THE AGGLUTINATION OF STANDARD AGGLUTINABLE CULTURES OF THE FLEXNER-GROUP OF DYSENTERY BACILLI BY NON-DYSENTERIC HUMAN SERA.

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In considering the agglutination-test for bacillary dysentery, the War Office Committee on Dysentery in 1918 decided, on the available evidence (Kruse, C. J. Martin, Andrewes and Inman, Gettings, Murray), that five strains of Flexner-*Y* bacilli, representing five separable, though closely related, serological types, might be taken as adequately covering the whole serological range of the species. The Vaccine Department of the R.A.M. College, Millbank, and the Standards Laboratory at Oxford accepted these findings as basis for practical work, and the latter institution proceeded to issue the five varieties of "Standard agglutinable cultures" and "Standard agglutinating sera."

At that time two types only were being produced, viz. "Flexner" and *Y*. These strains later on were relabelled *V* and *W* respectively in the new classification. Before any standard cultures of dysentery bacilli at all were issued, an adequate number (120) of sera of healthy human adults had been tested upon standard cultures of B. Shiga and B. Flexner (later *V*), and a calculation of the results made it possible to fix a limit above which the agglutination by a patient's serum of any standardized suspension of these bacilli could be taken as indicating a dysentery infection. The figures thus obtained cannot be applied to work with unstandardized suspensions, for not only do different strains of one serological type differ profoundly in agglutinability, but different suspensions of the same strain show considerable variations in this respect. The "normal limit," if it is to apply to all standardized suspensions of a type, must be expressed in standard units. Each serological type must be treated as an entirely separate entity.

The reduction-factor of agglutinability.

The absolute magnitude of the reduction-factor for agglutinability of the original arbitrarily chosen standard suspension of any bacillary type may be fixed at anything one pleases. In practice it is decided by convenience. For B. Shiga and B. Flexner the size of the reduction factor of the original standard suspension was arranged so that the normal human sera should not be found

to contain more than 10 standard units in the case of males, nor 20 in the case of females.

When standard cultures of another strain, a bacillus labelled *Y* from the Pasteur Institute, were added to the list, and later, when the number of Flexner-group types was increased to five (*V*, *W*, *X*, *Y*, *Z*) it was assumed that the limits of normal agglutination for the additional types would be similar to the limit for the original Flexner (*V*). This assumption was, however, not fully justifiable, for, as the experiments herein recorded will show, certain of the type-strains are more highly susceptible to agglutination by non-dysenteric human sera than the original Flexner (*V*). It is a simple matter to make the necessary correction for this higher agglutinability by increasing the reduction-factors for the affected strains.

The practical value of the "normal limit."

In the first place, the degree to which the sera of healthy persons agglutinate Flexner-group bacilli depends greatly on the technique employed and upon the sensitiveness of the strain agglutinated. A study of the literature from the earliest days down to the present time discloses a remarkable series of discrepant observations on this point. Suffice it to say that the figures have ranged between 1/20 (Pillsbury, 1903) and 1/2500 (Walker-Hall, 1916). This speaks clearly enough for the desirability of a constant and standardized method of measurement.

In this paper firstly we are only concerned with figures obtained by Dreyer's method. Secondly, the variations shown by individual "normal" sera are strikingly wide.

This is the greatest stumbling-block in the determination of a sound normal limit. For however many normal sera one may test, it is always possible that there may be some others that give higher titres than any of those tested. It is moreover quite probable that any batch of "normals" may include one or more cases of past infection.

With these considerations in view, it is clearly only possible to attach a relative value to any normal limit that may be established. It is, in fact, a question of proportion, and all we can do is to fix a line above which an extremely small percentage of healthy persons will be found to react. And in doing this we almost certainly cause many reactions that are really "positive" to be labelled "negative."

It is impossible to emphasize too strongly the fact that one or more repetitions of the agglutination test at intervals of three to four days are necessary in order to establish the presence of an active infection. This applies in varying degrees to all diseases in which the agglutination-test is employed (Courmont, 1897, 1900; Jórgensen, 1904; Schroeder, 1909; Dreyer, 1906).

EXPERIMENTS.

The Sera. Most of the sera were from blood-specimens sent for the Wassermann reaction. Some were from healthy students and members of the staff. None of the individuals yielding the blood was suffering from clinical dysentery.

Dilution of Serum. One in ten was the regular routine. Occasionally a higher dilution had to be used, owing to shortage of serum. For the same reason the first tube of the series had sometimes to be omitted.

The Types of Standard Cultures used:

(1) *V.* "Oxford Flexner." One suspension used throughout, of reduction-factor = 5·0.

(2) (*a*) W_1. "Logan" strain. One suspension only. Factor = 2·5.
(*b*) W_2. A number of sera were also tested with "Oxford *Y*," a strain originated from the Pasteur Institute, where it was known as *Y*. This was classed by Andrewes and Inman as *W*. One suspension used throughout. Factor = 3·6.

(3) *X.* "Toner" strain. Two suspensions employed. One factor = 3·0; the other = 3·2.

(4) *Y.* "Ledingham" strain. Earlier tests done with a suspension of factor = 4·7, later ones with a different suspension of factor = 6·5.

(5) *Z.* "Whittington" strain. Two suspensions, of factors = 3·0 and 3·7 respectively, employed at different times.

All these strains were used in the form of fully matured formalized sterile "standard agglutinable cultures" (Dreyer, 1906, 1909; Gardner, 1918).

Readings. The degree of agglutination in each tube was recorded after 4½ hours at 55° C. "Total," "Standard" or "trace." A second reading after a further 18–20 hours at room temperature was always taken.

EXPRESSION OF RESULTS.

With "Standard agglutination" (marked flocculation without sedimentation) at 4½ hours as the criterion, a calculation has been made of the number of sera which showed this degree of agglutination, or a higher one, at the different dilutions.

These are shown in Table I.

In Table II the number of sera which show 5, 10 and 20 standard units respectively are tabulated. The official reduction-factors were used for calculating the units, and Dreyer's reduction-table (Dreyer and Inman, 1917) was made use of when no tube showed an actual reading of standard, but only readings of total or trace were available.

In some cases it was difficult to assign a proper value in units to a given reading; for example, when such readings occurred as trace at 1/25, trace at 1/50, nothing at 1/125. Here, in order to err in the direction of safety, the highest of the possible values has always been recorded.

Table I. *Showing the number of sera per cent. which gave standard agglutination at various dilutions with the different types of Flexner standard cultures.*

	Dilution of serum	Percentage of sera showing standard agglutination with the various cultures					
		V	W_1	W_2	*X*	*Y*	*Z*
♂	1/25	8	2	16	0	33	16
	1/50	1	0	8	0	20	8
	1/125	0	0	0	0	0	0
♀	1/25	9	13	29	0	58	0
	1/50	6	6	13	0	24	0
	1/125	0	0	0	0	6	0
	Actual number of sera tested with the various cultures						
♂		76	69	25	76	76	76
♀		33	33	15	33	33	33

N.B. No serum gave standard agglutination at 1/250.

Table II. *Showing the number of non-dysenteric human sera per cent. giving from 5 to 20 standard agglutinin-units with the various types of Flexner standard cultures.*

	Number of standard units	Percentage of sera giving the number of units shown in left-hand columns with the various cultures					
		V	W_1	W_2	*X*	*Y*	*Z*
♂	5	15	13	28	0	28	4
	10	0	3	8	0	11	3
	15	0	0	4	0	0	3
	20	0	0	4	0	0	1
♀	5	12	19	50	0	55	23
	10	3	12	20	0	18	3
	15	0	9	13	0	6	0
	20	0	3	0	0	6	0

The total number tested with the various cultures is the same as in Table I.

Discussion of Tables I and II.

Table I sets out the proportion of cases in which agglutination of "standard" degree (marked flocculation without sedimentation) or more was found in dilutions of 1/25, 1/50, and 1/125. In no case did it occur at 1/250.

This table is of limited value, since it merely records the results found with the particular suspensions used. If the same sera were tested with other standard suspensions of the same types of bacilli, the results would be by no means necessarily the same. For none of the new suspensions would necessarily be of the same degree of sensitiveness as those actually employed.

This objection is removed in Table II where the results are set out after the reduction of the dilution-figures to standard-units by dividing with the agglutinability-factors of the suspensions used.

It must be stated at this point that when a suspension A has been shown, by means of specific agglutinating sera, to be more sensitive than a suspension B, the same relationship between them will be evident when the two are tested with "normal" sera. This is not to say that the exact numerical ratio is absolutely maintained in every test. In practice one always finds small

variations. One serum, specific or normal, may exaggerate the difference between two suspensions; another may minimise it. But these small differences do not appreciably diminish the value of the standard-unit of measurement.

Table II. In this table it is seen that a large proportion of non-dysenteric sera contain five standard units for one or other type of bacillus. Of the male sera a fair number contain 10, but only a few as much as 15, and very few 20. Of the females a fair sprinkling have 15 units, and a few reach 20.

Taking each type in turn:

V. No male serum reached 10 units nor any female serum 20 units.

W_1. 3 per cent. reach 10 units in the males, but none go higher. 3 per cent. of the females reach 20 units.

These figures show that the reduction-factors for this type of culture were made originally (arbitrarily) very slightly too low, with the result that the agglutination-limit of normal sera is very slightly above 10 and 20 units for males and females respectively. If the factor for the W_1 suspension be changed from 2·5 to 3·3, then all the male sera come out at less than 10 units, and all females at less than 20.

An increase of all constants of suspensions of this type in the ratio of 1·32/1·00 is therefore desirable.

W_2. This *W* race (*Y* "Pasteur") is not now employed in the making of standard cultures, so it need not be considered in detail. It is more highly agglutinated by normal sera than W_1, as it also is by specific sera made with either of the two races of *W*.

X is peculiar in that it is not affected by any normal serum at 1 in 25.

Y is the strain which exceeds the (♂ 10, ♀ 20) limit more than any other. 11 per cent. of males reach 10 units, and 6 per cent. of females reach 20.

A considerable enlargement of the reduction factors of suspensions of this type must be made in order to bring down the figures to the (♂ 10, ♀ 20) limit.

An increase of the factors in the proportion of 166/100 is sufficient to bring down all ♂ sera to less than 10 units, and all ♀ sera to less than 20.

Z is agglutinated by few male sera, but 3 per cent. of these agglutinate it to more than 10 units. No female serum reaches 15 units.

It is probably unnecessary in the light of these facts, to increase the factors of *Z* suspensions. For 10 units cannot, with any type of bacillus, be taken as diagnostic but only as suspicious. Without alteration of constants the probability here already amounts to 97 chances in every 100 of being right.

In view of the disconcerting variations in agglutinating power which are found in normal sera one is in a difficult position. To fix the constants of agglutinability too low, would inevitably cause false "positive" diagnoses. Whereas the alternative error of fixing the constants too high would tend to prevent the detection of numerous specific reactions of feeble intensity.

As we have already said, the whole principle of fixing a normal limit is

really unscientific and open to the objection that where the individual limits vary greatly, the fixing of one limit for all must necessarily fix it much too high for the majority.

The exigencies of clinical pathology nevertheless make it desirable to compromise with scientific truth and to agree on a limit which will be of some assistance to workers who are pressed for time.

The use of the limit in diagnosis should always be supplemented whenever possible by repetitions of the blood test and the establishment of a curve of agglutinin-production. When this is done fully, the limit can be entirely dispensed with, and a diagnosis made on the same lines as in the diagnosis of enteric fever in inoculated individuals (Dreyer, Gibson and Walker, 1916; Dreyer and Inman, 1917).

Summary.

1. The results are recorded of the degrees to which standard agglutinable cultures of five serological types of Flexner-group bacilli (*V*, *W*, *X*, *Y*, *Z*) are agglutinated by non-dysenteric human sera.

2. Certain of these cultures are more highly agglutinated by some normal human sera than was anticipated.

3. In order that the sera of non-dysenteric subjects may all (or practically all) be found to contain less than 10 or less than 20 units in males and females respectively, the reduction-factors of agglutinability at present in use for certain of these cultures must be somewhat increased.

4. The conception of a limit, above which no "normal" serum will be found to react, is only valid as a compromise between truth and convenience. Repetition of agglutination-tests capable of measuring the fluctuation of agglutinins in active infection is the only safe and scientific procedure.

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