# STUDIES ON STREPTOCOCCUS BACTERIOPHAGE.

## II. The Influence of Lytic Principles upon the Agglutination of Hemolytic Streptococci.

By GREGORY SHWARTZMAN, M.D.

*(From the Laboratories of the Mount Sinai Hospital, New York.)*

(Received for publication, July 14, 1927.)

### INTRODUCTION.

Bacteriophage is capable of inducing considerable disturbance in susceptible bacterial cells. Especially interesting is the influence of this principle upon the agglutinative properties of bacteria. The studies on the subject reported by d'Hérelle (1), Bordet and Ciuca (2), Gratia (3), Flu (4), McKinley (5) and others were limited to the *coli*-typhoid-dysentery group of bacteria. The observed changes consisted of loss of agglutinability by specific sera with and without loss of agglutinogenic and agglutinin-absorbing properties. In one instance, as reported by Gratia (3), the effect of the lytic principle consisted in appearance of agglutinability in a previously inagglutinable strain of *B. coli*.

The author's object was to extend these studies to other bacteria in which the bacteriophage phenomenon was shown to exist. The hemolytic pathogenic streptococci were chosen for this work for the following reasons:

1. It was shown by Dutton (6) that many strains of human pathogenic streptococci existed symbiotically with phage of low potency. Two strains of human origin were shown to undergo lysis (Piorkowski (7) and Dutton (6)). The author demonstrated the classical bacteriophage phenomenon among several strains of erysipelas origin (8). Since, therefore, it can be safely accepted that the bacteriophage phenomenon exists among streptococci, it seemed possible that there might exist a definite influence of phage upon the phenomenon of agglutination of these microorganisms.

2. The complexity of the problems of serological specificity and affiliations of different groups of streptococci makes it important to determine the influence of the phage which represents a powerful factor in the life of many species of bacteria, including streptococci.

The plan of this work was influenced by the following considerations:

Since the effect of the phage upon agglutination of streptococci is entirely unknown it was decided to study the effect of the phage upon the entire strain. Later should a definite relation be established, these studies could be extended to strain components derived from single colonies or single cells.

In view of the fact that various strains of pathogenic streptococci differ in the degree of susceptibility to the phage it was presumed that the changes induced would vary as well. For this reason a large number of strains was employed.

Since various groups of hemolytic streptococci differ in the complexity of the antigen it was also thought that the same agent would induce different changes, the nature of which might depend upon the antigenic structure. This consideration led to the study of representatives of various serological groups of streptococci.

Moreover, since lytic principles from various sources differ in their influence upon streptococci (Shwartzman (8)) it was decided to limit the work to the study of one or two lytic principles and employ these throughout the work.

In order to be able to obtain a full conception of the various possible changes which this principle may induce in streptococci under the conditions outlined above, the studies were made first by means of direct *agglutinations*. The information gained was further substantiated by studies on *the agglutinin-absorbing and agglutinogenic properties* of each culture under discussion. To study the effect of phage upon the affiliations between various streptococci each serum was tested against the majority of strains on hand.

## *Methods.*

*Lytic Principles.*—SL bacteriophage, active against a streptococcus from rabbit's lung and also potentially active against streptococci of erysipelas origin; the C/2, anti-*B. coli* phage were used in these experiments. Cultures of various streptococci were grown in phosphate broth containing 1 to 10 dilution of lytic

principle for a number of generations. These cultures will be referred to as SL and C/2 respectively. *The strains* employed in this work were as follows:

1. The erysipelas group, represented by ten strains kindly sent to me by Dr. Konrad Birkhaug under the name $E_1$-$E_{10}$. All these strains were potentially susceptible to SL lytic principle only.

2. Scarlet fever strains (55, 108), sent to me by Dr. A. W. Williams, which were found resistant to both lytic principles.

3. A strain of *pyogenes* hemolytic streptococcus, isolated in this laboratory from a case of meningitis and called H.S. This was resistant to both principles.

4. A strain of green-producing streptococcus, isolated from the blood of a case of subacute bacterial endocarditis, under the name V. This was also resistant to the lytic principles employed.

5. Rb streptococcus, a pyogenic hemolytic strain isolated by Clark and Clark from rabbit lung infection. This streptococcus was highly susceptible to SL bacteriophage and resistant to C/2 lytic principle.

*Immunization.*—Two rabbits were immunized with each strain and the serum of higher titer was selected for the work. The method of immunization was that recommended by Dochez (9).

*Agglutination.*—Homogenous antigens were obtained by growing streptococci in 0.1 per cent glucose phosphate broth. Phage strains were passed through phosphate broth containing 1 to 10 dilution of lytic principle. Both normal and phage cultures were subcultured into phosphate broth and used for agglutination after 24 hours incubation. A considerable number of experiments was first performed with the washed sediment of these cultures. The process of centrifuging, however, brought about frequent spontaneous agglutination of previously homogenous antigens. This procedure was then given up. Care was always taken to use cultures not older than 24 hours. Parallel experiments did not show any difference in agglutination of washed and unwashed *24 hour* old cultures. No formaldehyde was added to the antigens. Serum dilutions were made in phosphate broth. Readings were made after 4 hours incubation in a water bath at 55°. The agglutinations registered in the protocols were examined by the naked eye.

*Absorption of Agglutinins.*—Bacterial cells for absorptions of agglutinins were prepared by centrifuging and washing the growth of 24 hour old broth cultures. A dose sufficient to absorb completely agglutinins from the homologous serum was used. In making comparative studies on the agglutinin-absorbing power of various antigens the quantitative relationship of absorbing cells to the amount of serum (usually diluted 1 to 10) was kept in mind. The mixture of given amounts of cells (heated previously to 60°) and serum was kept in the water bath at 37° for 2 hours and in the refrigerator overnight. In order to titrate the absorbed sera, dilutions from 1 to 20 up to 1 to 25,000 were always made. Serum diluted 1 to 10 but unabsorbed, to be used for control titration, was kept under the same temperature conditions as the serum for absorption.

The results reported below were grouped according to the changes produced by given lytic principles in the various strains of pathogenic streptococci.

## EXPERIMENTAL.

### *I. Inagglutinability and Partial Loss of Agglutinogenic and Agglutinin-Absorbing Properties of "Whole" Cultures.*[1]

Strain 55 which represented serologically a large group of scarlet fever streptococci was cultured for forty passages in phosphate broth and in phosphate broth containing SL phage. The effect of the phage is shown in Table I.

TABLE I.

*The Influence of Phage upon Agglutination of Strain 55.*

| Sera | Absorbed by strain | Agglutination titer before absorption and percentage of absorption | |
|---|---|---|---|
| | | 55 | 55 SL |
| I | = | 6400 | 0 |
| I | 55 | 100 per cent | — |
| I | 55 SL | 50 per cent | — |
| II | = | 400 | 0 |
| III | = | 800 | 0 |

0, no agglutination; = no absorption; — not tested.

I, 55 strain serum; II, 55 SL strain serum; III, 55 SL strain serum.

As is seen the phage strain was rendered completely inagglutinable by normal culture serum. This change was coincident with partial loss of agglutinin-absorbing properties and also with what appeared a certain loss in the agglutinogenic power. In fact the immunization of two animals with the phage cultures resulted in a distinctly lower titer of agglutinins. These sera agglutinated the normal culture but did not react with the homologous culture.

Similar but somewhat more marked changes were obtained in Rb streptococcus.

[1] The change demonstrated in this work occurred in entire cultures. It remains yet to determine whether this was due to actual changes in the cells bearing the agglutinogens or to simple elimination of these cells by means of the lytic agent.

This streptococcus highly susceptible to SL phage was made resistant to this principle by means of several passages through broth containing at first $1 \times 10^{-6}$ cc. and later $1 \times 10^{-1}$ cc. dilution of the phage. Observations on cross-agglutinations and cross-absorptions of sera prepared by immunization with normal Rb streptococcus, the resistant type and lysed culture of the same strain are given in Table II. As is seen, Rb streptococcus was considerably affected by passage through SL phage. The strain became inagglutinable by the normal culture serum as well as by heterologous sera which normally cross-

TABLE II.

*The Influence of Phage upon Agglutination of Rb Streptococcus.*

| Sera | Absorbed by strain | Agglutination titer before absorption and percentage of absorption | | | |
|---|---|---|---|---|---|
| | | Rb streptococcus | Resistant Rb strepto-coccus | 55 | 130 |
| IV | = | 6400 | 0 | 400 | 40 |
| IV | Rb streptococcus | 100 per cent | — | 0 per cent | 0 per cent |
| IV | Resistant Rb. streptococcus | 0 per cent | — | — | — |
| V | = | 50 | 0 | 0 | 0 |
| VI | = | 0 | 0 | 0 | 0 |
| VII | = | 0 | 0 | 0 | 0 |
| VIII | = | 0 | 0 | 0 | 0 |
| IX | = | 0 | 0 | 0 | 0 |
| Ia | = | 200 | 0 | 5120 | 400 |
| X | = | 200 | 0 | — | 3200 |

IV, Rb strain serum; V, VI, VII, lysed culture Rb strain serum; VIII, IX, resistant Rb strain sera; X, 130 strain serum (55 and 130 belong to the scarlet fever group); Ia, 55 strain serum.

agglutinated with this strain. The inagglutinability was coincident with considerably more marked changes of the antigen than those shown in the previous experiments. There was a very significant loss of agglutinin-absorbing power on the part of the resistant type and also what appeared as an entire loss of agglutinogenic properties in both lysed and resistant cultures. Five animals failed to respond to repeated immunization with these antigens. Additional attempts to immunize with such a strain are necessary before complete loss of the agglutinogenic properties can be safely accepted.

It should be pointed out that the group components of this strain underwent similar changes. In contrast to the normal Rb culture the resistant streptococcus failed to agglutinate with Strains 55 and 130 and also failed to stimulate the production of group agglutinins for the two strains.

### *II. Inagglutinability with Complete Preservation of Agglutinogenic and Agglutinin-Absorbing Properties. Appearance of Additional Components.*

Examples of partial modification of antigens under the influence of phage were afforded by representatives of various groups of streptococci:

*A. The Erysipelas Group of Streptococci.*—Table III represents the results of direct agglutinations of normal and phage cultures of erysipelas strains. As is shown by Birkhaug (10), Dochez (9) and Tunnicliff (11) this group of streptococci possesses a specific antigen. In this work the serum for one of these strains agglutinated to a high titer several representatives of this group. The bacteriophage had a definite effect upon these strains. SL lytic principle, potentially active against these strains, lowered the agglutinability of eight strains out of ten tested. This change, however, did not alter the specific component of the strains since the agglutinin-absorbing power of all the phage cultures remained unaffected. The specific agglutinins of Serum XI were completely absorbed by these cultures (not recorded in the tables). Moreover, Serum XII agglutinated the normal strains to a high titer, as is seen from Table III. However, another component was rendered prominent in the phage cultures, as evidenced by the following observation. The phage culture serum (XII) agglutinated all the phage cultures to a higher titer than the normal cultures when tested soon after bleeding (not given in Table III). Since these strains agglutinated to a lower titer with the normal culture serum it suggested that another component was rendered prominent in the phage cultures.

This assumption was further strengthened by another observation. As is seen from Table III, when sterile, non-preserved Serum XII was retested 3 weeks after bleeding, the phage cultures this time agglutinated to a lower titer than the normal cultures. A new phage culture serum (XIII) was then prepared and a part of it was added with

TABLE III.

*The Influence of Phage upon Agglutination of Erysipelas Streptococci.*

*Agglutination Titer.*

| Sera | $E_1$ | SL $E_1$ | $E_2$ | SL $E_2$ | $E_3$ | SL $E_3$ | $E_4$ | SL $E_4$ | $E_5$ | SL $E_5$ | $E_6$ | SL $E_6$ | $E_7$ | SL $E_7$ | $E_8$ | SL $E_8$ | $E_9$ | SL $E_9$ | $E_{10}$ | SL $E_{10}$ | 55 | SL 55 | 108 | SL 108 | Rb |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| XI | 6400 | 200 | 12800 | 800 | 3200 | 0 | 6400 | 1600 | 800 | 800 | 12800 | 1600 | 3200 | 0 | 6400 | 400 | 12800 | 1600 | 25600 | 25600 | 0 | 0 | 0 | 0 | 0 |
| Sterile non-preserved; tested 3 wks. after bleeding XII | 1600 | 200 | 1600 | 400 | 1600 | 400 | 3200 | 200 | 800 | 0 | 1600 | 400 | 1600 | 0 | 1600 | 0 | 1600 | 0 | 3200 | 3200 | 0 | 0 | 0 | 0 | 0 |
| 3 wks. after bleeding; phenolized XII | 6400 | 12800 | 6400 | 12800 | 3200 | 6400 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| 2 wks. after bleeding; phenolized XIII | 800 | 1600 | 800 | 6400 | 1600 | 6400 | 800 | 3200 | 800 | 800 | 800 | 3200 | 200 | 1600 | 800 | 800 | 400 | 3200 | 400 | 400 | — | — | — | — | — |
| Sterile non-preserved immediately after bleeding XIII | 800 | 1600 | 800 | 6400 | 1600 | 6400 | 800 | 3200 | 800 | 800 | 800 | 3200 | 200 | 1600 | 800 | 800 | 400 | 3200 | 400 | 400 | — | — | — | — | — |

XI, $E_1$ normal culture serum; XII, $E_1$ SL phage culture serum; XIII, $E_1$ SL phage culture serum.

phenol.[2] The new serum, sterile but not preserved, when tested the following day after bleeding showed agglutination of a higher titer with phage cultures. Part of Serum XII, to which phenol was added immediately after bleeding when tested 3 weeks after bleeding also showed higher agglutination with the phage cultures. Serum XIII, preserved with phenol, titrated 2 weeks after bleeding, showed the same titer as the same serum non-preserved but tested immediately after bleeding. It became thus evident that the phage culture stimulated production of agglutinins specific to all the phage cultures. These agglutinins were of unstable nature and unless the serum was preserved with phenol, they soon disappeared. The relation of this additional component to the specific erysipelas component was established by cross-absorption. Four normal cultures absorbed completely all the agglutinins from phenolized Sera XII and XIII. Each of four phage cultures employed absorbed agglutinins for all the phage cultures. It was clear that the additional phage culture component was closely related to the specific erysipelas streptococcus antigen and that the phage culture components of various erysipelas streptococcus strains were closely affiliated as well.

*B. The Scarlet Fever Group of Streptococci.*—Strain 108 was cultivated for twenty-two passages in phosphate broth and in phosphate broth containing SL phage. The phage culture was studied as shown in Table IV. As is seen, Strain 108 became under the influence of phage relatively inagglutinable by the normal culture serum (XIV). This change, however, was not coincident with loss of agglutinin-absorbing and agglutinogenic properties, since, 108 phage culture completely absorbed Serum XIV and also stimulated production of agglutinins for 108 normal culture. Parallel to the inagglutinability of the specific 108 component another antigen appeared in the phage culture. This was evident from the fact that Serum XV, in contrast to Serum XIV, agglutinated the phage culture as well as the normal culture of Strain 108. Since, however, the normal 108 culture was able to absorb completely agglutinins for the phage culture from Serum

[2] All the sera including Serum XII were collected under sterile precautions by heart puncture and not preserved by phenol. Then the animals were killed, the blood clot removed and its serum preserved with phenol.

XV it was concluded that the phage culture component was closely related to the 108 normal culture specific antigen.

To complete the investigation of the phage culture component, its relation to another strain of the scarlet fever group was studied by cross-agglutinations and cross-absorptions. Strain 55, chosen for this purpose, had common components with Strain 108 in addition to a heterologous antigen (Table IV). It was then observed that 108 phage culture serum agglutinated Strain 55 to a far higher titer than that shown by 108 normal culture serum. Normal culture 55 serum

TABLE IV.

*The Influence of SL Phage on Agglutination of 108 Strain.*

| Sera | Absorbed by strain | Agglutination titer before absorption and percentage of absorption | | |
|---|---|---|---|---|
| | | 55 | 108 | 108 SL |
| XIV | = | 400 | 3200 | 200 |
| XIV | 55 | 100 per cent | 0 per cent | 0 per cent |
| XIV | 108 | 100 per cent | 100 per cent | 100 per cent |
| XIV | 108 SL | 100 per cent | 100 per cent | 100 per cent |
| XV | = | 1600 | 1600 | 3200 |
| XV | 55 | 100 per cent | 0 per cent | 0 per cent |
| XV | 108 | 100 per cent | 100 per cent | 100 per cent |
| XV | 108 SL | 100 per cent | 100 per cent | 100 per cent |
| I | = | 6400 | 400 | 3200 |
| I | 55 | 100 per cent | 100 per cent | 100 per cent |
| I | 108 | 50 per cent | 100 per cent | 100 per cent |
| I | 108 SL | 25 per cent | 100 per cent | 100 per cent |

XIV, 108 normal culture serum; XV, 108 SL phage culture serum.

agglutinated the 108 phage culture to a considerably higher titer than the normal culture of the same strain. It became, therefore, apparent that the phage culture 108 components belonged to the "group" variety. However, Strain 55 absorbed agglutinins for itself but failed to absorb agglutinins for 108 phage culture from Serum XV. It had to be assumed that the phage components were of a more complex structure than the 55 strain group antigen.

The conclusion to be drawn was that the phage was able to render prominent additional components of a complex antigenic structure. These components were partially of the "group" variety and, there-

fore, made possible a certain measure of agglutination with a heterologous strain.

*C. Rb Streptococcus.*—Another example of partial modification was brought out by Rb streptococcus which was cultivated in C/2 phage. It should be pointed out that the strain was completely resistant to this phage. Table V represents the results obtained. As is seen, here again Rb streptococcus C/2 culture became inagglutinable by the normal culture serum. The inagglutinability was not coincident with any changes in agglutinin-absorbing and agglutinogenic proper-

TABLE V.

*The Effect of C/2 Phage upon Agglutination of Rb Streptococcus.*

| Sera | Absorbed by strain | Agglutination titer before absorption and percentage of absorption | | |
|---|---|---|---|---|
| | | Rb streptococcus | Rb C/2 strain | V |
| XVI | = | 6400 | 0 | 0 |
| XVI | Rb streptococcus | 100 per cent | — | — |
| XVI | Rb C/2 | 85 per cent | — | — |
| XVI | V | 0 per cent | — | — |
| XVII | = | 0 | 1600 | 3200 |
| XVII | Rb streptococcus | — | 0 per cent | 0 per cent |
| XVII | Rb C/2 | — | 100 per cent | 100 per cent |
| XVII | V | — | 100 per cent | 100 per cent |
| XVIII | = | 1600 | 800 | 800 |
| XVIII | Rb streptococcus | 100 per cent | 0 per cent | 0 per cent |
| XVIII | Rb C/2 | 100 per cent | 100 per cent | 100 per cent |
| XVIII | V | 0 per cent | 100 per cent | 100 per cent |

XVI, Rb streptococcus serum; XVII, V strain serum; XVIII, Rb C/2 strain serum.

ties since Serum XVIII showed agglutinins for Rb streptococcus and the Rb C/2 strain absorbed agglutinins from Serum XVI for the normal culture of this streptococcus. The change was, however, accompanied by the appearance of another component in the Rb C/2 strain, since its serum, in contrast to the normal Rb streptococcus serum, agglutinated the homologous as well as the normal culture to a high titer. The additional component of the phage culture was not related to the specific Rb streptococcus antigen, as judged from the inability of the Rb streptococcus to absorb agglutinins for Rb C/2 strain from Serum XVIII.

To investigate the nature of the additional phage components Serum XVIII was tested against several strains of streptococci. This serum was able to cross-agglutinate with a strain of green-producing streptococcus. No cross-agglutination was obtained with any hemolytic streptococci tested (the negative findings are not given in Table V). The green-producing streptococcus serum was then found to agglutinate the Rb C/2 strain. Moreover, cross-absorption experiments established the very close similarity of the V specific component with the additional phage culture components. In this case the added component was of the "group" variety. In the case of scarlet fever streptococci a similarly arising component was found related only to the "group" component of another strain of scarlet fever streptococci. In the present example, however, the phage component was related very closely to the specific antigen of a strain of heterologous streptococci.

### *III. Transformation of the Normal Culture Agglutinogens into Antigens of an Entirely Different Specificity.*

As is seen from Tables VI and VII, H.S. and V streptococci when treated with SL phage for a number of generations underwent an even more striking modification. The changes consisted of complete disappearance of normal antigen and appearance of a different antigen, which had the power of absorption, agglutinability and agglutinin-stimulating properties characteristic of a new specificity. The changes described here were in contrast to those of the first category reported above[3] and were of a more marked nature than those described under Paragraph II.

Before concluding this paper it was necessary to investigate whether the phenomenon of paragglutination played any rôle in the observations described above. For this purpose the following experiments were made.

1. The lytic principles employed in this work also contained products of bacteria, at the expense of which the phages were regenerated. It was decided to determine whether bacteriophage-free filtrates of these cultures would not induce similar changes. Filtrates of 1 week old

[3] Page 154.

Rb streptococcus and *B. coli* 42 were used. Several passages of 55, 108 and V strains were made in phosphate broth containing 1 to 10 dilutions of Rb streptococcus filtrate. Rb streptococcus was passed through *B. coli* 42 filtrate. Agglutination reactions of the cultures prepared in this manner with homologous normal culture sera did not differ from those obtained with homologous cultures. It thus became

TABLE VI.

*The Influence of SL Phage upon Agglutination of V Strain.*

| Sera | Absorbed by strain | Agglutination before absorption and percentage of absorption | |
|---|---|---|---|
| | | V | VSL |
| XVII | = | 3200 | 0 |
| XVII | V | 100 per cent | — |
| XVII | V SL | 0 per cent | — |
| XIX | = | 0 | 5120 |
| XIX | V | — | 0 per cent |
| XIX | V SL | — | 100 per cent |

XIX, V SL strain serum.

TABLE VII.

*The Effect of SL Phage upon Agglutination of H.S. Strain.*

| Sera | Absorbed by strain | Agglutination titer before absorption and percentage of absorption | |
|---|---|---|---|
| | | H.S. | H.S./SL |
| XX | = | 0 | 1280 |
| XX | H.S. | — | 0 per cent |
| XX | H.S. SL | — | 100 per cent |
| XXI | = | 5120 | 0 |
| XXI | H.S. | 100 per cent | — |
| XXI | H.S. SL | 0 per cent | — |

XX, H.S. SL phage serum; XXI, H.S. serum.

apparent that the phenomenon of paragglutination was not responsible for the changes described in this paper. This belief was further strengthened by the following additional observations.

2. Three animals immunized with SL and C/2 lytic principles failed to show agglutinins for the bacterial strains from which these principles were derived.

3. Sera prepared with SL phage cultures of various strains cross-agglutinated with Rb streptococcus only in those cases in which the normal culture sera cross-agglutinated as well.

4. Various strains of streptococci (erysipelas and scarlet fever groups) which normally cross-agglutinated with Rb streptococcus failed to do so when passed through SL phage.

5. Rb C/2 and V strains were not agglutinated by the anti-C/2 serum.

6. Rb C/2 strain serum failed to agglutinate *B. coli* 42.

## CONCLUSIONS AND SUMMARY.

A series of experiments was carried out on various strains of streptococci in order to ascertain the changes which bacteriophage produces in the phenomena of agglutination of these organisms. The results can be placed in the following categories.

1. Loss of specific agglutinability was observed with partial and with what appeared as complete loss of specific agglutinin absorption and agglutinogenic properties.

2. Partial modification of the antigen, bringing about inagglutinability of the strains with complete preservation of agglutinogenic and agglutinin-absorption properties. Coincidentally, additional components appeared. Some of these were related to the specific antigens of the organisms from which they were derived, while others were not. In two instances these components were of "group" character.

3. "Complete" modification observed with two strains consisting of complete transformation of the normal culture agglutinogens into antigens of an entirely different specificity.

It appears from these studies that the bacteriophage phenomenon may play an intricate rôle in serological grouping of various strains of pathogenic streptococci.

## BIBLIOGRAPHY.

1. d'Hérelle, F., The bacteriophage, Baltimore, English translation by Smith, G. H., 1926.
2. Bordet, J., and Ciuca, M., Autolyse microbienne et sérum antilytique, *Compt. rend. Soc. biol.*, 1921, lxxxiv, 280.

3. Gratia, A., The Twort-d'Hérelle phenomenon. II. Lysis and microbic variation, *J. Exp. Med.*, 1922, xxxv, 287.
4. Flu, P. C., Das Verhalten eines inagglutinablen Flexner-Bacterium gegenüber Anti-Flexner-Bakteriophagen, *Centr. Bakt., 1. Abt., Orig.*, 1923, xc, 374.
5. McKinley, E., Transformation, sous l'influence du principe lytique faible, de la specificité antigenique d'une culture, *Compt. rend. Soc. biol.*, 1925, xciii, 1053.
6. Dutton, L. O., The probable rôle of the bacteriophage in streptococcus infections, *J. Lab. and Clin. Med.*, 1925–26, xi, 763.
7. Piorkowski, G., Beitrag zur Streptokokkenfrage. Anwendung des d'Herelleschen Phänomens auf Streptokokken, *Med. Klin.*, 1922, xviii, 474.
8. Shwartzman, G., Studies on streptococcus bacteriophage. A powerful lytic principle against hemolytic streptococci of erysipelas origin, *J. Exp. Med.*, 1927, xlvi, 497.
9. Dochez, J., *J. Exp. Med.*, 1919, xxx, 179; *J. Am. Med. Assn.*, 1920, lxxiv, 1600.
10. Birkhaug, K. E., *Bull. Johns Hopkins Hosp.*, 1925, xxvi, 233.
11. Tunnicliff, *J. Infect Dis.*, 1922, xxxi, 373; *J. Am. Med. Assn.*, 1920, lxxv, 1339.