30. Artificial Transformation of O Antigens in *Salmonella* E Group. I. Transformation by Antiserum and Bacterial Autolysate

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In 1948, Bruner and Edwards¹⁾ cultured *S. anatum* (III, X, XXVI: e, h-1, 6) and *S. meleagridis* (III, X, XXVI: e, h-1, w) of subgroup E₁ of *Salmonella* E group in a semisolid agar medium containing anti-X, XXVI serum which was obtained by absorbing anti-III, X, XXVI serum with a bacillus of subgroup E₂ possessing III, XV antigen, and from the organisms spread through the medium, they isolated bacilli which had the same antigenic structures with *S. newington* (III, XV: e, h-1, 6) and *S. cambridge* (III, XV: e, h-1, w), respectively. And concerning the transformation mechanism of antigenic structure, they stated that, because cell components of the bacillus which was used for absorption were contained in the antiserum which was allowed to react, they could not deny the contribution of the cell components. Then Obara²⁾ cultured *S. newington* and *S. new-brunswick* (III, XV: l, v-1, 7) in a semisolid agar medium containing anti-XV serum which was obtained by absorbing rabbit serum, immunized with *S. newington* of subgroup E₂, with *S. london* (III, X, XXVI: l, w-1, 6) of subgroup E₁, and isolated bacilli which had the same antigenic structures as *S. anatum* and *S. give* (III, X, XXVI: l, v-1, 7), respectively. Further, Uetake et al.³⁾ cultured *S. senftenberg* (I, III, XIX: g, s, t--) in a semisolid agar medium containing anti-I, XIX serum which was obtained by absorbing rabbit serum, immunized with *S. niloese* (I, III, XIX: d-z₁) of subgroup E₃, with *S. london*, and isolated a bacillus possessing III, XV antigen. The transformation from *S. newington* to *S. anatum* was possible also when the reacting antiserum was made by absorption with the dead bacillus killed by heating at 100°C for an hour. And in the above-mentioned transformation from I, III, XIX antigen to III, XV antigen, a bacillus with III, XV antigen was obtained when the reacting antiserum was prepared by absorption with the bacillus possessing III, X, XXVI antigen. From these facts it was explained that antigenic transformation was not caused by the cell components of the bacillus used for absorption. Yamada and Sakazaki⁴⁾ reported a case, in which *S. newington* was spontaneously transformed to *S. anatum* during storage, and they stated that they could retransform *S. anatum* to *S. newington* by the Bruner and Edward method¹⁾.

We also performed experiments on transformation of O antigens with some bacilli of subgroups E_1 , E_2 and E_3 , using antisera or bacterial autolysates, with the results as shown below. In this report, O antigen of bacilli of subgroup E_1 , for example *S. anatum*, was indicated by III, X according to the Kauffmann-White's new diagnostic antigenic schema⁷⁾.

Materials and Methods

1) Strains: The strains used in the experiments were *S. london* 1446, *S. give* 316 and *S. anatum* 293 of subgroup E_1 , *S. newington* C_2 , *S. selandia* 7482 and *S. new-brunswick* 5411 of subgroup E_2 , and *S. senftenberg* 3007 and *S. niloese* 1236 of subgroup E_3 .

2) Factor sera: Anti-X serum was made by absorbing anti-*S. anatum* immune rabbit serum with *S. newington*; anti-XV serum, by absorbing anti-*S. newington* immune rabbit serum with *S. anatum*; anti-I, XIX serum, by absorbing anti-*S. senftenberg* immune rabbit serum with *S. anatum*. In preparation of these factor sera, the immunizations were performed with the dead bacilli killed by heating at 100°C for 2.5 hours, and the absorptions also by the dead bacilli killed by heating at 100°C for an hour.

3) Bacterial autolysate: Bacterial cells were suspended in sterile physiological saline solution at a rate of 30 mg. to 1 cc., allowed to stand at 37°C for 48 hours, and centrifuged. The supernatant was diluted twofold and filtrated with a Berkefeld filter of the Shōfū. The sterile filtrate was used for the experiment.

4) Method of culture: The bacilli were cultured through successive generations in an ordinary broth containing the factor sera or autolysates. In the course of culture parts were inoculated on agar plates, and with the bacterial colonies, we investigated the presence or absence of antigenic transformation.

5) Method of investigation: For the investigation we performed slide agglutination tests mixing the colonies with the factor serum. And with those which proved negative we made further broth cultures, and then with them performed tube agglutination tests against the factor serum, and also absorption tests.

Results

1. Transformation of O antigen of subgroup E_1 : *S. london* 1446, *S. give* 316 and *S. anatum* 293 were each cultured through successive generations in broth media containing anti-X serum, autolysates of E_2 and E_3 , respectively. And it was found that only in the broth culture which contained the autolysate of subgroup E_2 , were produced bacilli which reacted not with anti-X factor serum but with anti-XV factor

serum. Result of examination of 20 colonies of each bacillus showed that in 15 colonies of *S. london*, 2 of *S. give*, 12 of *S. anatum*, antigenic transformation occurred at the first generation of culture. Those cultured in broth with anti-X serum or autolysate of E_3 did not exhibit antigenic transformation even after 10 generations.

With the above-mentioned bacilli of subgroup E_1 which came to have the antigen of subgroup E_2 by antigenic transformation, we performed absorption tests against each immune serum. The result showed, as seen in Table I, that with all of them only anti-III, XV antibody was completely absorbed.

Table I

Absorption tests of immune sera with the bacilli which had antigens transformed by bacterial autolysate

Subgroup			E_1	E_2	E_3
Immunogen			<i>S. anatum</i>	<i>S. newington</i>	<i>S. senftenberg</i>
Antibody			Anti-III, X	Anti-III, XV	Anti-I, III, XIX
Agglutinin titer			2560	640	2560
After absorption	Original strains	<i>S. london</i> 1446	—	320	640
		<i>S. give</i> 316	—	320	640
		<i>S. anatum</i> 293	—	320	640
	Transformed strains	<i>S. london</i>	1280	—	640
		<i>S. give</i>	1280	—	640
		<i>S. anatum</i>	1280	—	640

2. Transformation of O antigen of subgroup E_2 : *S. newington* C₂, *S. selandia* 7482 and *S. new-brunswick* 5411 were each cultured through successive generations in broth media containing anti-XV serum, autolysates of E_1 and E_3 , respectively. And it was found that only in the broth culture which contained the anti-XV antiserum, were produced bacilli which reacted not with anti-XV factor serum but with anti-X factor serum. Result of the examination of 20 colonies of each bacillus showed that antigenic transformation occurred in 2 colonies of *S. new-brunswick* after 2 generations, 2 of *S. selandia* after 4 generations, and in none of *S. newington* even after 10 generations. When the bacilli were cultured through generations in broth with the autolysate of E_1 or E_3 none exhibited antigenic transformation.

3. Transformation of O antigen of subgroup E_3 : *S. senftenberg* 3007 and *S. niloese* 1236 were each cultured through successive generations in broth media containing anti-I, XIX serum, autolysates of E_1

and E₃, respectively. And it was found that only in the broth culture which contained the anti-I, XIX serum, were produced bacilli which had antigens transformed. After 10 generations of culture, 20 colonies of each bacillus were investigated, with the following result. Two of *S. senftenberg* were found to react with anti-XV factor serum, one of them had lost I, XIX antigen but the other reacted also with anti-I, XIX factor serum, thus showing that it had an antigenic structure of I, III, XV, XIX. This one which possessed antigens of both subgroups E₂ and E₃ continued to have the same antigenic structure through several broth cultures. But when it was cultured through successive generations in medium containing anti-XV factor serum, a bacillus of subgroup E₃ was produced which had the antigenic structure of I, III, XIX. When it was cultured through successive generations in medium containing anti-I, XIX serum, a bacillus of subgroup E₂ which had the antigenic structure of III, XV was obtained. None of *S. niloese* was found to have antigen transformed. When the bacilli were cultured through successive generations in broth containing the autolysate of E₂ or E₃, antigenic transformation was observed in none of them.

With the above-mentioned bacilli which had their antigens transformed by the antisera, absorption tests were performed to each immune serum, with the result shown in Table II. From the bacilli of subgroup E₂, we obtained bacilli which contained the antigen of sub-

Table II
Absorption tests of immune sera with the bacilli which
had antigens transformed by antisera

Subgroup			E ₁	E ₂	E ₃
Immunogen			<i>S. anatum</i>	<i>S. newington</i>	<i>S. senftenberg</i>
Antibody			Anti-III, X	Anti-III, XV	Anti-I, III, XIX
Agglutinin titer			2560	640	2560
After absorption	Original strains	<i>S. selandia</i> 7482	1280	—	640
		<i>S. new-brunswick</i> 5411	1280	—	640
	Trans-formed strains	<i>S. selandia</i>	—	320	640
		<i>S. new-brunswick</i>	—	320	640
	Original strain	<i>S. senftenberg</i> 3007	1280	320	—
	Trans-formed strain	<i>S. senftenberg</i>	1280	—	640

group E_1 , and from the bacilli of subgroup E_2 , bacilli possessing the antigen of subgroup E_3 .

4. Flagellar antigen of transformed strain: When the autolysate of *S. new-brunswick* (e, v-1, 7) of subgroup E_2 transformed the O antigen of *S. anatum* (e, h-1, 6) of subgroup E_1 , the flagellar antigen in the latter still possessed the structure of e, h-1, 6. And when anti-XV serum transformed the O antigens of *S. new-brunswick* (e, v-1, 7) and *S. selandia* (e, h-1, 7) of subgroup E_2 their flagellar antigens still possessed the structures of e, v-1, 7 and e, h-1, 7, respectively. Consequently, it is known that although O antigen was transformed by antiserum or bacterial autolysate, flagellar antigen remained unchanged.

Summary

Transformation of O antigens of subgroups E_1 , E_2 and E_3 of Salmonella E group was attempted by the actions of antiserum and autolysate, with the following results:

1. When *S. london* 1446, *S. give* 316 and *S. anatum* 293 of subgroup E_1 were cultured in broth with the autolysate of subgroup E_2 , bacilli possessing III, XV antigen of subgroup E_2 were obtained. But when anti-X serum, specific to subgroup E_1 , was allowed to react, no bacillus which had antigen transformed was produced. Namely, O antigen was transformed in the direction $E_1 \rightarrow E_2$ by the action of bacterial autolysate of subgroup E_2 .

2. When *S. selandia* 7482 and *S. new-brunswick* 5411 of subgroup E_2 were cultured through successive generations in broth containing anti-XV serum, bacilli which possessed III, X antigen of subgroup E_1 were obtained. When *S. senftenberg* 3007 of subgroup E_3 was cultured in broth containing anti-I, XIX serum, a bacillus possessing III, XV antigen of subgroup E_2 was obtained. But when bacillus of subgroup E_2 was cultured in broth containing autolysates of E_1 and E_3 , respectively, and when bacillus of subgroup E_3 was cultured in broth containing autolysates of E_1 and E_2 , respectively, no bacilli which had antigens transformed were observed. Namely, by the action of antiserum, transformation of antigen was caused in the direction $E_3 \rightarrow E_2 \rightarrow E_1$.

3. Thus, transformation of O antigens can be produced between subgroups of Salmonella E group, but flagellar antigens in the transformed bacilli retained the same structures as in the original bacilli.

References

- 1) Bruner, D. W. and Edwards, P. R.: Changes induced in the O antigens of Salmonella. *J. Bact.*, **55** (3), 449 (1948).

- 2) Obara, T.: Artificial transformation of O antigens in Salmonella. Sapporo-Ikedaigaku-Kiyō, **1** (2), 68-78 (1950) (Japanese).
- 3) Uetake, H., Nakagawa, T., Sasaki, T., Ise, T., Mizushima, N., Togashi, S., Nagai, T., Koike, H., and Akiba, T.: Artificial transformation of O antigens in Salmonella. Second report. 25th Nippon-Saikin-Gakkai-Shoroku, 27-28 (1952) (Japanese).
- 4) Yamada, C. and Sakazaki, R.: The spontaneous transformation of Salmonella newington to Salmonella anatum. Med. and Biol., **2** (5), 209-211 (1951) (Japanese).
- 5) Kauffmann, F.: Enterobacteriaceae, 95-103 (1951). Ejnar Munksgaard Publisher, Copenhagen.