THE TRANSFORMATION OF PNEUMOCOCCAL TYPES

II. THE INTERCONVERTIBILITY OF TYPE-SPECIFIC S PNEUMOCOCCI

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In the previous communication (1) it was shown that avirulent, non-type-specific, R forms of Pneumococcus could be converted into virulent, type-specific, S forms of the original type by the following procedures as employed by Griffith (2): 1) The subcutaneous injection, in white mice, of large amounts of living R forms; 2) The subcutaneous injection, in white mice of small amounts of living R forms together with the heat-killed bacteria from large quantities of the homologous S culture. By these procedures the R forms were invariably converted to S forms of the same specific type as that from which they had been originally derived.

Griffith further reported (2) that R forms of Pneumococcus, derived from S forms of any specific type, could be converted into type-specific organisms of heterologous S types by the following procedure:—the subcutaneous injection, in white mice, of small amounts of living R forms together with the heat-killed bacteria from large quantities of heterologous S cultures. In other words, he stated that it was possible to transform S Pneumococci of one specific type into other specific types through the intermediate stage of the R form. The present communication is concerned with the question of such transformation of Pneumococcal types.

Methods

The suspensions of heat-killed organisms were prepared in the same manner as described in the preceding paper (1). Similar controls were employed to eliminate the possibility of the existence of viable organisms in the vaccines.¹ In all critical experiments the number of control animals injected was equal to the number of

¹ The term vaccine indicates a suspension of heat-killed pneumococci.
test animals. The bacteria obtained from large quantities of culture, killed by heating, were also injected into animals together with cultures of other live organisms. In addition large amounts of vaccine were injected into animals intoxicated with alcohol. In no instance were viable pneumococci recovered from the controls. Notwithstanding the conclusive nature of the controls employed, even more convincing evidence as to the improbability of the existence of any viable organisms in the heat-killed suspensions will be offered in the subsequent description of the experiments.

The R strains which were used were derived in all instances from typical, type-specific, S pneumococci by growth in homologous immune serum. The possibility of the cultures containing a mixture of R forms, derived from S forms of more than one type, was eliminated in many experiments by the use of single-cell strains. In addition, proof of the nature of the R strains was obtained by converting them to the S form by growth in media containing anti-R serum (3). Under these conditions the R forms invariably reverted to the S form of the same specific type as that from which they were originally derived.

EXPERIMENTAL

Conversion of R Forms of Pneumococcus into S Forms of Heterologous Type by the Subcutaneous Injection, in White Mice, of Small Amounts of Living R Forms, Together with Large Amounts of Heat-Killed S Forms of Heterologous Type

In the course of early experiments it became apparent that the selection of the R strain played an important role in determining the results obtained. The particular 2 R culture (strain D/39/R) which was first chosen could be readily converted to the S form of the original type by all the methods which have been described: 1) animal passage: 2) growth in media containing anti-R serum; 3) the subcutaneous injection, in white mice, of large amounts of living R forms: 4) the subcutaneous injection, in white mice, of small amounts of living R forms together with the heat killed bacteria from large amounts of the homologous S culture.

In the first experiment twelve mice were injected with 0.25 cc. of living 2 R culture together with the bacteria from 90 cc. of a Type I S culture, heated for 15' at 60°C. All the mice died after an interval of 24 to 48 hours and typical Type II S pneumococci were recovered from the heart's blood of each animal. In all cases the R forms were converted to S forms of the same type as that from which they were originally derived.

Another 2 R culture, (strain N/D/39/R), was then obtained by growing a
typical Type II S pneumococcus in its homologous immune serum. After six transfers the culture was plated. An individual colony was then selected and transferred to blood broth. This 2 R culture could likewise be converted into S forms of the original type; but greater difficulty was experienced in bringing about the change than in the case of the 2 R culture previously employed. This second 2 R culture was injected into a series of twelve mice together with aliquot portions of the Type I S vaccine used in the preceding experiment. Quite different results were obtained. Ten of the twelve animals died after an interval of 24 to 48 hours and from the heart's blood of nine of these typical Type I S pneumococci were recovered. Cultures made from the heart's blood of the tenth mouse yielded only R forms. Two mice survived.

**TABLE I**

*Conversion of R Forms of Pneumococcus into S Forms (1) of the Original Type (2) of the Type of the Vaccine*

<table>
<thead>
<tr>
<th>Type and amount of vaccine</th>
<th>Amount of living R culture</th>
<th>Number of mice</th>
<th>Result</th>
<th>Pneumococci recovered by culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria obtained from 90 cc. Type I S culture, heated at 60°C for 15'.</td>
<td>Nil.</td>
<td>10</td>
<td>All survived; sacrificed 7 days.</td>
<td>All cultures negative.</td>
</tr>
<tr>
<td>ditto.</td>
<td>0.25 cc. 2 R (strain D/39/R)</td>
<td>12</td>
<td>All died; 1-2 days.</td>
<td>Type II S</td>
</tr>
<tr>
<td>ditto.</td>
<td>0.25 cc. 2 R (strain N/D/39/R)</td>
<td>12</td>
<td>10 died 1-2 days.</td>
<td>Type I S</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2 survived; sacrificed 7 days.</td>
<td>R only. 1</td>
</tr>
</tbody>
</table>

The results obtained by injecting these two different 2 R strains, together with the same Type I S vaccine, appear in Table I.

*Abstract of Protocol.—*

2 R culture, (strain D/39/R), + I S Vaccine, heated for 15' at 60°C.
Number of mice injected—12.
Number of mice died—12.
Reversion to S forms of the original type—12.
Reversion to S forms of the same type as the vaccine—0.

2 R culture, (strain N/D/39/R), + I S Vaccine, heated for 15' at 60°C.
Number of mice injected—12.
Number of mice died—10.
Reversion to S forms of the original type—0.  
Reversion to S forms of the same type as the vaccine—9.  
No reversion, only R organisms recovered—1.  
Number of mice survived—2.

The Type I S organisms which were recovered from the heart's blood of the second series of mice possessed all the attributes of typical Type I S pneumococci. The cultures agglutinated specifically in Type I serum; they elaborated the specific soluble substance characteristic of Type I pneumococci, and were highly virulent for white mice. Subcultures made through twenty transfers retained the same properties and the organisms showed no tendency to revert to their original S type.

Since the same Type I S vaccine was used in the two experiments the variation in results obtained must have been referable to a difference in the R cultures employed. This difference was reflected in the greater difficulty experienced in causing the second 2 R culture to revert to the S form of its original type. Apparently in order that an R culture may revert to the S form of a heterologous type it must first be reduced to a definite stage in the "degradation" process.

This experiment also offers further proof of the absence of any viable organisms in the vaccine. It is highly improbable that the Type I S organisms recovered from the second series of mice could have developed from surviving forms in the vaccine; for no such forms were recovered from either the control mice or from the first series of mice which received the same vaccine.

An interesting result was obtained when still another 2 R culture was injected into mice along with Type I S vaccine. This third 2 R culture was obtained in the usual way by growing a typical Type II S pneumococcus in its homologous immune serum. When grown in anti-R serum, or injected subcutaneously in white mice in large amounts by itself, this R culture invariably reverted to the S form of the same type as that from which it was originally derived. Eleven mice were injected subcutaneously with 0.25 cc. of living R forms together with the bacteria from 100 cc. of Type I S culture heated for 15' at 60°C. Eight out of the twelve animals died after a period of one to two days, and Type I organisms were recovered from the heart's blood. In certain respects, however, the organisms recovered were not typical S forms. Although the cultures agglutinated specifically in Type I serum and produced the specific soluble substance characteristic of Type I pneumococcus, they did not possess maximal virulence for white mice nor
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did they produce typical Smooth colonies. As a rule the cultures proved fatal to white mice in dilutions of $10^{-8}$ cc., occasionally in dilutions of $10^{-6}$ cc., but never in dilutions of $10^{-7}$ cc. The colonies were atypical in appearance, having neither the glossy, shiny surface of Smooth colonies nor the finely granular, ground-glass appearance of Rough colonies. For the most part the colonies were irregular in outline with nibbled margins. The outer zone was usually slightly rough; toward the center the majority presented a smooth wavy appearance; and in the middle of the colony there was frequently a crater-like depression. Other colonies more closely resembled typical R colonies and still others were suggestive of those of the S variety. That such colonies were not composed of a mixture of Rough and Smooth organisms was repeatedly proven by selecting individual colonies, sub-culturing, and plating for several transfers. Even when passed through mice colonies with the same characteristics were recovered from the heart's blood. These forms most probably represented incompletely developed S organisms partially "stabilized" at this phase of the reversion process. Such cultures could be converted into typical S forms by the subcutaneous injection in white mice of large amounts of the cultures alone.

In the course of the same experiment a 3 R culture, obtained by growing a typical Type III S pneumococcus in Type III serum, was injected into a series of eleven mice, together with aliquot portions of the same Type I S vaccine. Seven of the eleven animals succumbed and typical Type I S organisms were recovered from the heart's blood in each instance. All the colonies obtained from this series of animals were of the typical Smooth variety, and no "intermediate" forms were observed. All the cultures possessed maximal virulence for white mice.

Since the heat-killed suspension used in both series was the same the conclusion must be drawn that the atypical intermediate colonies recovered from the first series of mice developed from the 2 R culture and not from the vaccine. This observation offers still further proof of the absence of viable forms in the heat-killed suspensions.

In certain other experiments the injection of an R culture together with a heterologous S vaccine resulted in the recovery of a mixture of S forms from the animals. Such mixtures were composed exclusively of S organisms of the same type as the vaccine and the type from which the R forms had been originally derived. In all such cases S organisms were not obtained from any of the control mice. It must therefore be concluded that, in these instances, conditions were equally suitable for reversion of the R forms to the S forms of the original type, or to the type of the vaccine.

When a plate is composed of a mixture of colonies of various S types the Type III colonies are usually readily identified by their large size and clear, watery appearance. It is also possible, as a rule, to identify colonies composed of Type I S organisms. When examined against a dark background through a plate culture microscope Type I colonies are usually denser, more opaque and whiter than Type II colonies. Colonies composed of Type II and Group IV organisms, on the other
hand, are paler, more transparent and "watery." They also appear to undergo autolysis more readily than Type I colonies and frequently present a 'ring' or 'life-saver' appearance.

Experiments were next undertaken to determine whether one and the same R culture could be successively transformed into the S form of each of the specific types of pneumococcus.

A 2 R culture was obtained by growing a typical Type II pneumococcus in its homologous immune serum. After four transfers the culture was plated. A single R colony was selected and subcultured in blood broth. A single-cell strain was then obtained from this culture by the method of Avery and Leland (4). Four mice were injected with 0.25 cc. of the single-cell culture together with the bacteria from 100 cc. of a Type III S culture heated for 30' at 60°C. Three of the animals succumbed, and typical Type III S pneumococci, possessing all the characteristics of that type, were obtained from the heart's blood of each. One of these III S cultures was then converted into the R form by growth in Type III serum. After five transfers the culture was plated. A single R colony was selected and subcultured in blood broth. The resultant growth was plated and again a single colony was selected and subcultured. This process was repeated four times and the final culture was injected into four mice together with a Type I S vaccine heated for 30' at 60°C. All four mice died and typical Type I S organisms were recovered from the heart's blood in each instance. One of these typical Type I S cultures was again converted to the R form in the same manner as previously described. The resulting R culture was injected into four mice together with a vaccine prepared from a Group IV S culture. Two of the mice died yielding S organisms in the heart's blood. Cultures from the heart's blood of these two animals did not agglutinate specifically in Types I, II or III sera but were highly virulent for mice. Specific anti-serum was not available to test the agglutination of the Group IV S strains, but in view of the preceding results it was highly probable that the cultures were of the same variety as the Group IV vaccine.

During the various stages of this experiment whenever an R culture was obtained it was grown in media containing 10 per cent anti-R serum. In all cases, after a variable number of transfers, the R form was converted to the S form of that type from which it had last been derived. This observation lends support to the contention that in no instance was a mixture of R forms, derived from S forms of more than one type, present in the culture.

In summary, a typical Type II S pneumococcus was successively transformed, through the intermediate stage of the R form, into a Type III S pneumococcus, a Type I S pneumococcus, and a Group
### TABLE II

*The Effect of the Temperature at Which a Type I S Vaccine is Heated upon Its Efficacy in Inducing Transformation of Type*

<table>
<thead>
<tr>
<th>Type and amount of vaccine</th>
<th>Temp. at which vaccine was heated for 15'</th>
<th>Amount of living R culture</th>
<th>Number of mice</th>
<th>Result</th>
<th>Pneumococcus recovered by culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria obtained from 100 cc. Type I S culture</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ditto</td>
<td>60°</td>
<td>Nil</td>
<td>4</td>
<td>All survived; sacrificed 8-10 days</td>
<td>All cultures negative</td>
</tr>
<tr>
<td>ditto</td>
<td>65°</td>
<td>0.25 cc. 2R</td>
<td>5</td>
<td>All died 1-2 days</td>
<td>Type I S</td>
</tr>
<tr>
<td>ditto</td>
<td>65°</td>
<td>Nil</td>
<td>2</td>
<td>Both survived; sacrificed 8-10 days</td>
<td>All cultures negative</td>
</tr>
<tr>
<td>ditto</td>
<td>70°</td>
<td>0.25 cc. 2R</td>
<td>5</td>
<td>All died 1-2 days</td>
<td>Type I S</td>
</tr>
<tr>
<td>ditto</td>
<td>70°</td>
<td>Nil</td>
<td>2</td>
<td>Both survived; sacrificed 8-10 days</td>
<td>All cultures negative</td>
</tr>
<tr>
<td>ditto</td>
<td>75°</td>
<td>0.25 cc. 2R</td>
<td>5</td>
<td>All died 1-2 days</td>
<td>Type I S</td>
</tr>
<tr>
<td>ditto</td>
<td>75°</td>
<td>Nil</td>
<td>2</td>
<td>Both survived; sacrificed 8-10 days</td>
<td>All cultures negative</td>
</tr>
<tr>
<td>ditto</td>
<td>80°</td>
<td>0.25 cc. 2R</td>
<td>5</td>
<td>All died 1-2 days</td>
<td>Type I S</td>
</tr>
<tr>
<td>ditto</td>
<td>80°</td>
<td>Nil</td>
<td>2</td>
<td>Both survived; sacrificed 8-10 days</td>
<td>All cultures negative</td>
</tr>
<tr>
<td>ditto</td>
<td>100°</td>
<td>0.25 cc. 2R</td>
<td>5</td>
<td>Three died 1-2 days</td>
<td>R only</td>
</tr>
<tr>
<td>ditto</td>
<td>100°</td>
<td>Nil</td>
<td>2</td>
<td>Two survived; sacrificed 8-10 days</td>
<td>All cultures negative</td>
</tr>
</tbody>
</table>

IV S pneumococcus. At any stage of the cycle the R form could be converted to the S form of that type from which it had last been derived by growth in anti-R serum.
The Effect of the Temperature at Which an S Vaccine Is Heated upon Its Efficacy in Causing an R Culture, Derived from a Heterologous S Type to Revert to the Type of the Vaccine

Griffith reported (2) that S vaccines, when heated at temperatures higher than 70°C., were rarely effective in causing R forms, derived from heterologous S types, to revert to the type of the vaccine. To verify this finding the following experiment was devised.

The bacteria obtained by centrifuging 4600 cc. of a Type I S culture were suspended in 23 cc. of plain broth. This suspension was divided into four equal portions of 3.5 cc. each, and two portions of 4.5 cc. each. (The larger portions were heated at the lower temperatures and a greater quantity of suspension was required for additional controls.) Each of these six portions of the same vaccine was heated for fifteen minutes at varying temperatures between 60°C. and 100°C., as outlined in the accompanying table. After heating, a volume of 2 cc. from each of the larger samples, and 1 cc. from each of the smaller samples was reserved for injection into control mice. To the remaining amounts of each portion of vaccine 1.25 cc. of a blood broth culture of a 2 R pneumococcus was then added. The quantities were so arranged that all mice, including the controls, received the heat-killed bacteria from 100 cc. of culture. In addition, each test animal received 0.25 cc. of living 2 R culture.

The results of the experiment appear in Table II.

Abstract of Protocol.—Type I S vaccine, heated for a period of 15' at temperatures between 60°C. and 80°C., when injected subcutaneously in white mice together with a living 2 R culture, apparently possesses the ability to cause the 2 R culture to revert to a Type I S pneumococcus. Type I S vaccine heated for 15' at 100°C. does not possess this property.

Attention should be called to certain features in this experiment. In the first place an unusually large number of transformations was obtained. Other experiments were not so uniformly successful. In another experiment a Type I S vaccine, heated for 15' at 70°C. caused a 2 R culture to revert to the type of the vaccine but did not do so when heated for 15' at 80°C. Again, in the experiment recorded above, the Type I S vaccine heated at 100°C. apparently had no effect on the 2 R culture. It did not even cause the R culture to revert to its original S type. In other experiments, however, a Type I S vaccine, heated for 15' at temperatures of 90° and 100°C., caused both 2 R and 3 R cultures to revert to their original S types. The results of such an experiment are reported in Table III.

2 More correctly this should read “derived from the S form of a heterologous type.” For the sake of brevity in this and succeeding instances the above expression has been adopted.
Abstract of Protocol.—In the experiment detailed in Table III Type I S vaccine, heated for 15' at 70°C., was effective in causing a 2 R culture to revert to a Type I S pneumococcus. When heated for the same period at a higher temperature than 70°C. Type I S vaccine was not effective in causing a 2 R culture to revert to the type of the vaccine. However, in several mice which received the Type I S vaccine heated at 80°, 90° and 100°C. the 2 R forms reverted to their original S type.

TABLE III
The Effect of Temperature at Which a Type I S Vaccine Is Heated upon Its Efficacy in Inducing Transformation of Type. (Second Experiment)

<table>
<thead>
<tr>
<th>Type and amount of vaccine</th>
<th>Temp, at which vaccine was heated for 15'</th>
<th>Amount of living R culture</th>
<th>Number of mice</th>
<th>Result</th>
<th>Pneumococci recovered by culture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacteria obtained from 80 cc. Type I S culture.</td>
<td>60°</td>
<td>Nil</td>
<td>4</td>
<td>All survived; sacrificed 7 days</td>
<td>All cultures negative</td>
</tr>
<tr>
<td>ditto</td>
<td>60°</td>
<td>0.25 cc. 2 R</td>
<td>4</td>
<td>All died 1-2 days</td>
<td>Type I S R only</td>
</tr>
<tr>
<td>ditto</td>
<td>70°</td>
<td>Nil</td>
<td>2</td>
<td>Both survived; sacrificed 7 days</td>
<td>Type I S R only</td>
</tr>
<tr>
<td>ditto</td>
<td>70°</td>
<td>0.25 cc. 2 R</td>
<td>4</td>
<td>All died 1-2 days</td>
<td>Type I S R only</td>
</tr>
<tr>
<td>ditto</td>
<td>80°</td>
<td>Nil</td>
<td>2</td>
<td>Both survived; sacrificed 7 days</td>
<td>Type I S R only</td>
</tr>
<tr>
<td>ditto</td>
<td>80°</td>
<td>0.25 cc. 2 R</td>
<td>4</td>
<td>3 died 1-2 days; 1 survived</td>
<td>Type II S R only</td>
</tr>
<tr>
<td>ditto</td>
<td>90°</td>
<td>Nil</td>
<td>2</td>
<td>Both survived; sacrificed 7 days</td>
<td>Type II S R only</td>
</tr>
<tr>
<td>ditto</td>
<td>90°</td>
<td>0.25 cc. 2 R</td>
<td>4</td>
<td>All died 1-2 days</td>
<td>Type II S R only</td>
</tr>
<tr>
<td>ditto</td>
<td>100°</td>
<td>Nil</td>
<td>2</td>
<td>Both survived; sacrificed 7 days</td>
<td>Type II S R only</td>
</tr>
<tr>
<td>ditto</td>
<td>100°</td>
<td>0.25 cc. 2 R</td>
<td>4</td>
<td>3 died 1-2 days; 1 survived</td>
<td>Type II S R only</td>
</tr>
</tbody>
</table>

Similar results were obtained when a 3 R culture was injected into mice together with a Type I S vaccine heated at various temperatures. From the majority of the animals which received the Type I S vaccine heated at temperatures up to 80°C. S organisms of the same type as that of the vaccine were obtained. From the animals which received the Type I S vaccine heated at higher temperatures than
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80°C. no Type I S organisms were recovered. Several of these mice, however, did yield typical Type III S pneumococci.

These findings may be summarized as follows:—Type I S vaccine, heated for 15' at various temperatures between 60° and 80°C. is effective in causing an R culture derived from a heterologous S type to revert to the type of the vaccine. When heated at temperatures higher than 80°C. Type I S vaccine does not cause an R culture derived from a heterologous S type to revert to the type of the vaccine; but frequently causes the R culture to revert to its original S type.

Experiments were then undertaken to determine whether vaccines prepared from S organisms other than Type I were subject to the same thermal differentiation as a Type I S vaccine.

### TABLE IV
The Effect of the Temperature at Which Types II S and III S Vaccines Are Heated upon Their Efficacy in Inducing Transformation of Type

<table>
<thead>
<tr>
<th>Type and amount of vaccine</th>
<th>Temp. at which vaccine was heated for 15'</th>
<th>Amount of living R culture</th>
<th>Number of mice</th>
<th>Result</th>
<th>Pneumococci recovered by culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria obtained from 90 cc. Type II S culture.</td>
<td>40°</td>
<td>0.25 cc.</td>
<td>6</td>
<td>3 died</td>
<td>Type II S</td>
</tr>
<tr>
<td>ditto</td>
<td>60°</td>
<td>0.25 cc.</td>
<td>8</td>
<td>All died</td>
<td>Type II S</td>
</tr>
<tr>
<td>ditto</td>
<td>100°</td>
<td>0.25 cc.</td>
<td>6</td>
<td>All survived</td>
<td>Type II S</td>
</tr>
<tr>
<td>ditto</td>
<td>100°</td>
<td>0.25 cc.</td>
<td>6</td>
<td>5 died</td>
<td>Type III S</td>
</tr>
<tr>
<td>ditto</td>
<td>100°</td>
<td>0.25 cc.</td>
<td>6</td>
<td>4 died</td>
<td>Type III S</td>
</tr>
<tr>
<td>ditto</td>
<td>100°</td>
<td>0.25 cc.</td>
<td>6</td>
<td>2 survived</td>
<td>Type III S</td>
</tr>
<tr>
<td>ditto</td>
<td>100°</td>
<td>0.25 cc.</td>
<td>6</td>
<td>3 died</td>
<td>Type II S</td>
</tr>
</tbody>
</table>

To revert to the type of the vaccine. When heated at temperatures higher than 80°C. Type I S vaccine does not cause an R culture derived from a heterologous S type to revert to the type of the vaccine; but frequently causes the R culture to revert to its original S type.
(In the previous paper it was shown that vaccines prepared from cultures of Types II S and III S pneumococcus, whether heated at 60°C. or 100°C., were equally effective in causing R organisms, derived from the same S type as that of the vaccine, to revert to their original S type. It was also shown that vaccines prepared by heating cultures of Type I S pneumococcus at 60°C. were effective in causing a 1 R culture to revert to its original S type. However, when a Type I S vaccine was heated at 100°C. this property was destroyed and reversion failed to occur.)

Vaccines were prepared by heating a culture of Type II S pneumococcus at temperatures of 60°C. and 100°C. Equal portions of each lot of vaccine were injected into two series of mice together with 1 R and 3 R cultures, respectively. Similarly, vaccines were prepared by heating a culture of Type III S pneumococcus at 60°C. and at 100°C. Two series of mice were injected with these vaccines together with 1 R and 2 R cultures, respectively.

The results of these experiments appear in Table IV.

From the foregoing results and from those obtained in previous experiments the following conclusions may be drawn:

1. Vaccines prepared by heating cultures of each of the three S types of pneumococcus at 60°C. are effective in causing R forms, derived from heterologous S types, to revert to the type of the vaccine.
2. Vaccines prepared by heating cultures of each of the three S types at 100°C. are not effective in causing R forms, derived from heterologous S types, to revert to the type of the vaccine.
3. Vaccines prepared by heating cultures of each of the three S types at 100°C. are frequently effective in causing 2 R and 3 R cultures to revert to the same specific type from which they were originally derived.
4. Vaccines prepared by heating cultures of any S type, including Type I, at 100°C. are not effective in causing a 1 R culture to revert to its original S type.

The Effect of the Duration of Heating upon the Efficacy of a Type I S Vaccine in Causing an R Culture, Derived from a Heterologous S Type, to Revert to the Type of the Vaccine

Griffith reported that vaccines heated for long periods, even at temperatures as low as 60°C., were less effective than vaccines heated for short periods in causing R forms, derived from heterologous S types, to revert to the type of the vaccine. To test the effect of heating an S vaccine for varying periods the following experiment was arranged.
The bacteria obtained by centrifuging 4000 cc. of a Type I S culture were taken up in 20 cc. of plain broth and heated for 15' at 60°C. A quantity of 8 cc. of suspension was withdrawn and used in the first part of the experiment. The remaining 12 cc. of suspension were heated for a further period of 15' at the same temperature. Three cc. were then withdrawn and a like amount at half-hour intervals thereafter on three occasions. Five cc. of the first 8 cc. sample withdrawn were used for control purposes, a volume of 0.5 cc. being injected subcutaneously into each of ten mice. To each of the five 3 cc. samples 1.5 cc. of a living 2 R culture was added. Six test mice were inoculated in each part of the experiment with the heat-killed bacteria from 100 cc. of Type I S culture, together with 0.25 cc. of living 2 R culture.

The details of the experiment are given in Table V.

Abstract of Protocol.—Type I S vaccine, heated for as long a period as two hours at 60°C., when injected subcutaneously in white mice together with a living 2 R culture, apparently possesses the ability to cause the 2 R forms to revert to Type I S organisms. A Type I S vaccine heated for 2 hours at 60°C. is just as effective in producing reversion to the type of the vaccine as that heated for 15' at 60°C.

### Table V

The Effect of the Duration of Heating upon the Efficacy of a Type I S Vaccine in Inducing Transformation of Type

<table>
<thead>
<tr>
<th>Type and amount of vaccine</th>
<th>Length of time during which vaccine was heated at 60°C.</th>
<th>Amount of living 2 R culture</th>
<th>Number of mice</th>
<th>Result</th>
<th>Pneumococci recovered by culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria obtained from 100 cc. Type I S culture.</td>
<td>15'</td>
<td>Nil</td>
<td>10</td>
<td>All survived; sacrificed at intervals up to 17 days</td>
<td>All cultures negative</td>
</tr>
<tr>
<td>ditto</td>
<td>15'</td>
<td>2 R</td>
<td>6</td>
<td>5 died</td>
<td>Type I S</td>
</tr>
<tr>
<td>ditto</td>
<td>30'</td>
<td>2 R</td>
<td>6</td>
<td>4 died</td>
<td>Type I S</td>
</tr>
<tr>
<td>ditto</td>
<td>1 hour</td>
<td>2 R</td>
<td>6</td>
<td>5 died</td>
<td>Type I S</td>
</tr>
<tr>
<td>ditto</td>
<td>1½ hours</td>
<td>2 R</td>
<td>6</td>
<td>4 died</td>
<td>Type I S</td>
</tr>
<tr>
<td>ditto</td>
<td>2 hours</td>
<td>2 R</td>
<td>6</td>
<td>5 died</td>
<td>Type I S</td>
</tr>
</tbody>
</table>
Mention should again be made of the fact that this also was an unusually successful lot of vaccine. In other experiments a smaller number of positive results was obtained and there appeared to be a slight falling off in the effectiveness of the vaccine when heated for prolonged periods at 60°C.

The Amount of Heat Killed S Organisms Necessary to Cause R Forms Derived from a Heterologous S Type, to Revert to the Type of the Vaccine

The minimal amount of vaccine capable of inducing transformation of type was then ascertained. An experiment was devised in which

### TABLE VI

<table>
<thead>
<tr>
<th>Amount of culture from which Type I S vaccine was prepared</th>
<th>Amount of living R culture</th>
<th>Number of mice</th>
<th>Result</th>
<th>Pneumococci recovered on culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 cc.</td>
<td>0.25 cc.</td>
<td>4</td>
<td>4 died</td>
<td>Type I S</td>
</tr>
<tr>
<td></td>
<td>2 R</td>
<td></td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>50 cc.</td>
<td>0.25 cc.</td>
<td>4</td>
<td>3 died</td>
<td>Type I S</td>
</tr>
<tr>
<td></td>
<td>2 R</td>
<td></td>
<td>1 survived</td>
<td>3</td>
</tr>
<tr>
<td>25 cc.</td>
<td>0.25 cc.</td>
<td>4</td>
<td>2 died</td>
<td>Type I S</td>
</tr>
<tr>
<td></td>
<td>2 R</td>
<td></td>
<td>2 survived</td>
<td>2</td>
</tr>
<tr>
<td>10 cc.</td>
<td>0.25 cc.</td>
<td>4</td>
<td>All survived</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>2 R</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 cc.</td>
<td>Nil</td>
<td>4</td>
<td>All survived; sacrificed 7 days.</td>
<td>All cultures negative.</td>
</tr>
</tbody>
</table>

four series of mice received a living 2 R culture together with varying amounts of Type I S vaccine. In addition to the living R culture the first series of animals received the heat-killed bacteria from 100 cc. of S culture; the second from 50 cc.; the third from 25 cc. and the fourth from 10 cc. Four control mice received only the heat-killed bacteria from 100 cc. of S culture.

The results of this experiment appear in Table VI.

**Abstract of Protocol.**—From the heart's blood of all 4 mice which received the living 2 R culture together with the heat-killed bacteria from 100 cc. of Type I S culture, S organisms of the same type as the vaccine were obtained. Similarly
Type I S organisms were recovered from 3 out of 4 mice which received the heat-killed bacteria from 50 cc. and from 2 out of 4 which received the bacteria from 25 cc. From those which received the bacteria from 10 cc. no Type I S organisms were obtained. The control mice which were injected with the heat-killed bacteria from 100 cc. of Type I culture all survived. They were sacrificed at the end of 7 days and cultures from the blood and viscera were negative.

In many other experiments it has constantly been found that large amounts of vaccine are necessary to effect transformation of type by this procedure.

The Effect of Autolysis on the Efficacy of an S Vaccine in Causing R Forms, Derived from Heterologous S Types, to Revert to the Type of the Vaccine

Early in the work it was found that many lots of vaccine were relatively ineffective in causing R forms to revert to the Type of the vaccine. In searching for an explanation of these failures it was noticed that many mice in such unsuccessful experiments developed purpura to a marked degree. Julianelle and Reimann (5) have shown that the purpura-producing fraction of pneumococcus is released only during autolysis and is not present in heat-killed cultures in which autolysis has not taken place. This fact suggested a possible explanation for the ineffectiveness of certain lots of vaccine. It seemed possible that the cultures might have undergone partial autolysis before being heat-killed and, as a consequence, the vaccines made from such cultures were no longer effective in producing reversion. To test this hypothesis the following experiment was done:

Cultures of Type I S and Type II S organisms were centrifuged and the deposit divided into two equal portions. One-half of the deposit from each culture was immediately heated at 60°C. for 15': the other half was allowed to autolyze at 37°C. for 48 hours. At the end of this period the autolysate was subjected to a temperature of 60°C. for 15'. The two preparations were injected into a series of mice together with cultures of heterologous R organisms as detailed in Table VII.

Abstract of Protocol.—Autolysates of S cultures, when injected subcutaneously in white mice together with living R cultures, are not effective in causing R forms to revert, either to their original S type, or to the S type from which the autolysate was prepared. (Heat-killed suspensions of S organisms, however, kept in the ice-box for periods up to three weeks, have been found to be effective in causing R forms derived from heterologous S types, to revert to the type of the vaccine.)
This experiment offers evidence that it is not the specific soluble substance as such that is responsible for transformation of type in these procedures. The specific soluble substance of pneumococcus is not altered during autolysis and is present both in the autolysate and in the heat-killed suspension (6).

**TABLE VII**

*The Effect of Autolysis on the Efficacy of an S Vaccine in Inducing Transformation of Type*

<table>
<thead>
<tr>
<th>Type and amount of vaccine</th>
<th>Type and amount of autolysate</th>
<th>Amount of living R culture</th>
<th>Number of mice</th>
<th>Result</th>
<th>Pneumococci recovered on culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria obtained from 80 cc. Type I S culture heated for 15' at 60°C.</td>
<td>Nil</td>
<td>0.25 cc.</td>
<td>2 R</td>
<td>4</td>
<td>4 died</td>
</tr>
<tr>
<td>ditto</td>
<td>Nil</td>
<td>0.25 cc.</td>
<td>3 R</td>
<td>4</td>
<td>3 died</td>
</tr>
<tr>
<td>Nil</td>
<td>0.25 cc.</td>
<td>2 R</td>
<td>4</td>
<td>All survived; sacrificed 7 days.</td>
<td>All cultures negative</td>
</tr>
<tr>
<td>Bacteria obtained from 80 cc. Type I S culture allowed to autolyze for 48 hrs. at 37°C.</td>
<td>Nil</td>
<td>0.25 cc.</td>
<td>3 R</td>
<td>4</td>
<td>All survived; sacrificed 7 days.</td>
</tr>
</tbody>
</table>

**The Effect of Injecting S Cultures Killed by Other Agents than Heat Together with R Cultures Derived from Heterologous S Types**

In the experiments reported up to this point the S cultures were invariably killed by heat.

In order to determine whether or not S organisms killed by other agents than heat possessed similar properties, cultures of S pneumococci were killed with the following substances:—formalin, iodine,
chloroform, tricresol, alcohol and acetone. None of the reagents selected destroy the specific soluble substance of pneumococcus (7). The smallest possible concentrations were employed to eliminate the toxic effect of the reagents themselves.

Six series of five mice each were injected with a living 2 R culture together with the bacteria from 100 cc. of Type I S culture which had been killed by adding minimal amounts of each of the above mentioned bactericidal substances. Reversion to the type of the vaccine did not occur in a single instance. At the same time five animals were injected with the bacteria from 100 cc. of the same Type I S culture, heated for 15' at 60°C. together with the same living 2 R culture. Type I S organisms were recovered from the heart’s blood in each instance. Four control mice which received only the heat-killed vaccine survived.

The failure to induce reversion by the use of suspensions of S organisms killed by other agents than heat suggests one of two possibilities; either that portion of the vaccine responsible for reversion may have been destroyed by the bactericidal substances; or, the toxic effect of the reagents may have created unfavorable conditions in the tissues of the animals.

Results Obtained by the Intraperitoneal Injection of Small Amounts of Living R Forms Together with the Heat-Killed Bacteria from Large Amounts of Heterologous S Cultures

In all the preceding experiments living R forms and S vaccines were injected subcutaneously in white mice. Experiments were undertaken to determine whether similar transformations could be effected by the intraperitoneal injection of living R forms together with vaccines of S cultures. It was hoped that it would be possible during this procedure to follow the transformation process by withdrawing and examining portions of the peritoneal contents from time to time. The results were not uniformly satisfactory. In one experiment eight mice were injected intraperitoneally with a living 2 R culture together with a Type III S vaccine. Four of the animals died and Type III S organisms were recovered from the heart’s blood. However, these organisms were only slightly agglutinable in Type III serum and the colonies were not of the large, typical Type III S variety. Typical Type III organisms were obtained by passing these cultures through second
mice. The first cultures were apparently composed of incompletely
developed Type III S forms.

In another experiment varying amounts of a living 2 R culture were
injected intraperitoneally together with large quantities of a Type I S
vaccine. In no case did the R form revert to the type of the vaccine.
It was therefore concluded that, while reversion to the type of the
vaccine could be effected intraperitoneally, the subcutaneous route was
the method of choice.

Attempts to Determine the Minimal Time Required to Effect Reversion of
R Forms into Organisms of Heterologous S Type

In different experiments there was a great variation in the interval
required to effect reversion. The shortest time in which an animal
succumbed, yielding S organisms of the same type as that of the
vaccine, was eighteen hours; the longest nine days. In some experi-
ments virulent S organisms were recovered at the site of injection
from apparently healthy animals which were sacrificed at the end of
seven days. The finding of virulent bacteria in local abscesses in
otherwise healthy animals after such an interval suggested that the
mice had acquired considerable general immunity before the R forms
had had the opportunity to develop into the S form. In the most suc-
cessful experiments the usual time at which the animals succumbed
was from one and one-half to two days after injection. However, if,
in this period, S forms had developed in sufficient numbers to over-
whelm the animal the actual time necessary for the reversion process
to occur at the site of injection was probably much shorter. An at-
ttempt was made to determine the minimal time required in the
following way.

A series of mice was injected subcutaneously with an S vaccine. Thereafter,
at intervals of two, four, eight, twelve, and twenty-four hours living R forms were
introduced into the same animals at the same location. Reversion to the type of
the vaccine occurred in a moderate number of animals which received the living
R forms as late as eight hours after the injection of the vaccine. Reversion did
not occur in any animal which received the living culture after an interval longer
than eight hours. However, only a small number of animals was employed in this
experiment and for this reason the results cannot be considered conclusive. The
procedure of introducing the living R forms simultaneously with the vaccines
uniformly gave a higher proportion of positive results.
At&mits to Effect Reversion by the Injection of Living R Forms and S Vaccines in Different Locations in the Same Animals

Various ways in which S vaccines might act in causing reversion of R forms in the animal body were considered. One of the possibilities was that the general conditions created in the animal by the injection of such large amounts of vaccine might be suitable for the development of organisms of the S variety. If the reversion process depended upon the existence of such general conditions it might be possible to produce a comparable state by the injection of S vaccines and living R forms into different locations in the same animals. Six mice were injected with a Type I S vaccine in one inguinal region. Simultaneously a living 2 R culture was injected into the opposite inguinal region. One of the six mice died in two days and organisms of the same type as the vaccine were recovered from the heart's blood. The other five mice survived. However, such experiments are not conclusive for it is always possible that no matter where the living R forms are injected some organisms may find their way to the site of the vaccine. Because of this difficulty no further experiments were attempted along these lines.

Attempts to Effect Reversion by the Injection of Living R Forms of Pneumococcus Together with S Vaccines of Friedländer's Bacillus

It has been demonstrated in this laboratory that Type II pneumococcus and Type B Friedländer's bacillus elaborate specific soluble substances which are chemically and immunologically similar, although not identical (8). Experiments were therefore undertaken to determine whether it was possible to convert R pneumococci, derived from other S types than Type II, into Type II S organisms, by the use of a Friedländer Type B vaccine. R forms of pneumococci, derived from both Type I S and Type III S organisms, were injected into a series of mice together with a Type B Friedländer vaccine. The experiments were accompanied by certain difficulties, for it was found that the primary toxicity of S Friedländer vaccine was considerable.

In the first experiment a series of four mice was injected with the bacteria from 80 cc. of Type B Friedländer culture along with a living culture of 3 R pneumococcus. A similar number of animals was injected with the same Friedländer
vaccine and a culture of 1 R pneumococci. All the animals died after an interval of less than twenty-four hours but only R forms of pneumococci were recovered from the heart's blood.

The converse of the above experiment was then done.

R forms of Friedländer bacilli were injected into mice along with the heat-killed bacteria from a Type II S pneumococcus culture. R forms derived from each of the three specific types A, B, C, and from a strain of the heterogeneous group X were employed. Four mice were injected in each experiment. Two animals died after a period of twelve hours; the remainder survived and were sacrificed after a period of six days. Cultures made from the site of injection and from the heart's blood did not yield S Friedländer bacilli in a single instance. R forms were found at the site of injection in a large proportion of cases. Cultures of the recovered R forms were passed through a second series of mice and the virulence of the organisms was found to remain unchanged. Unfortunately many of the mice in this experiment developed ulcers at the place of injection. It is possible that this fact may have had some effect in determining the results obtained. It is also possible that the particular R strains of Friedländer's bacillus which were selected were not suitable for reversion.

Attempts to Convert R Forms of Pneumococci into Organisms of the Heterologous S Type by in Vitro Methods

The in vitro methods which were employed in attempts to convert R forms into organisms of the homologous S type, by the use of vaccines, have been described in a preceding paper (1). All the procedures adopted gave negative results. Similar attempts were made to convert R forms into S organisms of heterologous types. All such experiments were unsuccessful. It must therefore be concluded that, either the in vitro conditions, as provided, were inadequate, or that living tissues are essential for the reversion process. An attempt to partially reproduce in vivo conditions in the test tube was made in the following way.

Large quantities of a Type I S vaccine were injected intraperitoneally into five mice. The animals were sacrificed at intervals of 2, 4, 8, 12 and 24 hours and the peritoneal contents washed out with plain broth. The washings were transferred to test tubes and seeded with a 2 R culture. Plates made from the resulting growth, however, yielded only R colonies and the cultures remained avirulent for white mice.
The negative results of this experiment suggest that living tissues may play a part in the reversion process. However, it should be pointed out that in previous experiments the intraperitoneal route was not found as suitable as the subcutaneous route. The possibility remains that if the conditions obtaining in the subcutaneous tissues of the mouse could be reproduced in the test tube transformation of type might be effected in vitro.

Attempts to Convert S Pneumococci of One Specific Type Directly into S Organisms of Another Specific Type

In all the experiments described in which pneumococci have been converted from one specific S type into other specific S types the transformation has been effected through the intermediate stage of the R form. Inasmuch as the phenomenon of transformation of type had never been observed in type-specific S cultures under artificial cultivation it seemed most unlikely that S organisms could be transformed directly from one specific type to another specific type. An attempt to effect such a direct transformation of type was made in the following manner. Mice were injected subcutaneously with the smallest possible dilutions of living S cultures together with the heat-killed bacteria from large amounts of heterologous S cultures. For example a Type II S culture, in dilutions of $10^{-7}$, $10^{-8}$, and $10^{-9}$ cc. was injected into a series of mice together with a Type I S vaccine. In all cases the animals succumbed and only S organisms of the same type as those introduced in the living cultures were recovered from the heart's blood. Direct transformation from one type to another did not occur in a single instance. These experiments also prove that large quantities of vaccine have no inhibitory effect on any viable forms introduced with the vaccine. On the contrary the animals that received both the living culture and the vaccine succumbed in a much shorter period of time than those which received only the dilutions of living culture.

DISCUSSION

The transformation of pneumococci from one specific type into other specific types is a phenomenon of wide bacteriological and epidemiological significance. It has not been conclusively demonstrated
that transformation of type actually occurs under natural conditions. Griffith (2) has presented certain evidence which indicates the possibility of such an occurrence during disease processes, but further work is required to establish definitely the validity of these observations. In any case the demonstration that transformation of type may be effected experimentally shows that the various types of pneumococcus are closely related biologically. Indeed it is possible to think that these various types may represent attempts on the part of the organism to adapt itself to varying environmental conditions.

It is important to note that it was found impossible to transform S organisms directly from one specific type into other specific types. Change of type was invariably brought about through the intermediate stage of the R form. The R form of the organism is most readily produced by growing S organisms in their homologous immune serum. It may also be produced by subjecting S pneumococci to unfavorable environmental conditions,—such as, growth in poor media, growth at temperatures between 40° and 42°C., and growth in media containing small traces of bile. The R form, therefore, probably results from attempts of S bacteria to adapt themselves to unfavorable environmental conditions. Once reduced to the R state the organisms potentially have the capacity to develop the S structure of any of the various specific S types. They most readily assume the characteristics of that S type from which they were last derived; but under the influence of certain conditions they may also develop the S structure of other specific types.

What conditions determine the development of S characteristics? The change may be induced experimentally by subcutaneously injecting, in white mice, large amounts of an S vaccine together with living R forms. The type of S structure which the R forms assume under these conditions is apparently dictated by two circumstances; (1) The degree of “degradation” to which the R forms have been subjected; (2) The degree of heat to which the S vaccine has been exposed. If the R forms have been reduced to a definite state in the “degradation” process they assume the characteristics of the same S type as the vaccine. If the R forms have been only partially “degraded” they assume the characteristics of that S type from which they were originally derived. Similarly, if the vaccine is heated at a tempera-
ure between 60° and 80° C. the R forms revert to the type of the vaccine: if the vaccine is heated at a temperature higher than 80°C, the R forms revert to the S type from which they were originally derived.

What are the causes responsible for transformation of type as induced by this procedure? In the previous paper various possibilities were considered to explain the way in which S vaccines might act in causing R forms, derived from the same S type as the vaccine, to revert to their original S type. It was pointed out that the precise factor responsible for reversion, as brought about by this procedure, was not understood. If the causes determining reversion of R forms to their original S type are not understood it is even more difficult to interpret the conditions under which R forms assume the characteristics of S organisms of the same type as the vaccine. That property of the vaccine responsible for reversion does not exactly correspond with any known substance or property of S organisms. It cannot be the S substance itself, for it has been shown that the carbohydrate fraction of pneumococcus is not altered by heating at 100°C. (9), and its specificity is not destroyed during autolysis. Moreover, the efficacy of an S vaccine in inducing transformation of type does not parallel the antigenic properties of the vaccine. It has been shown that vaccines of S pneumococci are equally good antigens whether heated at 60°C or at 100°C.

It is possible that S vaccines may exert their effect in one of two ways:—(1) Directly on the R forms themselves; (2) On the tissues of the animals in which they are injected.

The failure of all in vitro attempts to secure transformation of type suggests that, if the vaccine exerts its influence directly on the living R forms, it does so only under very precise conditions. If it were possible to reproduce in the test tube the conditions obtaining in the subcutaneous tissues of the mouse, transformation of type might be effected in vitro. However, it would be difficult to duplicate experimentally the conditions created by the disintegration and digestion of large amounts of vaccine in the living tissues of an animal.

A second possibility is that the conditions created in the subcutaneous tissues of the mouse offer a suitable environment in which the R forms may build up their S structure. In the previous paper it was pointed out that, under natural conditions, the white mouse "possessed
some capacity to overcome infection by organisms producing minimal amounts of S substance.” It was further suggested that “the injection of S vaccines might destroy or inhibit this limited ability of the mouse and under such conditions the R forms might develop into S organisms.” May it not be possible that the injection of an S vaccine only inhibits or destroys the capacity of the mouse to overcome infection by that particular S type? Under such circumstances may not the R organism, potentially capable of synthesizing any type of polysaccharide, be able to elaborate that particular S substance most suitable for the survival of the organism in its environment?

In this connection reference is again made to the work of Sia (10). Employing serum-leucocyte mixtures in a specially constructed apparatus, he reported the following observation.

“...The presence of a small amount of the purified soluble substance of the homologous type markedly altered the conditions in the mixtures so that even a small number of avirulent pneumococci were enabled to grow in the serum and leucocytes of animals which ordinarily possess the power to destroy such pneumococci in relatively large numbers.” Sia further reported that this effect was highly type-specific for “a Type II substance assisted the growth of only pneumococcus Type II; likewise a Type III substance, the growth of pneumococcus Type III only.”

Any such explanation, however, fails to account for the different effects produced by vaccines heated at temperatures above and below 80°C. Further work is therefore required to understand clearly the causes responsible for transformation of type as induced by Griffith’s technique.

In the previous paper it was pointed out that R forms of pneumococcus could be found in the flora of the upper respiratory tract of many normal individuals. It was suggested that these forms resulted from attempts of the bacteria to adapt themselves to unfavorable environmental conditions. Although degraded to the R form these organisms still retained the capacity of again developing into virulent, type-specific, S pneumococci. Any such development would appear to be dictated by conditions in the environment. Those environmental conditions would also determine the particular S type which the R organisms may assume. In the absence of more precise data concerning such transformations further speculation is unprofitable. However, the possibilities of alteration in type under natural and
disease conditions cannot be ignored and may attain proportions of much significance in infectious and epidemiological problems.

CONCLUSIONS

1. Type-specific S pneumococci may be transformed from one specific S type into other specific S types through the intermediate stage of the R form.

2. R forms of pneumococci, derived from any specific S type, may be transformed into S organisms of other specific types by the following procedure:—The subcutaneous injection, in white mice, of small amounts of living R forms together with vaccines of heterologous S cultures.

   (i) S vaccines heated for 15' at temperatures between 60° and 80°C., are effective in causing R forms, derived from heterologous S types, to revert to the type of the vaccine.

   (ii) S vaccines heated for 15' at temperatures between 80° and 100°C., are not effective in causing R forms, derived from heterologous S types, to revert to the type of the vaccine.

   (iii) S vaccines heated for 15' at temperatures between 80° and 100°C., may cause 2 R and 3 R cultures to revert to their original S type.

   (iv) S vaccines of any type, including Type I, heated for 15' at temperatures between 80° and 100°C., are not effective in causing 1 R cultures to revert to their original S type.

   (v) S vaccines heated for periods as long as two hours at 60°C. are effective in causing R forms, derived from heterologous types, to revert to the type of the vaccine.

3. A single cell R strain, derived from a Type II S pneumococcus, has been successively transformed into a Type III S, a Type I S and a Group IV S culture.

4. Corresponding with the various degrees of "degradation" of the R form there are varying degrees of "development" of the S form.

5. The nature of the conditions responsible for alteration of type as induced by these procedures has been investigated and the causes responsible for the transformations are discussed.

6. All attempts to produce transformation of type in vitro have been unsuccessful.
7. The rôle which the phenomenon of transformation of type may play in problems of infection and epidemiology is indicated.

BIBLIOGRAPHY