

**SEROLOGICAL ANALYSIS OF THE ANTIGENIC STRUCTURE  
OF « SALMONELLA EILBEK »,  
A STRAIN INTERMEDIATE  
BETWEEN « SALMONELLA » AND « ARIZONA »**

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**RÉSUMÉ**

**ANALYSE SÉROLOGIQUE DE LA STRUCTURE ANTIGÉNIQUE  
DE *Salmonella eilbek*,  
SOUCHE INTERMÉDIAIRE ENTRE *Salmonella* ET *Arizona***

*Salmonella eilbek* (61:i:z) a été isolée de matières fécales d'un lézard en Thaïlande en 1963 et sa formule antigénique établie à Hambourg. La souche a reçu le nom de *eilbek* qui est un district de cette ville.

*S. eilbek* est considérée comme une souche intermédiaire entre le genre *Salmonella* et le genre *Arizona*. Elle ne fermente pas le lactose, le dulcitol ni le mucate; son antigène O est 61<sub>1</sub>, 61<sub>2</sub>.

*S. eilbek* est identique à *Arizona* 26a,26b:29:30, mais non à *Arizona* 26a,26c:23:30.

L'antigène i de *S. eilbek* n'est pas identique à l'antigène i de *S. typhimurium*, mais est identique à l'antigène H:33 d'*Arizona* 26:33:25.

L'antigène z de *S. eilbek* est identique à l'antigène H:31 d'*Arizona* 16:24:31; mais non à l'antigène z de *S. poona*, *S. greenside* ou *S. constantia*.

Nous avons mis en évidence des différences concernant l'antigène z dans le sous-genre II et dans *Arizona* H:31. Les immunsérums spécifiques anti-26b et anti-26c ont été préparés, et à l'aide de ces sérums, tous les *Arizona* connus du groupe 26 ont été classés dans les sous-groupes 26a,26b et 26a,26c.

**MOTS-CLÉS :** *Salmonella eilbek*, *Arizona*, Antigènes.

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An atypical organism was isolated from a stool specimen of a lizard in Thailand. The biochemical behaviour and the antigenic formula were determined at the National Salmonella Centre, Hamburg, in 1963 and confirmed by Kauffmann as a new atypical member of the *Salmonella sub-genus II* and as a representative of a new O:group, numbered 61 [3]. The strain was named *Salmonella eilbek* after a district in the town of Hamburg, and it was registered by Kauffmann in the Supplement VII to the Kauffmann-White scheme [3].

A detailed biochemical and serological study have not previously been available and the characteristics of the strain are described in the present report.

#### A. BIOCHEMICAL BEHAVIOUR

The strain promptly fermented glucose (+ gas), arabinose, trehalose, xylose, maltose, mannitol (+ gas), and sorbitol. Lactose, sucrose, salicine, dulcitol, inositol, and rhamnose were not fermented.

Indol and acetyl-carbinol were not produced. The methyl red test was positive.

The organism produced a positive reaction in Simmon's citrate agar and in Stern's glycerol-fuchsin-broth. H<sub>2</sub>S was produced. Nitrates were reduced. There was no decomposition of urea and no growth in KCN-medium. The LDC reaction was positive but fermentation of D-tartrate (= x) was irregular and delayed whereas there was no fermentation of l- and i-tartrate and mucate.

The reactions in sodium citrate (+ 2) and sodium malonate were positive. Gelatine was liquified after 3 days (Kohn-Lautrop's method). The  $\beta$ -galactosidase-test was weakly positive after 24 hours (when examining 24 single colonies, 22 were weak positive after 24 hours, whereas 2 colonies were negative after 3 days).

According to these reactions, *S. eilbek* has an intermediate position between *Salmonella sub-genus II* and *Arizona* (= *sub-genus III*). The negative reactions in dulcitol and mucate are more in favour of *Arizona*, but the inability to ferment lactose after 30 days is decisive for classifying it as an atypical member of *Salmonella sub-genus II*.

#### B. SEROLOGICAL BEHAVIOUR

In slide agglutination tests the strain was negative with a polyvalent *Salmonella* anti O-serum which contained antibodies against all O-groups known at that time (O-groups: A-60).

On the other hand, a positive agglutination with a polyvalent serum anti those O-groups of *Arizona* which were not related to *Salmonella* was obtained. By using these different *Arizona* anti O-sera, a strong positive agglutination with an *Arizona* serum anti O-group 26 (prepared from *Arizona* 26:32:21) was demonstrated.

In view of the biochemical characteristics which placed the strain in *Salmonella sub-genus II* (atypical), and on account of the positive agglutination only with anti O:26-serum of *Arizona*, which did not correspond to *Salmonella* O-groups, it was necessary to establish a new *Salmonella* O-group:61.

The flagella antigens of *S. eilbek* were verified as i for the first phase and z for the second phase. Thus the complete formula was 61:i:z.

The combination of any specific H-phase (for example « i ») with « z » as a second phase is mainly typical for *Arizona (sub-genus III)*. This finding demonstrated, once again, the intermediate position of *S. eilbek*.

When testing the H-antigens with the specific factor-sera developed by Rohde [4], a negative reaction was found with factor-sera iI (= serum anti *S. aberdeen*, phase 1, absorbed by i of *S. eilbek* + H:33 of *Arizona* 34:33:28 + r of *S. heidelberg*) and zI (= serum anti *S. poona*, phase 1, absorbed by z of *S. greenside* + z<sub>44</sub> of *S. guinhon*) and a positive reaction was found with factor-sera iII (= serum anti *S. eilbek*, phase 1, absorbed by i of *S. typhi-murium*) and zII (= serum anti *S. greenside*, phase 1, absorbed by z of *S. poona* + z of *S. worthington* + z of *S. schæneberg*). The iI and zI factor-sera are characteristic only for the *sub-genus I* strains of *Salmonella*, whereas the iII and zII factor-sera are characteristic for *sub-genus II* strains of *Salmonella* as well as for *Arizona* strains (*sub-genus III*).

### C. SEROLOGICAL ANALYSIS OF THE O-ANTIGEN OF *S. EILBEK* IN COMPARISON WITH THE *ARIZONA* O-GROUP 26

Due to close collaboration with the *Arizona*-Laboratory in Atlanta, all the *Arizona* types belonging to O-group 26 were received from Dr. Martin and from Dr. Hermann.

With the help of serological experiments it was intended to determine

- 1) whether O-antigen 61 corresponds to *Arizona* sub-groups 26a, 26b, or 26a, 26c ;
- 2) to differentiate all the known *Arizona* types belonging to O-group 26, which are in the Hamburg collection, into sub-groups 26a, 26b and 26a, 26c.

In the various monographs concerning *Arizona*, types are registered only with simplified O-formula 26.

### MATERIAL AND METHODS

In addition to *S. eilbek*, 15 *Arizona* types belonging to O-group 26 were also tested.

Two O:26 factor-sera (26b and 26c) were used. These were prepared as follows:

- 1) Serum anti O:26b = O-serum anti *Arizona* Minn 240 = 26a,26b:29:30 absorbed by *Arizona* 4850-52 = 26a, 26c:23:30.
- 2) Serum anti O: 26c: O-serum anti *Arizona* 4850-52 = 26a,26c:23:30 absorbed by *Arizona* Minn 240 = 26a,26b:29:30.

## RESULTS

By using the above defined anti *Arizona* O:26b and O:26c factor-sera it was found that *S. eilbek* had the detailed O-antigen 26a,26b because it was positive with the O:26b and negative with the O:26c factor-serum. In a *Salmonella* formula, the detailed *S. eilbek* O-antigen would be: 61<sub>1</sub>, 61<sub>2</sub>.

Table 1 shows the *Arizona* serotypes which belong to the detailed O-group 26a,26b (with *Salmonella*-formula = 61<sub>1</sub>,61<sub>2</sub>) or to 26a,26c (= 61<sub>1</sub>,61<sub>3</sub>). The strain no. 5128-62, also belonging to group O:26, could not be serologically determined because of its rough colonial morphology.

From the cross absorption tests it was clear that the O-antigens of *S. eilbek* and *Arizona* 26a,26b:29:30 were identical, whereas the O-antigens of *S. eilbek* and *Arizona* 26a,26c:23:30 were not identical.

### D. SEROLOGICAL EXAMINATION OF THE H-ANTIGENS OF *S. EILBEK* IN COMPARISON WITH i- AND z-PHASES OF OTHER *SALMONELLA* OF SUB-GENERA I,II, RESPECTIVELY WITH *ARIZONA* H:33 (= i) AND H:31 (= z) PHASE

The following antigens and sera were used: i antigen of a *sub-genus I* strain (= *S. typhi-murium*) and of an *Arizona* strain (= *Arizona* 26:33:25), and the z antigen of 4 different *Salmonella sub-genus I* strains and of 32 *Salmonella sub-genus II* strains, and also of 4 *Arizona* strains (= H:31) and sera against i-antigens of *S. eilbek (sub-genus II)*, *S. typhi-murium (sub-genus I)*, and H:33 (= i) of *Arizona* 26:33:25 (*sub-genus III*), as well as against z-antigens of *S. eilbek (sub-genus II)*, *S. poona (sub-genus I)*, *S. greenside*, and *S. constantia (sub-genus II)*, and against H:31 (= z) of *Arizona* 16:24:31 (*sub-genus III*).

The results of cross absorption and agglutination tests of the H-phases of *S. eilbek* with i- and z-phases of representatives of the different *sub-genera (I, II, and III)* are summarised in table II.

These results show that the i-antigen of *S. eilbek* was not identical with the i-antigen of the *sub-genus I*-strain, *S. typhi-murium*, but that it was identical with the H:33-antigen of *Arizona* 26:33:25.

On the other hand, the z-antigen of *S. eilbek* was identical with *Arizona* H:31-antigen of *Arizona* 16:24:31, but was not identical with the z-antigen of *S. poona (sub-genus I)*.

TABLE I. — Detailed antigenic structure of all known « Arizona » serotypes of the O-group 26.

Strain No.	<i>Arizona</i> -formulae (Edwards <i>et al.</i> )			<i>Salmonella</i> -formulae (transposed by Rohde)				
	O-antigens	H-antigens			O-antigens	H-antigens		
		Phase 1	Phase 2	Phase 3		Phase 1	Phase 2	Phase 3
2081-64	26a,26b	22	25	—	61,61 <sub>2</sub>	(k)	Z <sub>53</sub>	—
251-58	26a,26b	23	21	—	61,61 <sub>2</sub>	1,(v)	Z <sub>35</sub>	—
3538-54	26a,26b	23	30	—	61,61 <sub>2</sub>	1,(v)	1,5,7	—
3068-61	26a,26b	23	30	40a,40c	61,61 <sub>2</sub>	1,(v)	1,5,7	Z <sub>57</sub>
3765	26a,26b	23	30	42	61,61 <sub>2</sub>	1,(v)	1,5,7	Z <sub>50</sub>
706-64	26a,26b	23	31	—	61,61 <sub>2</sub>	1,(v)	z	—
6174-60	26a,26b	24	30	—	61,61 <sub>2</sub>	1,(v)	1,5,7	—
Minn 240	26a,26b	29	30	—	61,61 <sub>2</sub>	1,(v)	1,5,7	—
261-58	26a,26b	32	21	—	61,61 <sub>2</sub>	k	Z <sub>35</sub>	—
6655-59	26a,26b	32	30	—	61,61 <sub>2</sub>	c	1,5,7	—
4850-52	26a,26c	23	30	—	61,61 <sub>3</sub>	c	1,5,7	—
1574-61	26a,26c	24	25	—	61,61 <sub>3</sub>	r	Z <sub>53</sub>	—
1481-61	26a,26c	26	25	—	61,61 <sub>3</sub>	Z <sub>32</sub>	Z <sub>53</sub>	—
3595-56	26a,26c	33	25	—	61,61 <sub>3</sub>	i	Z <sub>53</sub>	—
5128-62	26:33	28	(61:r:e,n,x,z <sub>15</sub> ) = rough					



With respect to the z-antigens of *sub-genus II* strains, it was observed that there was no identity of the z-antigens of *S. eilbek* with z antigens of either *S. greenseide* or of *S. constantia*. The two strains, although both belonging to *sub-genus II*, had a slight difference in their z antigenic structure, but were jointly able to remove from the anti z serum of *S. eilbek*, the z-receptors for the z antigens of *Salmonella sub-genus I*, of all *sub-genus II* strains, and also for most of the H:33 antigens of *Arizona*. There remained a small amount of antibodies for the homologous z-factor and for the z-phase of *Arizona* 16:24:31.

## DISCUSSION

### I. CONCERNING THE O:61 (RESPECT. *Arizona* O:26) GROUPS

In supplement VII to the K.-W. scheme *Kauffmann* had already described a close relationship between the *S. eilbek* O-antigen and the O:26 antigen of an *Arizona* strain, but without indicating its complete formula, and therefore the identity of the *Arizona* type he used to make the comparison is not clear. He designated the detailed O-antigen of *S. eilbek* as 61<sub>1</sub>,61<sub>2</sub> and the O-antigen of the *Arizona* strain he used as 61<sub>1</sub>,61<sub>3</sub>, and mentioned that the 61<sub>3</sub> agglutinations were very weak.

*Kauffmann's* cross absorption tests therefore showed a non-identity of *S. eilbek* with a certain *Arizona* strain having O:26 [3].

Because *Fife et al.* [2], had made a serological differentiation of the *Arizona* O:26-antigen in two sub-groups: 26a,26b and 26a,26c, it was interesting to make a detailed investigation of *S. eilbek* = O:61 in relation to the *Arizona* O-group 26 [2].

The tests made with single factor sera 26b and 26c showed an evident prevalence of the sub-group 26a,26b (= 61<sub>1</sub>,61<sub>2</sub>) to which, beside *S. eilbek*, also belonged 9 *Arizona*-serotypes, whilst sub-group 26a,26c (= 61<sub>1</sub>,61<sub>3</sub>) consisted of only 4 types.

One type of the Hamburg *Arizona* collection could not be placed into one of the sub-groups as it was of rough morphology.

As we have been able to prove the identity of *S. eilbek* O-antigen with *Arizona* O:26a,26b, we can conclude that the non-identity between *S. eilbek* and *Arizona* O:26, as stated by *Kauffmann*, may be explained because he probably used an *Arizona* strain of the sub-group 26a,26c (= 61<sub>1</sub>,61<sub>3</sub>) for comparison purposes.

Further, our serological analysis of O-group 61 demonstrates the necessity for considering the existence of two O:61 sub-groups, as far as the production of a suitable anti O:61 serum is concerned. With O-groups divided in sub-groups it is important to use representatives of the various sub-groups for the preparation of anti group O-sera or polyvalent O-sera containing antibodies against such sub-divided groups. Consequently,

the preparation of an anti O:61 serum against *S. eilbek* (= 61<sub>1</sub>,61<sub>2</sub>) + *Arizona* 26a,26c:23:30 (= 61<sub>1</sub>,61<sub>3</sub>:1,(v):1,5,7) is to be recommended.

We would like to emphasize that *S. eilbek* is also a good example of the close biochemical and serological relationship between *Salmonella*—especially *sub-genus II*—and *Arizona*, so that it seems reasonable to include *Arizona* as a special *sub-genus III* of *Salmonella*, and to use the same antigenic scheme for both.

As the K.-W.-Scheme is widely and generally used in practice, the *Arizona* antigenic formulæ could also be expressed with the symbols of this Scheme. Rhode has transferred the *Arizona*-formulæ of all known types in the K.-W.-Scheme; this translation Schedule will be published in detail later [4]. The International Salmonella Centre, Pasteur Institute, Paris, will also publish a complete K.-W.-Scheme which will include all *Arizona*-serotypes translated into the corresponding *Salmonella* formulæ.

## II. CONCERNING THE H-ANTIGENS « i » AND « z »

Rohde [4] showed that a rapid differentiation of the *Salmonella subgenera* is possible on a serological basis. He was able to determine certain factors in most H-phases which are characteristic for *sub-genus I* and others characteristic for *subgenera II* and *III*. This method applies also to the antigens i and z; the preparation of the analogous anti-sera was described above.

The results, obtained by Rohde [4], were once again confirmed by our serological cross absorption tests which we performed for a detailed analysis of the H-phases of *S. eilbek*. From this work it is clear that the i-antigen of *S. typhi-murium* (*sub-genus I*) was unable to exhaust an anti *S. eilbek* i-serum and *vice versa*. But the i-antigens of *S. eilbek* (*sub-genus II* atypical) and H:33-antigen of *Arizona* 26:33:25 (*sub-genus III*) absorbed the antisera of each other completely.

With regard to the z-phase, Bøecker and Kauffmann [1] have already discovered certain serological differences in *sub-genus I* strains which they defined as z<sub>a</sub>, z<sub>b</sub>, z<sub>d</sub> or as z<sub>a</sub>, z<sub>c</sub>, z<sub>d</sub> [1]. Similarly, we have shown differences in the z-antigens of *sub-genus II* and *III* (*Arizona*) strains.

For example: z-serum anti *S. eilbek* minus z-antigen of *S. greenside* was still positive with all tested z-phases of *sub-genus I* and H:31 of *Arizona* (*III*) and also with all *sub-genus II* strains, with the exception of *S. greenside*, *S. haarlem*, and *S. lousvbester*.

An other absorption test of *S. eilbek* anti z-serum by z of *S. constantia* (*sub-genus II*) removed z-antibodies for the tested *sub-genus I* strains, and from *sub-genus II* only the z-antibodies for *S. constantia* and *Salmonella* 53:z:z<sub>6</sub>.

All other known *sub-genus II* z-phases and all tested *Arizona* H:31-phases remained positive.

Each of these two strains *S. greenside* and *S. constantia* could remove a small amount of the *S. eilbek* z-antibodies, both together did absorb most



of the antibodies with the result that all z-phases of the *sub-genus II* and the tested z-phases of *sub-genus I*, and most H:31-phases of *Arizona* were negative.

There remained a small amount which agglutinated in addition to the z-antigen of *S. eilbek* and the H:31-antigens of the *Arizona* types 1,4:24:31—16:24:31—19:23:31 and 22:27:31. Twenty other *Arizona* types with H-phase 31 were negative. This proves that type-specific differences exist not only between the z-phases of *sub-genus I* and *II* but also between the *Arizona* H:31 (= z)-antigens.

Besides these type-specific differences in the z-phases of the 3 *sub-genera*, the z-phases of all *sub-genus I* strains possess—as Rohde demonstrated—a common z-factor specific for the whole z-phases. Similarly, in all z-phases of *sub-genera II* and *III* (H:31 *Arizona*) together, there exists—independent of certain type-specific-factors mentioned above—also a common and a specific z-factor.

During the last years we have used for our routine work *sub-genus* specific anti i I and i II or z I and z II-sera, and have been successful in differentiating the *sub-genera* without exception by slide agglutination tests. The serological differentiation of the *sub-genera* is more rapid than the biochemical differentiation. It is also more reliable and almost immediate.

#### SUMMARY

*S. eilbek* (61:i:z) was isolated from a stool specimen of a lizard in Thailand in 1963, and its serological formula was determined in Hamburg. The strain was named after a district called « Eilbek » in the town of Hamburg.

*S. eilbek* is regarded as an intermediate strain between *Salmonella* and *Arizona* in view of the fact that it does not ferment lactose, dulcitol or mucate. The detailed O-antigen is 61<sub>1</sub>,61<sub>2</sub>.

It was found that *S. eilbek* is identical with *Arizona* 26a,26b:29:30, but not with *Arizona* 26a,26c:23:30. The i-antigen of *S. eilbek* is not identical with the i-antigen of *S. typhi-murium*, but it is identical with the H:33-antigen of *Arizona* 26:33:25.

The z-antigen of *S. eilbek* is identical with *Arizona* H:31-antigen of *Arizona* 16:24:31 but not with the z-antigen of *S. poona*, *S. greenside* or *S. constantia*.

We showed differences in the z-antigens of *sub-genus II* and of *Arizona* H 31.

The 26b and 26c specific antisera have been prepared, and with the assistance of these sera all known *Arizona* of the group 26 have been classified in subgroups 26a,26b and 26a,26c.

**KEY-WORDS:** *Salmonella eilbek*, *Arizona*, Antigens.

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