THE ANTI-GLOBULIN TECHNIQUE APPLIED TO THE DETECTION OF NON-AGGLUTINATING ANTIBODY AGAINST *SALMONELLA TYPHI* O IN HUMAN SERA

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The anti-globulin technique introduced by Coombs, Mourant & Race (1945) is now widely used in the detection of the non-agglutinating (hyper-immune) form of anti-*Rh* antibody. The essence of this technique is the exposure of cells which have been coated with antibody to the action of rabbit antiserum containing an antibody against human globulin. The test is, in principle, similar to one developed by Moreschi (1908), for the detection of sensitization of rabbit red cells by goat anti-rabbit red-cell serum, in which the washed, sensitized cells were exposed to the action of rabbit anti-goat serum. In the preparation of suitable antiserum for the test, Coombs & Mourant (1947) found that sera from rabbits immunized with whole serum were as effective in demonstrating sensitization as were those from rabbits immunized with γ -globulin. The capacity of these sera to detect sensitization appeared to be due to the presence of a specific anti- γ -globulin antibody.

The anti-globulin test has been used by Morgan & Schutze (1946) for the demonstration of non-agglutinating antibody in human sera to *Shigella shigae* and *Salmonella typhi* Vi antigens. These workers, using a rabbit anti-human γ -globulin serum, obtained in all the sera examined by them a higher titre by the anti-globulin technique than by a direct agglutination technique. They showed that this amplification, which was appreciably greater immediately following artificial immunization, was apparently not the result of a non-specific adsorption process; from the different ratios of agglutinating to anti-globulin titres obtained in different sera they concluded that it was probably due to a type of antibody distinct from agglutinating antibody.

The present communication reports the results of tests which have been carried out with human sera and *Salm. typhi* O suspension. Instead of the rabbit anti- γ -globulin serum used by Morgan & Schutze, rabbit antiserum prepared by intravenous immunization with whole human serum has been employed.

The technique adopted was as follows: serial dilutions of the serum under examination were made in saline, and to 1 c.c. of each dilution in Kahn type tubes (75 × 10 mm.) was added 1 c.c. of *Salm. typhi*, 901/0 suspension. This suspension was harvested and preserved in 1 in 1000 HgI₂ saline, and was approximately equivalent in opacity and agglutinability by *Salm. typhi* O rabbit antiserum (Standards Laboratory) with standard agglutinable *Salm. typhi* O suspension. After incubation for 1 hr. at 37° C. the cells were centrifuged, washed twice in saline and resuspended in four drops of saline. Two drops of the resulting suspension were transferred to a second row of tubes; to each tube of the first series

was then added two drops of 1 in 40 dilution (in 1 in 1000 HgI₂ saline) of the rabbit antiserum previously absorbed with T 901/O suspension; and to each tube of the second series, two drops of normal rabbit serum similarly absorbed and diluted. The tubes were incubated for a further hour at 37°C., and following the addition of 1 c.c. of 1 in 2000 HgI₂ saline to each, were allowed to stand at room temperature overnight; the results were read by examination of the sediment with a magnifying mirror. The titre was taken as the dilution of serum in the tube immediately preceding that showing a clean spot, and will be referred to as the indirect titre. At the same time as the anti-globulin test, a direct agglutination test was also carried out; in these the total volume of fluid used was 1 c.c. and the tubes were incubated for 2 hr. at 37°C. before standing at room temperature overnight.

Table 1. *Indirect titres*

Group	Serum dilutions								Averages
	20-320	640	1280	2560	5120	10,240	20,480	40,960	
	Number of sera with end-points at each dilution								
Normal	19	3	—	—	1	—	—	—	434
T.A.B.	19	7	3	2	1	—	—	—	694
Infection immune	1	1	—	2	2	2	2	1	10,778

Titres expressed as reciprocals of serum dilutions. Direct titres of normal and T.A.B. sera fell within the range 20-320.

The results obtained with sixty-six sera are shown in the accompanying tables. The sera were derived from three main groups of individuals:

(1) Normal individuals who had not been immunized with T.A.B. vaccine and who did not give a history of a previous attack of typhoid fever.

(2) Individuals immunized with T.A.B. vaccine; the interval between immunization and examination ranged from 1 to 18 months. Two sera in this group with a direct titre of less than 40 are excluded as the exact titre was not determined.

(3) This group, which will be referred to as the 'infection immune' group, comprises carriers and other individuals with a past history of typhoid infection, from whom *Salmonella typhi* was isolated. The four carriers exhibited a carrier state for periods varying from 8 months to 3 years; in three, *Salmonella typhi* was isolated either immediately prior to or following serological examination, and in one 3 months prior to examination. The three individuals classified as 'cured carriers' had, prior to treatment with penicillin and sulphathiazole approximately 17 months before examination (Bigger & Daly, 1949), been carriers for 3-6 years; for 1 year following treatment, *Salmonella typhi* had not been isolated from repeated faecal specimens. The four non-carriers were individuals with bacteriologically proved typhoid infection, occurring 1-3 years before the time of serological examination. In each of these, three separate cultural examinations of both faeces and urine, following serological examination, had given negative results.

From the results presented, it will be seen that the majority of sera have shown an amplification of twofold or greater by the indirect technique; furthermore, the degree of amplification found is not constant for all sera.

Table 2. Degrees of amplification

Group	Amplification								Averages
	< 2	2	4	8	16	32	64	128	
	Number of sera exhibiting each degree								
Normal	10	7	2	1	2	—	—	1	4.1
T.A.B.	7	8	8	5	1	3	—	—	6.1
Infection immune	1	—	1	—	—	4	4	1	41.1

Table 3. Titres of sera from 'infection immune' individuals

Serum	Category	Direct titre	Indirect titre	Amplification
Ev.	Non-carrier	80	5,120	64
Art.	Non-carrier	160	640	4
S.D.	Non-carrier	160	160	—
A.D.	Non-carrier	80	2,560	32
Mul.	Carrier	320	20,480	64
McH.	Carrier	1,280	40,960	32
Smi.	Carrier	320	20,480	64
Beg.	Carrier	160	10,240	64
Hen.	Cured carrier	160	5,120	32
Gle.	Cured carrier	80	10,240	128
Fitz.	Cured carrier	80	2,560	32

Titres expressed as reciprocals of serum dilutions.

The highest figures for both amplification and indirect titres occur in the 'infection immune' group—a correlation shown not only in the averages of the values obtained but also in their frequency distribution. Thus, out of ten sera in this group in which the direct agglutination titre was in the normal range of 320 or less, eight gave an indirect titre of 2560 or greater, and an amplification of 32-fold or greater as compared with 1 in 23 of the normal group.

The T.A.B. immunized group show, on the other hand, only slightly greater indirect titres and amplifications than the normal—a finding in substantial agreement with that of Morgan & Schutze with sera examined 2-3 years after immunization.

In addition to the sera shown above, we have examined sera from five cases of bacteriologically proven typhoid fever, four taken in the second and one in the seventh week. With each of these an indirect titre of 10,240 was obtained; the amplifications found were 4, 8, 8, 16, 32, with an average amplification of 8.4.

The fact that some of the ex-cases examined show low, indirect titres, while those of the carriers are consistently high suggests that the indirect test may have a value as a screen test in the examination of suspected carriers. It would seem probable that the carrier condition is unlikely if the indirect titre is low, e.g. 640 or less. The test might have a similar exclusion value in the diagnosis of typhoid fever. In general, in view of the independent variation of direct and indirect titres, the indirect test should be of considerable utility in experimental work in the evaluation of antibody response.

It has been shown by Hayes (1947), that certain sera have a low titre against heated T 901/O suspension, while the globulin fraction alone may give a high titre.

Hayes suggested that, in such sera, agglutination of the sensitized bacteria was inhibited by some substance present in the albumen fraction of normal serum. It was thought that some such inhibition might explain the differences between indirect titres and agglutinin titres in our sera. On fractionation of a number of sera showing high indirect titres it was found, however, that the globulin fraction was still capable of sensitizing and was not transformed by fractionation into agglutinating antibody.

SUMMARY

A technique for carrying out the anti-globulin test with *Salm. typhi* O suspension is described; this technique has been used to examine a number of human sera. The degree of amplification of agglutinin titre and the final titres obtained were appreciably greater in the sera of individuals with a history of *Salm. typhi* infection, some of whom were carriers, than in the sera of normal individuals.

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