laryngologist is in the very centre of his own domain, but even here there is a territory which has too long been left unexplored.

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VI AGGLUTINATION IN THE DIAGNOSIS OF TYPHOID FEVER AND THE TYPHOID CARRIER CONDITION

BY

S. S. BHATNAGAR, M.D.Laus., Ph.D., M.R.C.P.,
Major I.M.S.

Deputy Assistant Director of Pathology, Deccan District, and Officer in Charge, Deccan District Laboratory, Trimulgherry, India

Our conception of the diagnostic significance of a Widal reaction has materially altered since it became known that the typhoid-paratyphoid group of organisms are possessed of more than one antigenic component—notably the flagellar H antigens and the somatic O antigens, which produce their serum counterparts on human infection. Since the great war a complicating factor in the serological diagnosis of the enteric group of fevers has been introduced by the widespread use of T.A.B. inoculation.

The relative importance of the two types of antibody (O and H) in the diagnosis of typhoid fever, and of other enteric infections, in the inoculated and the uninoculated individual, first demonstrated by Felix (1924), has been the subject of a large number of studies, the literature being fully summarized by Topley and Wilson (1936). As a result it is being realized that in the interpretation of serological tests such diverse factors as the natural level of O and H agglutinins, the state of the subject in relation to previous inoculation, the agglutinin curves, the individual titres of the antibodies, and the possibility of non-specific stimulation of antibody formation, have to be taken into consideration.

In the laboratories of the Army in India Widal reactions are carried out as a routine every fourth day throughout the course of a continued fever, against standard O and H suspensions supplied by a central enteric laboratory at Kasauli. From personal experience and from a study of the carefully kept records of cases of typhoid fever for the past five years, it was observed that in a typhoid infection in the inoculated the trouble taken in estimating the O and the H agglutinins could not be justified when the value of the results obtained was assessed. In a large

majority of cases the rise in the titre of these agglutinins offered no tangible evidence from which alone a positive diagnosis could be arrived at. Reliance had therefore to be placed on the isolation of the infecting organism from the blood, the urine, or the faeces. Where this failed, one was commonly confronted with a positive clinical picture and doubtful serological findings. The results proved to be so disappointing that the practice of carrying out tests for the detection of H agglutinins has recently been entirely discontinued in the Army in India.

It is now generally recognized that, apart from the O and the H antigen, the great majority of strains of *Bact. typhosum* possess a third antigen—the Vi antigen—described and designated as such by Felix and his co-workers. A number of strains alleged to be free from Vi antigen were collected by us and grown on a medium consisting of mutton digest agar (2 per cent.) to which 1 per cent. saccharose was added. They all showed the presence of this antigen and gave rise to Vi antibody on animal inoculation.

Serological analysis of sera over the past three years from patients suspected to be suffering from typhoid fever suggested the possibility that Vi agglutination may prove to be a more reliable method of serological diagnosis of typhoid infection than the time-honoured O and H types of agglutination. This view was further strengthened by the observation that in the examination of thousands of sera from both inoculated and uninoculated persons no Vi antibody could be detected. The explanation why this antibody is not produced in spite of the inclusion of a Vi-containing typhoid strain in a T.A.B. vaccine has already been given (Felix and Bhatnagar, 1935; Bhatnagar *et al.*, 1937). Thus no interference was anticipated in the interpretation of serological tests from Vi antibody which could be accounted for either as a natural or as an inoculation agglutinin.

The present communication deals with the demonstration of Vi antibody in 134 cases of bacteriologically proved typhoid fever. The material was collected from both the inoculated and the uninoculated. In addition, information obtained on the bearing of Vi agglutination on the typhoid carrier condition is included.

Detection of Vi Antigen in a Typhoid Organism and of Vi Antibody in a Serum

When an organism with the morphology and the biochemical reactions of *Bact. typhosum* is found to agglutinate with a typhoid O serum much below the established titre of the serum the presence of Vi antigen in the culture is to be suspected. This is confirmed if it gives a positive reaction in agglutination with a pure Vi serum—that is, a serum prepared against a highly virulent strain of *Bact. typhosum* and from which the O and the H antibodies have been completely removed by absorption.

While the detection of Vi antigen can thus be carried out comparatively easily, the titration of Vi antibody in a serum, on the other hand, is complicated by the simultaneous presence in it of O and H agglutinins. Two methods are usually employed to overcome this difficulty: (a) Complete absorption of O and H antibodies from the serum by the use of a strain devoid of Vi antigen. In our experience very few strains indeed are completely devoid of Vi antigen; moreover, this method is a laborious process, hardly of practical applicability to an investigation which involves the serological analysis of a large number of samples. (b) Agglutination against selected strains or their chemically treated suspensions sensitive to one type of antibody only. While alcoholized

and formalized suspensions have proved to be excellent reagents for the titration of the O and the H agglutinins respectively, the real difficulty met with in the routine practice of Vi agglutination has been the want of a strain which either in the live state or as a preserved suspension would react only with the Vi antibody. In the course of the antigenic analysis of 235 typhoid strains, collected from different parts of the world, we were able to identify one culture which in its agglutinating reactions behaved as a pure Vi variant of *Bact. typhosum*. This strain has been designated "Vi I" and is described elsewhere (Bhatnagar *et al.*, 1938). Its agglutination reactions with pure O, H, and Vi sera are exemplified in Table I. This strain is not only insensitive to O and H agglutinins but is also more sensitive to Vi antibody than the well-recognized typhoid strains hitherto employed for the purpose of Vi agglutination—namely, "Ty 2," "Watson," and "6 S."

TABLE I.—Agglutination Reactions of Strain "Vi I" of *Bact. typhosum*

	Serum Dilutions	Typhoid Sera		
		Pure H Serum	Pure O Serum	Pure Vi Serum
Strain "Vi I"	1:10	—	—	+++
	1:25	—	—	+++
	1:50	—	—	+++
	1:100	—	—	++±
	1:200	—	—	++
	1:500	—	—	+±
	1:1,000	—	—	+

Vi Antibody in the Blood Serum of Typhoid Patients

The 134 cases of typhoid fever described here consist of fifty-six inoculated and seventy-eight uninoculated individuals. The serum from each case was collected every fourth day and titrated for the presence of the three types of antibody (O, H, and Vi). Table II summarizes the highest titres obtained during the course of typhoid infection; the "titre" represents the dilution of the serum which gives a *one plus* (+) reading according to the agglutination technique of Felix. The O and H agglutinin titres are omitted, as they are intended to form part of a subsequent communication.

TABLE II.—Vi Titre in Typhoid Patients

Titre	Number showing a Particular Titre		Height of Titre per 100 Individuals	
	Inoculated	Uninoculated	Inoculated	Uninoculated
1:10	—	—	—	—
1:25	—	27	—	34.6
1:50	10	45	17.8	57.7
1:100	37	6	66.1	7.7
1:200	9	—	16.1	—
1:500	—	—	—	—
Totals ..	56	78		

It is evident from Table II that Vi antibody was present in the serum from every case of typhoid fever, whether previously inoculated or not. A distinct difference was, however, seen to have been caused by inoculation, so far as the titre of this antibody is concerned, in that the inoculated individuals generally gave a higher reading. Whereas in the uninoculated more than 90 per cent. of the cases examined did not show a titre higher than

1 in 50—34.6 per cent. of these reacting only in the dilution 1 in 25—among the inoculated, on the other hand, a titre of 1 in 100 was obtained in as high a percentage as 66.1 and that of 1 in 200 in another 16.1 per cent. It was noticed that those individuals who have had the benefit of multiple inoculations recorded, as a rule, a higher titre than those who had undergone a single course.

Advantage was taken in sixteen cases (eight inoculated and eight uninoculated) to bleed the patient every third morning so that the development of Vi antibody could be studied every forty-eight hours. Two sample cases are illustrated in Charts I and II.

CHART I.—Showing the development of Vi antibody in an inoculated person.

CHART II.—Showing the development of Vi antibody in an uninoculated person.

It is seen from the charts that the Vi antibody response was different in the two types of individual. In the inoculated the rise of the Vi agglutinin curve was steeper, more Vi antibody was produced, and the highest point was reached much earlier (on the tenth day in this case); in the uninoculated the rise was much more gradual, and the highest titre (only 1 in 50, compared with 1 in 100 of the inoculated person) was not reached till the eighteenth day. It is of interest to note, however, that the fall of the curve was rapid in both cases.

These two curves exemplify what has been observed by us in an average inoculated and an average uninoculated case in which the typhoid fever takes what may be described as a normal course. Departures from the normal in the clinical course of the disease were noticed to produce variations in the elaboration of the Vi antibody as well. Thus in an inoculated community like the Army

it was common to see the infection follow a modified course in which the symptoms were mild and the pyrexia did not last more than eight to ten days. Here the rise of the Vi antibody curve was observed to be much more steep, and so was the fall, the difference in the titre in the course of twenty-four hours being as much as four times. Thus it was not unusual to find a titre of 1 in 100 one morning to be followed by a reading of 1 in 25 the day after.

Typhoid fever in an uninoculated individual in India commonly presents a textbook description of this disease—namely, a step-ladder type of pyrexia, the typical rash, the tympanitic abdomen with “pea-soup” stools, and complications like severe mental disturbances, haemorrhage, and relapse. It was noticed as a rule that the titre of Vi antibody continued to be low till late in the disease in those cases where either the clinical course was severe or the pyrexia prolonged, or a relapse set in later.

From the point of view of the diagnosis of acute typhoid condition with the aid of Vi agglutination, it has been our experience that in an inoculated community a definite conclusion can be arrived at by the end of the first week. The Vi titre was found by then to have been in the region of 1 in 25 to 1 in 50, which could be easily detected with the help of the strain “Vi I.”

In an uninoculated population a positive Vi agglutination reaction is of definite diagnostic value. A negative result, on the other hand, cannot be relied upon to exclude the presence of typhoid infection, since the Vi antibody is produced with difficulty in this type of individual and at a much later date during the course of the infection. In a case which runs an uninterrupted course frequent serological tests will, however, detect the presence of this antibody at a titre of 1 in 10 to 1 in 25 during the second week. In cases which relapsed it was noticed that generally no Vi antibody could be found during the primary rise of temperature, a positive Vi agglutination being obtained only during the course of the secondary rise.

Before the identification of the strain “Vi I” the Vi titre of a serum was determined by testing it against three different strains of *Bact. typhosum*, sensitive to O, H, and Vi agglutinins respectively, and the reading of the Vi reaction was taken in the simultaneous presence of O and H agglutination. Where the Vi titre was low the alternative method of completely absorbing the serum of its O and H antibodies was resorted to. This procedure inevitably resulted in the reduction of the already low Vi titre of the serum due to non-specific factors. Further loss took place owing to the absorption of a certain amount of Vi antibody by the small amount of Vi antigen present in the so-called Vi-free organisms that are employed for this purpose.

In view of these technical difficulties in assessing the Vi antibody content of a serum it can be easily understood why the study of this antibody in human infection has not progressed beyond its simple recognition by a few observers (Felix *et al.*, 1935; Bensted, 1937; Lewin, 1938). To start with, the impression was gaining ground with us that the Vi antibody is produced in a large number of inoculated individuals but very rarely in those who are not inoculated. It has only been possible to demonstrate the presence of this antibody in every case of typhoid fever since pure Vi agglutination has been obtained with the help of the strain “Vi I,” which is sensitive to the Vi antibody but is insensitive to the O and the H agglutinins in as low a dilution as 1 in 10 (see Table I).

Up to the present we have not failed to correlate a positive Vi agglutination reaction with either the isola-

tion of the infecting organism or with a positive clinical picture of typhoid fever where bacteriological proof of the presence of typhoid infection could not be obtained. The great advantage of this type of agglutination lies in the fact that a positive result clinches the diagnosis of typhoid fever, there being no interference in the interpretation of the serological test from either the normal or the inoculation agglutinins such as occurs with the O and the H types of agglutination. In a recent personal communication Bensted expressed the opinion that T.A.B. inoculation does give rise to Vi antibody, but only in very small amounts, not large enough to be taken account of in Vi agglutination. On this basis he compares the altered Vi antibody reactivity of an inoculated individual to typhoid infection to the very rapid response to diphtheria toxin when there are minimal amounts of antitoxin in the blood.

It is necessary to emphasize certain technical points in connexion with the quantitative estimation of the Vi antibody. Dreyer's tubes are not suitable for Vi agglutination tests. The type of tube with a round bottom, employed in Felix's technique for the differentiation between O and H agglutination, is most suitable for Vi agglutination. The tubes are about 5 cm. long and have a uniform internal diameter of about 1.25 cm. The difference between the agglutinated bacteria and those that have simply settled down can be easily made out in these tubes, since they permit the shaking of the whole serum-suspension mixture. The clumped bacilli in Vi agglutination have a tendency to distribute themselves round about the lowest quarter of an inch of the agglutination tube, where they appear as a general haze to the naked eye. When the tube is shaken these agglutinated bacteria become readily visible as a considerable mass of somatic agglutination.

With a view to preparing sterile suspensions of strain “Vi I” suitable for Vi agglutination various chemical reagents were tried. None of them has so far proved to be entirely satisfactory. The agglutinability of the organisms so treated diminishes rapidly. Formalized suspensions (0.5 c.cm. of pure formalin added to every 80 c.cm. of the suspension) or those prepared with mercury perchloride (1 in 1,000 HgCl₂ in normal saline) can, however, be trusted to give reliable results for a period varying from four to ten weeks. It is therefore necessary that the Vi sensitivity of the suspension should be tested every few days against a pure Vi serum of known titre.

Vi Agglutination in Detection of the Typhoid Carrier Condition

During convalescence from typhoid fever it was observed that, as a rule, only traces of Vi antibody could be found in the serum. In a certain number of cases, however, the Vi titre continued to be high in spite of the termination of the acute infection. Further investigations revealed that this variation connoted a tendency towards the typhoid carrier condition. It was possible to study the serum reactions of four such individuals over a period of one year side by side with the examination of their stools and urine for the presence of the typhoid organism. None of the patients gave a history of previous T.A.B. inoculation. Three of them were faecal carriers and one urinary. Specimens were obtainable at approximately one-month intervals. The results are reproduced in Table III.

A correlation between the typhoid carrier condition and the presence of Vi antibody in the serum is evident from this table. Individual No. 1 proved to be only a tem-

TABLE III

Time After Acute Typhoid Infection	Temporary and Chronic Typhoid Carriers															
	No. 1				No. 2				No. 3				No. 4			
	Isolation of Typhoid Bacilli	Agglutinin Titres			Isolation of Typhoid Bacilli	Agglutinin Titres			Isolation of Typhoid Bacilli	Agglutinin Titres			Isolation of Typhoid Bacilli	Agglutinin Titres		
		Vi	O	H		Vi	O	H		Vi	O	H		Vi	O	H
1 month	+	50	100	100	+	100	200	100	+	50	200	200	+	100	200	50
2 months	+	25	100	50	+	50	100	25	+	50	200	50	+	100	100	25
3 "	+	10	50	50	+	50	50	25	+	50	100	25	+	50	50	25
4 "	—	—	50	25	+	50	50	25	+	50	100	25	+	50	100	25
5 "	—	—	50	25	+	50	100	25	+	50	100	25	+	50	100	25
6 "	—	—	50	25	+	50	100	25	+	50	100	25	+	50	100	25
7 "	—	—	50	50	+	50	100	25	+	50	100	25	+	50	100	25
8 "	—	—	50	50	+	50	100	25	+	50	100	25	+	50	50	25
9 "	—	—	50	50	+	50	100	25	+	50	50	25	+	50	50	25
10 "	—	—	50	50	+	50	100	25	+	50	50	25	+	50	50	25
11 "	—	—	50	50	+	50	100	25	+	50	50	25	+	50	50	25
12 "	—	—	50	50	+	50	100	25	+	50	50	25	+	50	50	25

porary carrier. After the third month no typhoid bacilli could be isolated from his urine or his faeces, nor could the Vi antibody be demonstrated in his serum. The three persons whose serum continued to give a positive Vi reaction proved to be true chronic carriers. The typhoid bacilli isolated from their faeces were invariably found to be virulent Vi-containing organisms.

Pijper (1930), Ashby (1931), Wyllie (1933), and others have suggested the estimation of O agglutinins for the detection of the typhoid carrier condition. The O titres in our series appear to be significantly high. But when this observation is considered in conjunction with the normal level of the typhoid O agglutinin in India, which has been shown to be as high as 1 in 100 in 64 per cent. of the individuals examined (see Bhatnagar *et al.*, 1937), the figures reproduced in Table III, obtained by the same technique of agglutination, cannot be taken to be more than suggestive of the chronic typhoid carrier condition.

The suggestion that possibly Vi agglutination may be a useful method of detecting chronic typhoid carriers was made by Felix, Krikorian, and Reitler (1935), and was followed up by Giovanardi (1936, 1937) in Italy and by Pijper and Crocker (1937) in South Africa. More recently the results of the examination of a considerable number of chronic carriers were published by Felix (1938). I myself have been using Vi agglutination in the routine carrier examination of a large number of individuals, such as is done in the Army in India, and in tracing the source of infection of a particular case or a group of cases of typhoid fever. The detection of Vi antibody in a serum is taken to be an indication for a thorough search for typhoid bacilli in the stools and urine. We have not yet come across an individual who while excreting typhoid bacilli failed to produce evidence of the presence of Vi antibody in his serum. The procedure not only led to the saving of a considerable amount of time and material, but also reduced the chances of missing a carrier in whom the discharge of bacilli was intermittent.

Summary

A strain of *Bact. typhosum* which gives rise to pure Vi agglutination has been identified.

With the help of this strain the Vi antibody is shown to be produced in every case of typhoid fever, the inocu-

lated individuals producing a higher Vi titre than the uninoculated.

Vi agglutination is shown to be a more reliable method of diagnosing typhoid fever in the inoculated than the time-honoured O and H types of agglutination.

A correlation between the typhoid carrier condition and the presence of Vi antibody in the serum is described. The employment of Vi agglutination in the routine carrier examination and in tracing the source of typhoid infection is suggested.

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