THE OCCURRENCE OF TYPHOID BACILLI CONTAINING Vi ANTIGEN IN CASES OF TYPHOID FEVER AND OF Vi ANTIBODY IN THEIR SERA

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The investigation recorded in the present paper was undertaken as a corollary to the clinical trials with a new antityphoid serum, the results of which were recently published (Felix, 1935). Its object was to enquire into:

(i) the occurrence of Vi antigen in strains of *B. typhosus* freshly isolated from cases of typhoid fever;

(ii) the occurrence of Vi antibody in the blood serum of typhoid patients and convalescents.

Vi antigen in freshly isolated strains of *B. typhosus*

All the strains of *B. typhosus* isolated at the Government laboratories in Jerusalem and Jaffa during October-November 1934, were tested for their agglutinability by pure O and pure Vi antiserum, as soon as possible after they had been identified by the routine methods. The cultures were transplanted daily on agar slopes and were kept constantly at 37° C., since the effect on the formation of Vi antigen of temperatures other than 37° C. was already known (Felix, Bhatnagar and Pitt, 1934). For the same reason the agglutination tests were performed on the spot, to avoid variation in the content of Vi antigen of the cultures occurring during transport from one laboratory to the other. The technique of the agglutination tests was as described in a previous paper (Felix and Pitt, 1934*a*), and the procedure adopted in the present investigation is illustrated in Table I.

It is seen from Table I that the four freshly isolated strains showed very marked differences in susceptibility to pure O and pure Vi antiserum, and the well-known antagonism between Vi and O agglutination is clearly brought out in this table. The intensity of either of the two reactions was independent of the number of daily transplants, to which the strains had been subjected since first isolated from the blood of typhoid patients. The Table also shows that none of the four strains was entirely devoid of Vi antigen in contrast to the control strain H901, and that none of them was resistant to the action of the O agglutinin to the same degree as the control strain Watson. While the two control strains represent the two extreme types of *B. typhosus*, the

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four freshly isolated strains were all to be classed as belonging to the type of intermediate agglutinability and virulence.

Table I. *Agglutination tests with strains of* B. typhosus *freshly isolated from the blood of typhoid patients*

Strain No.	Number of daily subcultures since isolated	Agglutination with immune sera: Pure H serum In dilution 1 : 1000	Pure O serum In dilutions 1 : 1000 / 1 : 5000 / 1 : 20,000	Pure Vi serum In dilution 1 : 250
717	2	+	+ + + + + ± +	(±)
1196	2	±	+ + + + + ± +	+ ±
1139	5	+	+ ± (±)	+ + +
814	7	±	± [±] –	+ + +
Controls				
Strain Watson		+	– – –	+ + +
Strain H 901		+	+ + + + + + + +	–

Note. ± = weakest degree of agglutination which could be estimated with the naked eye.
(±) = trace
[±] = faint trace } estimated by means of a magnifying lens.

Ninety strains of *B. typhosus*, isolated from ninety patients, were examined in the manner indicated in Table I, and two strains of *B. paratyphosus* A and three strains of *B. paratyphosus* B were also included in these tests. The results are summarised in Table II.

Table II. *Occurrence of strains of* B. typhosus *containing Vi antigen*

	Isolated from	Number of strains tested	Vi antigen of *B. typhosus*: Present	Absent
B. typhosus	Blood	86	84	2
	Urine	2	2	0
	Faeces	2	2	0
	Total	90	88	2
B. paratyphosus A	Blood	2	0	2
B. paratyphosus B	Blood	3	0	3

Only two out of ninety strains of *B. typhosus* tested were found to be devoid of Vi antigen. The two cultures had been isolated from the blood and the suspensions used for the agglutination tests were derived from the seventh and eighth agar subculture, respectively. With all the other strains of *B. typhosus* the presence of Vi antigen was demonstrated by agglutination

with the pure anti-Vi serum. Kauffmann (1935) recently reported similar observations from Denmark.

According to the degree of agglutination with the pure O antiserum the eighty-eight strains which contained Vi antigen were all of the type of intermediate agglutinability and virulence. Not a single strain of the type of extreme inagglutinability and virulence, described in previous papers (Felix and Pitt, 1934*a* and *b*), was met with in this Palestinian series. In this connection it may be of interest to mention that the death-rate from typhoid fever in Palestine, according to the annual reports of the Department of Health, is lower than that known from many other countries. No attempt could be made to investigate whether or not there is any correlation between the severity of the disease and the type of strain isolated from the patient, since the one extreme type, viz. that of high agglutinability and low virulence, was found only in two cases, while the other extreme type, viz. that of high virulence, was not represented at all in this series.

Table II shows that the specific Vi antigen of *B. typhosus* could not be detected in freshly isolated strains of *B. paratyphosus* A and B. The same result has been obtained by one of the present writers (A. F.) in similar tests with a considerable number of strains of *B. paratyphosus* A and B which had been supplied by the National Collection of Type Cultures and included strains from various parts of the world.

Unlike the highly virulent and inagglutinable strains of *B. typhosus* those of the intermediate type do not maintain their relative inagglutinability on prolonged subculture on plain agar. This is illustrated in Table III.

Table III. *Decrease in inagglutinability by pure O serum on prolonged subculture*

		Subcultures on plain agar of Strain 18904			
Serum	Dilution	6th	14th	22nd	32nd
Pure O serum	1 : 1000	±	±	+ + ±	+ + ±
	1 : 5000	(±)	(±)	+	+
	1 : 20,000	−	−	(±)	(±)
Pure Vi serum	1 : 250	+ + +	+ + +	+ + ±	+ + ±
		Strain 19398			
		5th	11th	19th	25th
Pure O serum	1 : 1000	±	+ +	+ + ±	+ + ±
	1 : 5000	(±)	+ ±	+ ±	+ +
	1 : 20,000	[±]	±	±	±
Pure Vi serum	1 : 250	+ + +	+ + ±	+ + ±	+ + ±

The two strains described in Table III, when first tested shortly after they had been isolated, showed about the highest degree of inagglutinability by pure O antiserum, that was observed amongst the Palestinian strains. It is seen that this relative inagglutinability was lost after twenty-two subcultures of the one strain and after eleven subcultures of the other. The contrast in this respect between strains of the intermediate type and those of the highly virulent type is very sharp. It may well be recalled that one of the

most virulent and inagglutinable strain (Ty2), used in the experiments of Felix and Pitt (1934*a*) was isolated so long ago as 1918 and has since been maintained on plain agar in many hundreds of subcultures.

From previous experiments with old laboratory strains (Felix and Pitt, 1934*b*) evidence has already been obtained of the presence of Vi antigen in strains of intermediate agglutinability and virulence, which represent the most common type of *B. typhosus*. This is now corroborated by the results of the investigation of cultures freshly isolated from typhoid patients. From these observations the following conclusions of practical importance seem to be quite obvious:

(*a*) To prevent infection with the most common type of *B. typhosus* the vaccine used must contain Vi antigen.

(*b*) To be efficient in the treatment of the most common type of typhoid case an antityphoid serum is required containing Vi antibody.

(*c*) No beneficial effect is to be expected from the use of an antityphoid serum in patients suffering from paratyphoid fever A or B.

Vi ANTIBODY IN THE BLOOD SERUM OF PATIENTS AND CONVALESCENTS

Two series of patients' sera were tested for the presence of Vi antibody. The first series comprised 100 sera taken from the blood specimens that were sent to the laboratories for diagnostic purposes and were found to have been derived from bacteriologically or serologically proved cases of typhoid fever. The great majority of these sera had been taken during the early stages of the disease, mostly before the end of the second week, a small proportion only came from patients in the later stages.

Table IV. *Occurrence of Vi antibody in the sera of typhoid patients and convalescents*

Sera from	Total number of cases tested	Vi agglutination	
		Negative	Positive
Patients tested during pyrexial period (majority of cases before the end of the second week)	100	92	8
Convalescents tested on eve of discharge from hospital (4–10 weeks from onset)	17	12	5

It is seen from Table IV that in the series of 100 sera only eight were found to contain Vi antibodies, whereas in ninety-two cases Vi antibodies were not demonstrable in dilutions of 1 in 10 or even 1 in 5. Table V shows that most of the positive results were obtained with sera taken late in the course of the disease, although such sera formed a small minority of the total investigated. Consequently another series of sera was tested, taken from convalescents on the eve of their discharge from hospital, the dates of the bleedings varying from 4 to 10 weeks from the onset of the disease. In this series only seventeen sera were available, owing to the fact that the seasonal

outbreak had come to an end. Table IV shows that in this group five sera were found to contain Vi antibodies, which is a proportion about three to four times greater than that observed in the first series.

Table V. *Agglutination titres in the sera from typhoid patients, convalescents and carriers*

Case No.	Clinical course	Number of days from onset	Titres of the antibodies present in the sera		
			H	O	Vi
		Patients			
1	Very severe	22	1000	500	10
2	Moderately severe	18	<50	50	10
3	,, ,,	28	1000	1000	20
4	,, ,,	6	200	200	20
5	Mild	21	<50	1000	20
6	Very severe	47	5000	500	100
7	Extremely severe	13	1000	500	200
8	Very severe	56	1000	500	<10
		66	1000	500	200
		77	1000	500	500
		Convalescents			
9	Very severe	45	1000	1000	10
10	Moderately severe	29	<50	2000	10
11	,, ,,	27	500	1000	10
12	,, ,,	34	1000	200	20
13	Mild	30	5000	500	50
		Carrier			
14	*B. typhosus* in gall-bladder	4 years	2000	200	20

In Table V are recorded the titres of H, O and Vi agglutination observed in all those patients and convalescents, in whose blood serum the presence of Vi antibody has been detected. Particular care was taken in the exact estimation of the titre of this antibody. All the positive sera were examined repeatedly against several strains of the inagglutinable type and most of them were also tested for Vi agglutination after the H and O agglutinins had been removed by absorption with the strain H901, which is devoid of Vi antigen. The Vi titres recorded in the table represent those determined with the strain "Watson" as test strain and are therefore directly comparable with the titres of the rabbit and horse immune sera published in previous papers (Felix and Pitt, 1934*b*; Felix, 1935).

It is seen from Table V that most of the Vi titres observed in patients and convalescents were comparatively low, few only exceeding a dilution of 1 in 20. The outstanding exception, patient No. 8, was the most interesting case. The patient, a boy of 8 years, had a relapse of his typhoid fever lasting from the 22nd to the 47th day from the onset of the disease. Owing to the gravity of his condition he was given two intramuscular injections of antityphoid serum (each 20 c.c.) on 40th and 42nd day, after which the symptoms ameliorated and the child, who was extremely emaciated, gradually progressed towards complete recovery. It is seen from the table that on the 56th day of the disease, *i.e.* 2 weeks after the second dose of the therapeutic serum, when

presumably passively transferred antibodies had disappeared from the blood, Vi antibodies were not yet demonstrable in a dilution of 1 in 10 of the patient's serum, but on the 66th and 77th day they had reached remarkably high titres, that on the latter date being actually higher than the titre of the therapeutic serum.

Table V also shows that there is no correlation between the titres of the Vi antibody and those of either the O or H antibodies. Furthermore, no constant relationship is apparent between the clinical course and the appearance or the titre of Vi antibodies in the patients' blood serum, since cases exhibiting all degrees of severity, including mild and extremely severe cases, have been found amongst those containing Vi antibodies in their blood serum. However, it would be premature to draw any definite conclusion from the scanty figures collected in the course of this preliminary investigation. Specifically planned and detailed studies are required to assess the relative importance of the effect on the course of the disease exerted by the presence of Vi antibody in the patient's serum.

The observations recorded in Tables IV and V clearly indicate that the Vi antibody is not readily elaborated as a result of an attack of typhoid fever. They further strengthen the conclusion that early application of a therapeutic serum containing Vi antibody as one of its constituents is an essential requirement of typhoid patients.

Note on Vi agglutination with the serum from a carrier

The blood serum from a typhoid carrier came up also for examination. The patient was a woman who was suffering from cholecystitis and *B. typhosus* was isolated from the duodenal juice. She had had an attack of typhoid fever 4 years previously and apparently harboured the bacilli in her gall-bladder throughout this period. Her blood serum contained Vi antibodies in a significant titre (see Table V, case No. 14). This observation, if confirmed on a greater number of sera from typhoid carriers, may prove to be of practical importance. It is known that no reliable serological method for diagnosing the carrier condition has yet been evolved, though the estimation of the O agglutinin titre has been suggested by some workers (Pijper, 1930; Ashby, 1931; Wyllie, 1933). It is not impossible that Vi agglutination, while at present apparently of no diagnostic value in typhoid patients, may contribute a useful method of diagnosing typhoid carriers.

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