THE Vi ANTIGEN OF *SALMONELLA PARATYPHI B*

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CONTENTS

INTRODUCTION

In the first paper of this series (Felix, 1952*a*) the labile Vi antigen of *Salmonella paratyphi B* and *Salm. typhi-murium* was compared with the Vi antigens of *Salm. typhi* and *Salm. paratyphi A* with regard to the effect on them of various chemical and physical agents. In the second paper (Felix, 1952*b*) the behaviour of the Vi antigen of *Salm. paratyphi A* under varying conditions of growth was described, with the part it plays in virulence as tested in the mouse.

This paper is concerned with a similar investigation of the role of the Vi antigen in the pathogenic and immunogenic activities of *Salm. paratyphi B*.

VIRULENCE AND AGGLUTINATION WITH O AND Vi SERA

It was shown in the preceding paper (Felix, 1952*b*) that the difference in the O-agglutinability of sensitive and non-sensitive strains of *Salm. paratyphi A* is much less striking than that between O-agglutinable and O-inagglutinable strains of *Salm. typhi*. When strains of *Salm. paratyphi B* are tested against pure O antisera the differences observed are still less conspicuous. To select suitable strains for the experiments described in this paper twenty-four strains maintained at the National Collection of Type Cultures, London, were examined in 1934. Eighteen of the cultures were smooth and two of these, nos. 158 and 359, were selected as showing the greatest difference in O agglutinability. Another pair of strains was selected from a number of cultures isolated only a few weeks before the experiments were started. The four strains are listed in Table 1.

Table 2 shows that the two O-sensitive strains 158 and 18142 were agglutinated by the O serum to titres only two or three times higher than those recorded for the

* Miss R. Margaret Pitt collaborated in some of the early experiments described in this paper.

https://doi.org/10.1017/S002217240001980X Published online by Cambridge University Press

two non-sensitive strains 359 and HB3. For comparison it may be recalled that in *Salm. paratyphi A* the difference was approximately tenfold (Felix, 1952*b*, Table 2). On the other hand, in Vi agglutination *Salm. paratyphi B* behaved in the

Table 1. *Details of strains of* Salmonella paratyphi B *used in the present investigation*

Strain	Sensitiveness to O agglutinins	Isolated: Locality	Isolated: Year	Reference
158	Sensitive	Unknown	About 1908	Savage: culture received in 1934 from the National Collection of Type Cultures, London
359	Non-sensitive	Southampton	1917	Fletcher: culture received in 1934 from the National Collection of Type Cultures, London
HB3	Non-sensitive	Palestine	1934	Cultures received in 1934 from Dr K. S. Krikorian, Jerusalem
18142	Sensitive	Palestine	1934	

Table 2. *Agglutination reactions and virulence of strains of* Salmonella paratyphi B

Serum	Dilution	Strains: 158	359	HB3	18142
Agglutination of living organisms					
BH serum (spec.)	1:10,000	+ +	+ +	+ +	+ +
BO serum	1:200	+ + +	+ + +	+ + +	+ + +
	1:500	+ + +	+ + +	+ + +	+ + +
	1:1000	+ +	+ ±	+ ±	+ +
	1:2000	+	±	±	+
	1:5000	(±)	−	−	±
	1:10,000	−	−	−	−
BVi serum (rabbit no. 94, *versus* strain HB3 live, absorbed with acid-treated organisms of strain HB3)	1:200	+ + +	+ + +	+ + +	+ + +
	1:500	+ + +	+ +	+ +	+ + +
	1:1000	+ + +	±	±	+ + +
	1:2000	+	−	−	+
	1:5000	((±))	−	−	(±)
Virulence for mice					
Dose 100×10^6 organisms intraperitoneally		1/6	5/6	6/6	0/6

The technique of the agglutination and virulence tests has been described in a previous paper (Felix & Pitt, 1951).

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

Numerators of fractions indicate the number of mice that died, denominators the number inoculated.

same manner as *Salm. paratyphi A*. Table 2 shows that the Vi serum agglutinated the two O-sensitive strains 158 and 18142 to titres approximately four times higher than those obtained with the two O-insensitive strains 359 and HB3.

https://doi.org/10.1017/S002217240001980X Published online by Cambridge University Press

From what is known of *Salm. typhi* and *Salm. paratyphi A* regarding the inverse ratio between Vi-agglutinability and the Vi-antigen content of a culture, it was to be expected that the strains exhibiting the lower titres of Vi agglutination would be more virulent for mice than those giving the higher titres. Table 2 shows that this was so.

CONTENT OF Vi ANTIGEN AND VIRULENCE OF STRAINS

The relative Vi-antigen content of the four strains of *Salm. paratyphi B* was estimated by quantitative absorption of Vi agglutinins by means of the technique recently described by Felix & Pitt (1951) in experiments with *Salm. typhi*. The results of an absorption and virulence test are shown in Table 3. For reasons that will become apparent in the subsequent discussion, the table has been reprinted from the original paper on this subject (Felix & Pitt, 1936).

Table 3. *Virulence of strains of* Salmonella paratyphi B *in relation to their content of Vi antigen*

[The table is reprinted from the paper by Felix & Pitt (1936, Table III, p. 84)]

Absorption of Vi antibody

		Vi serum made with live organisms of strain 18142 and absorbed with acid-treated organisms of strain 18142				
			Further absorbed with living organisms of strains			
	Serum dilutions	Control not further absorbed	158	359	3	18142
Agglutination of living organisms of strain 18142	1:100	+ + +	+ + +	+	+	+ + +
	1:200	+ + +	+ +	−	−	+ +
	1:500	+ + +	+	−	−	+
	1:1000	+	−	−	−	−
	1:2000	−	−	−	−	−

		Virulence to mice			
		Organisms of strains			
		158	359	3	18142
Test dose intraperitoneally	200×10^6	1/4	4/4	4/4	0/4
	100×10^6	0/6	6/6	6/6	0/6

Numerators of fractions denote the number of animals that died, denominators the number inoculated.

Strain 3 of this table is the same as strain HB3 of the present paper.

The absorption tests in Table 3, as originally printed, were recorded in less detail than the corresponding tests with *Salm. paratyphi A* described in the preceding paper (Felix, 1952*b*, Table 3, p. 543), though the same technique was used in the two sets of experiments. The results shown in Table 3 for the four strains of *Salm. paratyphi B* were those obtained from the absorption of 2·5 ml. of serum dilution 1:100 with 1000×10^6 organisms/ml. A larger absorbing dose of 4000×10^6 organisms/ml. had also been employed at the same time, but for economy of space the results were omitted from the original table.

https://doi.org/10.1017/S002217240001980X Published online by Cambridge University Press

A comparison of Table 3 with the corresponding table in the preceding paper (Felix, 1952*b*) shows that the association of virulence for mice with the quantity of the Vi antigen in the culture is as intimate with *Salm. paratyphi B* as it is with *Salm. paratyphi A*.

EFFECTS OF VARYING CONDITIONS OF GROWTH

Like the *Salm. paratyphi A* strains dealt with in the preceding paper the four *Salm. paratyphi B* strains were also studied under varying conditions of growth. The relatively virulent strains 359 and HB 3 did not show any decrease in virulence or Vi-antigen content when grown at 21° C. on trypsin-digest agar, or at 37° C. on agar containing 1 in 900 phenol. Prolonged subculture of the relatively avirulent strains 158 and 18142 on agar containing 20% normal rabbit serum or 20% ascitic fluid did not enhance development of Vi antigen nor raise the virulence of the cultures for mice. Growth on these media in an atmosphere of 5% CO_2 was also ineffective in this respect.

THE Vi ANTIGEN OF *SALMONELLA TYPHI-MURIUM*

It was established in the early investigation that the Vi antigen of *Salm. paratyphi B* was identical with that of *Salm. typhi-murium*. The original paper also contained the following paragraph: 'Now the question arises, on what grounds are these salmonella antigens classified as Vi antigens, together with that known in *S. typhi*, where the relationship to virulence has been well established? So far evidence of a similar relationship between the specific Vi antigen and the virulence of the organisms has been obtained in the case of *S. paratyphi A* and *B*. We do not yet commit ourselves with regard to *S. aertrycke*, though with this species, too, some preliminary tests seem to point in the same direction. Some strains of *S. aertrycke* were found to contain Vi antigen, others to be devoid of it' (Felix & Pitt, 1936, p. 84).

THE RELATION OF THE Vi ANTIGEN OF *SALMONELLA PARATYPHI B* TO O ANTIGEN V OF THE KAUFFMANN-WHITE SCHEMA

It is interesting to inquire how the Vi antigen of *Salm. paratyphi B* was disposed of in Kauffmann's papers.

Kauffmann (1936*a*, p. 786), using the technique of treatment with dilute HCl which Felix & Pitt (1936) recommended as a method of separating the 'labile' Vi antigens from the 'stable' O antigens, found that the O-factor V (five) of the Kauffmann-White schema was acid-labile, whereas the O-factor IV, like all the other O factors, was acid-stable. He therefore assigned to O-factor V a 'special position' ('Sonderstellung').

Kauffmann (1936*b*, p. 320) extended the 'special position' of the O-factor V by postulating that it is complex and is composed of a heat-stable factor V_1 and a heat-labile factor V_2. Table 4 is an English version of Kauffmann's table in which the properties of factors V_1 and V_2 were summarized (Kauffmann, 1936*b*, Table 2, p. 320).

This table was again printed in Kauffmann's monograph on the *Salmonella* group (Kauffmann, 1941, Table 6, p. 84) and incorporated in a more comprehensive table in a later paper (Kauffmann, 1943, Table 2, p. 25).

At the Fourth International Congress for Microbiology (1947) Kauffmann reported that heating at about 125° C. completely destroys the agglutinogenic property of the V antigen and strongly damages its agglutinable and binding property. 'Therefore Felix and Pitt's so-called "Vi antigens" of *S. paratyphi B* and *S. typhi-murium* must be identical with the V antigen of the Kauffmann-White schema' (Kauffmann, 1949, p. 331). In the discussion Felix (1949, p. 332) stated that it was not justifiable to classify the O-factor V, which is both acid- and alkali-labile, as an O antigen. He suggested that the symbols V_1 and V_2 be discontinued and this antigenic component be classed as the Vi antigen of *S. paratyphi B* and *S. typhi-murium*. Kauffmann's final remarks were: 'But in spite of this

Table 4. *Antigenic differences between factors V_1 and V_2 of antigen V*

(This is an English version of Kauffmann's (1936*b*) Table 2 published in *Z. Hyg. InfektKr.* **118**, 320.)

		V_1	V_2
Living, formalin, alcohol or 60° C.	1	+	+
	2	+	+
	3	+	+
2 hr. 100° C.	1	+	—
	2	+	—
	3	+	—
HCl	1	—	—
	2	—	—
	3	—	—

Key: 1 = agglutinability.
2 = agglutinin-binding capacity.
3 = agglutinogenic capacity.
+ = preserved.
— = is destroyed.

special behaviour we will keep the name V antigen and its position as an O antigen in our antigenic table. By heating at 100° C. the behaviour of this somatic antigen is almost like an O antigen. I repeat, there is no reason to change the name' (Kauffmann, 1949, p. 332).

In a paper published after the Copenhagen Congress, Kauffmann (1947, p. 593) stated: 'It seems no longer necessary to assume the existence of two different parts of the V antigen (V_1 and V_2); we may regard this antigen as a homogeneous somatic antigen, much more labile than the other O antigens and for that reason occupying a special position.'... 'The designation "Vi" antigen for the V antigen (somatic antigen no. five) is rejected' (see p. 594).

In his latest monograph Kauffmann (1951) bestowed upon the *Salmonella* group a new class of antigens, namely, the K antigens, and elevated the V antigen (antigen no. five) to the dignity of first member of this group. 'This antigen, earlier designated as O antigen, has a special position and differs from other somatic antigens' (see p. 40). 'The V antigen always occurs with the IV antigen in smooth strains and can be the cause of partial O inagglutinability in agglutination tests

with pure IV serum. The lability of the V antigen and its relation to the IV antigen indicate that the V antigen is a K antigen like the Vi antigen' (see p. 41).

There seems to be no doubt that the V antigen of the Kauffmann-White schema is identical with the Vi antigen of Felix & Pitt, and that there is no need to postulate the existence of a new class of K antigens.

DISCUSSION

The conclusion to be drawn from the experiments recorded in this paper is that the role of the BVi antigen in the pathogenic and immunogenic activities of *Salm. paratyphi B* is analogous to that of the AVi antigen in *Salm. paratyphi A* (Felix, 1952*b*). In these two species the presence in the cultures of their respective Vi antigens has much less striking effects than those of the Vi antigen of *Salm. typhi*. Extreme O-inagglutinability, so common in freshly isolated strains of *Salm. typhi*, is not met with in *Salm. paratyphi A* and *B*. Even those strains of *Salm. paratyphi A* and *B* that develop their specific Vi antigen in maximum quantity are only partially resistant to the action of the O antibody when examined immediately after isolation. Such strains, therefore, correspond to *Salm. typhi* strains of the intermediate type which are susceptible to both the O and the Vi antibody.

In earlier experiments on passive immunization of mice pure BO sera as well as pure BVi sera were found to protect mice against infection with the most virulent strains of *Salm. paratyphi B* available. The BVi antibody acting alone was at least as potent as, if not more so than, the BO antibody in protective action (Felix & Pitt, 1936). An obvious conclusion of practical importance was that the culture of *Salm. paratyphi B* to be included in T.A.B. vaccine should contain maximum amounts of both the Vi and the O antigens (Felix, 1941, 1951*a*).

The strain HB3, selected in 1936 on the basis of quantitative absorption of Vi antibody (see Table 3, reprinted from Felix & Pitt, 1936), is still widely used as a vaccine strain in this and many other countries. Early in 1940 the 'Rowlands' strain of *Salm. paratyphi B*, the vaccine strain then employed at the Army Vaccine Laboratory, was compared with the strain HB3. Two cultures of the 'Rowlands' strain, immediately after passage through mice (made by Brigadier (then Colonel) J. S. K. Boyd), were found to contain less of the BVi antigen than the strain HB3 that had been maintained on nutrient agar since its isolation in 1934. Dr J. H. Mason, of the South African Institute for Medical Research, Johannesburg, who adopted the strain HB3 as the vaccine strain in 1947, found the culture to be much more virulent for mice than any of the strains that had been in current use at the Johannesburg Institute (Mason, 1947, personal communication). In this respect, therefore, *Salm. paratyphi B* resembles *Salm. typhi* and *Salm. paratyphi A*; in all three species it is an inherent property of certain strains to maintain their specific Vi antigen at a high level almost indefinitely when grown on a simple nutrient agar medium.

Cultures of *Salm. paratyphi B* maintained for a long time under unfavourable conditions of growth do not lose their specific Vi antigen as readily as do cultures of *Salm. typhi*. This seems to be paralleled by the fact that Vi-negative variants of

Salm. paratyphi B are not encountered as often as those of *Salm. typhi*. These observations have been made during the past ten years in connexion with the phage typing of *Salm. typhi* and *Salm. paratyphi B* by means of adapted Vi phages (Felix, 1951*b*). It is possible, however, that Vi-negative strains of *Salm. paratyphi B* are less uncommon in other parts of the world than they are in Britain.

Salm. typhi-murium possesses the same Vi antigen as *Salm. paratyphi B*. The presence of this antigen in strains of *Salm. typhi-murium* has, however, no significant effect on the resistance of this organism to the action of the O antibody. Differences in the O-agglutinability of Vi-positive and Vi-negative strains of *Salm. typhi-murium* are hardly perceptible. It is, therefore, not to be expected that Vi-positive strains of *Salm. typhi-murium* should be more virulent than Vi-negative variants. The earlier observation (Felix & Pitt, 1936) that a considerable proportion of strains of *Salm. typhi-murium* do not contain the Vi antigen was confirmed in later work. This limited to a certain extent the practical value of the Vi-phage typing of this organism (Felix & Callow, 1943; Felix, 1951*b*).

The persistent claim by Kauffmann that *Salm. paratyphi B* does not possess a Vi antigen analogous to that of *Salm. typhi* has been reviewed in the preceding section. One of the criticisms levelled by Kauffmann on many occasions against Felix & Pitt's (1936) conclusion was that these workers 'did not recognize that the so-called "Vi" antigen is in reality the V antigen' (Kauffmann, 1947, p. 594). There may be some truth in this argument. On the other hand, there was at the beginning of this work no reason for suspecting that one of the recognized O antigens of the Kauffmann-White schema was in reality not an O antigen. The definition of what constitutes an O antigen was derived from the work of Weil & Felix (1920), who based it not only on resistance to heat but also on resistance to treatment with alcohol, dilute acid and dilute alkali. From the experiments described in this and the two preceding papers of this series (Felix, 1952*a*, *b*) it is abundantly clear that *Salm. paratyphi B* possesses a labile Vi antigen analogous to those of *Salm. typhi* and *Salm. paratyphi A*. From Kauffmann's work it is equally clear that the so-called O-factor V is identical with this labile somatic antigen of *Salm. paratyphi B*. The suggestion that the symbol V be abandoned and this labile antigen be classified as the Vi antigen of *Salm. paratyphi B* seems to be the only logical conclusion possible.*

It may be appropriate to recall that the particular antigen now known as factor V has changed its label in the past more than once. It was first designated by the Roman numeral I by Bruce White (1926) at the time when Kauffmann (1926) was unable even to confirm the existence of 'pure O' antisera. Kauffmann himself first assigned to this antigen the Roman numeral III (Kauffmann, 1930*a*, p. 522). Subsequently he changed the number to V (Kauffmann, 1930*b*). The Salmonella Subcommittee (1934) agreed to accept Kauffmann's notation V. Kauffmann (1936*b*, 1941, 1943) employed the symbols V_1 and V_2 to cover the supposedly complex antigen V, but later discontinued the use of the symbols V_1 and V_2 and

* Seven of the *Salmonella* types listed in the Kauffmann-White schema as containing O-factor V were found to be agglutinated by pure B Vi serum; twelve of the *Salmonella* types containing factor IV but not factor V were negative with B Vi serum.

reverted to V. It is, therefore, no time-hallowed name which it is now suggested should be abandoned.

Kauffmann's latest innovation in *Salmonella* serology, namely, the formation of a 'new' class of K antigens comprising the V antigen, the Vi antigen and the M antigen (Kauffmann, 1951, p. 40), will be discussed in the concluding paper of this series.

SUMMARY

1. *Salmonella paratyphi B* possesses a Vi antigen essentially similar to the Vi antigens of *Salm. typhi* and *Salm. paratyphi A*.

2. The biological function of the Vi antigen of *Salm. paratyphi B* is to protect the O antigen against the action of the natural or immune O antibody, thereby increasing the virulence of the microorganism.

3. The most suitable paratyphoid-*B* vaccine strains are those that develop both the Vi and the O antigens in maximum quantities. The methods of testing vaccine strains of *Salm. paratyphi B* are essentially the same as those applied to the virulent Vi + O form of *Salm. typhi*.

4. Evidence is brought to show that Kauffmann's conclusion that *Salm. paratyphi B* does not develop Vi antigen is unfounded. The so-called O-factors V_1 and V_2 obviously cannot be classified as O antigens.

5. It is suggested that the symbol V of the Kauffmann-White schema be abandoned and this antigenic component be classed as the Vi antigen of *Salm. paratyphi B*.

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(*MS. received for publication* 1. v. 52)