REACTIONS OF T Vi AND BALLERUP Vi HAPTENES WITH ANTISERA AGAINST Vi-COATED ERYTHROCYTES

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Since salmonella types are identified by their reactions with antisera, it is a matter of some importance to have typing sera of sharp specificity and suitably high titre. Such sera are usually produced by immunization followed by adsorption; in some instances dilution of an immune serum makes it possible to produce a satisfactory reagent. The studies reported here were made in an attempt to produce a pure Vi antiserum by the injection of an antigen from which other somatic and flagellar components had been eliminated. Such a possibility was suggested by the work of Spaun (1951), who showed that erythrocytes can be coated with Vi antigen extracted from *Salmonella typhi*. He further found that under certain conditions the coated cells appear to carry only Vi antigen as shown by agglutination reactions with Vi and O antisera. It seemed that immunization with such Vi-coated red cells prepared under conditions which provide the selective adsorption of the Vi antigen might yield a pure Vi antiserum.

MATERIALS AND METHODS

The following organisms were used during the study: *Ballerup* strain of paracolon bacillus, Vi form; *S. typhi* Vi 1; *S. typhi* 2, Vi form; *S. typhi* Watson, Vi form; *S. typhi* 0901, Vi-negative form; *S. typhi* 813, Vi-negative form; *S. typhi* 897, Vi-negative form.

Bacterial extracts for coating red cells were prepared from cultures grown on yeast-beef extract agar for 24 hr. at 37° C. After 24 hr. incubation at 37° C. the Vi extracts of *S. typhi* were made in the following way, based on the studies of Felix (1952*a*). The organisms were washed off the Blake bottles in N/1-HCl, 50–60 ml. per Blake bottle. After freezing and thawing twice, the extract was neutralized with N/1-NaOH. The cells were thrown down by centrifuging for 2 hr. at 3500 r.p.m. Rabbit erythrocytes were added to the supernatant to make a 5% suspension. After coating for 15 min. at 37° C. the cells were washed twice and resuspended in 5% strength.

Rabbits selected for immunization were subjected to a preliminary bleeding. Those showing no antibodies against Vi strains were used. Injections of coated cells were made every other day, with doses increasing as follows: 0·2, 0·5, 1·0, 1·5, and 2·0 ml. One week after the last injection a trial bleeding was made. If the antibody titre was found to be suitable, the animal was bled 2 weeks after the last injection. The serum from these animals was preserved with merthiolate, 2 mg./100 ml.

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I. *Antibody response to injection of coated cells*

Felix (1952*a*) has shown that the typhoid Vi antigen is resistant to dilute acid. We have made use of this fact in preparing extracts for immunization, as described under the section on methods. *S. typhi* Vi 1 was selected for extraction. Rabbit red cells coated with the HCl extract were agglutinated by anti-Vi serum but not by anti-O serum (IX, XII). Sera from rabbits immunized against the Vi-coated cells were tested for antibody by the rapid slide method using the organisms listed in Table 1.

Table 1. *Agglutinating action of Vi antisera*

	Antigens			
Vi-antisera	Vi-coated red cells	Ballerup	*S. typhi* Vi 1	*S. typhi* 813*
Rabbit, group I	1/640	1/1280	1/1280	1/10 neg.
Rabbit, group II	1/640	1/1280	1/1280	1/10 neg.

* A Vi-negative strain, giving no agglutination with Vi antiserum.

Table 2. *Agglutination reactions of various Vi-positive and Vi-negative forms with antisera*

	Antigens						
					Vi-negative forms		
Antiserum rabbits immunized with	Vi-coated R.B.C.	Ballerup	*S. typhi* Vi1	*S. typhi* Watson Vi	*S. typhi* 0901	*S. typhi* 813	*S. typhi* 897
Vi-coated R.B.C.	1/640	1/1280	1/1280	1/1280	1/10 neg.	1/10 neg.	1/10 neg.
Vi-extract (HCl) alone	1/10 neg.	1/10 neg.	1/160	1/160	1/160	1/160	1/160
Sediment from HCl extract	1/2560	1/2560	1/2560	1/2560	1/2560	1/2560	1/2560

Here it is seen that such a serum agglutinates organisms containing the Vi antigen, as well as the Vi-coated red cells, but does not agglutinate the strains of *S. typhi* which lack the Vi antigen.

Rabbits immunized with the sediment from the HCl extraction developed antibodies against both O and Vi antigens. The HCl extract alone (not adsorbed on red cells) immunized against O antigens in low titre but not against the Vi factor. This confirms the observations of Felix (1952*b*) that the Vi antigen, when separated from the cell, is not a good antigen but rather functions as a haptene.

The foregoing observations are summarized in Table 2.

These experiments show that the Vi haptene when properly attached to rabbit erythrocytes produces antibodies which agglutinate organisms carrying the Vi haptene, but do not react with the somatic antigens—in other words, a pure Vi antiserum.

II. *Heat stability of the Vi haptene*

Different opinions have been expressed regarding the inactivation of Vi antigens by heat. The subject has been studied in detail by Felix (1952*a*) who concluded

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that Vi antigens from different salmonellae differ in their lability, and further, that under various environmental conditions, the Vi antigen may be more or less labile. He also found that agglutinability and agglutinogenic properties are readily lost by heating in aqueous suspension, while agglutinin binding was observed after heating at 100° C. for 1 hr.

Our own experiences indicate that the Vi haptene has great resistance to heat, as shown by its ability to render coated red cells agglutinable. A sample of the *S. typhi* Vi 1 extract was autoclaved for 30 min. at 20 lb. pressure. The cooled extract was used to coat rabbit erythrocytes, which were then tested against Vi antiserum, using the rapid slide method. Prompt heavy agglutination showed that the combining property of the haptene was not destroyed by this treatment. It seems probable that heating may bring about the rapid extraction of the Vi antigen, thus producing a situation where the organisms are lacking in haptene while the supernatant contains the free substance in a form which can combine with antiserum and presumably neutralize it. The fact that the haptene, when adsorbed on a suitable carrier, can still combine specifically shows that its immunological specificity has not been lost. The Vi haptene was also shown to be agglutinogenic after autoclaving at 20 lb. pressure for 30 min. Red cells were coated with a Vi extract treated in this way and used to immunize rabbits as described in the section on methods. The sera of animals injected with these heated extracts agglutinated Vi cultures of *S. typhi* Vi 1 and Vi-coated cells as did the antiserum prepared against the unheated Vi haptene. These observations show that the agglutinogenic, agglutinin binding and agglutinating properties of the Vi haptene are not impaired by prolonged heating, and the haptene must be regarded as heat stable. The solubility of the Vi haptene is further shown by the fact that the supernatant fluid of a 24 hr. broth culture of *S. typhi* Vi 1 renders red cells agglutinable by Vi antiserum when it is used as a coating extract. On the other hand, the absolute alcohol extract of *S. typhi* Vi 1 was not able to coat red cells. This agrees with the observation that Vi antigens are best dried from alcohol suspensions when whole organisms are used to prepare Vi antisera.

III. *Relationship between Vi antigens of* S. typhi *and Ballerup*

Because Ballerup strains lack the O antigens of the salmonellae (Ballerup XXIX, Vi), this organism is often used in preparing Vi antisera, since no absorptions are necessary to remove anti-O antibodies. The antiserum produced in this way agglutinates Vi strains, both of salmonella types and of the Ballerup organism, and it is generally supposed that the Vi antigens are the same in both organisms.

Pure antisera were prepared against rabbit erythrocytes coated by extracts of *S. typhi* Vi 1 and by extracts of the Ballerup organisms. Each serum agglutinated Vi-coated red cells and bacterial suspensions of Vi strains. However, when the sera were absorbed, using homologous and heterologous antigens, an antigenic difference became apparent (Table 3). When the Vi-Ballerup antiserum was absorbed with either Ballerup or *typhi* suspensions, the Vi antibodies were completely removed. Likewise, when the Vi-*typhi* serum was absorbed by *S. typhi*, all Vi antibodies were removed. However, when the Vi-*typhi* anti-serum was absorbed

with a Ballerup suspension, antibodies against Ballerup were removed, but agglutinins active against *S. typhi* remained. When the titres of absorbed and unabsorbed serum are compared (Table 4), it is apparent that the Vi-*ballerup* antigen leaves about a quarter of the original antibody in the serum.

This unilateral absorption shows that the specificity of the Ballerup Vi haptene overlaps the *S. typhi* Vi, while the *S. typhi* Vi carries an additional specificity not shared with Ballerup.

Table 3. *Cross-agglutination reactions of absorbed Vi antisera*

	Absorbed by			
	S. ballerup		*S. typhi*-Vi 1	
Anti-serum	Against Ballerup	Against *S. typhi*	Against Ballerup	Against *S. typhi*
Anti-Vi, Ballerup	1/10 neg.	1/10 neg.	1/10 neg.	1/10 neg.
Anti-Vi, *S. typhi*	1/10 neg.	1/320	1/10 neg.	1/10 neg.

Sera were absorbed undiluted, using sedimented bacteria.

Table 4. *Absorption of anti-typhi-Vi serum*

T Vi antiserum	Titre against *S. typhi*	Titre against Ballerup
Before absorption	1/1280	1/1280
After absorption with Ballerup	1/320	1/10 neg.
After absorption with *S. typhi*	1/10 neg.	1/10 neg.

DISCUSSION

The nature of the Vi antigen and its relation to the schematic structure of the bacterial cell have been the subject of controversy since the discovery of this antigen by Felix in 1934 (Felix, 1952*a*). Without reviewing the different opinions expressed, it is fair to say that until precise chemical methods can be applied to the problem, serological techniques must be used in these investigations. Therefore, it is essential that the antisera used be of greatest possible purity and of high titre. The method we have used provides immune serum of satisfactory titre and excellent specificity, as shown by the agglutination tests summarized in Table 2. The good results obtained using Vi-coated cells suggests that the technique may be very useful in studying other antigenic constituents of the bacterial cell.

The observations on heat stability show that the T Vi haptene can not be considered heat-labile, since after 30 min. in the autoclave at 20 lb. pressure it was still able to react with Vi antiserum and was antigenic when adsorbed on rabbit red cells. This agrees with the work of Felix (1952*b*) who showed that the saline extract of Vi-positive O strains gives precipitation with Vi antisera, and that the extract resists heating for 2 hr. at 100° C.

It seems abundantly clear that the Vi antigen consists of a heat-stable haptene which may, if heated, become dissociated from its antigenic complex. Thus, the earlier work characterizing this fraction as heat-labile is not entirely correct, although heating in saline does eliminate the ability of a Vi form organism to react

with Vi antiserum. We are inclined to agree with Felix (1952*b*), who objects to accepting resistance to heat as a definitive test in differentiating supposedly different antigens. At any rate, if the response to heat is to be given the emphasis which Kauffmann gives it (Kauffmann, 1951), one ought to distinguish between destruction of a heat-labile antigenic substance and extraction of heat-stable haptene from the bacterial cell. With respect to the Vi antigen, the distinction is easily made using the coated cell technique.

SUMMARY

1. T Vi-coated rabbit erythrocytes, when injected into rabbits, stimulate the production of a (pure) Vi antiserum.

2. The T Vi extract was found to be heat-stable with respect to antigenicity and agglutinability, as shown by means of coated erythrocytes.

3. By means of cross-absorption tests it was shown that the Vi antigen of *S. typhi* differs from the Vi antigen of the Ballerup organism.

4. The need for caution in relying on resistance to heat as a character of groups of antigens is emphasized.

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REFERENCES

SPAUN, J. (1951). *Acta path. microbiol. scand.* **29**, 416.
FELIX, A. (1952*a*). *J. Hyg., Camb.*, **50**, 515.
FELIX, A. (1952*b*). *J. Hyg., Camb.*, **50**, 557.
KAUFMANN, F. (1951). *Enterobacteriaceae*. Copenhagen: Einar Munksgaard.

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