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SEROLOGICAL DIAGNOSIS OF THE ENTERICA BY THE METHOD OF QUALITATIVE RECEPTOR ANALYSIS.

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(With a Diagram.)

I. INTRODUCTION.

BACTERIOLOGICAL diagnosis of the Enterica is only established with certainty on recovery of the infecting organism by recognised laboratory methods. In default, recourse must be made to a serological investigation into the agglutinating power of the patient's serum, when, theoretically, little difficulty ought to be experienced. In enterica suspects, unvaccinated or uninfluenced by previous enterica disease, demonstration of agglutinins reacting with the *Bact. typhosum*, *paratyphosum* A, B, or C[1] in relatively high dilutions of their sera should be considered adequate evidence. In the vaccinated, elevation of agglutination titre—as determined by repeated examinations during the course of the disease—has been generally accepted as criterion for existence and type of infection. The principle of ascending dilutions, on which such "quantitative serum analysis" is based, is explained by the fact that in an infection with one organism a specific agglutinin is formed for that particular organism and group agglutinins for other organisms closely related biologically; the specificity, therefore, of the agglutination reaction must be wholly dependent on the basis of dilution, the specific agglutinin being present in greatest quantity and operative in a range much exceeding that of the secondary group agglutinins. Reasonable as this view may seem in principle, it is far from easy of application in practice. Lack of accurate information as to the day of disease on which serum was taken, unreliable case histories in regard to vaccination or previous enterica infections, and inability of private practitioners to induce patients to submit to repeated blood collection constituted here primary difficulties.

In Palestine the prophylactic vaccination of all immigrants and of contacts of actual enterica cases has been in force since 1920. Added to this, a majority of the male population still retain effects of the repeated vaccinations enforced by military service. It is not surprising, therefore, that the reading and interpretation of agglutination reactions during the years when quantitative

[1] The term *Bact. paratyphosum* C, often used loosely to cover several varieties of paratyphoid organisms, refers throughout this article to the *Bact. paratyphosum* β, serologically identical with *Bact. suipestifer*.

The bacteriological nomenclature used throughout this article is that recently adopted by the *Bulletin of Hygiene* (February 1928) as being most suitable for universal use "pending some form of international agreement."

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serum analysis alone was practised frequently presented a problem incapable of accurate solution. The case reported in 1924 of a patient admitted to hospital three times within six months and thrice diagnosed typhoid fever well illustrates the type of error likely to be made under such conditions.

Unexpected results of the Gruber-Widal test became increasingly common and the occurrences outlined below served still further to complicate diagnosis.

(*a*) The finding of the end titre of a serum to be identical for typhoid and paratyphoid organisms alike precluded, especially in the absence of case history, differentiation between enterica disease and "anamnestic reaction" due to previous vaccination. Attempts at differentiation by the routine employment of Gaertner strains, as recommended by Dawson in 1915, were not, on the whole, attended by satisfactory results. Group agglutination with the sera of enterica patients—deciding factor in the test—frequently failed to occur, doubtless due to a hyposensitiveness of the Gaertner organisms then in use.

(*b*) *The occurrence of the "paradox reaction."* Here a serum re-submitted for examination shows height of agglutination titre quite at variance with the previous finding. Thus a serum giving an end titre of 1 : 500 dilution for *Bact. typhosum* on the tenth day of disease proved, on second examination in these laboratories ten days later, able to agglutinate the *Bact. paratyphosum* B in a 1 : 2000 dilution. At the time of first examination B was not agglutinated even in a dilution of 1 : 50 serum. Subsequent recovery of B from the stool confirmed the diagnosis of paratyphoid B.

(*c*) Cases in which diagnosis of an infecting organism by estimation of end titre of the serum does not correspond with that established by isolation of the true causal agent by cultural methods from blood, stool, urine or cerebro-spinal fluid.

One case described by us in 1926 will suffice to indicate the inefficiency of the quantitative method of analysis alone in diagnosis. Patient's serum submitted on the eighth day of disease agglutinated organisms of the T.A.B.C.G. group in the dilutions indicated: T 1 : 50, A 1 : 50, B 1 : 100, C 1 : 50, G 1 : 50. A tentative diagnosis of B infection resulted. Re-examination of the serum on the 17th day of illness showed agglutination to be effected as follows: T 1 : 50, A 1 : 200, B 1 : 100, C 1 : 50, G 1 : 50. In that, however, meningeal symptoms had by then become predominant, lumbar puncture was carried out and on laboratory examination *Bact. enteritidis* Gaertner was isolated in pure culture from the cerebro-spinal fluid. On account of its rarity, the finding in this instance was controlled and verified by Dr A. Felix. It followed, therefore, that the infecting organism was a pure G strain, and that the agglutination tests performed failed entirely to indicate the true relative proportions of primary and secondary agglutinin content in the patient's serum.

Now, by enabling a distinction to be drawn between primary, specific, homologous agglutinins and secondary, non-specific, group, heterologous agglutinins the absorption test of Castellani has, admittedly, proved of utmost

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value in the diagnosis of closely related organisms. For example, the occurrence of marked co-agglutination of *Bact. paratyphosum* B by the *Bact. paratyphosum* C (serologically akin to *Bact. suipestifer*) so complicates differential diagnosis as ordinarily to compel application of absorption tests. This test, however, is generally considered too cumbersome and complicated for routine laboratory procedure, and, as will be instanced later, proves also on occasion to be ineffective.

Any method, therefore, in the light of the foregoing, able to distinguish between agglutination due to vaccination and that occurring during enterica disease, able to recognise "anamnestic reaction" and eliminate "paradox reactions," able to supersede quantitative serum analysis with its many pitfalls, able to render Castellani's absorption test unnecessary, and capable of being carried out by any experienced worker in a well-equipped laboratory, must obviously be of utmost value in serological diagnosis. Such a method is that designated "qualitative receptor analysis." Study of the theory since 1917—its year of discovery by Weil and Felix—and the demonstrations to us of its practical value by Felix, our former colleague, resulted in a trial being given to the method in 1925. At first quantitative serum analysis in accordance with Dreyer's technique was performed in parallel, but this was shortly found to be unnecessary.

From a consideration of our results during the past two years we now feel that the claims advanced in favour of the qualitative method have received sufficient confirmation to justify its adoption by well-staffed and well-equipped laboratories as standard in routine serological diagnosis of the enterica.

II. THE THEORY OF QUALITATIVE RECEPTOR ANALYSIS.

(*a*) *Its origin and development.*

During investigations into the nature of sero-diagnosis in typhus fever, it was observed by Weil and Felix (1916) that while *Proteus vulgaris* X_{19} and *Proteus vulgaris* X_2 each reacted specifically with the sera of typhus cases or convalescents, such reaction was by no means equally marked, the X_{19} strain commonly being agglutinated by very much higher dilutions of serum than the X_2. Rabbits' artificially produced immune serum, on the other hand, was found to agglutinate both X_{19} and X_2 strains in equal titre. The nature of agglutination was dissimilar, however, for whereas that brought about by patients' serum showed itself as small, firm, granular, slowly forming flakes, with immune serum agglutination occurred rapidly and was characterised by the appearance of large, voluminous, loose, coarse flakes. Again, while patients' serum agglutinated only X strains, immune serum reacted often to the end of titre with X strains and ordinary saprophytic strains of *Proteus* alike. So striking was the difference between the effects of the two types of sera on various species of *Proteus*, that it was generally concluded that the agglutinins of typhus patients' serum did not owe their origin to the antigens of the

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X strains. Weil and Felix (1917), by proving that in the antigenic apparatus of X strains there exist two different types of receptor, did much to clear up the question. From the character of their colonies the X strains were found capable of differentiation into H- (Hauch = hazy) and O- (ohne Hauch = clear) forms, of which the H-form possessed two receptors, the O-form only one. Now the receptor of the O-form evokes the small-flaking agglutinins which react only with the X strains, while the H-receptor forms the large-flaking agglutinins, which react with the X strains as well as with ordinary saprophytic strains of *Proteus*. It was shown further that, whereas in typhus patients' serum only O-agglutinins (small-flaking) occurred, in rabbits' immune serum produced by the X strains H-agglutinins were present in addition.

Serologically considered, therefore, there was great difference between the H- and O-forms; immune serum of the O-form (OX_{19} or OX_2) contained only one agglutinin which, reacting specifically with the homologous bacteria alone, agglutinated them in small flakes (O-agglutinin); immune serum of the H-form, on the other hand, possessed two agglutinins, viz.: a specific small-flaking O-agglutinin, and a non-specific, large-flaking H-agglutinin reacting with homologous and heterologous bacteria alike.

Briefly then: the antigenic structure of the O-form consists of one type of receptor (O-receptor), that of the H-form of two types (H- and O-receptors). The serum of typhus patients being in all respects identical with an immune serum produced by OX_{19} (containing only O-agglutinins) and in artificial immune serum produced by the H-form there being both O- and H-agglutinins present, the difference between the behaviour of patients' serum and artificial immune serum to *Proteus* X organisms could be readily accounted for.

It was later demonstrated that all strains of *Proteus* were equipped with double "binding groups," O- and H-receptors, and that in the entire *Proteus* genus specific agglutination was effected by the O-receptors, group agglutination by the H-receptors, agglutination in the former being of the small flake variety, in the latter of the large.

The discovery by Sachs (1918) that HX strains, if heated to 80° C., behaved exactly like O-forms of these strains, permitted the deduction that the H-receptor had disappeared and that the H-receptors were thermolabile, the O-receptors thermostable. Felix and Mitzenmacher (1918) then showed that if the H-forms of *Proteus* were heated for two hours at 100° C. the H-receptors were so affected that the heated antigen in its behaviour corresponded exactly with the O-form obtained by culture.

The terminology H- and O-receptors, founded as it was on the cultural characteristics of the *Proteus* group, was thereafter superseded by one dependent on the inherent qualities of the receptors themselves, and so the use of "labile" instead of H- and of "stable" instead of O- became general. At the same time the agglutinins corresponding to the two types of receptors, the essential characteristics of which determined the nature of the agglutination, were named large-flaking (interacting with the labile receptors) and small-flaking

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(interacting with the stable receptors). More recently the O-agglutinins reacting with the O-antigen have been termed "stabilotropic," the H-agglutinins combining with the H-antigen "labilotropic."

Weil, Felix and Mitzenmacher (1918) were next in a position to assert that all bacteria of the typhoid-paratyphoid-enteritidis groups possessed the double type of receptors.

The following summary indicates the more important findings in regard to the organisms with the diagnosis of which this paper is mainly concerned:

1. In rabbits' immune sera produced against the various organisms of the typhoid-paratyphoid-enteritidis groups two well-defined types of agglutinins are present, large-flaking and small-flaking.

2. Each organism of the group, *Bact. typhosum*, *enteritidis* Gaertner, *paratyphosum* A and B, has two forms of "receptor" or "binding group" in its antigenic structure:

   (*a*) Labile receptors reacting with the large-flaking labilotropic agglutinins of the corresponding immune serum.

   (*b*) Stable receptors interacting with the small-flaking stabilotropic agglutinins.

3. The labile receptors are specific for each of the members of the group.

4. The stable receptor apparatus is complex:

   (*a*) Main receptors which, like the labile main receptors, are specific for each of these types; and

   (*b*) Secondary receptors (group receptors) which possess close relationship.

5. Specific agglutination is primarily dependent on the large-flaking labilotropic agglutinins reacting with the labile receptor of the particular organism,

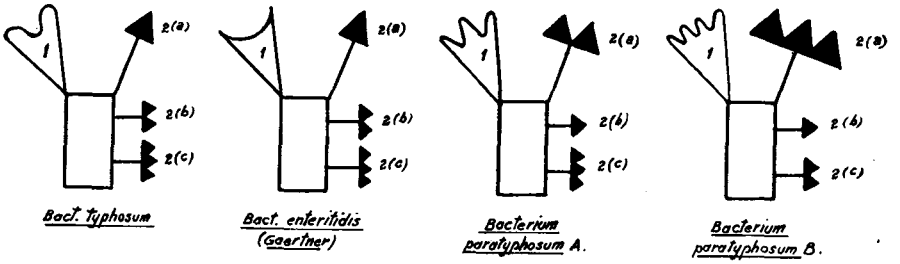

Diagram to illustrate double receptor type of antigenic apparatus possessed by the typhoid-paratyphoid-enteritidis group.

1 = labile (specific) receptors, interacting with large-flaking agglutinins of corresponding immune serum.

2 = stable receptors:

2 (*a*) = main receptors, specific for each member of the group.

2 (*b*) + 2 (*c*) = secondary or group receptors showing close inter-relationship among the various organisms.

Note. The stable antigenic apparatus of *Bact. typhosum* is identical with that of *Bact. enteritidis* (Gaertner), the two organisms differing only in respect of their labile (specific) receptors.

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while group agglutination is determined by the combination of small-flaking stabilotropic group agglutinins with the stable receptor apparatus.

6. The stable apparatus of *Bact. typhosum* and of *Bact. enteritidis* Gaertner is identical, the two bacteria differing only in respect of their labile receptors.

The diagram inserted will, it is hoped, serve to make clear the relation the organisms of this group bear to each other.

It will be noted that here the specificity is dependent upon conditions the converse of those obtaining in the case of *Proteus*. While in the *Proteus* group small-flaking agglutination plays a strictly specific rôle in regard to the differentiation of strains, in the typhoid-paratyphoid group specificity is wholly bound to the large-flaking agglutination.

Table I. *Agglutination of homologous strains with specific immune sera.*

(*a*) *Bact. typhosum* and *Bact. enteritidis* Gaertner.

		Agglutination			
		Typhoid R		Gaertner$_3$	
Rabbit	Serum dilution	Immune serum Ty. R obtained by heating Ty. R culture to 53° C.	Immune serum Ty. R obtained by heating Ty. R culture to 100° C.	Immune serum G_3 obtained by heating G_3 culture to 53° C.	Immune serum G_3 obtained by heating G_3 culture to 100° C.
1	1 : 50	+ + +l+s	+ + +s	+ + +l+s	+ + +s
	1 : 100	+ + +l+s	+ + +s	+ + +l+s	+ + +s
	1 : 200	+ + +l+s	+ + ±s	+ + +l+s	+ + +s
	1 : 500	+ + +l+s	+ +s	+ + +l+s	+ + ±s
	1 : 1000	+ + ±l+s	±s	+ + ±l+s	+s
	1 : 2000	+ ±l	—	+ +l	—
	1 : 5000	+l	—	+l	—
2	1 : 50	+ + +l+s	+ + +s	+ + +l+s	+ + +s
	1 : 100	+ + +l+s	+ + +s	+ + +l+s	+ + +s
	1 : 200	+ + +l+s	+ +s	+ + +l+s	+ + +s
	1 : 500	+ + ±l+s	+s	+ + +l+s	+ +s
	1 : 1000	+ +l	—	+ +l	—
	1 : 2000	+l	—	+l	—
	1 : 5000	—	—	±l	—
3	1 : 50	+ + +l+s	+ + +s	+ + +l+s	+ + +s
	1 : 100	+ + +l+s	+ + +s	+ + +l+s	+ + +s
	1 : 200	+ + +l+s	+ + +s	+ + +l+s	+ +s
	1 : 500	+ + +l+s	+ + ±s	+ +l+s	±s
	1 : 1000	+ + +l+s	+s	+ ±l	—
	1 : 2000	+ +l+s	—	+l	—
	1 : 5000	+l	—	—	—
4	1 : 50	+ + +l+s	+ + +s	+ + +l+s	+ + +s
	1 : 100	+ + +l+s	+ + +s	+ + +l+s	+ + +s
	1 : 200	+ + +l+s	+ + +s	+ + +l+s	+ + +s
	1 : 500	+ + +l+s	+ + +s	+ + +l+s	+ + ±s
	1 : 1000	+ + +l+s	+ +s	+ + +l+s	+ ±s
	1 : 2000	+ + ±l+s	+ ±s	+ +l	—
	1 : 5000	+ ±l	—	+l	—

+ + + = bacteria completely agglutinated and sedimented, supernatant fluid clear.
+ + = much sediment, supernatant fluid turbid.
+ = little sediment, flakes in supernatant fluid visible on use of an aplanatic lens × 10– × 20.
± = agglutination in fluid visible on similar magnification.
l = large flakes.
s = small flakes.
l+s = large and small flakes.
53° C. = suspension of organisms heated in water bath for ½ hour at 53° C.
100° C. = suspension of organisms heated for 2 hours at 100° C. (in the autoclave without pressure).
Readings made after 2 hours at 37° C. and 22 hours at room temperature.

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Table I—*continued.*

(*b*) *Bact. paratyphosum* A and *Bact. paratyphosum* B.

Rabbit	Serum dilution	Agglutination: Paratyphoid A_1: Immune serum Para. A_1 obtained by heating Para. A_1 culture to 53° C.	Agglutination: Paratyphoid A_1: Immune serum Para. A_1 obtained by heating Para. A_1 culture to 100° C.	Agglutination: Paratyphoid B_2: Immune serum Para. B_2 obtained by heating Para. B_2 culture to 53° C.	Agglutination: Paratyphoid B_2: Immune serum Para. B_2 obtained by heating Para. B_2 culture to 100° C.
5	1 : 50	+ + +l+s	+ + +s	+ + +l+s	+ + +s
	1 : 100	+ + +l+s	+ + +s	+ + +l+s	+ + +s
	1 : 200	+ + +l+s	+ +s	+ + +l+s	+ + +s
	1 : 500	+ +l+s	±s	+ + +l+s	+ +s
	1 : 1000	+l	—	+ + ±l+s	+s
	1 : 2000	±l	—	+ +l+s	±s
	1 : 5000	—	—	+l	—
6	1 : 50	+ + +l+s	+ + +s	+ + +l+s	+ + +s
	1 : 100	+ + +l+s	+ + +s	+ + +l+s	+ + +s
	1 : 200	+ + +l+s	+ +s	+ + +l+s	+ + +s
	1 : 500	+ + ±l+s	+s	+ + +l+s	+ +s
	1 : 1000	+ ±l	—	+ +l+s	±s
	1 : 2000	±l	—	+l	—
	1 : 5000	—	—	±l	—
7	1 : 50	+ + +l+s	+ + +s	+ + +l+s	+ + +s
	1 : 100	+ + +l+s	+ + +s	+ + +l+s	+ + +s
	1 : 200	+ + +l+s	+ +s	+ + +l+s	+ + ±s
	1 : 500	+ +l+s	±s	+ + +l+s	+ +s
	1 : 1000	+l	—	+ +l+s	±s
	1 : 2000	±l	—	+l	—
	1 : 5000	—	—	—	—
8	1 : 50	+ + +l+s	+ + +s	+ + +l+s	+ + +s
	1 : 100	+ + +l+s	+ + ±s	+ + +l+s	+ + +s
	1 : 200	+ + +l+s	+ ±s	+ + +l+s	+ + +s
	1 : 500	+ +l	—	+ + +l+s	+ +s
	1 : 1000	+l	—	+ +l+s	±s
	1 : 2000	±l	—	+ ±l	—
	1 : 5000	—	—	±l	—

Note. The monovalent rabbit immune sera used throughout were obtained by three intravenous injections of saline suspensions of bacteria.

It is of interest to record at this point that the H-receptor, in addition to its thermolability, is easily destroyed by appropriate treatment with alcohol, acid and alkali, agents which do not affect adversely the O-receptor. Curiously enough the behaviour of the two agglutinins is the converse of their corresponding antigens in respect of these physical and chemical influences.

Further, and this is of utmost importance from serological considerations, the H-receptor may disappear completely under normal conditions in patients infected with the enterica just as in artificial culture media—cf. H- and O-forms of *Proteus*—a loss of antigenic substance leading to growth in culture of the O-form with its single antigen in contradistinction to the normal H-culture possessing both O- and H-antigens.

Experiments conducted in these laboratories during 1926 (unpublished) substantiate the essentials of the above summary. They are recorded in Tables I, II and III.

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Table II. *Experiments showing that large-flaked agglutination occurs only with the homologous strain while group agglutinins are exclusively small-flaking.*

(*a*) *Bact. typhosum* and *Bact. enteritidis* Gaertner.

Rabbit	Monovalent immune serum	Serum dilution	Agglutination with living emulsion of the strains: Ty. R	G_3	A_1	B_2
1	*Bact. typhosum* at 53° C.	1 : 50	+ + +l+s	+ + +s	+ +s	+ +s
		1 : 100	+ + +l+s	+ + +s	+ ±s	+ +s
		1 : 200	+ + +l+s	+ + +s	+s	+ ±s
		1 : 500	+ + ±l+s	+ +s	—	±s
		1 : 1000	+ +l+s	+s	—	—
		1 : 2000	+ +l	—	—	—
		1 : 5000	+ ±l	—	—	—
2	*Bact. typhosum* at 100° C.	1 : 50	+ + +s	+ + +s	+s	+ ±s
		1 : 100	+ + +s	+ + +s	±s	±s
		1 : 200	+ +s	+ +s	—	—
		1 : 500	+ ±s	+s	—	—
		1 : 1000	+s	—	—	—
		1 : 2000	—	—	—	—
3	*Bact. enteritidis* Gaertner at 53° C.	1 : 50	+ + +s	+ + +l+s	+s	+ ±s
		1 : 100	+ + +s	+ + +l+s	+s	+s
		1 : 200	+ + +s	+ + +l+s	±s	±s
		1 : 500	+ +s	+ +l+s	—	—
		1 : 1000	+s	+l+s	—	—
		1 : 2000	—	+l	—	—
		1 : 5000	—	+l	—	—
4	*Bact. enteritidis* Gaertner at 100° C.	1 : 50	+ + +s	+ + +s	+s	+s
		1 : 100	+ + +s	+ + +s	±s	±s
		1 : 200	+ + +s	+ + +s	—	—
		1 : 500	+s	+s	—	—
		1 : 1000	±s	—	—	—
		1 : 2000	—	—	—	—

Note. The monovalent immune sera used in this experiment were obtained by three intravenous injections of saline suspensions of bacteria heated as indicated.

(*b*) *Bact. paratyphosum* A and *Bact. paratyphosum* B.

Rabbit	Monovalent immune serum	Serum dilution	Agglutination with living emulsion of the strains: Ty. R	G_3	A_1	B_2
5	*Bact. paratyphosum* A at 53° C.	1 : 50	+ + +s	+ + +s	+ + +l+s	+ +s
		1 : 100	+ + +s	+ + +s	+ + +l+s	+ ±s
		1 : 200	+ +s	+ +s	+ + +l+s	+s
		1 : 500	+s	+s	+ + +l+s	—
		1 : 1000	—	—	+ +l	—
		1 : 2000	—	—	+l	—
		1 : 5000	—	—	+l	—
6	*Bact. paratyphosum* A at 100° C.	1 : 50	+ + +s	+ + +s	+ + +s	+ ±s
		1 : 100	+ + +s	+ + +s	+ + +s	+s
		1 : 200	+ ±s	+ ±s	+ + ±s	—
		1 : 500	±s	±s	+ ±s	—
		1 : 1000	—	—	—	—
		1 : 2000	—	—	—	—
7	*Bact. paratyphosum* B at 53° C.	1 : 50	+ + +s	+ + +s	+s	+ + +l+s
		1 : 100	+ +s	+ +s	±s	+ + +l+s
		1 : 200	+s	+s	—	+ + +l+s
		1 : 500	—	—	—	+ + +l+s
		1 : 1000	—	—	—	+ +l
		1 : 2000	—	—	—	+ +l
		1 : 5000	—	—	—	+l
8	*Bact. paratyphosum* B at 100° C.	1 : 50	+ + +s	+ + +s	±s	+ + +s
		1 : 100	+s	+s	—	+ + +s
		1 : 200	±s	±s	—	+ +s
		1 : 500	—	—	—	+s
		1 : 1000	—	—	—	—
		1 : 2000	—	—	—	—

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Table III. *Absorption Tests.*

To prove that the stable antigenic apparatus of *Bact. typhosum* is identical with that of *Bact. enteritidis* Gaertner.

Culture	Serum dilution	Typhoid immune serum 1 : 50 dilution saturated with emulsions made from 3 agar cultures of Ty. O_{901}	Typhoid immune serum 1 : 50 dilution saturated with emulsions made from 3 agar cultures of G_3	Typhoid immune serum untreated
Ty. O_{901}	1 : 50	—	—	+ + + s
	1 : 100	—	—	+ + + s
	1 : 200	—	—	+ + s
	1 : 500	—	—	+ s
	1 : 1000	—	—	—
G_3	1 : 50	—	—	+ + + s
	1 : 100	—	—	+ + + s
	1 : 200	—	—	+ + ± s
	1 : 500	—	—	+ ± s
	1 : 1000	—	—	± s

Culture	Serum dilution	Gaertner immune serum 1 : 50 dilution saturated with emulsions made from 3 agar cultures of Ty. O_{901}	Gaertner immune serum 1 : 50 dilution saturated with emulsions made from 3 agar cultures of G_3	Gaertner immune serum untreated
G_3	1 : 50	+ + + l	—	+ + + l + s
	1 : 100	+ + + l	—	+ + + l + s
	1 : 200	+ + + l	—	+ + + l + s
	1 : 500	+ + + l	—	+ + + l + s
	1 : 1000	+ + + l	—	+ + + l + s
Ty. O_{901}	1 : 50	—	—	+ + + s
	1 : 100	—	—	+ + + s
	1 : 200	—	—	+ + ± s
	1 : 500	—	—	+ + s
	1 : 1000	—	—	± s

Note. Typhoid culture O_{901} possesses only one receptor—a small flake producing antigen—and is therefore similar in its action to a strain of typhoid heated for two hours at 100° C. without pressure.

(*b*) *Laboratory method of application.*

Each member of the group under review has now been proved to possess a complex antigenic structure, a stable O-antigen and a labile H-antigen. So very constant and characteristic differences do these two antigens and their corresponding antibodies present that, in regard to agglutination tests, a qualitative instead of a quantitative analysis of serum can be substituted for diagnostic purposes. The "Qualitative Receptor Analysis" method has been so named from its essential ability to distinguish between the two qualitatively different receptor types of antigen normally found in organisms of a group such as the typhoid-paratyphoid-enteritidis.

Technique of the Reaction. This may, at first sight, appear somewhat complicated. Certain cultures are selected of which the receptor content and power of reacting with immune sera have been accurately measured. Such cultures, with a known content of receptors, will detect the presence of small- and large-flaking agglutinins in any serum submitted for examination. The receptor content of each organism employed must of necessity be maintained at a

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constant level if observations are to be accurate and comparable, and this is ensured by each strain being carefully tested at short intervals against varying dilutions of immune sera of which the agglutinin content is known.

Bacterial strains for routine investigation will be found of service only if particular attention is given to the culture media employed; thus, the bouillon forming the basis of agar medium is made from fresh meat, not from meat extracts; the reaction of the media is to be *p*H 7·4 exactly; sterilisation is effected by steam at 100° C. without pressure, to obviate the occurrence of hydrolytic changes; agar slants for sub-culture may only be used if water of condensation is present. Upon accurate titration, upon the degree of humidity and upon the nature of the sugar content depends chiefly a normal development of labile and stable receptors, and it is on the relative development of these that the agglutinability of the various strains rests. The various organisms having been tested and proved satisfactory, the main test may be proceeded with.

To a young (18–24 hours) agar slant culture of each strain selected for routine investigation 2·5 c.c. normal saline solution are added, and suspension is aided by gently rubbing the surface growth with a sterile pipette which afterwards remains in the culture tube. All pipettes used in this test deliver, when held vertical, 20 drops to 1 c.c. Bacterial suspensions are allowed to stand 15–30 minutes before use, so that recognition of the occurrence of spontaneous agglutination may enable "rough" and unsuitable forms to be discarded[1].

Primary dilutions of patient's serum with normal saline 1 : 10 are now prepared in 6 × $\frac{5}{8}$ in. tubes.

Three rows of tubes $3\frac{1}{2} \times \frac{3}{8}$ in. are arranged in a suitable metal rack, each horizontal row containing the same number of tubes as bacterial suspensions prepared. Here the number is nine.

Into each of the nine tubes of the third row are placed 20 drops (= 1 c.c.) N.S.S., into each tube in the second row 19 drops N.S.S., into each tube in the first row 18 drops N.S.S.

Of the 1 : 10 dilution of patient's serum in the 6 × $\frac{5}{8}$ in. tubes two drops are added to each first row tube and one drop to each second row tube. To the third row tubes no serum is added.

As performed here, therefore, the reaction requires the employment of nine series of tubes, three tubes in each series—each series corresponding to the particular bacterium of the nine strains with which it is proposed to analyse the patient's serum.

The nine bacterial cultures being now so placed that each one is directly behind its corresponding series, from each culture tube, by means of the pipette

[1] Cultures showing evidence of "going rough" must be plated out on agar medium and "smooth" colonies picked from the resultant growth. Cultures made from such "smooth" colonies, however, may not be employed until their bacteria have shown by the ordinary tests with immune sera indicated above that they possess the normal receptor content of the strain.

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originally used to emulsify the surface growth, one drop is transferred to each tube of its series.

The first tube of each of the nine series thus contains 1 c.c. of 1 : 100 P.S. + 1 drop of bacterial emulsion, the second tube 1 c.c. of 1 : 200 P.S. + 1 drop of bacterial emulsion, the third tube 1 c.c. N.S.S. + 1 drop of bacterial emulsion. The tubes in the third row control the various strains employed.

The tubes are now shaken and the rack is placed in the incubator for two hours at 37° C.; after removal it is left to stand at room temperature for from 18–20 hours. Although final readings should not be made until at least 24 hours have elapsed since the setting up of the tubes, it is of importance to be able to detect early signs of agglutination, often visible within the first few hours, and to differentiate the type of "flaking" which then occurs.

As has already been observed, "flaking" is of two kinds, large and small, and in the case of the latter, particularly when just in process of formation, the use of an aplanatic lens × 10–× 20 is imperative in reading results. Although it has to be admitted that certain difficulties in reading can only be overcome by experience, especially when, as so frequently happens, both types of agglutination occur together, yet, for the purposes of the present article, the undermentioned characteristics should make differentiation between large and small flakes possible.

Large-flake agglutination.	*Small-flake agglutination.*
1. Forms rapidly.	1. Forms slowly.
2. Settles rapidly.	2. Settles slowly.
3. Flakes large and of varying size.	3. Flakes small and uniform.
4. Sediment voluminous.	4. Sediment scanty.
5. Sediment easily dislodged.	5. Sediment very difficult to dislodge.
6. Supernatant fluid turbid.	6. Supernatant fluid clear.

The strains used here in routine agglutination tests are as follows:

1. An O-form of *Bact. typhosum* isolated by Felix and named Ty. O_{901}. It is of remarkable value in the early detection of small-flaking agglutinins, being not only much more sensitive for the homologous main agglutinins than other strains of typhoid or Gaertner, but in heterologous sera also for the group agglutinins of A and B, of which the stable receptors are commonly much less sensitive. As, normally, small-flaking agglutinins appear considerably earlier in sera than the large-flaking variety, it is only to be expected that this strain reacts with the small-flaking agglutinins contained in typhoid patients' sera several days before the occurrence of large-flaking agglutination, but it interacts also, often much more readily, with the heterologous agglutinins produced in the course of A and B infections than do the respective homologous strains A and B.

2. The Rawlings strain of *Bact. typhosum*, referred to in the text as Ty. R. This form is equipped with the normal content of labile and stable receptors possessed by typhoid bacteria.

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3. A strain of *Bact. typhosum* in which the labile receptor alone is developed far in excess of normal; it is known as Ty. L.

The routine use of these three strains furnishes adequate control for all agglutinations performed with typhoid patients' sera, and permits, in conjunction with the Gaertner strain (reacting only with small-flaking agglutinins), absolutely accurate estimation of large- and small-flaking agglutinins coexisting in the same serum.

4. A strain of *Bact. enteritidis* Gaertner, G_3, which has, especially in respect of its stable antigen, preserved a most satisfactory receptor content. The importance of maintaining this G_3 strain at constant receptor level cannot be over-emphasised because, as will be seen later, the whole value of the test depends on the normal development of its receptors under certain conditions.

5. A strain of *Bact. paratyphosum* A.

6. A strain of *Bact. paratyphosum* B.

It has been already noted that the stable receptors of these two organisms are relatively much less sensitive in their appreciation of their respective homologous agglutinins than the heterologous Ty. O_{901}.

7. A strain of the *Bact. paratyphosum* C. (C is *sensu restricto* = β, an organism serologically identical with the *Bact. suipestifer* Voldagsen, and one closely allied to the *Bact. paratyphosum* B.)

This strain serves to control the large-flaking agglutination taking place in a patient's serum with B. It is considered necessary for the sake of completeness to offer an explanation of the antigenic structure of this organism (here termed C) and of its true relation to the *Bact. paratyphosum* B.

Normal C bacteria are also equipped with a double receptor type of antigen. Sera of patients infected with such C strains agglutinate homologous bacteria completely in granular, small flakes, the supernatant fluid becoming perfectly clear, but, in addition, they agglutinate B in scanty, coarse, large flakes, the supernatant fluid in this case remaining turbid.

Certain C strains, however, do not possess the typical double receptor apparatus; these are to be regarded as O-forms. Sera of patients infected with these strains do not agglutinate B at all. Again, whereas B immune serum agglutinates the normal C strains in a coarse, large-flaking and incomplete manner, it fails entirely to agglutinate the O-forms.

These differences go to prove that the receptors possessed by normal C strains and which react with B strains are absent in the O strains. The O strains stand, then, in the same relation to normal C strains as the O-forms of *Proteus* to the H-form, while the binding groups, lacking in the O strains of C, which react in the case of normal C strains with the non-specific B-agglutinins in large flakes and incompletely, correspond with the H-receptors of the *Proteus* strains.

The relation of normal C, C-O-form, and normal B becomes apparent from the results of agglutination tests performed with sera derived from patients suffering from a typical C infection.

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Absorption tests with patients' serum confirm the results of agglutination, since saturation with an O-form of C removes only the small-flaking agglutinins, while saturation with normal C strains is followed by the absorption of small-flaking C-agglutinins, large-flaking C-agglutinins and large-flaking B-agglutinins.

Further absorption tests, however, carried out with immune serum prove that the agglutinins which precipitate the C strains and the B strains in large flakes are not identical, for whereas B immune serum is rendered inert for all

Table IV.

Serum dilution	Normal C strain	O-form C strain	Normal B strain
1 : 100	+ + +l+s	+ + +s	+ +l
1 : 200	+ + +l+s	+ + +s	+ +l
1 : 500	+ + +l+s	+ + +s	+ +l
1 : 1000	+ +l+s	+ +s	+l

strains through saturation with B, saturation with a C strain removes the large-flaking C-agglutinins but not the B-agglutinins. In other words, the homologous strain can combine with both agglutinins but the heterologous only with that agglutinin corresponding to itself. It follows, therefore, that in the antigenic structure of B exist not only a main receptor, which reacts with the large-flaking B-agglutinins, but a secondary receptor capable of combining with C-agglutinins and which is qualitatively identical with the analogous C main receptor.

In the same way it can be shown by saturating a rabbit's immune normal-C serum with C and B respectively that the large-flaking C- and B-agglutinins cannot be identical. But, because saturation with normal C removes the large-flaking B-agglutinins from the serum, it is clear that the receptor apparatus of C must possess in addition to the main receptor corresponding to the large-flaking C-agglutinins another secondary receptor qualitatively identical with the analogous B main receptor[1].

In the serum of a patient suffering from a typical C infection are found two agglutinins, a specific small-flaking O-agglutinin which reacts with the homologous C bacterium only and a non-specific large-flaking H-agglutinin which reacts with the heterologous and homologous bacteria alike.

The *Bact. paratyphosum* C, therefore, is in respect of its antigenic structure and serological reactions in an "intermediate" position in regard to the members of the enterica group.

8. A strain of *Proteus vulgaris* HX_{19}.

9. A strain of *Proteus vulgaris* OX_{19}.

The two strains of *Proteus* are in routine use because a request for the performance of both Widal and Weil-Felix reactions accompanies practically every serum submitted for laboratory examination. Small-flaked agglutina-

[1] The whole subject of the relationship between B and C will be found discussed in detail by Weil and Felix (1920) in the *Zeitschrift für Immunitätsforschung.* Orig. **29**, 24–91, under the title "Doppeltypus der Rezeptoren in der Typhus-Paratyphus-Gruppe."

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tion, as has already been indicated, constitutes a positive reaction in the Weil-Felix test.

(*c*) *The method applied to diagnosis of the enterica.*

Nearly 2000 sera have now been examined here by the method of qualitative receptor analysis, with the result that of the total 694 positive findings[1] 465 were diagnosed typhoid fever, 5 paratyphoid A, 41 paratyphoid B, 3 paratyphoid C and 180 "enterica, type not differentiated." In addition, the reactions given by 42 sera could be ascribed to the effects of previous vaccination.

Accurate estimation of its true value is, however, only possible if the results obtained by this method are analysed in relation to the principal varieties of sera submitted for examination, and such sera fall naturally into three categories:

1. Sera of unvaccinated persons actually suffering from enterica infection.
2. Sera of vaccinated persons not suffering from enterica infection.
3. Sera of vaccinated persons actually suffering from enterica infection.

The applicability of the method to each of these categories will, therefore, now be considered.

1. *Sera of unvaccinated persons actually suffering from enterica infection.*

In the case of patients with present enterica disease, who have never suffered from previous similar infection nor been inoculated with anti-enterica vaccines, serological diagnosis is generally a simple matter. Here large-flaking agglutination takes place only with the homologous strain, whereas, with the heterologous organisms of the group, agglutination is small-flaking. The occurrence of large-flaking agglutination in a patient's serum determines therefore the type of bacterial infection. Such a qualitative method of analysis will obviously facilitate differential diagnosis by rendering unnecessary the performance of complicated absorption tests or agglutinations involving quantitative estimation of end-titre; it further obviates misinterpretation of readings encountered in "paradox reactions." (Examples of diagnosis made in this category are to be found in Table V, numbers 1, 2, 3, 4, 5, 6, 8, 9, 11, 12.)

It has to be admitted, however, that in certain exceptional cases identification of the causal organism is impossible by this or any other method of serological investigation alone.

Although normally infection with a bacterium of the enterica group leads to the appearance in the serum of both large- and small-flaking agglutinins, it will be appreciated that infection with an organism from which the labile component has disappeared (= O-form) must result in the production of purely small-flaking agglutinins. Diagnosis is limited in such small-flaking sera to "enterica, type not differentiated"—a finding, however, not without value to the physician. (See Table V, 7, 10, 13. It may be mentioned that if these types of organisms are isolated they are, at least for the first few generations, inagglutinable or hypagglutinable, since the ordinary immune sera

[1] The large number of positive findings is due to epidemic occurrence.

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used in agglutination tests show for the enterica group almost without exception a very high titre for large-flaking agglutination and a very low titre for small-flaking agglutination.)

In our experience on only one occasion in the serum of an unvaccinated person suffering from typhoid fever has the presence of large-flaking agglutinins been observed without any accompanying formation of small-flaking agglutinins. In view of the important rôle assigned by certain workers to the small-flaking stabilotropic agglutinin in the production of immunity, it is of interest to record that the case terminated fatally. (See Table V, number 14.)

2. *Sera of vaccinated persons not suffering from enterica infection.*

Anti-enterica vaccination has undoubtedly introduced a most complicating factor into serological diagnosis. The agglutinins developed as a result of vaccination lead, by their persistence for a variable period in the circulating blood, to uncertainty in the interpretation of "positive results." Further, it has been shown by Conradi and Bieling (1916) that agglutinins, after complete disappearance from the blood, may reappear under the influence of some non-specific intercurrent infection such as tuberculosis and render ordinary agglutination tests without value. In the previously vaccinated a reappearance of agglutinins brought about by the stimulus of some presently-active non-enterica disease is known as "anamnestic reaction."

The question, then, naturally arises: Can the agglutination caused by prophylactic inoculation be distinguished from that due to enterica disease? The answer is that in practically every case such differentiation is possible by means of "qualitative receptor analysis."

In Table VI a comparison is made between the results of agglutination tests carried out here in 1926 on healthy individuals immediately before inoculation with mono- or tri-valent vaccines and those of tests performed ten days after the second dose had been administered, at a time when the agglutinating power of the sera should have been nearing its maximum. It will be observed that the effects of vaccination in human beings afford striking contrast to those following animal inoculation with similar anti-enterica vaccines. (Compare Tables I and VI.) In the sera of vaccinated human beings there is a production of large-flaking labilotropic agglutinins only, and in no dilution is there any evidence of small-flaking stabilotropic agglutinin formation.

Sera of persons inoculated with monovalent typhoid vaccine show with the *Bact. typhosum* large-flaking agglutination only; sera of persons treated with T.A.B. vaccine agglutinate all three bacteria in large flakes, each labilotropic agglutinin combining with its homologous bacterium. No heterologous group agglutination takes place in such sera under any circumstances.

Now *Bact. enteritidis* Gaertner is not included in anti-enterica vaccines and no specific large-flaking agglutination with that organism is, therefore, possible in the sera of vaccinated persons. But small-flaking agglutinins are

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Table V. *Agglutination Reactions. Non-vaccinated Patients.*

Case	Blood culture	Day of illness	Serum dilution	Agglutination against: Ty. O_{901}	Ty. Rawlings	Ty. L	G_8	A_1	B_2	Remarks
1	*Bact. typhosum*	9th	1 : 100	++±s	++±l+s	++±l	++s	+s	++s	
			1 : 200	+s	++l+s	++l	+s	±s	+s	
			1 : 500	±s	+l	±l	—	—	±s	
			1 : 1000	—	±l	—	—	—	—	
2	Sterile	12th	1 : 100	+++s	+++l+s	++±l	+±s	++s	+++s	Paradox reaction. Judging by titre alone, such a case would have been diagnosed as a para. B infection
			1 : 200	+±s	++l+s	+l	+s	+s	+++s	
			1 : 500	±s	+l	±l	—	±s	++±s	
			1 : 1000	—	±l	—	—	±s	++s	The typhoid infection was confirmed by isolation of *Bact. typhosum* from urine on 30th day of illness
			1 : 2000	—	—	—	—	—	++s	
3	Paratyphoid B	14th	1 : 100	++s	+±s	±s	±s	+s	+++l+s	
			1 : 200	±s	±s	—	—	±s	++l+s	
			1 : 500	—	—	—	—	—	+l	
			1 : 1000	—	—	—	—	—	—	
4	Not done	21st	1 : 100	+s	++l+s	+l	+s	—	+s	Blood culture positive for *Bact. typhosum* after relapse on the 35th day after the start of the illness—ending fatally
			1 : 200	—	+l	±l	—	—	—	
			1 : 500	—	—	—	—	—	—	
			1 : 1000	—	—	—	—	—	—	
5	*Bact. typhosum*	8th	1 : 100	+++s	+++l+s	+++l	++s	+s	++s	
			1 : 200	++s	++±l+s	++l	+s	—	+s	
			1 : 500	+s	++l+s	+l	—	—	—	
			1 : 1000	—	+l	—	—	—	—	
6	*Bact. typhosum*	10th	1 : 100	++s	+++l+s	+±l	+s	+s	++s	
			1 : 200	+s	++l+s	+l	—	—	+s	
			1 : 500	—	+l	—	—	—	±s	
			1 : 1000	—	—	—	—	—	—	
7	Sterile	15th	1 : 100	++±s	++±s	±s	++s	+±s	++±s	Enteric fever. Impossible to differentiate type serologically
			1 : 200	++s	+±s	—	+s	+s	++s	
			1 : 500	+s	±s	—	—	—	+±s	
			1 : 1000	+s	—	—	—	—	±s	

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8	P.	oid B	10th	1 : 100	+ + s	+ + s	± s	+ ±s	+ s	+ + + l + s	
				1 : 200	+ + s	+ + s	—	+ s	± s	+ + ± l + s	
				1 : 500	± s	± s	—	—	—	+ + l + s	
				1 : 1000	—	—	—	—	—	+ l	
9	P.	oid A	8th	1 : 100	+ s	+ s	± s	± s	+ + ± l + s	+ s	
				1 : 200	± s	± s	—	—	+ + ± l	± s	
				1 : 500	—	—	—	—	± l	—	
				1 : 1000	—	—	—	—	—	—	
10	St.		18th	1 : 100	+ + + s	+ + + s	± s	+ + s	+ s	+ + + s	Enteric fever. Impossible to differentiate type serologically
				1 : 200	+ + + s	+ + + s	—	+ + s	± s	+ + s	
				1 : 500	+ s	± s	—	± s	—	+ s	
				1 : 1000	—	—	—	—	—	± s	
11	N.		14th	1 : 100	+ + + l + s	+ + + l + s	± l	+ ± s	+ s	+ + s	*Bact. typhosum* isolated from stool at end of third week of illness
				1 : 200	+ + + s	+ + + s	+ + l	± s	—	+ s	
				1 : 500	+ s	+ l	—	—	—	± s	
				1 : 1000	—	—	—	—	—	—	
12	*Ba*		13th	1 : 100	+ s	+ + l + s	+ + l	+ ± s	—	+ ± s	
				1 : 200	± s	+ + l + s	+ + l	—	—	± s	
				1 : 500	—	+ l	± l	—	—	—	
				1 : 1000	—	± l	—	—	—	—	
13	Pa.	oid A	10th	1 : 100	+ s	+ ± s	± s	+ s	± s	+ s	From results of agglutination alone this case would have been classed "Enteric," not differentiated serologically
				1 : 200	± s	+ s	—	—	—	± s	
				1 : 500	—	—	—	—	—	—	
				1 : 1000	—	—	—	—	—	—	
14	*Bo*	*vosum*	12th	1 : 100		+ + l	+ + l	—	—	—	Case died
				1 : 200		+ l	+ l	—	—	—	

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Table VI. *Agglutination Reactions in Healthy Vaccinated Persons.*

Two injections of vaccine given, 0·5 c.c. and 1 c.c. at a week's interval. Sera tested 10 days after the second injection.
(Typhoid vaccine contains 1000 millions per 1 c.c.; T.A.B. = T. 1000, A. 750, B. 750 millions per 1 c.c.)

No. of serum	Serum dilution	Before vaccination. Agglutinated against						After vaccination. Agglutinated against						Vaccine used
		Ty. O_{901}	Ty. R	Ty. L	G_3	A_1	B_2	Ty. O_{901}	Ty. R	Ty. L	G_3	A_1	B_2	
1	1 : 100	—	±1	±1	—	—	—	—	+ + +1	+ + +1	—	—	—	Monovalent
	1 : 200	—	—	—	—	—	—	—	+ + +1	+ + +1	—	—	—	typhoid vaccine
	1 : 500	—	—	—	—	—	—	—	+ + +1	+ + +1	—	—	—	
	1 : 1000	—	—	—	—	—	—	—	+ +1	+ +1	—	—	—	
2	1 : 100	—	—	—	—	—	—	—	+ + +1	+ + +1	—	—	—	Monovalent
	1 : 200	—	—	—	—	—	—	—	+ + +1	+ + +1	—	—	—	typhoid vaccine
	1 : 500	—	—	—	—	—	—	—	+ + +1	+ + +1	—	—	—	
	1 : 1000	—	—	—	—	—	—	—	+ + +1	+ + +1	—	—	—	
3	1 : 100	—	—	—	—	—	—	—	+ + +1	+ + +1	—	—	—	Monovalent
	1 : 200	—	—	—	—	—	—	—	+ + +1	+ + +1	—	—	—	typhoid vaccine
	1 : 500	—	—	—	—	—	—	—	+ + +1	+ + +1	—	—	—	
	1 : 1000	—	—	—	—	—	—	—	+ + +1	+ + +1	—	—	—	
4	1 : 100	—	—	±1	—	—	—	—	+ + +1	+ + +1	—	+ + +1	+ + +1	T.A.B. vaccine
	1 : 200	—	—	—	—	—	—	—	+ + +1	+ + +1	—	+ + +1	+ + +1	
	1 : 500	—	—	—	—	—	—	—	+ + +1	+ + +1	—	+ +1	+ + +1	
	1 : 1000	—	—	—	—	—	—	—	+ + +1*	+ + +1*	—	+1	+ +1	
5	1 : 100	—	—	—	—	—	—	—	+ + +1	+ + +1	—	+ + +1	+ + +1	T.A.B. vaccine
	1 : 200	—	—	—	—	—	—	—	+ + +1	+ + +1	—	+ + +1	+ + +1	
	1 : 500	—	—	—	—	—	—	—	+ + +1	+ + +1	—	+ + +1	+ + +1	
	1 : 1000	—	—	—	—	—	—	—	+ + +1*	+ + +1*	—	+ + +1*	+ + +1*	
6	1 : 100	—	—	—	—	—	—	—	+ + +1	+ + +1	—	+ + +1	+ + +1	T.A.B. vaccine
	1 : 200	—	—	—	—	—	—	—	+ + +1	+ + +1	—	+ + +1	+ + +1	
	1 : 500	—	—	—	—	—	—	—	+ + +1	+ + +1	—	+ +1	+ + +1	
	1 : 1000	—	—	—	—	—	—	—	+ + +1*	+ + +1*	—	±1	+ +1	

* Not end of titre.

Note. To avoid unnecessary complication in the reading of results, reactions with *Bact. paratyphosum* C have been omitted.

absent for typhoid bacteria in sera following monovalent, and for all three (T.A.B.) bacteria in sera following trivalent vaccine administration; there can be, therefore, no group agglutinins developed in such sera to react with Gaertner organisms. For these reasons the *Bact. enteritidis* Gaertner is not, and cannot be, agglutinated by the sera of vaccinated persons, and this fact alone is sufficient to allow distinction to be drawn between agglutination due to specific enterica infection and that due to prophylactic vaccination.

Table VII gives selected examples of "anamnestic reaction" in sera derived from patients suspected of enterica infection. Little difficulty is experienced by this method in the recognition of such cases. The tabulated results show that here also there is a constant absence of small-flaking agglutinins; no matter at what stage of the disease or how often serological examination was carried out, agglutination was invariably large-flaked. As in the reactions given by the sera of healthy vaccinated persons, no small-flaking agglutination with the *Bact. enteritidis* Gaertner occurred. It is possible, therefore, to subscribe to the opinion of Felix (1924) that in the complete absence of small-flaking agglutinins for all species of typhoid-paratyphoid-enteritidis bacteria used for carrying out the Gruber-Widal reaction, the disease under investigation could, with certainty, be designated non-enterica.

3. *Sera of vaccinated persons actually suffering from enterica infection.*

Although Tidy (1916) advanced the opinion that febrile conditions destroy the agglutinins produced by vaccination and that a positive agglutination with the *Bact. typhosum* after the fifth day of pyrexia is a definite proof of typhoid fever in the inoculated and uninoculated alike, the consensus of opinion is that this view is erroneous; indeed, as we have seen, Conradi and Bieling have proved the reverse to occur. Table VIII gives examples of typical agglutination reactions obtained with sera of patients who, despite prophylactic vaccination, have developed enterica disease. Small-flaking agglutinins are, it will be observed, again in evidence, in addition to the large-flaking variety due to inoculation. Further, the results tabulated under *Bact. enteritidis* Gaertner are exactly comparable with those shown in Table V, which deals with cases of enterica disease among the unvaccinated. Application of this method, therefore, allows sera of vaccinated patients infected with the enterica readily to be distinguished from those of healthy vaccinated persons, since in the former, during the course of the disease, recognition is made possible of the constant occurrence of small-flaking agglutinins for all four bacteria of the group used in the test, viz.: *Bact. typhosum*, *paratyphosum* A and B, and *enteritidis* Gaertner. Whereas, however, demonstration of small-flaking agglutinins in the serum justifies a diagnosis of "enterica disease" being given, identification of the actual infecting organism must rest, under these circumstances, on the results of cultural examination. It follows, therefore, that in the laboratory investigations of enterica disease, the occurrence of large-flaking labilotropic agglutinins in unvaccinated patients' sera is

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Table VII. *Agglutination due to anamnestic reactions in sera submitted for the sero-diagnosis of the enterica.*

No. of serum	Day of illness	Serum dilution	Agglutination against								Remarks
			Ty. O_{901}	Ty. R	Ty. L	G_3	A_1	B_2	OX_{19}	HX_{19}	
1	12th	1 : 100	—	+++l	+++l	—	—	—	—	—	Agglutination due to monovalent typhoid inoculation. Nature of illness—unknown
		1 : 200	—	++l	++l	—	—	—	—	—	
		1 : 500	—	+l	±l	—	—	—	—	—	
2	8th	1 : 100	—	+++l	+++l	—	+l	++l	—	—	Agglutination due to inoculation with T.A.B. Nature of illness—unknown
		1 : 200	—	+++l	+++l	—	—	+l	—	—	
		1 : 500	—	+++l	+++l	—	—	±l	—	—	
3	18th	1 : 100	—	++l	++l	—	±l	+l	—	—	Agglutination due to inoculation with T.A.B. Nature of illness—unknown
		1 : 200	—	+l	±l	—	—	±l	—	—	
		1 : 500	—	—	—	—	—	—	—	—	
4	14th	1 : 100	—	++l	++l	—	±l	+l	+++s	+++s	Typhus fever. The agglutination in the enterica group is due to previous vaccination
		1 : 200	—	+l	+±l	—	—	+l	+++s	+++s	
		1 : 500	—	—	—	—	—	—	+++s	+++s	
5	10th	1 : 100	—	++l	+l	—	±l	+l	++s	+++s	Typhus fever. The agglutination in the enterica group is due to previous vaccination
		1 : 200	—	±l	±l	—	—	±l	+s	+±s	
		1 : 500	—	—	—	—	—	—	—	±s	
6	22nd	1 : 100	—	+++l	+++l	—	—	+l	—	—	Agglutination due to vaccination. Subsequent tests showed the patient to be suffering from undulant fever
		1 : 200	—	+++l	++l	—	—	±l	—	—	
		1 : 500	—	+l	+l	—	—	—	—	—	

Note. To avoid unnecessary complication in the reading of results, reactions with *Bact. paratyphosum* C have been omitted.

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Table VIII. *Agglutination reactions in persons suffering from the enterica who have been previously vaccinated.*

(Diagnosis confirmed by cultural method.)

No. of serum	Day of illness	Serum dilution	Agglutination against: Ty. O_{901}	Ty. R	Ty. L	G_3	A_1	B_2	Remarks
1	12th	1 : 100	+s	++l+s	++l+s	+s	+l	++l+s	Typhoid fever in a person previously inoculated with T.A.B. *Bact. typhosum* isolated from blood
		1 : 200	±s	++l+s	++l	±s	±l	+l	
		1 : 500	—	+l	+l	—	—	±l	
2	25th	1 : 100	++s	++l+s	++l+s	+s	++l+s	++l+s	Typhoid fever in a person previously inoculated with T.A.B. *Bact. typhosum* isolated from stool
		1 : 200	+s	++l+s	++l+s	±s	+l	++l	
		1 : 500	—	++l	+l	—	—	+l	
3	9th	1 : 100	±s	++l	++l	±s	±s	++l+s	Paratyphoid B infection in person previously inoculated with T.A.B. *Bact. paratyphosum* B in blood
		1 : 200	—	+l	±l	—	—	++l+s	
		1 : 500	—	—	—	—	—	+l+s	
4	18th	1 : 100	+s	++l+s	++l	+s	±l	++l+s	Typhoid fever in a person previously inoculated with T.A.B. *Bact. typhosum* in urine
		1 : 200	±s	++l	+l	±s	—	++l	
		1 : 500	—	+l	—	—	—	+l	
5	10th	1 : 100	+s	++l+s	++l	+s	±s	++l+s	Paratyphoid B infection in a person previously inoculated with monovalent typhoid vaccine. Paratyphoid B in blood
		1 : 200	±s	++l	++l	—	—	++l+s	
		1 : 500	—	++l	++l	—	—	++l+s	
6	15th	1 : 100	+++s	++l+s	++l+s	++s	++l+s	++l+s	Enteric fever in a person previously vaccinated with T.A.B. Failure to obtain evidence from cultural methods makes it impossible to type this infection
		1 : 200	++s	++l+s	++l+s	+s	+l	++l+s	
		1 : 500	+s	++l+s	++l	±s	—	++l+s	

Note. To avoid unnecessary complication in the reading of results, reactions with *Bact. paratyphosum* C have been omitted.

diagnostic of specific infection with the homologous bacterium; in the case of the vaccinated, however, it is entirely on the occurrence of small-flaking agglutinins that diagnosis must be based, as here the specific importance of large-flaking agglutinins has been lost. From the sera, then, of vaccinated persons suffering from enterica infection, a diagnosis of "enterica, type not differentiated" alone can be given in the absence of cultural results. (See Table VIII, case 6.) Case numbers 1–4 in Table VIII exemplify agglutination reactions when the infecting organism has also been isolated by culture. Diagnosis of specific infection in this category of sera, however, presents no difficulty in the case of patients previously inoculated with a monovalent typhoid instead of a T.A.B. trivalent vaccine. For example, the development in a patient's serum of specific labilotropic agglutinins against *Bact. paratyphosum* B (as evidenced by large-flake agglutination of that organism), taken in conjunction with the occurrence of small-flaking agglutinins for all four bacteria of the group, permits the diagnosis of paratyphoid B fever with certainty, the presence of large-flaking agglutination in the ordinary typhoid tubes notwithstanding. (See Table VIII, case 5.)

It would appear desirable, now that qualitative receptor analysis has been considered from both theoretical and practical standpoints, briefly to touch upon two conditions which might be urged against adoption of the method.

In certain cases of enterica infection the absence of large-flaking agglutinins leads to the occurrence of purely small-flaking sera. The presence of stabilotropic agglutinins, both in vaccinated and in unvaccinated persons, certainly allows a diagnosis of "enterica" to be made, but only as a result of successful cultural investigation can the specific infecting organism be identified.

Again, although among the typhoid, paratyphoid A and B, and Gaertner organisms group agglutination is normally caused by small-flaking agglutinins, most exceptionally in about perhaps 0·2 per cent. of cases it may be brought about by heterologous large-flaking agglutinins. In these latter cases the absorption test of Castellani is also unserviceable, just as it proves to be when attempts are made to differentiate between the typhoid and Gaertner bacteria in sera containing only small-flaking agglutinins.

The writers are convinced, after two years' fair trial of the method, that qualitative receptor analysis, whatever its drawbacks, marks a very definite advance in the serological investigation of the enterica.

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