A TYPHOID VARIANT AND A NEW SEROLOGICAL VARIATION IN THE SALMONELLA GROUP

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Recently Almon and Stovall (1939) reported on two cultures of *Salmonella typhi* which lacked an O-antigen. The cultures were described in the following manner:

Cross absorption tests showed that the missing antigen was not the one which *E. typhosa* shares with *Salmonella schottmuelleri*, but was one which is normally present in *S. enteritidis*. On the assumption that the somatic antigenic fractions of *E. typhosa* are limited in number to two (since there was no evidence to the contrary apparent from these studies), the component which was lacking was tentatively identified as antigen IX of the Kauffmann scheme of classification.

When this publication arrived I was engaged on work connected with serological variation within the O-antigens and had demonstrated a variation involving antigen XII that corresponded in principle to the "I-variation" (i.e. "I-Formenwechsel") which I had previously described. These new discoveries, of which Almon and Stovall were ignorant, necessitated a fresh study of these typhoid cultures with due regard for the XII-variation.

Variation in antigen I was summarized by Kauffmann (1940) as follows:

The development of antigen I varies in most of the *Salmonella* strains possessing this antigen. By means of a strong O-serum of *Salmonella senftenberg* and slide agglutination it is possible sometimes within the same strain to differentiate three forms which I should call the I++ form, the I+ form, and the I± form. *S. senftenberg* and *Salmonella niloese* occur in the I++ form only. Most of the other strains possessing antigen I contain both the I++ form and the I±

form, whilst the I+ form occurs more seldom. Continued cultivation of single colonies results in production of the other forms, for instance:

Salmonella rostock, original culture: 2++ colonies, 18± colonies
One ++ colony from *S. rostock:* 5++ colonies, 5 ± colonies
One ± colony from *S. rostock:* 1++ colony, 9± colonies

If rabbits are immunized with the I++ form, immune sera with a high I-titer are obtained, while immunization of rabbits with the I± gives sera with low I-titer.

XII-VARIATION

There is a XII-variation fundamentally similar to I-variation. Within the same strain there occur colonies with a well-developed antigen XII (form ++), moderately well developed antigen XII (form +) and a weakly developed antigen XII (form ±), which forms dissociate each other. Within the *Salmonella* B-group (IV.XII) these different forms of XII are easy to differentiate in a slide of agglutination with the aid of *S. typhi* O-serum (IX.XII). For example, with an immume serum prepared from the strain O 901, having an O-titer of about 5000 and diluted to 1:5 for slide agglutination, it is possible from 20-hour agar plates of a *Salmonella paratyphi B* strain to find the following: Of 20 colonies from the original culture there will be, for example, 2 colonies in the XII++ form, whereas 18 colonies are in the XII± form. On subcultivating a ++ colony, 9 of the 10 colonies examined are in the ++ form and one in the ± form. Conversely, of 10 colonies examined on subcultivating a ± colony, 9 are in the ± form and one in the ++ form (see table 1).

In a similar manner the XII-variation was induced in five cultures of *Salmonella enteritidis*, an O-serum of *S. reading* (IV.XII) being used in slide agglutination. Two kinds of colonies could be differentiated by means of this serum, one giving a positive reaction in IV.XII-serum, whereas the other gave a completely negative reaction. In tube agglutination, however, it was possible to demonstrate the weakly developed antigen XII in the latter. It was the XII± form.

With cultures possessing antigen I and antigen XII both variations may occur simultaneously; this the writer demonstrated with a strain of *S. paratyphi B*. After spreading from the mass

culture on agar plates we found 20 colonies distributed as follows: 9 colonies of the I++, XII± form, 1 colony of the I++, XII++ form, 5 colonies of the I±, XII± form, 5 colonies of the I±, XII++ form. On transplanting these four different serological forms there were further dissociations, though all four forms were not always present at the same time.

Taking these two antigens (I and XII) into consideration, we are thus able to distinguish four serological forms. If antigen V is considered, then there are 8 forms since some strains of *S. paratyphi B* contain this antigen while in others it is lacking.

TABLE 1

Results of slide agglutination (in IX.XII serum diluted 1:5)

STRAIN	FROM	NUMBER OF COLONIES		STRAIN	FROM	NUMBER OF COLONIES	
		±	++			±	++
S. paratyphi B	Mass	18	2	*S. typhi-murium*	Mass	17	3
	± colony	9	1		± colony	19	1
	++ colony	1	9		++ colony	4	6
S. essen 173	Mass	16	4	*S. reading*	Mass	6	4
	± colony	9	1		± colony	8	2
	++ colony	2	8		++ colony	1	9

Explanation of table 1: "Mass: 18±, 2++" means that after spreading on agar plates from the original culture, 18 colonies belong to the XII± form and 2 colonies to the XII++ form.

"± colony: 9±, 1++" means that from the transplantation of a ± colony, 9 colonies are of the XII± form and 1 colony of the XII++ form.

To assist in the correct appraisal of the following results and tables the writer would draw attention to the fact that the XII-variation does not involve the entire antigen XII, but merely a partial antigen which he calls XII_2. The partial antigens designated XII_1 and XII_3 are not subject to any variation, as far as it has been possible to discover. Since various *Salmonella* types possess only the antigens XII_1 and XII_3, no variation could be observed within these types; on the other hand there was a variation in various types possessing $XII_1.XII_2$ or $XII_1.XII_2.XII_3$ antigens. Table 2 presents some *Salmonella* types of which the various XII-partial antigens were examined more closely.

One strain, "cerdo 25/1," sent to the writer by E. Hormaeche as "*S. bredeney*," differs from *Salmonella bredeney* in its O-antigen, which has the formula [I].IV.XII$_1$..., that is to say it lacks the characteristic antigen XXVII of *S. bredeney*. Possibly this is a serological variant of *S. bredeney* in which antigen XXVII has been lost. On the other hand, as antigen XII$_3$ could not be

TABLE 2

O-antigenic formulae under consideration of the XII partial antigens

TYPE	O-ANTIGENS
S. paratyphi A	[I].II.XII$_1$.XII$_3$
S. paratyphi A var. *durazzo*	II.XII$_1$.XII$_3$
S. paratyphi B	[I].IV.V.XII$_1$.[XII$_2$]
S. abony	[I].IV.V.XII$_1$.[XII$_2$]
S. typhi-murium	[I].IV.V.XII$_1$.[XII$_2$]
S. reading	IV.XII$_1$.[XII$_2$]
S. derby	IV.XII$_1$.[XII$_2$]
S. essen 173	IV.XII$_1$.[XII$_2$]
S. brandenburg	IV.XII$_1$.[XII$_2$]
S. bispebjerg	[I].IV.XII$_1$.[XII$_2$]
S. bredeney	[I].IV.XXVII.XII$_1$.XII$_3$
S. abortus bovis	[I].IV.XXVII.XII$_1$.XII$_3$
S. schleissheim	IV.XXVII.XII$_1$.XII$_3$
S. abortus-equi	IV.XII$_1$.XII$_3$
S. abortus-ovis	IV.XII$_1$.[XII$_2$].XII$_3$
S. typhi T4	IX.XII$_1$.XII$_2$.XII$_3$
S. typhi T2	IX.XII$_1$.XII$_3$
S. enteritidis	[I].IX.XII$_1$.[XII$_2$].XII$_3$

Explanation of table 2: [ ] means that these antigens are subject to form-variation, whereby ±, +, or ++ forms may occur. An O 901 serum absorbed with strain T2 was used for demonstrating antigen XII$_2$, whereas an O-serum of *S. durazzo* absorbed with *S. reading* was used for demonstrating antigen XII$_3$. The agglutinations were carried out with alcohol cultures in test-tubes; they were read after 20 hours in a water-bath at 50°C.

demonstrated in this strain, the probability is that it is a separate type with the formula [I].IV.XII$_1$...l, v ↔ 1, 7... Furthermore, the writer has hitherto been unable to find the antigens XII$_2$ and XII$_3$ in the original strain of *Salmonella chester* IV.V.XII$_1$... and a strain of *Salmonella derby* [I].IV.XII^1... It is possible that the *S. derby* strains may be divided into two groups by their XII-antigenic structures.

It should be pointed out that *Salmonella abortus-equi* has an O-antigen differing from that of *Salmonella abortus-ovis*, as the first-named possesses the O-antigens IV.XII_1.XII_3, whereas the latter has IV.XII_1.[XII_2].XII_3. Again, the O-antigen of *Salmonella bispebjerg* is different from that of *S. abortus-equi*, as *S. bispebjerg* has the O-formula [I].IV.XII_1.[XII_2].

Further O analyses will probably reveal more differences in the antigenic structure, as the studies so far made are to be regarded only as the commencement of the O-antigen analysis. However, as it was not the intention of this paper to clear up the XII-variation within the entire *Salmonella* group, but only so far as seemed necessary for the present purpose, the various results need not be gone into here; the writer will proceed with the examination of the typhoid cultures concerned.

EXAMINATION OF TYPHOID CULTURES T2 AND T4

To obviate misunderstanding it should be observed that strain "T2" of Almon and Stovall is not identical with the familar typhoid strain "Ty 2" of Felix, which possesses the full O-antigen and also occurs in the V form. To distinguish it from the Felix strain, the strain of Almon and Stovall is designated as "T2". The cultures T2 and T4 were kindly sent to me by Stovall, and I was able to establish the fact that they were cultures with a well-developed Vi-antigen, occurring in the V form and therefore O-inagglutinable in the live state. The H-antigen was well-developed in both cultures. The analysis of the O-antigen showed (confirming the report of Almon and Stovall) that strain T2 lacked an O-antigen present in strain T4 which, as regards its O-antigen, conforms to the well-known strain H 901. Culturally there were the following differences between T2 and T4: Whereas strain T4 fermented xylose at once, it took T2 twelve days to do so. T2 did not produce hydrogen sulphide, which was produced promptly by T4.

Tube agglutinations with alcohol-treated cultures from strains T2 and T4 in different O-sera, partly shown in table 3, gave results indicative of the importance of antigen XII, for they behaved inversely in the Durazzo serum (II.XII_1.XII_3) and Reading serum (IV.XII_1.XII_2). In Durazzo serum strain T2 agglutinated

up to titer 2560, whereas strain T4 agglutinated only up to 1:160. In Reading serum (IV.XII$_1$.XII$_2$) the cultures behaved conversely, for strain T2 agglutinated only up to 1:80, but T4 up to 1:2560, even higher than the titer of 1280 for the homologous strain. In *S. typhi* O-serum (IX.XII$_1$.XII$_2$.XII$_3$) both cultures

TABLE 3

O tube agglutination with alcohol cultures

CULTURE	20	40	80	160	320	640	1,280	2,560	5,120	10,240	TITER
Serum *S. paratyphi* A var. *durazzo* = II.XII$_1$.XII$_3$											
S. typhi T2	++	++	++	++	++	++	++	++	±	−	2,560
S. typhi T4	++	++	++	+	−	−	−	−	−	−	160
S. durazzo	++	++	++	++	++	++	++	+	−	−	2,560
S. reading XII++	+	±	±	±	−	−	−	−	−	−	20
Serum *S. reading* = IV.XII$_1$.XII$_2$											
S. typhi T2	++	++	+	±	−	−	−	−	−	−	80
S. typhi T4	++	++	++	++	++	++	++	++	±	−	2,560
S. durazzo	++	++	++	++	+	±	−	−	−	−	320
S. reading XII++	++	++	++	++	++	++	+	−	−	−	1,280
Serum *S. typhi* T2 = IX.XII$_1$.XII$_3$											
S. typhi T2	++	++	++	++	++	++	++	++	+	±	5,120
S. typhi T4	++	++	++	++	++	++	++	++	±	−	2,560
S. durazzo	++	++	++	++	++	±	−	−	−	−	320
S. reading XII++	+	±	−	−	−	−	−	−	−	−	20
Serum *S. typhi* T4 = IX.XII$_1$.XII$_2$.XII$_3$											
S. typhi T2	++	++	++	++	++	++	++	+	−	−	2,560
S. typhi T4	++	++	++	++	++	++	++	++	+	−	5,120
S. durazzo	++	++	++	+	±	−	−	−	−	−	160
S. reading XII++	++	++	++	+	−	−	−	−	−	−	160

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

behaved similarly, the only difference being that T4 was agglutinated in somewhat higher dilution than was T2.

This result, especially the high agglutination of strain T4 in Reading serum, indicated that T4 possessed antigen XII$_2$, which seemed to be lacking in strain T2. The high agglutination given

by T2 in Durazzo serum also argues for this explanation, as after the disappearance of antigen XII_2 there is a stronger agglutination in $XII_1.XII_3$-serum. This may be either because $XII_1.XII_3$-antigens are more strongly developed in strain T2 than in strain T4, or that the presence of antigen XII_2, inhibits agglutination of T4 in Durazzo serum. Accordingly, this inhibitive factor (antigen XII_2) should be lacking in the T2 strain.

In order to ascertain more nearly the exact relationships of the strains, a number of absorption tests were then done; only the more important of which need be discussed here. Naturally these experiments must be carried out with due reference to the

TABLE 4

O tube agglutination with alcohol cultures

CULTURE	20	40	80	160	320	640	1,280	2,560	5,120	TITER
Serum *S. paratyphi* B XII++ form = IV.V.$XII_1.XII_2$										
S. paratyphi B XII++	++	++	++	++	++	++	+	±	−	1,280
S. paratyphi B XII±	++	++	++	++	++	+	+	−	−	1,280
S. typhi T2	++	++	++	+	−	−	−	−	−	160
S. typhi T4	++	++	++	++	++	++	++	+	−	2,560
Serum *S. paratyphi* B XII± form = IV.V.XII_1										
S. paratyphi B XII++	++	++	++	++	++	+	±	−	−	640
S. paratyphi B XII±	++	++	++	++	++	+	−	−	−	640
S. typhi T2	++	++	++	+	−	−	−	−	−	160
S. typhi T4	++	++	±	±	−	−	−	−	−	40

XII-variation, i.e. one must know whether the sera employed possess antigen XII_2 and whether it is contained in the cultures used for absorption and agglutination. If this serological variation is not taken into consideration, the experiments will be subject to chance and must lead to contradictory results, as conditions here are almost the same as in the phase variation displayed by the H-antigens.

In order to show that the strong agglutination of strain T4 in Reading serum is due to the agglutinin XII_2, the writer prepared immune sera with two different forms of *S. paratyphi B*, viz. 1) the XII++ form, i.e. the form with the XII_2-antigen

strongly developed, and 2) the XII ± form, in which this antigen is only weakly developed. Table 4 shows some agglutination results obtained with these sera.

It will be seen from this table that the titers of both forms of *S. paratyphi B* are equally high in both sera, XII ± serum happening to have a lower titer than XII + + serum. Culture T2 is also agglutinated to the same level by both sera, as it is acted on only by the XII_1-agglutinin of these sera. On the other hand, there is a considerable difference in the titers of the two *S. para-*

TABLE 5

CULTURE	40	80	160	320	640	1,280	2,560	5,120	10,240	TITER
Serum *S. typhi* O 901 absorbed with T2 = XII_2										
S. typhi T2	−	−	−	−	−	−	−	−	−	
S. typhi T4	++	++	++	++	++	++	++	+	−	5,120
S. enteritidis XII++	++	++	++	++	++	++	−	−	−	1,280
S. enteritidis XII±	+	+	±	−	−	−	−	−	−	80
S. paratyphi B XII++	++	++	++	++	++	±	−	−	−	640
S. paratyphi B XII±	±	−	−	−	−	−	−	−	−	
Serum *S. typhi* O 901 not absorbed = IX.XII_1.XII_2.XII_3										
S. typhi T2	++	++	++	++	++	++	++	±	−	2,560
S. typhi T4	++	++	++	++	++	++	++	+	±	5,120
S. enteritidis XII++	++	++	++	++	++	++	−	−	−	1,280
S. enteritidis XII±	++	++	++	++	++	±	−	−	−	640
S. paratyphi B XII++	++	++	++	++	++	±	−	−	−	640
S. paratyphi B XII±	±	−	−	−	−	−	−	−	−	

typhi B sera when one turns to the agglutination of strain T4. Here it is seen that serum XII + + possesses a strongly developed cross-agglutinin, the XII_2-agglutinin, which is only faintly present in XII ± serum. The difference revealed by these tests between cultures T2 and T4 is due to antigen XII_2, which strain T2 lacks in contradistinction to strain T4; for if O-serum of T4 or strain 901 is absorbed with strain T2, the XII_2-agglutinin remains, as table 5 shows.

It will be seen from Table 5 that the absorption of serum derived from *S. typhi* 901 with culture T2 does not exhaust the

serum, as there remain very strong agglutinins for several cultures. Strain 901 is agglutinated in exactly the same manner as strain T4 and is therefore omitted from the table. Distinct differences in the agglutination of the XII++ and XII± forms of *S. enteritidis* and *S. paratyphi B* are apparent. These differences are the result of the degree of development of antigen XII.

TABLE 6

Absorption and agglutination results

O-sera

CULTURES	S. PARATYPHI A I.II.XII_1.XII_3					S. READING IV.XII_1.XII_2				S. TYPHI T2 IX.XII_1.XII_3				S. TYPHI T4 IX.XII_1.XII_2.XII_3				
	Not absorbed	Absorbed with				Not absorbed	Absorbed with			Not absorbed	Absorbed with			Not absorbed	Absorbed with			
		Durazzo	Reading	T2	T4		Durazzo	T2	T4		Durazzo	Reading	T4		Durazzo	Reading	T2	T2 + Reading
S. paratyphi A.....	++	+	++	++	++	+	−	−	−	+	−	±	−	+	−	±	−	−
S. reading XII++.	+	−	−	−	−	++	++	++	++	±	−	−	−	++	++	−	++	−
S. typhi T2.....	++	−	+	−	−	+	−	−	−	++	++	++	−	++	++	++	−	−
S. typhi T4.....	+	−	+	−	−	++	++	++	−	++	++	++	−	++	++	++	++	+
S. enteritidis XII++.....	+	−	±	−	−	++	++	++	−	++	++	++	−	++	++	++	++	±
S. enteritidis XII±.	+	−	±	−	−	±	−	−	−	++	++	++	−	++	++	++	−	−

Explanation of table 6: S. durazzo = II.XII_1.XII_3; *S. enteritidis* XII++ = IX.XII_1.XII_2.XII_3; *S. enteritidis* XII± = IX.XII_1.XII_3.

The signs ++, + or ± indicate the strength of the agglutination. − = negative.

Absorption with the homologous cultures led to the complete exhaustion of serum.

Table 6 summarizes further absorption experiments, from which the following may be gathered:

(1) Serum: *S. paratyphi A* (I.II. XII_1.XII_3)

(a) After absorption with *S. paratyphi A* var. *durazzo* (II.XII_1.XII_3), the I-agglutinin remains in the serum, which indicates that the difference between cultures T2 and T4 is not due to I antigen.

(b) After absorption with *S. reading* (XII++ form) the serum is exhausted of agglutinins for *S. reading* only, as I.II.XII_3 agglutinins are still residual in the serum. *S. typhi* and *S. enteritidis* contain antigen XII_3.

(c) After absorption with cultures T2 and T4 there only remain agglutinins for *S. paratyphi A* (I.II).

(2) Serum: *S. reading* (IV.XII_1.XII_2).

(a) After absorption with *S. paratyphi A* var. *durazzo* the IV.XII_2 agglutinins remain in the serum, so that besides *S. reading* the XII_2 forms of *S. typhi* and *S. enteritidis* are also agglutinated. Here the T2 culture behaves exactly as the XII± form of *S. enteritidis*, which clearly shows that it lacks the antigen XII_2.

(b) Absorption with strain T2 must lead to the same result as in (a), for the IV.XII_2-agglutinins remain here too.

(c) On the other hand, absorption with strain T4 leads to the complete removal of all cross-agglutinins, so that the IV-agglutinin is the only one left.

(3) Serum: *S. typhi* T2 (IX.XII_1.XII_3).

(a) After absorption with *S. paratyphi A* var. *durazzo*, all the XII-agglutinins are removed, only the IX-agglutinin remaining in the serum.

(b) After absorption with *S. reading* there remains, in addition to the IX-agglutinin, the XII_3-agglutinin, by which *S. paratyphi A* is weakly agglutinated.

(c) Absorption with strain T4 leads to complete exhaustion of the serum, thus confirming the report of Almon and Stovall.

(4) Serum: *S. typhi* T4 (IX. XII_1.XII_2.XII_3).

(a) After absorption with *S. paratyphi A* var. *durazzo*, the IX.XII_2-agglutinins remain in the serum, so that the agglutination of the Reading culture is a result of the presence of XII_2-agglutinins, which are lacking in T2 serum.

(b) Absorption with *S. reading* must lead to the same result as with T2 serum, as the IX.XII_3-agglutinins also remain here.

(c) After absorption with culture T2 there are XII_2-agglutinins left in the serum, so that *S. reading*, *S. typhi* T4 and the XII++ form of *S. enteritis* are agglutinated.

(d) Absorption of T4 serum with the culture T2 + Reading should theoretically lead to complete exhaustion of the serum; yet there remain slight agglutinins for T4 and the XII++ form of *S. enteritidis*. These slight agglu-

tinins, which by the way cannot be demonstrated in all IX.XII-sera, have not been taken into consideration in the formulae. The explanation of this low residual agglutination is, presumably, that *S. reading* does not possess the complete XII_2-antigen, so that a small part of the XII_2-antigen remains in the serum. Another factor in favor of this explanation is the absence of agglutination of the XII± form of *S. enteritidis*, so that in this case it cannot be a residual of the IX-agglutinin.

So far it has not been possible to observe any XII-variation in the typhoid cultures T2, T4 and 901. Whereas with strain 901, which is of the W-form (i.e. contains no Vi-antigen), we can study single colonies in different O-sera by slide agglutination, the method fails with strains T2 and T4, which occur in the V-form and therefore are O-inagglutinable. The writer therefore started broth cultures from 20 single colonies of each of these two cultures, heated them for one hour at 100°C. and agglutinated them in various O-sera. All the colonies from strain T2 and T4 respectively, were uniform in their behavior, and in particular all T4 cultures contained antigen XII_2, which was lacking in the T2 cultures. An examination of strain 901 by means of slide agglutination revealed no variation, as antigen XII_2 was strongly developed in all colonies. An examination of various other typhoid cultures such as Ty_2 (Felix), Watson, Rawlings, Gigliolo and several others demonstrated the presence of antigen XII_2 in them all; no test for variation was made with any of these cultures, however.

Thus, all that we can say today is that there are certain typhoid strains which lack the XII_2-antigen. No O-variation such as that occurring in various other *Salmonella* types has so far been observable in the typhoid cultures; nevertheless, further investigation will be required before this question is completely settled.

The writer is aware that the demonstration of the I- and XII variations does not mean that all variations have been discovered; other serological variations of this kind may safely be anticipated. For example, in strains of *S. bredeney* and *S. abortus-bovis* the

writer has found part of antigen XIX of *S. seftenberg* which is coupled to antigen I and, with it, subject to variation.

DISCUSSION

The writer was able to confirm the observation of Almon and Stovall that an O-antigen is lacking in the typhoid strain T2. However, his studies of the XII-variation, which were unknown to Almon and Stovall, led to the demonstration that this loss of the O-antigen has to do with an essential part of antigen XII, i.e. antigen XII_2. In normal typhoid strains, such as strain T4 and Watson, this antigen is a very strongly developed O-antigen which must be regarded as an antigen shared by other species and one that occurs in many types of the *Salmonella* B-group. In all strains of the B-group, for example in *Salmonella paratyphi B, Salmonella typhimurium, Salmonella reading* and others in which this antigen XII_2 is present, it is possible to prove the occurrence of a form-variation, for colonies appear with strongly or weakly developed antigen XII_2. This *"XII-variation"* conforms in principle with what the writer has previously described as the *"I-variation."* The two forms of variation may occur simultaneously and independently within the same strain.

These newly discovered variations within the O-antigens, which may generally be called *"O-variations,"* are of importance in judging of serological reactions within the *Salmonella* group, just like the *"phase-variation"* within the H-antigens. We differentiate between:

I. A *"form-variation"* taking place within the somatic antigens, and divisible into an *"O-variation"* within the *O-antigens* and a *"V-W variation"* within the Vi-antigens. Variations beyond these are usually called *"smooth-rough variation"* (S-R variation), but they are not considered in the present paper.

II. A *"phase-variation"* within the H-antigens, for practical purposes divisible into a *"specific-nonspecific"* (for instance b ↔ 1, 2 ...) and an *"α-β"* (for instance b ↔ e, n, x) *variation.* As a term applicable to both phase-variations, the phases might be designated by the neutral terms, *"phase 1"* and *"phase 2."*

While in the O-variation quantitative differences within the normal O-antigens are involved, the S-R and phase variations are due to qualitative alterations of the antigens. The clearing up of all these serological variations, which proceed within the constant types and do *not* involve the transformation of one type to another, has placed the whole of *Salmonella* serology on a solid experimental basis and removed many divergences. *Within the Salmonella group the institution and valuation of serological reactions must be made with due regard to form- and phase-variations otherwise constant and useful results cannot be obtained.* In the example with the typhoid cultures T2 and T4 it was shown that the O-differences between these two is a result of the lack of antigen XII_2 in the T2 strain, but that these differences can be established with certainty only if the XII_2-variation within the *Salmonella* B-group is taken into consideration.

The present communication being occupied with purely serological problems, it cannot go deeper into the immunological significance of these new discoveries. We may take it, however, that serological loss-variants, as occurring in strain T2, may also be immunological loss-variants, in which case they should not be used as vaccines against typhoid. When preparing typhoid vaccines and therapeutic typhoid sera the worker should convince himself that the entire normal O-antigen $IX.XII_1.XII_2.XII_3$ is present in the cultures employed. However, this very important question of practical application is not yet ripe for discussion, for it requires special study.

SUMMARY

1. Confirming the report of Almon and Stovall it is found that typhoid strain T2 lacks part of the O-antigen.

2. This lack does *not* affect antigen IX, as assumed by Almon and Stovall, but a part of the complex common antigen XII, the antigen XII_2.

3. The XII_2-antigen in various *Salmonella* types (for instance *S. paratyphi B*) is subject to a "*XII-variation*," in which forms occur with a strongly or a weakly developed XII_2-antigen. This corresponds in principle to the "*I-variation.*"

4. The writer discusses the serological form variations and phase variations in general, with particular reference to the newly demonstrated "O-variation."

REFERENCES

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