latter. The agglutinability of the new suspension in relation to the standard can then be expressed numerically in the form of an "Agglutinability factor."

This factor, and its operation, form the subject of the experiments described in this paper—experiments in which an attempt has been made to estimate to what extent the agglutinability-factor succeeds in reducing to manageable order the numerical chaos that results from the variability of suspensions of an agglutinable bacillus (*B. typhosus*).

To this end, a number of specimens of blood were obtained from patients suffering from typhoid fever, and from persons who had been inoculated with *B. typhosus*.

From the stock of "standard" cultures of *B. typhosus*, which have been issued from the Standards Laboratory at one time or another, five were selected which showed a considerable range of agglutinability (*i.e.* a range of more than 100 per cent. difference), were of widely different ages (see list below), and included at least one suspension of relatively inferior quality (viz. T 32 which, though quite fit for routine use, had always been regarded as somewhat hyper-sensitive, and as an inferior clumper).

Each serum was suitably diluted, after a rough preliminary test, and titrated at one and the same time against each of the suspensions employed.

Since the full technique has been described in several recent articles (*e.g.* Gardner, A. D., 1918) and is identical in all essentials with the original method used by Dreyer and his co-workers since 1905 (see Schroeder, 1909), a detailed description of it will be omitted.

The long series of 12 tubes was used, except on a few occasions (when insufficient serum was available). Drop measurement was employed, for the sake of economy in material and labour. All readings were taken by the direct comparative method (Madsen). Three readings of each series were made; "Standard" agglutination and "trace" at two hours, and "trace" only at 24 hours.

SOURCE AND NATURE OF THE SERA TESTED.

Nos. 1 to 20 (inclusive), 26 and 27, were from persons in normal health who had been inoculated with *B. typhosus* during the war. Nos. 2, 3 and 20 had been inoculated with vaccine made in Oxford; No. 14 with American, and the remainder with Army vaccine. Three different "strains" of *B. typhosus* are therefore represented among this number. No. 26 was suffering from Paratyphoid B. fever at the time of bleeding. No. 31 was from a rabbit which had been inoculated with strain T. Lab.; and No. 32 from a rabbit inoculated with strain Edwin; the two last mentioned contained 0·3 per cent. Phenol. The remainder of the sera came from cases of *B. typhosus* infection drawn from N. England, London and Dublin. Two (Nos. 25 and 33) were old stored specimens to which antiseptic had been added in quantities unknown to the writer. The remainder were freshly drawn. Certain of the latter, as will be seen later, were spoiled or infected in the course of preparation and in transmission by post; and some of the few anomalous reactions observed were doubtless due to these accidents.

THE SUSPENSIONS USED.

All were standard cultures of *B. typhosus* which had at one time or another been issued from the Standards Laboratory for routine use. Their characteristics are given here in tabular form.

No.	Age	Strain	Agglutinability-factors at time of issue. (Later correction in brackets)		Character of agglutination
T 12	4 yrs	Typhoid Lab.	6·3	—	Good
T 18	3 yrs 2 mths	,,	5·8	—	Good
T 19	3 yrs 1 mth	,,	6·1	(5·1)	Good
T 21	3 yrs	,,	4·7	—	Good. (Rapid clumper)
T 26	2 yrs 6 mths	,,	6·7	—	Good
T 32	1 yr 4 mths	Typhoid Edwin	10·3	—	Not good. (Fine clumps; slow to reach maximum; hyper-sensitive)
T 35	1 yr		4·1	—	Good. (Very rapid clumper)

ARRANGEMENT OF TESTS.

It was intended to test all the sera with a single specimen of each of five cultures, T 18 to T 32; but the frequent opening of the large culture bottles, led eventually to the contamination of some of them. Subsequent sera were tested with three of the same cultures (T 18, T 21, T 32), from a number of smaller bottles, and two more cultures (T 12 and T 35) were added in place of T 19 and T 26 which had been all used up.

The experiments have therefore been arranged in two groups in accordance with the break in procedure.

The preparation and dilution of the sera and cultures, and all the readings, were done by the writer; while the measurements into agglutination-tubes were mostly carried out by Miss J. Jörgensen and Miss E. F. Stubington, both of whom were thoroughly familiar with the necessary technique, and to whom my thanks are due.

A single test only of each serum was made, in order to obtain figures comparable with those obtainable in routine practice.

CALCULATIONS.

In Tables I, II and III, are presented the relative quantities of each serum required to give the same degree of agglutination with each of the suspensions used.

Each figure was arrived at by taking the middle-term of the three readings, each of which was previously reduced in proportion to the corresponding figure

Table I.

Proportional quantities of serum required to give equal degrees of agglutination.

Sera No.	Suspensions					Sera No.	Suspensions				
	T 18	T 19	T 21	T 26	T 32		T 18	T 12	T 21	T 32	T 35
2	72	—	100	102	42	29	75	75	100	78	157
3	67	—	100	129	69	33	86	74	100	40	96
6	76	—	100	118	—	34	74	65	100	—	—
9	62	—	100	96	—	35	70	60	100	35	100
13	67	84	100	83	54	37	77	69	100	55	98
14	66	90	100	94	60	38	75	71	100	47	95
15	74	100	100	100	43	39	69	69	100	40	108
16	67	83	100	94	60	42	78	78	100	49	90
17	58	84	100	105	72	43	85	72	100	40	119
19	65	94	100	83	55	44	73	67	100	46	106
20	67	95	100	102	62	45	67	70	100	49	104
23	70	100	100	75	45	46	75	67	100	65	123
26	79	100	100	79	73	49	75	77	100	56	123
27	64	103	100	78	43	50	53	61	100	37	107
28	72	91	100	63	58	53	68	58	100	40	118
29	74	91	100	65	55	56	68	59	100	41	112
30	76	83	100	77	51	57	100	74	100	—	144
31	71	75	100	70	52	—	—	—	—	—	—
32	65	92	100	80	42	—	—	—	—	—	—
Mean	71·5	91	100	83	50		71·5	69	100	50	107·5

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Table II. Incomplete Tests.

Relative serum-quantities.

No.	T 18	T 12	T 21	T 26	T 32	T 35
7	85	—	100	100	—	—
8	54	—	100	75	50	—
12	61	—	100	122	61	—
36	66	60	100	—	40	100
40	96	85	100	—	58	71

Ratios of above figures to the means of Table I.

No.	T 18	T 12	T 21	T 26	T 32	T 35
7	1·19	—	1·00	1·21	—	—
8	0·76	—	1·00	0·90	1·00	—
12	0·85	—	1·00	1·47	1·22	—
36	0·92	0·87	1·00	—	0·80	0·85
40	1·36	1·23	1·00	—	1·16	0·66

NOTES.

No.

7. Incomplete readings.
8. Short series.
12. Short series. Haemolysis in serum.
36. Incomplete readings.
40. Short series.

Table III.

Aberrant figures.

No.	T 18	T 12	T 21	T 26	T 32	T 35
25	83	—	100	>200	200	81
41	341	318	100	—	545	<90
47	90	83	100	—	71	132
54	120	97	100	—	99	132
55	68	58	100	—	49	164
58	Cannot be stated in figures.					

NOTES.

No.

25. Old 1916 serum, containing unknown quantity of phenol. Slow acid reaction to neutral red. Agglutination very imperfect and peculiar.
41. Centrifuge accident. Contamination + +.
47. Haemoglobin + + in serum. (Centrifuge accident.)
54. T 21 bottle infected; ? others too.
55. Haemoglobin + + in serum.

for T 21, which was taken as unity. T 21 was chosen as the basic-standard for the series because it was used in every test. (T 18 would have done equally as well.)

At the base of the columns in Table VII is found the mean of all the observations for each suspension in the whole table.

In the few cases where the short series of tubes was used, the values of the readings have been calculated with Dreyer's reduction table.

In taking T 21 as unity, it is assumed that this culture gave always the exactly true figure. But this is of course not the case, as that suspension is susceptible of experimental and other uncontrolled variations to the same degree as the others. It should be remembered therefore that the actual deviations of T 21 from its mean behaviour with each serum is algebraically summed in each of the figures calculated for the proportional divergence of the other cultures from their mean ratio to T 21.

In other words, each ratio of a suspension to T 21 as unity throws on to the first suspension the whole burden of error, which should in reality be divided between the two. This slight weakness is inherent in all standard methods of comparison in which the standard itself has to be measured, by a method susceptible of some error, each time a test object or substance is to be compared with it.

The figures in Table IV are calculated thus:

The whole column (or columns) of figures in Table I under the heading of each suspension is added up and its arithmetical mean is found (see bottom of Table I). Each value in the columns is divided by the mean for the suspension, and the resulting figures are recorded in Table IV. In theory each should

Table IV.

Ratios of observations to their arithmetical means.

Serum	T 18	T 19	T 21	T 26	T 32	Serum	T 18	T 12	T 21	T 32	T 35
2	1·01	—	1·00	1·23	0·84	29a	1·05	1·09	1·00	1·56	1·46
3	0·94	—	1·00	1·56	1·38	33	1·20	1·07	1·00	0·80	0·89
6	1·06	—	1·00	1·42	—	34	1·03	0·94	1·00	—	—
9	0·87	—	1·00	1·16	—	35	0·98	0·87	1·00	0·70	0·93
13	0·94	0·92	1·00	1·00	1·08	37	1·08	1·00	1·00	1·10	0·91
14	0·92	0·99	1·00	1·13	1·20	38	1·05	1·03	1·00	0·97	0·88
15	1·03	1·10	1·00	1·20	0·86	39	0·97	1·00	1·00	0·80	1·00
16	0·94	0·91	1·00	1·13	1·20	42	1·09	1·13	1·00	0·98	0·84
17	0·81	0·92	1·00	1·27	1·44	43	1·19	1·04	1·00	0·80	1·11
18	0·91	1·03	1·00	1·00	1·10	44	1·02	0·97	1·00	0·92	0·99
20	0·94	1·04	1·00	1·23	1·24	45	0·94	1·01	1·00	0·98	0·97
23	0·98	1·10	1·00	0·90	0·90	46	1·05	0·97	1·00	1·30	1·14
26	1·10	1·10	1·00	0·95	1·46	49	1·05	1·12	1·00	1·12	1·14
27	0·89	1·13	1·00	0·94	0·86	50	0·74	0·88	1·00	0·74	0·99
28	1·01	1·00	1·00	0·76	1·16	53	0·95	0·84	1·00	0·80	1·10
29	1·04	1·00	1·00	0·78	1·10	56	0·95	0·85	1·00	0·82	1·04
30	1·06	0·91	1·00	0·93	1·02	57	1·40	1·07	1·00	—	1·34
31	0·99	0·82	1·00	0·84	1·04						
32	0·91	1·01	1·00	0·96	0·84						

be unity. The percentage deviations from theory may be directly read by observing the difference between 100 and each of the figures deprived of its decimal point.

The table may be interpreted thus: if each suspension gave results in every case in accordance with its true degree of agglutinability, all the figures in this table would be 1·00. But a number of unknown or uncontrollable factors

introduce an error into nearly every figure. Each represents the percentage divergence of the actual findings from the theoretical, in comparing each suspension with T 21, which is assumed to yield no error.

For example, since we found in Table I that in general 71·5 parts of serum give as high a degree of agglutination with T 18 as 100 parts do with T 21; this proportion should theoretically be found in every case. But in point of fact there is nearly always a greater or lesser divergence from this ratio.

This being so we must proceed to determine whether these variations are of manageable or of unmanageable proportions; whether, in fact, they throw credit or discredit upon the principle of standardisation, and the use of a reduction-factor.

It will be useful to consider the matter from a second point of view.

In the first place let us consider what is the average and what the maximal deviation from theory in these sets of figures. Analysing them we find a 10 per cent. or greater deviation in 38 per cent. cases; a 25 per cent. or greater in 8·2 per cent. cases; and a 50 per cent. or more (each time actually 56 per cent.) in two cases or 1·2 per cent. In other words nearly two-thirds of observations give less than 10 per cent. deviation; more than nine-tenths give less than 25 per cent., and about ninety-nine hundredths give less than 50 per cent. The mean deviation is about 6 per cent.

It is of considerable interest that the older suspensions T 12, T 18 and T 21 yield much more consistent results than the later ones. Age indeed is all to their advantage as regards stability, but I think that the chief explanation lies in certain imperfections of the war-time materials with which the later suspensions were made. The two most variable are T 26 and T 32, the former made with strain T. Lab. and the latter with strain T. Edwin. The notion of "strain-differences" in the usual sense finds support neither here nor in any other part of these experiments.

Since the agglutinability-factor claims to be an agency whereby any serum can be brought to give (within experimental limits) the same titre with all suspensions, a simple test of its value may now be applied.

If by use of factors calculated from these experiments we obtain a distinctly closer approximation to the ideal of identical titres than is attained without them, the value of the factor is established.

On applying the test to the figures in Table I, we find the results to be overwhelmingly in favour of the factor. In Tables V and VI are set out figures for two pairs of cultures, viz. (*a*) T 21 with the suspension (T 32) most unlike it in agglutinability, and (*b*) T 21 with the suspension most similar to it (T 19). The numbers represent the ratios of agglutinability found by comparison of T 32 or T 19 with T 21, the latter always taken as unity.

In the case of the pair T 21, T 32 (Table V) when the figures are unreduced (col. 1), the latter culture yields constantly a very much higher titre than the former, the ratio varying around a mean of 2 to 1.

The second column shows the percentage differences of the "titres" found

for the pair of cultures. *E.g.* the number 2·38 means that the titres were in the proportion of 238 to 100, *i.e.* the former titre exceeds the latter by 138 per cent. The process is then repeated but with the introduction of the reduction-factor, calculated from Table I (T 21 = 1·0, T 32 = 2·0). The figures in the first column are simply divided by the reduction-factor for T 32. In theory the result should be a column of ones; in fact, it is a column of figures varying on either side of one. At the bottom will be found the mean percentage differences, with and without use of the reduction-factor. Without it, the mean is 102 per cent.; with it, only 17·4 per cent. A method whereby titres

Table V.

No. of Serum	Titre found with T 32 / Titre found with T 21 (No reduction-factor used)	Percentage difference of the two titres	Titre found with T 32 / Titre found with T 21 (Reduction-factor used)	Percentage difference of the two titres
2	2·38	138	1·19	19
3	1·45	45	0·73	27
13	1·85	85	0·93	7
14	1·67	67	0·84	16
15	2·33	133	1·17	17
16	1·67	67	0·84	16
17	1·39	39	0·79	21
19	1·82	82	0·91	9
20	1·61	61	0·80	20
23	2·22	122	1·11	11
26	1·37	37	0·69	31
27	2·33	133	1·17	17
28	1·72	72	0·86	14
29	1·82	82	0·91	9
30	1·96	96	0·98	2
31	1·92	92	0·96	4
32	2·38	138	1·19	19
29a	1·28	28	0·64	36
33	2·50	150	1·25	25
35	2·86	186	1·43	43
37	1·82	82	0·91	9
38	2·13	113	1·07	7
39	2·50	150	1·25	25
42	2·04	104	1·02	2
43	2·50	150	1·25	25
44	2·17	117	1·09	9
45	2·04	104	1·02	2
46	1·54	54	0·77	23
49	1·78	78	0·89	11
50	2·70	170	1·35	35
53	2·50	150	1·25	25
56	2·44	144	1·22	22
Mean	2·02	102	1·01	17·4

Reduction-factor improves figures in 31 cases.
Reduction-factor fails to improve figures in one case (see text).

$$\text{Reduction-factor} = \frac{k \text{ of T 32}}{k \text{ of T 21}} = \frac{9·4}{4·7} = 2·0.$$

Table VI.

Serum No.	Titre found with T 19 / Titre found with T 21 (No reduction-factor used)	Percentage difference of the two titres	Titre found with T 19 / Titre found with T 21 (Reduction-factor used)	Percentage difference of the two titres
13	1·19	19	1·08	8
14	1·11	11	1·01	1
15	1·00	0	0·91	9
16	1·20	20	1·10	10
17	1·19	19	1·08	8
19	1·06	6	0·97	3
20	1·05	5	0·95	5
23	1·00	0	0·91	9
26	1·00	0	0·91	9
27	0·97	3	0·88	12
28	1·10	10	1·00	0
29	1·10	10	1·00	0
30	1·20	20	1·10	10
31	1·33	33	1·21	21
32	1·09	9	0·99	1
Mean	1·11	11	1·01	7

Reduction-factor improves figures in 67 % of cases.
Reduction-factor fails to improve figures in 33 % of cases.

are obtained within 17 per cent. of identity is obviously preferable to one which yields a more than 100 per cent. divergence.

In all cases in this table except that of serum 29a, there is a closer approach to the ideal identity of titre when the reduction-factor is used, than when it is not; and the gain is usually very great. In the case of serum 29a the reduction-factor has actually increased slightly the difference between the two cultures.

That this occurrence is a technical error is made almost certain by the fact that 29a is the same serum as 29, under which heading it showed the usual large improvement of ratio on use of the factor.

Table VI shows a similar series of calculations for the pair of suspensions T 21, T 19. Here the agglutinabilities of the two cultures are very close to one another, the relative agglutinability factors being in proportion of 1·0 to 1·1, and therefore no large improvement could be brought about by the use of the factor. Nevertheless, there is a definite improvement; for the divergence from theory is diminished in more than 70 per cent. of instances.

In the remaining 27 per cent. of cases there is an increased divergence, but never amounting to more than 9 per cent.

Tables for the remaining suspensions have not been included, for they all show similar phenomena. The necessary material for calculation is all to be found in Table I, should any reader care to check this statement.

The deductions can be put in another way:

1. If any two cultures differ in agglutinability by 50 per cent. or upwards, the use of a reduction-factor is indispensable for obtaining comparable figures (titres or units) with the two.

of Tables V and VI, comparing each suspension in turn with T 21, and determining what difference is made by the use of the factor, we find that:

The factor for T 12 improves the results greatly.

    ,,   ,,   T 18   ,,   ,,   ,,

    ,,   ,,   T 19 makes the results a little worse.

    ,,   ,,   T 26 improves the results slightly.

    ,,   ,,   T 32   ,,   ,,   enormously.

    ,,   ,,   T 35 makes no difference.

Some of these factors were recalculated, and improved, at some interval after they had been fixed for official use (sometimes rather hurriedly under war-pressure). Such was the case with T 19, whose factor was altered (for standardising purposes) to 5·1, a figure which agrees closely with the new factor 5·2.

To the figures given in this paper it will perhaps be objected that a more accurate method of comparison has been employed than that available in routine work, and that therefore a much greater margin of error must be allowed for such work.

No doubt that is true, but in actual fact a pretty close approximation is obtained by use of short-series of tubes, and the reduction-table, when suspensions are compared one with another.

Table II contains the few sera tested in this manner, and it will be seen that, though the average deviations are considerably greater than those in Table IV yet the maximum does not reach 50 per cent.

Since in the curve-tracing of agglutinins for diagnosis (the only "clinical" operation requiring much precision) variations of 100 per cent. are regarded as of dubious significance, and only 200 per cent. or more as of certain value (Dreyer), even a 50 per cent. wobble due to a change of suspension in the middle of the curve will not give rise to errors of judgment.

Nevertheless, as recommended by Topley, Platts and Imrie (1919), it is always well, in such cases, for the worker to do a simple restandardisation test in parallel of the two suspensions with the actual serum in use.

For this will eliminate all chance of confusion.

In conclusion, attention is drawn to Table III, in which I have placed the sera which gave results quite outside the scope of expectation.

The notes appended supply, in my opinion, sufficient proof that the materials were unreliable, and that the figures cannot be included in the main experimental results.

Serum No. 58 was found to give fine traces of flocculation with T 32 (the worst culture of my series), and also with a living saline suspension of *B. typhosus*, up to a dilution of some hundreds. With two other good suspensions it failed to react at all. The most probable explanation of this phenomenon seems to lie in a chemical contamination of the serum, giving rise to a partial clumping of some suspensions; but I could not obtain any actual evidence that this explanation is the true one.

My best thanks are due to the bacteriologists and medical officers who responded so helpfully to my requests for typhoid sera. The work was carried out at Prof. Dreyer's request.

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