A NEW TYPE OF *SALMONELLA* (*S. BALLERUP*) WITH VI-ANTIGEN*

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THE purpose of this paper is to describe a new type of *Salmonella* (*S. ballerup*) which is remarkable serologically in that it possesses the Vi-antigen of the typhoid bacillus.

By means of the combined enrichment method (Kauffmann) an organism belonging to the *Salmonella* group was isolated on 18 November 1939, from the faeces of a female patient who came from the town of Ballerup and was lying in the Copenhagen County Hospital at Gentofte. This patient, aged 69, had become ill at the beginning of October 1939 with nausea, vomiting and diarrhoea; her temperature was not taken. She was ill at home for several weeks and was only hospitalized in the middle of November. After the first faeces analysis on 18 November 1939 had proved to be positive, the second one on 2 December was negative. A Widal test was made on 28 November and 2 December, with negative result on both occasions. Beginning with a dilution of 1 : 2 the serum was tested with all likely antigens, especially with typhoid Vi-antigen and the live homologous culture. This negative result of the Widal test means little, as about two months had elapsed after the illness began. We can say nothing definite as to the pathogenity of this new type to man, but merely assume that it was the cause of a case of gastro-enteritis.

CULTURAL CHARACTERS

Motile, Gram-negative rods growing well on agar and in broth. Fermenting arabinose, dextrin, dextrose (with gas), dulcitol, maltose, mannitol, rhamnose, sorbitol, trehalose and xylose; not fermenting adonit, inositol, lactose and sucrose. Fermentation of salicin after 7–8 days. Positive reaction in Bitter, Weigmann & Habs's media with arabinose, dextrose, dulcitol, rhamnose and xylose. Negative reaction in Stern's glycerine fuchsin broth, which stains red after 8 days and violet only after 14 days. Fermenting *d*-tartrate after 7 or 8 days, sodium citrate after 2 days and mucate after 1 day; *l*-tartrate and *i*-tartrate were not attacked after 14 days' incubation. Produces hydrogen sulphide promptly; failing to produce indole, to liquefy gelatin or to form slime wall. Good growth on brilliant green agar and on Simmons's agar with arabinose, dextrose, dulcitol, sodium citrate and rhamnose, so that the strain may be described as "ammonstark".

As regards its cultural characters it may be stated that on agar plates the strain forms two kinds of colonies of macroscopically different appearance: one is opaque, the other translucent. Both kinds are smooth forms, which are stable in saline solution. Through series of subcultures the opaque form is very constant and only rarely dissociates the clear form, whereas the clear form is

[1] This work was undertaken with the support of the Commonwealth Fund.

much less stable, as in from 20 to 50 % of the colonies it regularly dissociates the opaque form again. It should be pointed out at this stage that the opaque form contains Vi-antigen (V form), whilst the clear form has no Vi-antigen (W form). Sometimes there are colonies of which one half or a segment is clear and the other opaque (VW form). Accordingly, here we have the same circumstances as in the typhoid bacillus, on whose V, W and VW forms Kauffmann has already reported. If it is desired to differentiate clearly between both kinds of typhoid colonies, it is best to make a mixture of pure V and pure W forms, for which the V form may be taken from the Watson strain and the W form from strain H901. If this mixture is spread on agar plates, whereby isolated colonies arise, it is possible by means of transmitted light (best against an electric bulb) to distinguish two kinds of colonies. One is opaque = the V form of Watson, whereas the other form is translucent = the W form of H901. Thus even without serological tests we can differentiate the V and W forms of the typhoid bacillus from each other:

S. typhi	Appearance of colonies	O-agglutination	Vi-agglutination
V form	Opaque	–	+
W form	Translucent	+	–

In the case of *S. paratyphi* C, too, we can distinguish the colonies with or without Vi-antigen from each other macroscopically, but not so clearly as with *S. typhi* and *S. ballerup*.

Serological characters

Serologically the strain (7851/39) was outstanding when isolated in that in slide agglutination with all the *Salmonella* sera available it gave positive agglutination only in Vi-serum. For this reason it might therefore have been an O- and H- inagglutinable strain of *S. typhi*; this diagnosis, however, was ruled out with certainty by means of the cultural tests and further serological tests.

When spread on agar plates the aforesaid two kinds of colonies were at once apparent, and only the opaque growths contained the Vi-antigen. These colonies, which as in the case of *S. typhi* we shall call "V forms", in slide agglutination reacted negatively in all O-sera. On the other hand the translucent forms, "W forms", which lacked the Vi-antigen, gave positive slide agglutination in an O-serum from *S. senftenberg* and weak agglutination in an O-serum of *S. typhi*. Both forms behaved identically in a cultural sense. In subcultures on agar plates the W form was only rarely dissociated from the V form, and in some passages it was entirely absent, whereas the V form frequently and regularly dissociated from the W form. This means that in order to obtain the purest possible suspensions of the W form these must be prepared directly from single colonies. With the V form, on the other hand, this is unnecessary, as we can employ a mass culture if it has been grown from a single V form colony.

Both forms gave negative H-agglutination with all *Salmonella* H-sera, so that beyond all doubt we had here a new H-antigen, both forms being very motile.

The H-antigen of *S. ballerup*

For producing the Ballerup H-serum five rabbits were immunized intravenously with a 6 hr. broth culture to which had been added 0·5 % formalin, incubated 20 hr. at 37° C. and then kept in the refrigerator. The sera of the five rabbits were mixed and supplemented with 0·5 % phenol. This serum had a titre of 6400.

Just as the *S. ballerup* culture failed to react to any of the *Salmonella* H-sera, *S. ballerup's* H-serum did not agglutinate any of the *Salmonella* cultures. We are thus concerned with a new H-antigen, which we call z_{14}. So far we have been unable to demonstrate any phase variation within the H-antigen, but no special tests were made, for example, with the swarming method (Schwärmmethode).

The O-antigen of *S. ballerup*

To obtain the Ballerup O-serum, five rabbits were immunized intravenously with a 20 hr. broth culture which was heated for 2½ hr. at 100° C. and then kept in the refrigerator. The sera of these five rabbits were mixed and 0·5 % phenol was added. This serum had an O-titre of 12800.

As its chief antigen the O-antigen of *S. ballerup* contains a new O-antigen, which we call XXIX. On this occasion we shall not dwell upon an overlapping O-antigen that is closely related to the XIX-antigen of *S. senftenberg*, as the structure of the XIX-antigen will be accounted for in another connexion later. To produce the pure XXIX-agglutinin, *S. ballerup's* O-serum is absorbed with *S. senftenberg*, thus removing the overlapping O-agglutinin, which to *S. senftenberg* has a titre of 160.

In the O-agglutination of *S. ballerup* as already stated we encounter the same circumstance as in *S. typhi*, for as a consequence of the Vi-antigen we get O-inagglutinability, which is removed on heating to 100° C. The V form of *S. ballerup*, live or killed with formalin, is not agglutinated either by the homologous serum or by other O-sera, whereas the W form of *S. ballerup* is O-agglutinable.

The Vi-antigen of *S. ballerup*

To obtain Ballerup Vi-serum five rabbits were immunized intravenously with live bacteria of the V form from 20 hr. agar plates, the number of bacteria per injection being 100, 200, 400 and 800 millions. The rabbits tolerated these injections well and were bled 6 days after the last injection. Phenol 0·5 % was added to the sera, which were first titrated separately. It having proved that the Vi-titre of these five sera lay between 640 and 1280, they were mixed and again titrated. The Vi-titre was now 1280, the O-titre 6400 and the H-titre 3200.

The Vi-antigen of *S. ballerup* is serologically identical with the Vi-antigen of *S. typhi* and *S. paratyphi* C

If a pure typhoid Vi-serum (i.e. an OH Vi-serum made from live typhoid bacilli of the V form and absorbed with the strain Ty H901 W form) is absorbed with the live V form of *S. ballerup*, this serum becomes completely exhausted. Conversely, *S. ballerup's* OH Vi-serum can be deprived completely of its Vi-agglutinins with the V form of *S. typhi*, so that only Ballerup

O- and H-agglutinins are left. Now, as the Vi-antigens of *S. typhi* and *S. paratyphi* C are serologically identical, the Vi-antigens of *S. paratyphi* C and *S. ballerup* must also be mutually identical. Accordingly, to-day we know of three different types of Salmonella with Vi-antigen:

S. paratyphi C	= VI. VII. [Vi].	$c \leftrightarrow 1, 5, \ldots$.
S. typhi	= IX. [Vi].	*d*.
S. ballerup	= XXIX. [Vi].	z_{14}.

The Vi-sensitivity of *S. ballerup* is not so high as that of the well-known typhoid strain Watson V form, as *S. ballerup's* V form is agglutinated by a pure Vi-serum up to the dilution of 1 : 160, whereas the Watson V form goes up to 1 : 320. Nevertheless, the Ballerup V form sensitivity and stability are great enough to serve to demonstrate the Vi-agglutinin. For example, if from a 20 hr. agar plate with Ballerup V form, which reacts negatively in Ballerup O-serum and positively in typhoid Vi-serum, we use a fresh suspension in 0·5 % formalinized saline solution, we have an excellent, sterile reagent for demonstrating Vi-agglutinin in patient serum. Owing to the lack of typhoid-patient sera we were unable to make Widal reactions, but had to work with rabbit immune sera. From these experiments it became clear that the V form of *S. ballerup* can be employed for the demonstration of Vi-agglutinin in serum (see Table 1), for as the live or formalin-killed Ballerup V form is agglutinated neither by O-sera nor by typhoid H-serum, a positive, granular agglutination evidences the presence of Vi-agglutinin.

In Table 1 we have set up some results of test-tube agglutination, in which the fundamentally most important speak for themselves.

The employment of slide agglutination with live agar cultures gives the results shown in Table 2. It will be seen from these that with the aid of *S. ballerup's* OH Vi-serum we can make a sharp differential diagnosis between the V and W forms of *S. typhi*, so that for this purpose we no longer need a pure Vi-serum of *S. typhi*.

Accordingly, *S. ballerup's* antigenic formula may be written as XXIX. [Vi]. z_{14}, the angular brackets indicating that the Vi-antigen is missing in the W forms.

With this we have simultaneously established one more example of the V–W change of form which Kauffmann first described in connexion with *S. typhi*; in this strain of *S. ballerup* it is characterized by the fact that the V form, in contrast to the W form, is very stable. As with *S. typhi*, both forms can be distinguished from each other macroscopically by the appearance of the colonies alone.

THE MOUSE PATHOGENITY OF *S. BALLERUP*

S. ballerup proved to be apathogenic, or almost apathogenic, in feeding experiments on white mice, for it was only after feeding with enormous quantities of bacteria that a few mice died (with positive findings of bacteria in the heart blood). In the case of intra-abdominal infection, too, the patho-

Table 1

Pure Vi-serum of S. typhi

	20	40	80	160	320	640	1280	Saline
S. ballerup V live	+ +	+ +	+ +	+	±	−	−	−
S. ballerup V 100°	−	−	−	−	−	−	−	−
S. typhi Watson V live	+ +	+ +	+ +	+	+	±	−	−

O-serum of S. typhi

	200	400	800	1600	3200	6400	12800	25600	51200	Saline
S. ballerup V live	−	−	−	−	−	−	−	−	−	−
S. ballerup V 100°	+	±	−	−	−	−	−	−	−	−
S. typhi H 901 alcohol	+ +	+ +	+ +	+ +	+ +	+ +	+	±	−	−

O-serum of S. ballerup

	200	400	800	1600	3200	6400	12800	25600	51200	Saline
S. ballerup V live	±	−	−	−	−	−	−	−	−	−
S. ballerup V 100°	+ +	+ +	+ +	+ +	+ +	+ +	+	±	−	−
S. typhi H 901 alcohol	−	−	−	−	−	−	−	−	−	−

Reading after 2 hr. at 37° C. and 20 hr. at room temperature.

H-serum of S. ballerup

	50	100	200	400	800	1600	3200	6400	12800	Saline
S. ballerup formalinized broth	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+	−	−
S. typhi H 901 formalinized broth	−	−	−	−	−	−	−	−	−	−

H-serum of S. typhi

	50	100	200	400	800	1600	3200	6400	12800	Saline
S. ballerup formalinized broth	−	−	−	−	−	−	−	−	−	−
S. typhi H 901 formalinized broth	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+	−	−

Reading after 2 hr. water-bath 50° C.

Table 2. *Slide agglutination with live agar cultures*

	Immune sera					
	S. typhi			*S. ballerup*		
Cultures	O	H	Vi	O	H	OH Vi
S. typhi H 901 W	+ +	+ +	−	−	−	−
S. typhi Watson V	−	+ +	+ +	−	−	+ +
S. ballerup W	±	−	−	+ +	+ +	+ +
S. ballerup V	−	−	+ +	−	+ +	+ +

Explanations: ±, +, + + = different strengths of agglutination. − = negative.

genity is very low, as some of the infected mice died only after the injection of large doses (400–800 millions). An injection of 200 millions is easily tolerated, regardless of whether the V or the W form is injected. We were unable to establish the existence of any difference in pathogenity between the V and W forms. The V forms were in no way more toxic or more virulent than the W forms; in fact, more mice died from the W form infection than of the V form infection.

Having regard to this low mouse pathogenity we did not make active immunization experiments against Ballerup infection on mice, but we did make

https://doi.org/10.1017/S0022172400027790 Published online by Cambridge University Press

an experiment for the purpose of testing the potency of the Ballerup V form against an infection with the typhoid V form.

With this in view we vaccinated twenty-four mice intra-abdominally with a vaccine of *S. ballerup's* V form (heated for 1 hr. at 100° C.) four times at intervals of 5 days (100, 200, 400, 800 millions per injection); 14 days after the last injection the vaccinated mice were infected with over 500 million bacteria of the V form of typhoid 2 strain. Though this trial-infection was very strong, being more than ten times the lethal dose, eleven mice survived this infection with live typhoid bacilli of the V form. All six controls, which received 500 millions, died after 1 day.

Thus we succeeded in immunizing mice actively against infection with the typhoid V form by means of a V form vaccine of *S. ballerup*.

SUMMARY

1. A description is given of a new *Salmonella* type (*S. ballerup*) with Vi-antigen which was probably the cause of an attack of gastro-enteritis in man.

2. *S. ballerup* contains a new O- and a new H-antigen, so that the antigenic formula reads XXIX.[Vi].z_{14}. There is also a slight O-antigen relationship with the XIX-antigen of *S. senftenberg*.

3. Like *S. typhi*, *S. ballerup* has the V–W change of form, as colonies occur with or without Vi-antigen; of these the V forms are much more stable than the W forms. The V forms are O-inagglutinable.

4. As with *S. typhi*, the V and W forms are already distinguishable macroscopically on agar plates, the V forms being opaque, whereas the W forms are translucent.

5. *S. ballerup's* Vi-antigen is serologically identical with *S. typhi* and *S. paratyphi* C's Vi-antigen.

6. By means of the V form of *S. ballerup* it is easy to demonstrate Vi-agglutinin in typhoid sera, as there is no need to fear disturbances from O- and H-antibodies.

7. By means of a V form vaccine of *S. ballerup* mice can be immunized actively against a lethal infection with the typhoid V form.

8. The mouse pathogenity of *S. ballerup* is very low. There is no distinct difference in the pathogenity of the V and W forms of *S. ballerup*.

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See also literature in above papers.

(*Received for publication* 25. I. 40.—Ed.)