A CONTRIBUTION TO THE PROBLEM OF THE VIRULENCE ("VI") ANTIGEN OF *B. TYPHOSUS*[1]

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In recent papers Felix and Pitt (1934*a*, *b*) and Felix, Bhatnager and Pitt (1934) described the presence in *B. typhosus* of a special antigen which they designate as virulence ("Vi") antigen. According to the writers, highly virulent strains of *B. typhosus* show a weak somatic O agglutination and high resistance to the action of O antibody. The resistance of live cultures to O agglutinins affords evidence of the presence of the Vi antigen.

Later investigations by Felix (1935) and McSweeney (1935) were directed towards determining the protective action of therapeutic sera obtained from horses immunised with Vi-containing strains of *B. typhosus*. Felix reports on forty-three cases of typhoid fever which were treated with the serum; of these cases twenty-one were "severe or very severe" and twelve "extremely severe". A beneficial action of the serum was observed by McSweeney in seven out of eight cases. In the control groups of patients treated with normal horse serum no amelioration whatever was observed, on the contrary the patients grew rather worse (Felix).

The line of investigation followed by Felix seems to promise interesting results: it opens new horizons in the field of serotherapy and prophylaxis of typhoid fever (vaccination with strains containing the Vi antigen), as well as in the analysis and demonstration of the antigen structure of other micro-organisms; moreover, it may be of considerable use in throwing light on the relationship between the generally recognised O and H antigens.

These reasons led us to investigate the subject. In the present study, following Felix's method, we sought to investigate a number of strains of *B. typhosus* in respect to the occurrence of the Vi antigen and to the consequences of its presence in the strains.

Selection of strains

In order to select extreme types as to agglutinability by O agglutinins, we subjected more than 500 strains to agglutination tests. Old laboratory strains as well as freshly isolated strains were used. Our choice fell upon the strains N 205 D (isolated from blood), N 1302 and "Viktorov" (Mechnikov Institute), belonging to the inagglutinable form, and upon the strains "Rawlings",

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"Botson" (Lister Institute), N 40 and N 2123, representing the highly agglutinable type. The last two strains had been used for the production of vaccine in the Bacteriological Institute of Kiev. With regard to morphology, biochemistry and serology they all proved to be typical *B. typhosus* strains.

TECHNIQUE

Agglutination tests were carried out according to Felix's procedure, using live cultures of the smooth (S) form, grown for 24 hours at 37° C. on a Japanese peptonised beef agar. Every strain under investigation was thoroughly tested for its being a smooth variant, and the tests carried out, as suggested by White (1929), showed that all the strains were typical S forms.

Virulence tests, according to Felix's method, were carried out on mice. Suspensions were standardised by opacity.

The experimental mice, males, weighing 25–28 g., were fed on a normal constant diet (milk, bread, oats). The infected mice died, as a rule, within 48 hours, but a few survived for 5–7 days.

VIRULENCE AND SOMATIC O AGGLUTINATION

Table I shows that the virulence of the strains under investigation was closely related to their reaction to the O agglutinins. The strains Viktorov, N 205 D and N 1302 showed much lower O agglutinin titres than the strains Botson, Rawlings, N 40 and N 2123. Flagellar H agglutination, on the other hand, was obtained with all the strains to almost equal titres.

With regard to virulence a striking difference was observed between the different strains. With a dose of 125×10^6 living organisms, the strains of low O agglutinability showed high virulence, namely all the mice inoculated with the strains 205 D and 1302 died; whereas none of the animals died as a result of the inoculation of the same dose of the agglutinable strains Rawlings, N 40 and N 2123. The strains Victorov and Botson apparently belong to the group of intermediate virulence.

In Tables I and II virulence is expressed as a fraction, the numerator indicating the number of animals that died, the denominator the number inoculated.

In order to compare the degree of virulence of the various strains the minimal lethal doses (M.L.D.) were determined (Table II). The experimental mice weighed 25–28 g., but were not of the same breed. The difference in the virulence of the two extreme types of strains is clearly seen from Table II, the M.L.D. being 125×10^6 for the strains 205 D and 1302, whereas it is more than 600×10^6 for the strains 40 and 2123.

Tables I and II thus give proof of a correlation between the virulence of the S forms of *B. typhosus* and their reaction to the O antibody. Strains inagglutinable by or non-sensitive to the O agglutinins prove to be virulent, whereas agglutinable or sensitive strains show a low virulence. Since all the

strains used were typical S forms, one may agree with Felix's opinion, that the virulence of a strain is not due to the smooth O antigen alone, but to an additional unknown factor, which he and his followers denominate as the "Vi antigen".

Table I. *Agglutination reaction and virulence of strains of* B. typhosus

Serum	Serum dilutions	Strains: 205 D	1302	Victorov	Botson	Rawling	40	2123
		Agglutination of living micro-organisms						
O serum No. 16	1 : 400	++++	++++	++++	++++	++++	++++	++++
	1 : 800	++++	++	+++	++++	++++	++++	++++
	1 : 1,600	++	±	+	++++	++++	++++	++++
	1 : 3,200	+	−	−	+++	++++	++++	++++
	1 : 6,400	−	−	−	+	++++	+++	++++
	1 : 12,800	−	−	−	+	++	+	+
	1 : 25,600	−	−	−	−	±	−	+
	1 : 51,250	−	−	−	−	−	−	−
H serum No. 15	1 : 800	++++	++++	++++	++++	++++	++++	++++
	1 : 1,600	++++	++++	++++	++++	++++	++++	++++
	1 : 3,200	+	±	+	++	+	++	++
	1 : 6,400	−	−	−	−	−	−	−
		Virulence to mice						
Dose 125 × 106 intraperitoneally		10/10	6/6	4/6	1/8	0/10	0/10	0/10

Signs: ++++ = highest degree of agglutination; + = different degrees of agglutination; ± = traces of agglutination; − = negative agglutination.

Table II. *Virulence of "S" forms of inagglutinable and agglutinable strains of* B. typhosus

Strain	Type of strain	Virulence to mice following intraperitoneal inoculation. Dose in millions of micro-organisms: 100	125	200	400	600
205 D	Inagglutinable	6/8	10/10	—	—	—
1302	,,	4/6	10/10	—	—	—
Victorov	,,	2/5	4/6	10/10	—	—
Botson	Agglutinable	—	0/4	1/10	6/10	—
Rawling	,,	—	0/6	0/8	5/10	—
40	,,	—	0/10	0/10	1/10	4/10
2123	,,	—	0/10	0/10	2/10	5/10

In order to demonstrate the presence of this antigenic component and to study its immunising capacity, experiments on active and passive immunisation were conducted. Five strains were used for active immunisation of mice: strain N 205 D, inagglutinable and highly virulent; strains Rawlings, Botson, N 40 and N 2123 of high agglutinability and low virulence. The mice

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were immunised with vaccines, prepared according to Felix and Pitt (1934*b*), which were administered subcutaneously at 3 days' intervals. The first dose contained 250×10^6 organisms, the second 500×10^6. Twenty-four days later, two M.L.D. (250×10^6 live organisms of virulent strain N 205 D) were inoculated intraperitoneally. At the same time two groups of normal mice received the full test dose (250×10^6) and half the test dose (125×10^6), respectively.

Table III. *Active immunisation of mice*

Vaccines used for immunisation of mice		Number of mice submitted to intraperitoneal inoculation of strain N 205 D (250×10 micro-organisms)	
Strain	Type	Tested	Surviving
205 D	Inagglutinable "S" (virulence high)	16	16
Botson	Agglutinable "S" (virulence low)	15	1
Rawling	,,	15	1
40	,,	14	0
2123	,,	15	0
Controls with normal mice:			
Two M.L.D. (250×10^6 micro-organisms)		10	0
One M.L.D. (125×10^6 micro-organisms)		12	0

Table III shows that none of the mice immunised with the vaccine from the virulent strain died, whereas amongst the four groups of mice immunised with the four agglutinable strains only two survived. In the two control groups all the mice died.

The five strains employed in the experiments on active immunisation were also used for the preparation of rabbit immune sera. The rabbits were inoculated with suspensions of live organisms, and the sterile sera, which did not contain any disinfectant, were used for the passive immunisation of mice. The sera were injected intramuscularly and 2 days later the mice were inoculated intraperitoneally with a test dose containing 200×10^6 organisms of the virulent strain "205 D". The results are given in Table IV.

Table IV. *Passive immunisation*

Rabbit sera injected into mice intramuscularly 2 days prior to test dose			Number of mice tested by intraperitoneal inoculation of strain N 205 D (200×10^6 micro-organisms)	
Strain	No. of corresponding serum	Dose of serum in c.c.	Tested	Survived
205 D	10	0·5	4	4
	10	0·25	5	4
	10 (absorbed O and H)	0·5	6	5
Botson	24	1·0	4	0
Rawling	21	1·0	4	0
40	22	1·0	4	0
2123	23	1·0	4	0
Control normal mice were inoculated with:				
Full test dose (200×10^6 micro-organisms)			4	0
Half the test dose (100×10^6 micro-organisms)			4	0

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Table IV clearly shows that the serum of the rabbit immunised with suspensions of the virulent strain "205 D" exercised a protective action, even when injected in a dose of 0·25 c.c. Only one out of five mice which were given this dose died, whereas a dose of 0·5 c.c. protected all the mice tested. Absorption of the O and H antibodies did not affect the protective action of this serum. On the other hand, the sera prepared against the four strains of low virulence did not exert any protective action whatever when injected in a dose of 1 c.c.

With a view to demonstrating the Vi antigen and ascertaining its relation to the O and H antigens, the rabbit sera employed in the preceding experiment were also tested for their content of the various types of agglutinins. According to the procedure adopted by Felix some control rabbits were immunised with suspensions of the virulent strain "205 D" heated at different temperatures (58, 70 and 100° C.). The total numbers of organisms inoculated in the course of the immunisation are recorded in Table V, together with the titres of the various rabbit sera.

Table V. *Agglutination reaction*

Rabbits immunised with strains of *B. typhosus*						
No. of rabbit	Type of strain	Strain	Dose in millions of micro-organisms	Titre of antibodies in sera		
				H	O	Vi
10	Inagglutinable	205 D living suspension	800	12,800	6,400	320
12		205 D heated at 58° 1½ hr.	10,000	25,600	25,600	0
13		205 D heated at 70° 1 hr.	10,000	640	25,600	0
14		205 D heated at 100° 1 hr.	10,000	320	12,800	0
24	Agglutinable	Botson living suspension	1,600	3,200	6,400	0
21		Rawling living suspension	1,600	4,800	21,000	0
22		40 living suspension	1,600	4,800	21,000	0
23		2123 living suspension	1,600	6,400	25,600	0

In order to prove that the Vi antibody is not dependent upon the H and O antibodies, the serum of rabbit N 10 (immunised with the living culture strain N 205 D) was absorbed in a dilution of 1 in 20 with both inagglutinable and agglutinable strains. After the absorption of the O and H agglutinins with highly agglutinable strains, the presence of the Vi antibody, although in low concentration, could be readily demonstrated in the serum (Table V). No Vi antibody could be traced in sera obtained by immunisation with agglutinable strains, or with inagglutinable strains heated at 58, 70 and 100° C.

This gives us reason to believe that the high virulence of strains of *B. typhosus*, their inagglutinability and protective value is due to the Vi antigen.

The present experiments seem to confirm the suggestions advanced by Felix and his followers, and we propose to continue our researches more especially upon other properties of Vi antigen.

SUMMARY

1. The strains of *B. typhosus*, which are inagglutinable, or non-sensitive to somatic O agglutinins, are virulent, whereas the agglutinable, or sensitive strains, show a low virulence.

2. Virulence and inagglutinability of strains seem to depend upon the presence in them of a virulence (Vi) antigen.

3. A considerable protective action, observed by means of active and passive immunisation, is exerted by the inagglutinable strain used (205 D), because it contains Vi antigen.

4. When the O and H agglutinins are eliminated through absorption, the agglutination reaction may serve to demonstrate *in vitro* the presence of the Vi antibody.

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