

LYSOGENICITY OF SALMONELLA E₂ GROUP ORGANISMS AND RELATIONSHIP
BETWEEN BACTERIOPHAGE AND ANTIGENIC CHANGES IN SALMONELLAS
OF GROUP E

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INTRODUCTION

Though there had been many papers on induced changes in H antigens of Salmonella, no report had appeared on induced changes in O antigens until Bruner and Edwards described a short note in J. of Bacteriology, 1948, in which O antigens of *S. anatum* and *S. meleagridis* were reported to undergo a change from 3,10 to 3,15 and vice versa by antiserum. This note attracted the authors' attention as they were interested in induced antigenic changes. Uetake and Obara (1950), Obara (1950), and Nakagawa (1951a; 1951b; 1953a; 1953b) extended this experiment and found that O antigens of *S. newington* and *S. new-brunswick* were converted from 3,15 to 3,10 by cultivation in semi-solid medium containing 15 antiserum, and vice versa. Further Nakagawa (1951b; 1953b) and Uetake et al. (1952) pointed out that the antigenic changes from 3,15 to 3,10 are not analogous to transformation reactions (Austrian, 1952a), which have been observed in *Pneumococcus* (Avery et al., 1944; Austrian, 1952b; Hotchkiss, 1952; McLeod and Krauss, 1953), *Escherichia coli* (Boivin, 1947), *Hemophilus influenzae* (Alexander and Leidy, 1950; 1951a; 1951b; Leidy et al., 1953; Zamenhof et al., 1953), and *Neisseria meningitidis* (Alexander and Redman, 1953), but is rather similar to the mechanism of antigenic changes observed in *Pneumococcus* (Laros, 1949), *Paramecium aurelia* (Sonneborn, 1947) and/or phase variation in H antigens of Salmonella. Soon thereafter it was observed that O antigens of *S. senftenberg* were changed from 1,3,19 to 3,15 by 1,19 antiserum and further spontaneously changed to 3 alone. (Uetake et al., 1952; Ise, 1953a, 1953b).

Shortly thereafter Zinder and Lederberg (1952) reported a new phenomenon, transduction. In 1953 Iseki and Sakai (1953a; 1953b), using *S. london*, *S. give*, *S. anatum*, *S. newington*, *S. selandia*, *S. new-brunswick* and *S. senftenberg*, confirmed the authors' findings and further added that the antigenic change from 3,10 to 3,15 could be induced by infection with phages which were obtained from E₂ group organisms.

As this phenomenon is related to transduction and ^{is} noticeable, the authors have confirmed, extended the experiments, have been and are continuing the study at present. An outline of the results was reported at the 27th General Meeting of Japan Bacteriological Society, in April 1954, which will be described in this paper.

During the above research, Iseki and Sakai (1953b) reported that the antigenic changes from 3,15 to 3,10 were induced by phage antiserum. Lederberg and Edwards (1953), and Storker, Zinder and Lederberg (1953) reported on transduction of flagellar characters in *Salmonella*. Another related phenomenon has been observed in *Corynebacterium diphtheriae*. It has been reported that nontoxic strain of *Corynebacterium diphtheriae* was changed to toxic by infection with phages obtained from toxic strain. In this case it is pointed out that the induced variant cells were lysogenic and variation rate was much higher in transduction (Freeman, 1951; Freeman and Morse, 1952; Groman, 1953). The authors' findings on antigenic changes in E₁, E₂, and E₃ ^{group} organisms of *Salmonella* are similar to the latter findings rather than to transduction ~~as~~ far as hitherto studied.

MATERIALS AND METHODS

1. Strains:

S. senftenberg Aa' is a strain possessing 3 factor alone as O antigen, which Ise(1953b) obtained as a variant of S. senftenberg A. S. senftenberg HS-1 through HS-10 are strains received from Bact. Dept., Faculty of Veterinary Medicine, Hokkaido University, all of which were isolated from chicken eggs.

All other strains of E₁, E₂ and E₃ groups are international standard strains which were sent from Kauffmann to Enterobacteriaceae Committee of Japan, the donor.

2. Immune sera: All of H and O sera were prepared according to the routine methods. (Kauffmann, 1951)

3. Flagellar and somatic antigens were tested by slide agglutination, using suitably diluted absorbed sera. When necessary, tube agglutination and cross absorption test were carried out.

4. Phage suspensions were prepared by autolysis of lysogenic cells or by mixed cultivation of lysogenic and susceptible cells.

Thus, about 300mg (wet weight) of 24-hour agar culture of lysogenic strain were suspended in 10ml of saline and kept at 37°C for 48 hours. The bacteria were removed by centrifugation and the supernate was filtered by Chamberland L₃. In some cases, mixed broth cultures of lysogenic and susceptible cells were centrifuged and the supernate was heated at 58°C for 30 to 40 minutes and then filtered by Chamberland L₃.

The filtrates were used as phage suspensions, and tested for sterility, both at the time of preparation and when used.

5. Induction of antigenic changes by phage

Each phage suspension was mixed with broth in proportions of 3 to 7. Susceptible cells of E₁ and E₃ group were cultured in phage broth mixtures, and when necessary, serially subcultured everyday up till the 10th passage. At appropriate intervals, the cultures were plated on nutrient agar and single colonies were subjected to serological diagnostic test. Further repeated single colony isolations were made if the results were at all ambiguous. In many cases single cell culture technique was applied by using Peterfi's type of micromanipulator.

6. Plaque counting was conducted by agar layer method.

7. Test for lysogenicity of induced variant strains

1) After soaking the culture in phage antiserum, single cell isolations of induced variant strains were made with Peterfi micromanipulator. From each single cell culture autolysate was prepared and its lytic activity was tested by plaque counting and by nephelometric determination of the turbidity of broth culture of mixture of the autolysate and E₁ group organisms.

2) Each autolysate was also tested for its capacity of inducing antigenic changes in E₁ group organisms.

3) It was also tested whether each single cell culture was immune or susceptible to the phages obtained from E₂ group organisms.

8. Induction of antigenic changes by antiserum

The 15 antiserum was prepared by absorbing S. newington "0" antiserum with S. london. The variant strains to be tested, possessing 15 antigenic factor, were cultured in broth containing 15 antiserum and when necessary, serially subcultured. At appropriate

intervals the culture was plated on agar and single colonies were tested by monofactor serums.

9. Enzymatic treatment

For enzymatic treatments, the phage suspension obtained from ^{S. cambridge} was employed, which contained about 4×10^9 phage particles per ml, calculated from plaque counting by using *S. anatum* as an indicator strain.

For pepsin digestion test, Merck's crystallized hog gastric pepsin was used, and casein was used as a control substrate. The pH of phage suspensions was corrected to 4.8 by adding phosphate buffer solution, though the optimal pH for pepsin digestion is at 2.0 through 4.0. It is because the phages used were found to be inactivated by standing overnight in refrigerator at pH 3.0.

For trypsin digestion Merck's crystallized trypsin was used and casein as a control substrate. The pH of phage suspensions was kept at 8.0, the optimal pH, during digestion.

As a desoxyribonuclease preparation, varidase (Lederle) was used, which was a commercial preparation, containing streptokinase and streptodornase. Lytic activity and antigenic change inducing activity of phage suspension were tested after keeping the phage-varidase mixture at 30°C for 5 hours.

EXPERIMENTAL RESULTS

I. Lysogenicity of E₂ group strains and phage susceptibility of E₁ group strains.

Autolysates were prepared from *S. newington*, *S. new-brunswick*, *S. selandia*, *S. cambridge*, *S. kinshase*, *S. canoga*, *S. illinois* and *S. thomasville*, and 0.3ml of each autolysate were mixed with 0.7ml of nutrient broth, in turn. In autolysate broth mixture, various type strains of E₁ group were separately cultivated in turn and, when necessary, serially subcultured up¹ till 10th passage. Each cultures were separately plated out in turn on nutrient agar and the colonies were serologically examined by monofactor serum. The results are summarized in table 1, which indicates the followings.

1) Whatever the strain may be, the organisms of E₂ group, possessing 15 antigenic factor as a part of O antigens, were found to be lysogenic and the autolysates were found to be capable of inducing changes of O antigens of E₁ group cells from 3, 10 to 3,15.

2) The susceptibility to a definite phage of E₁ group organisms varied with strains, some of which were readily changed in their O antigenic structure (for example; *S. anatum*, *S. butantan*, *S. simi*) while others were not so readily altered (for example; *S. london*, *S. meleagridis*).

3) The capacity of inducing antigenic variation in a definite strain of E₁ group seems to vary with phage strains. This may, however, depend upon the amount of phage, though the details have not been made clear as yet.

Beside the above, it was observed during these experiments that

culture of E₁ group organisms was less turbid in the autolysate-broth than in broth alone. This is due to partial bacteriolysis.

II. Antigenic changes in S. lexington and S. macallen.

In both S. lexington and S. macallen, O antigens are 3,10, which are the same as those of the type strains described above, but they were changed to 3,10,15 by the phage obtained from S. cambridge. This type of change was also induced by the phage obtained from other type of organisms of E₂ group (S. kinshasa, S. canoga).

Since this finding is very important in that two antigenic factors, 10, and 15, are able to co-exist in a single cell, the antigenic structures of parent strains and induced variants were reexamined by single cell culture technique with micromanipulator, and the above findings were reconfirmed.

In the previous papers and the data described in the preceding paragraph, only the changes from 3,10 to 3,15 or from 3,15 to 3,10 had been observed, but the coexistence of 10 and 15 in a single cell had not been observed as yet. Therefore, this was the first evidence of the coexistence of 10 and 15.

III. Antigenic changes in S. chittagong.

The O antigenic structure of S. chittagong^x is shown as 1,3,10, 19. As the phages from E₂ group organisms seemed to attack the organisms, possessing 10 antigenic factor, S. chittagong^x was exposed to the phage. By each of the phages from S. newington and from S. canoga, O antigens of S. chittagong^x were converted from 1,3,19 to 3,15 (table 2). In this case, it is noteworthy that the antigenic changes involved the loss of 10 as well as 1,19 anti-

genic factors. The following experiments, however, showed the possibility of coexistence of 15 and 1,19 in a single cell.

IV. Antigenic changes in S. niloese and S. senftenberg-simsbury

When these two strains were serially subcultured in broth containing the autolysate of S. canoga or the mixture of autolysates of S. newington, S. new-brunswick and S. selandia and/or when serially subcultured in broth containing 1,19 antiserum, which was prepared by absorbing S. niloese O antiserum with S. london, the induction of antigenic changes was not observed in so far as examined. However, when these two strains were subcultured in broth containing both the mixture of autolysates of the above-mentioned three strains and 1,19 antiserum, antigenic variants were obtained from each of them, which were agglutinable by 15 monofactor serum. As these variants were agglutinated not only by 15 antiserum but also by 1,19 antiserum, antigenic structures were reexamined by single cell culture technique, which revealed that the O antigens of the variants were 1,3,15,19 (table 2). In short, it was confirmed that O antigens of S. niloese and S. senftenberg-simsbury were converted from 1,3,19 to 1,3,15,19.

In these cases it is noteworthy that the induction of formation of 15 antigenic factor did not result in the disappearance of 1,19 antigenic factors, when compared with antigenic variation of S. chittagong, as described above. The reason, however, has not been made clear as yet, wherefrom this difference comes.

V. Antigenic variation of S. senftenberg 87Aa'

S. senftenberg 87Aa' is a strain which was obtained from S. senftenberg A by Ise and has only a 3 factor as a major O antigen.

O antigenic structure of this strain was changed from 3 to 3,15 by being exposed to the phage from *S. canoga*. This change was accomplished without subculture (table 2).

VI. Lysogenicity of antigenic variants.

As described above all strains of E₂ group, possessing 15 antigenic factor, ~~were~~ been found to be lysogenic and the phages obtained from such strains ~~have~~ been found to be capable of inducing antigenic changes in E₁ group cells, resulting in changes from 3,10 to 3,15 or to 3,10,15. Then experiments were conducted to determine whether the induced antigenic variants possessing 15 factor are lysogenic or not and, if any, whether or not phages obtained from such variants are capable of inducing the same antigenic changes as those obtained from E₂ group organisms. The results were as follows.

1) Variants possessing 15 factor, derived from E₁ group strains, were found to be lysogenic and the phages obtained from such variants were found to be capable of inducing antigenic changes from 3,10 to 3,15 in E₁ group organisms. These observations are consistent with Iseki and Sakai's report.

The antigenic variants were found also to be resistant to the phages obtained from E₂ group organisms.

2) Variants of *S. chittagong*, possessing antigenic structure of 3,15, were also found to be lysogenic and the phages obtained from them were found to be capable of inducing the same antigenic changes.

3) In variants of *S. lexington* and *S. macallen* the states were found to be the same as described above, as to the lysogenicity

and the activity of the phages.

4) In variants of *S. niloese* and *S. senftenberg-simsbury* the states were also found to be the same as described above, as to the lysogenicity and the activity of the phages.

These findings indicates the close correlation between lysogenicity and formation of 15 antigenic factor in *Salmonella* organisms of E_1 , E_2 and E_3 group and/or their antigenic variants.

VII. Correlation between lytic activity and activity inducing antigenic changes

1) Effect of pH

Aliquots of phage suspensions obtained from *S. cambridge* were separated into several portions, in each of which pH was corrected to pH 1.0, 2.0, 3.0, 4.0, 5.0, 8.0, 9.0, 10.0 and 11.0 respectively and kept in refrigerator for 20 hours.

Both the lytic activity and the activity of inducing antigenic change of the phage were found to be destroyed at pH 3.0 or below and at pH 11.0 or over.

2) Effect of temperature

The results of plaque counting showed a considerable reduction in number of active phage by heating at 60°C for 30 minutes and a complete inactivation of phages by heating at 65°C for 30 minutes or at 70°C for 15 minutes. The activity inducing antigenic change was found to be retained after heating at 60°C for 30 minutes, but not at 65°C for 30 minutes or at 70°C for 15 minutes.

3) Effect of enzymes

(1) Phage suspension obtained from *S. cambridge* was treated with pepsin at pH 4.8 at 37°C overnight, without any reduction

of lytic activity or destruction of activity inducing antigenic changes.

(2) Phage suspension was treated with trypsin at pH 8.0 at 37°C for 20 hours. The results were the same as those in pepsin digestion.

(3) Varidase treatment resulted in ^{no} destruction of neither lytic activity nor activity of inducing antigenic changes.

4) Turbidity of broth cultures

The cultures of susceptible cells in phage-broth mixture, from which antigenic variants were isolated, were always less turbid than those in broth alone. Though the grade of turbidity was not exactly measured, it is clear that there is a certain correlation between bacteriolysis and antigenic changes.

All of the above findings indicate that the lytic activity is parallel to the activity of inducing antigenic changes, and no evidence has been obtained by which to indicate the separation of the two activities.

VIII. Induction or selection ?

The possibility that the antigenic variants were spontaneous mutants selected by the phage minimized by the following.

Even when *S. anatum* or *S. butantan* was exposed to the phage for only one minute and plated out, many antigenic variant colonies were detected on the plates. And the number of colonies of antigenic variant were so numerous and the time of exposure to the phage was so short that ^{variants} they could not have been selected but ^{they} could reasonably be considered as induced by phage infection.

In the course of this experiment another noticeable phenomenon was observed. Thus, it was noted for the first time that the antigenic variants formed translucent colonies which could be easily distinguished from the colonies of parent cells. Such translucent colonies were formed ~~only~~ when the parent cells were exposed to the phage for a short time and plated out at once, and pure cultures obtained from such translucent colonies did not show the aforesaid transparency. This is a very interesting phenomenon, though the mechanism involved has not been made clear as yet.

IX. Reversion of converted antigens.

The O antigens 3,15 of the phage-induced variants of E₁ group organisms were reverted to the original structure 3,10 by cultivating the variants in broth containing 15 antiserum.

In the variants of S. chittagong, O antigens were reverted to 1,3,19, the structure of the parent strain, by 15 antiserum.

In the variants of S. senftenberg-simsbury and S. niloese, O antigens were reverted to 1,3,19, the structure of the parent strain, by 15 antiserum.

X. H antigens

In each of the O antigenic variants, H antigens were found to be the same as those of the parent strains respectively.

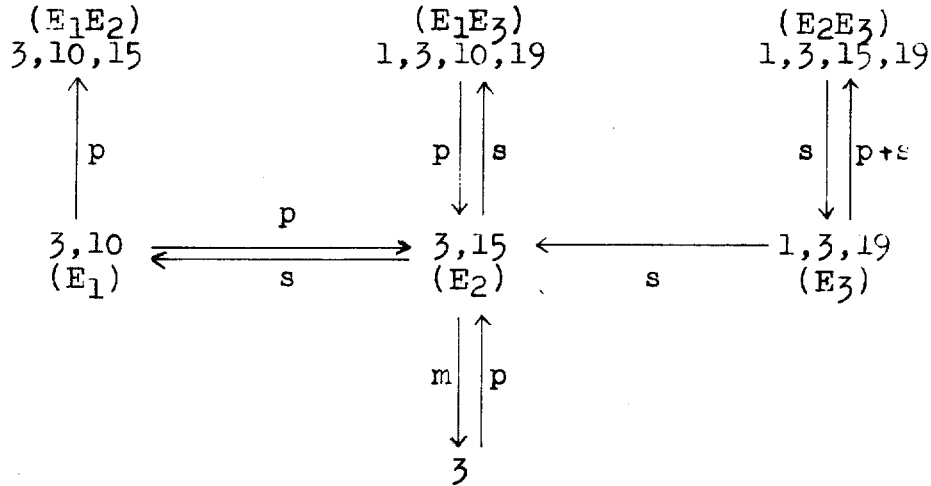
XI. Biochemical behaviors

Biochemical behaviors of the antigenic variants were found to be the same as those of the parent strains respectively.

DISCUSSION

The above findings may be summarized as follows.

- 1) The antigenic changes hitherto observed, are summarized as below.



\overleftarrow{p} = Direction of antigenic changes induced by phage infection.

\overleftarrow{s} = Direction of antigenic changes induced by antisera.

\overleftarrow{m} = Direction of spontaneous mutation.

- 2) As far as tested, all of the E₂ group strains were found to be lysogenic and the phages obtained from them were capable of inducing antigenic changes from 3,10 to 3,15 or to 3,10,15 in E₁ group organisms. They were also capable of inducing antigenic changes in E₃ group organisms from 1,3,19 to 1,3,15,19. In special cases, they induced antigenic changes in *S. chittagong* from 1,3,10,19 to 3,15 and in *S. senftenberg* 87Aa' from 3 to 3,15.

- 3) In so far as tested, all of the induced variants, possessing 15 factor as a part of O antigens, were found to be lysogenic and the phages obtained from them were found to have the capacity of inducing antigenic changes, in connection with the formation

of 15 factor, in E₁ group organisms. This point seems to be similar to Groman's findings in *Corynebacterium diphtheriae*, and to be different from transduction which was observed in *Salmonellas* by using bacteriophage of *S. typhimurium*. In transduction it is pointed out that the transmission of any given character can be readily separated from the action of the phage. (Lederberg and Edwasrd, 1953; Stocker, Zinder and Lederberg, 1953)

- 4) In transduction, phages which were propagated on one strain, transmitted some genetic trait of this donor strain to the recipient strain, while in the authors' experiments phages invariably induced the activity forming 15 factor in E₁ group organisms, even when they were propagated on E₁ group cells.
- 5) In many of the E₁ group organisms O antigenic structures were altered from 3,10 to 3,15, but it was suggested that they still retained their latent activity forming 10 antigenic factor, because when the variants were cultured in broth containing 15 antiserum, O antigenic structures were reverted to 3,10. This is also a very important point of difference which exists between transduction and the authors' observations. In transduction, "a replacement of genetic traits" has been pointed out (Stocker, Zinder and Lederberg, 1953), while in the authors' experiments there has been no evidence as yet that the antigenic changes from 3,10 to 3,15 are considered to involve the replacement of genetic traits for formation of 10 antigenic factor or some other character by genetic traits for formation of 15 factor.

In antigenic changes of *S. chittagong*, the states seems to be the same as described above.

6) In the previous papers antigenic changes were reported to occur~~ed~~ only from 3,10 to 3,15 or vice versa, and the formation of 15 antigenic factor seemed to be followed by the disappearance of 10 factor. The states were the same in many of E₁ group strains used in the experiments herein, but the coexistence of 10 and 15 antigenic factors was observed for the first time in two strains. Thus in *S. lexington* and *S. macallen*, 0 antigenic structures were changed from 3,10 to 3,10,15. This fact and the findings, that 3,15 were changed to 3,10 by antiserum, suggest that the antigenic changes from 3,10 to 3,15 do not involve such "a replacement of genetic traits" as observed in transduction.

7) Antigenic changes from 1,3,19 to 1,3,15,19 suggest the same as mentioned above.

In this experiment, the changes from 1,3,19 to 1,3,15,19 were observed only when the test strains were exposed to a mixture of phage and 1,19 antiserum, and not when exposed to the phage alone or to the antiserum singly. However, the role of antibodies is not clear as yet, because the variants still retain 1,3,19 factors. Besides, it can not be denied that the difference among results of the above experiments might be only **an** apparent^{one}, depending upon the number of colonies examined in this kind of experiment that has to be limited to a certain small number.

8) In *S. anatum* and *S. butantan*, both of which are liable to

undergo antigenic changes by phage infection, many antigenic variant colonies were detected even after exposing the cells to the phage suspension for only a very short time. This high variation rate is in contrast with the low frequency in transduction, and is rather similar to Groman's findings. This difference, however, could be quantitative rather than qualitative in nature, as pointed out by Groman (1953).

- 9) When phage was inactivated by the change of pH or heating, the activity inducing antigenic changes was lost simultaneously.
- 10) Deoxyribonuclease did not inactivate the activity inducing antigenic variations of phage suspension. This indicates that the said phenomenon differs from transformation which has been observed in antigenic changes of Pneumococcus, H. influenzae, E. coli and ^Nmeningitidis. In transformation, the active principle is inactivated by desoxyribonuclease.
- 11) Among the induced variants, those possessing O antigenic structures of 3,10,15 and 1,3,15,19 have not been isolated as yet from natural sources. Furthermore, the immediate practical contributions of these variants are probably in the availability of the above for use as antigens for serum production.
- 12) In the authors' experiments antigenic changes have been observed in O antigens only but not in H antigens. These facts, however, do not exclude the possibility that changes in H antigens may occur by phage infection. It depends on the method of detecting the variants, whether or not H antigenic variants can be discovered. If H antigenic variants should be sought, it can not be denied that they might be discovered. Experiments

along these lines are now under study.

- 13) It has been observed that anti-15 monofactor serum induced antigenic changes from 3,15 to 3,10, from 1,3,15,19 to 1,3,19 and from 3,15 to ¹3,10,19. Iseki and Sakai⁽¹⁹⁵³⁾ reported that phage antiserum induced a change from 3,15 to 3,10. As anti-15 monofactor serum was prepared by absorbing S. newington "C" antiserum with E₁ group organisms, it can not be denied that it might have contained antiphage antibodies. This is now under investigation. Even though Iseki and Sakai's report were correct, there remains another question to be determined, as to how the anti-phage antibodies induced the variation from 3,15 to 3,10. Sakai and Iseki (1953) reported that the phage antibodies combined with prophages within E₂ group cells. This explanation, however, is difficult for the authors to accept for the following reasons:

The first reason is that there has been no evidence as yet that prophages can be neutralized by phage antiserum or that they possess any antigen which reacts with antiphage antibodies. (McKinley, 1925; Freeman and Morse, 1952; Lwoff, 1953)

The second reason is that even when cells of E. coli B were soaked in anti-T₂ serum for a 5 days period after infection with phage T₂, neutralization of phage (gonophage) was not observed within the cells. (Okada, **personal communication**).

Now, if the prophages can not be neutralized by antiphage antibodies, what would be the appropriate explanation of the mechanism? It has been reported that lysogenic strain may yield nonlysogenic cells in its progeny though in^a very small

number (Lwoff, 1953). This may be true in E₂ group strains. If so, it should be possible that such nonlysogenic cells would be selected by phage antiserum. Nonlysogenic cells are supposed to be yielded, if any, in a very small number and to be infected by free phages present unless antiphage antibodies are added. This may be one possible explanation, and further study will be required in the future.

- 14) In the early stage of the research, the phages obtained from E₂ group organisms seemed to be adsorbed by strains which carry the 10 somatic antigen. However, from the findings that they were also adsorbed by E₃ group strains, it is doubtful whether the receptor for the bacteriophage can be the 10 antigenic factor itself, though the 10 factor has been reported to be of a complex nature (Kauffmann, 1938).
- 15) Taking H antigens beside O antigens into consideration, when O antigens of *S. anatum*, *S. nyborg*, *S. meleagridis*, *S. give* and *S. uganda* are altered from 3,10 to 3,15, these strains are changed to forms which are indistinguishable from *S. newington*, *S. selandia*, *S. cambridge*, *S. new brunswick* and *S. kinshasa* respectively. Table 3 shows a comparison of the sources from which they were isolated. Though the summarized data in table 3 are not complete as not all of the references concerned were available, they show that each pair of *S. anatum* and *S. newington*, *S. nyborg* and *S. selandia*, *S. give* and *S. new-brunswick*, and *S. lexington* and *S. illinois* has been isolated from similar sources respectively. Besides, the corresponding two types are identical or almost identical in biochemical behaviors with each other.

Though between *S. meleagridis* and *S. cambridge* no definite similarity of sources has been found, further accumulation of data will be ^{re}quired in the future, as it has not been long since *S. cambridge* was discovered. The state seems to be the same in the relationship between *S. canoga* and *S. senftenberg* var. *newcastle*, both of which are identical in biochemical behaviours. The details of *S. kinshasa* could not be surveyed because of the unavailability of the references.

Anyhow, in the strains which have been isolated not so recently, it is noteworthy that each of the above pairs are not only identical in antigenic structure but also are identical or almost identical in biochemical behaviors and have been isolated from similar sources. These findings seem to suggest that one of the pair was yielded from the other as a result of antigenic variation, in nature. This might be supported by the fact that more types have been isolated in E_1 group than E_2 group and each of the E_2 group types has its corresponding type in E_1 group.

From the above it might be assumed that E_2 group type strains might have been yielded as a result of infection of E_1 group organisms with the phages which were liberated from some unknown strain, though there has been no direct evidence as yet.

SUMMARY

1. *S. newington*, *S. new-brunswick*, *S. selandia*, *S. cambridge*, *S. kinshase*, *S. canoga*, *S. illinois* and *S. thomasville* of E₂ group were found to be lysogenic, and the phages obtained from them were found to be capable of infecting E₁ or E₃ group organisms, resulting in establishment of lysogenicity and antigenic changes from 3,10 to 3,15 or 3,10,15 and/or from 1,3,19 to 1,3,15,19.
2. *S. london*, *S. give*, *S. anatum*, *S. amager*, *S. zanzibar*, *S. shangani*, *S. butantan*, *S. veile*, *S. meleagridis*, *S. elisabethville*, *S. simi*, *S. weltevreden*, *S. orion*, *S. lexington* and *S. macallen* of E₁ group were found to be receptive to the phages obtained from E₂ group organisms and to be lysogenized, resulting in the said antigenic changes. The antigens of the variants can be reverted to 3,10 by cultivating the variants in broth containing 15 antiserum.
3. *S. chittagong* was also lysogenized by the phage and its O antigens were converted from 1,3,10,19 to 3,15, which were reverted to the original structure by cultivating the variant in broth containing 15 antiserum.
4. *S. senftenberg* Aa', possessing 3 alone as a major O antigen, was lysogenized by the phage, resulting in antigenic changes from 3 to 3,15.
5. *S. senftenberg-simsbury* and *S. niloese* were lysogenized by the phage, resulting in antigenic changes from 1,3,19 to 1,3,15,19, which ^{were} reverted to the original structure by cultivating the variants in broth containing 15 antiserum.

6. Thus, when the organisms possessing 15 factor, whether they may be type strains of E₂ group or phage-induced antigenic variants, were cultured in broth containing anti-15 monofactor serum, antigenic changes were induced from 3,15 to 3,10, from 3,15 to 1,3,10,19 and/or from 1,3,15,19 to 1,3,19.
7. The phages obtained from the antigenic variants possessing 15 factor were found to have the same activity as those from E₂ group organisms, in infecting E₁ group organisms and inducing antigenic changes in them.
8. Desoxyribonuclease, trypsin or pepsin treatment affected the phage in neither lytic activity nor capacity of inducing antigenic changes.
9. The loss of lytic activity of the phages by heating or change of pH was accompanied by the loss of capacity inducing antigenic changes.
10. The observed phenomenon was compared with transduction and a few differences were focused and discussed.
11. The bearing of the present findings on taxonomic problems was discussed.

REFERENCES

1. Alexander, H.E. and Leidy, G. 1950 Transformation type specificity of *H. influenzae*. Proc. Soc. Exptl. Biol. Med., 73, 485-487.
2. Alexander, H.E. and Leidy G. 1951a Induction of heritable new type in type specific strains of *H. influenzae*. Proc. Soc. Exptl. Biol. Med., 78, 625-626.
3. Alexander, H.E. and Leidy, G. 1951b Determination of inherited traits of *H. influenzae* by desoxyribonucleic acid fractions isolated from typespecific cells. J. Exptl. Med., 93, 345-359.
4. Alexander, H.E. and Redman, W. 1953 Transformation of type specificity of meningococci. J. Exptl. Med., 97, 797-806.
5. Austrian, R. 1952b Observation on the possible role of nucleic acid exchange reaction in pneumococcal capsular type transformation. A preliminary note. Bull. Johns Hopkins Hcsp., 90, 170-174.
6. Austrian, R. 1952a Bacterial transformation reactions. Bact. Revs., 16, 31-50.
7. Avery, O.T., MacLeod, C.M. and McCarty, M. 1944 Studies on the chemical nature of the substance inducing transformation of pneumococcal types. Induction of transformation by a desoxyribonucleic acid fraction isolated from pneumococcus Type II. J. Exptl. Med., 79, 137-158.
8. Boivin, A. 1947 Directed mutation in colon bacilli by an inducing principle of desoxyribonucleic nature: its meaning for the general biochemistry of heredity. Cold Spring Harbor Symposia Quant. Biol., 12, 7-17.
9. Breed, R.S., Murray, E.G.D. and Hitchens, A.P. 1948 Bergey's manual of determinative bacteriology. 6th Ed., 492-534.
10. Brunner, D.W. and Edwards, P.R. 1948 Changes induced in the C antigens of *Salmonella*. J. Bact., 55, 449.
11. Freeman, V.J. 1951 Studies on the virulence of bacteriophage-infected strains of *Corynebacterium diphtheriae*. J. Bact., 61, 675-688.
12. Freeman, V.J. and Morse, I.U. 1952 Further observations on the change to virulence of bacteriophage-infected avirulent strains of *Corynebacterium diphtheriae*. J. Bact., 63, 407-414.

13. Groman, N.B. 1953 Evidence for the induced nature of the change from nontoxigenicity to toxigenicity in *Corynebacterium diphtheriae* as a result of exposure to specific bacteriophage. *J. Bact.*, 66, 184-191.
14. Hotchkiss, R.D. 1952 The role of desoxyribonucleates in bacterial transformations. *Phosphorus metabolism*, II, 426-439, Johns Hopkins Press, London.
15. Ise, T. 1953a Antigenic variation in *S. senftenberg*. *Jap. J. Bact.*, 8, 359. (In Japanese)
16. Ise, T. 1953b Changes induced in the O antigens of *S. senftenberg* and chemical analysis of specific O antigens. (Changes induced in the antigens of *Salmonella* IV). *Sapporo Med. J.*, 4, 395-400. (In Japanese)
17. Iseki, S. and Sakai, T. 1953a Immunogenetic studies on microorganisms. II. Transformation of O antigens in *Salmonella* E group by the action of antiserum and bacterial autolysate. *Med. and Biol.*, 28, 77-80. (In Japanese)
18. Iseki, S. and Sakai, T. 1953b Immunogenetic studies on microorganisms. III. Antigen-transforming factor in bacilli of *Salmonella* E₂ group. *Med. and Biol.*, 28, 193-196. (In Japanese)
19. Kauffmann, F. 1938 *Salmonellaproblem*. *Z. Hyg.*, 120, 177-197.
20. Kauffmann, F. 1951 *Enterobacteriaceae*. E. Munksgaard, Copenhagen.
21. Laros, B. 1949 Induced modifications in the antigenic reactivity of the pneumococcus. *J. Infectious Diseases*, 85, 263-267.
22. Lederberg, J. and Edwards, P.R. 1953 Serotypic recombination in *Salmonella*. *J. Immunol.*, 71, 232-240.
23. Leidy, G., Hahn, E. and Alexander, H.E. 1953 In vitro production of new type of *H. influenzae*. *J. Exptl. Med.*, 97, 467-482.
24. Lwoff, A. 1953 Lysogeny. *Bact. Revs.*, 17, 269-337.
25. McKinley, E.B. 1925 Sérum antilytique obtenu par immunisation contre une bactérie normale. *Compt. rend. soc. biol.*, 93, 1050-1052.
26. McLeod, C.M. and Krauss, M.R. 1953 Control of factors distinct from specific transforming principle of the amount of capsular polysaccharide produced by the III pneumococci. *J. Exptl. Med.*, 97, 767-771.

27. Nakagawa, T. 1951a Changes induced in the antigens of Salmonella (II). Reversion of antigenic changes. Record of the 3rd Meeting of the Hokkaido Branch of Japan Bact. Soc., 7.; 1953a Sapporo Med. J., 4, 318-325. (In Japanese)
28. Nakagawa, T. 1951b Changes induced in the antigens of Salmonella (III). Studies on the mechanism of changes induced in the "O" antigens of Salmonella. Acta Med. Hokkaidonensia, 28, 13-14; 1953b Sapporo Med. J., 4, 386-394. (In Japanese)
29. Obara, T. 1950 Changes induced in the antigens of Salmonella. Induced changes of O antigens from 3,15 to 3,10,26. J. Sapporo Univ. Med., 1, 68-78. (In Japanese)
30. Sakai, T. and Iseki, S. 1953 Immunogenetic studies on microorganisms. IV. Physicochemical properties of antigen-transforming factor in Salmonella E group. Med. and Biol., 29, 199-201. (In Japanese)
31. Sonneborn, T.M. 1947 Beyond the gene. Am. Scientist, 37, 33-59.
32. Stocker, B.A.D., Zinder, N.D. and Lederberg, J. 1953 Transduction of flagellar characters in Salmonella. J. Gen. Microbiol., 9, 410-433.
33. Uetake, H. and Obara, T. 1950 Changes induced in the antigens of Salmonella. Jap. J. Bact., 5, 164. (In Japanese)
34. Uetake, H. et al. 1952 Changes induced in the O antigens of Salmonella. Studies on reversion and mechanism of changes and chemical analysis of monosaccharide composition of specific polysaccharides of O antigens. Jap. J. Bact., 7, 415. (In Japanese)
35. Wilson, G.S. and Miles, A.A. 1948 Topley and Wilson's Principles of bacteriology and immunity, 3rd Ed., 702-749.
36. Zamenhof, S., Alexander, H.E. and Leidy, G. 1953 Studies on the chemistry of the transforming activity. I. Resistance to physical and chemical agents. J. Exptl. Med., 98, 373-397.
37. Zinder, N.D. and Lederberg, J. 1952 Genetic exchange in Salmonella. J. Bact., 64, 679-699.