# 7235 P

## Chemical Studies in Bacterial Agglutination.*

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### I. A micro method for the quantitative estimation of agglutinins.

The agglutination of bacteria may be regarded as a precipitin reaction at the surface of the cells.[1, 2] A method had been worked out in this laboratory for the quantitative estimation of precipitins,[3] and it has been found possible to adapt the procedure to the micro determination of agglutinins. Thoroughly washed, heavy bacterial suspensions are standardized by measuring their nitrogen content per cc. by the micro-Kjeldahl method. Accurately measured quantities of bacteria and an appropriate antibody solution or serum are then mixed and allowed to stand, with occasional mixing, at 37° for 2 hours and overnight in the ice-box. The remainder of the analysis is carried out as in the precipitin reaction,[3] except that it has been found convenient to make use of the higher speed possible with the angle centrifuge.† This was run in an ice-box compartment. Agglutinin nitrogen is determined by subtracting from the total nitrogen of the washed precipitate the amount of nitrogen contained in the bacteria used. Agglutinin N may also be determined from the difference between a blank without bacteria, and the supernatant from the agglutination. Both methods are illustrated in Table I. Agglutinin = agglutinin N × 6.25, under the assumption that agglutinin is protein. If the relative amounts of bacteria and serum are so chosen that the supernatant contains no more agglutinin the result obtained (divided by the volume) is the agglutinin content of the serum in milligrams per cc. In the table are given typical data for pneumococcus agglutinins. Application of the method to other systems is in progress.

An immediate outcome of the method is given in the following

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1 For a review, *cf.* Wells, H. G., Chemical Aspects of Immunity, 2nd Edition, New York 1929.

2 Francis, T., Jr., *J. Exp. Med.*, 1932, **55**, 55.

3 Heidelberger, M., Sia, R. H. P., and Kendall, F. E., *J. Exp. Med.*, 1930, **52**, 477; Heidelberger, M., Kendall, F. E., and Soo Hoo, C. M., *Ibid.*, 1933, **58**, 137.

† Furnished by the Standard Scientific Supply Company, New York.

TABLE I.

| Vol. bacterial suspension | Supernatant: Vol. antibody sol'n | Supernatant: Original N content | Supernatant: N after agglutination | Supernatant: Agglutinin N found | Supernatant: Aggl. N per cc. antibody sol'n | Precipitates: Total N found | Precipitates: N of bacteria | Precipitates: Aggl. N found | Aggl. N per cc. antibody sol'n |
|---|---|---|---|---|---|---|---|---|---|
| cc. | cc. | mg. | mg. | mg. | mg./cc. | mg. | mg. | mg. | mg./cc. |
| Pn I S B75* (Dilution Susp. B 8:25) | | | | | | | | | |
| 0 | 1.0 | .87 | | | | | | | |
| 3.0 | 0 | .01 | | | | | .42 | | |
| 3.0 | 0.5† | .44 | .15 | .29 | .58 | .67 | .42 | .25 | .50 |
| Susp. C | | | | | | | | | |
| 2.0 | 0 | .02 | | | | | .33 | | |
| 2.0 | 0.5 | .44 | .15 | .29 | .58 | .61 | .33 | .28 | .56 |
| 2.0 | 1.0 | .87 | .38 | .49 | | .80 | .33 | .47 | |
| 2.0 | 1.5 | 1.31 | .74 | .57 | | .90 | .33 | .57 | |
| | Anti-Egg-albumin serum | | | | | | | | |
| 1.0 | 2.0 | | | | | .16 | .17 | .00 | .00 |
| PnII-R. susp. | Anti-"R" protein serum‡ | | | | | | | | |
| 2.0 | 0 | | | | | | .34 | | |
| | Serum 231 | | | | | | | | |
| 2.0 | 0.5 | | | | | .37 | .34 | .03 | .06 |
| | Serum 235 | | | | | | | | |
| 2.0 | 0.5 | | | | | .37 | .34 | .03 | .06 |
| | Supernatant 231 anal. | | | | | | | | |
| 2.0 | 2.0 cc. | | | | | .34 | .34 | .00 | |
| | Supernatant 235 anal. | | | | | | | | |
| 2.0 | 2.0 cc. | | | | | .34 | .34 | .00 | |

* Pneumococcus I antibody solution prepared according to Felton.[4]

† The supernatant from this experiment, set up again with 0.5 cc. of PnI suspension, gave 0.01 mg. additional agglutinin N—a negative result within the limit of error of the method.

‡ Serum 231 agglutinated PnII"R" suspensions to a dilution of 1:160.
Serum 235 agglutinated PnII"R" suspensions to a dilution of 1:320.

notice. Quantitative evidence bearing on the mechanism of bacterial agglutination has also been obtained, and will be published later.

**II. Quantitative relation between agglutinin and precipitin.**

Although there is much in favor of the unitarian theory of antibodies, quantitative evidence for the identity of precipitins and agglutinins has hitherto been lacking.[5] This may now be supplied

[4] Felton, L. D., *J. Infectious Dis.*, 1928, **43**, 543.

[5] *Cf.* Zinsser, H., *J. Immunol.*, 1930, **18**, 483; also Felton, L. D., *J. Immunol.*, 1931, **21**, 341.

through the quantitative method for agglutinins outlined in the preceding note and the method for precipitins given in reference 3.

In order to minimize complications due to a multiplicity of antigens and antibodies, Type I anti-pneumococcus horse serum was absorbed with pneumococcus "C" substance and then with pneumococcus I "nucleoprotein" until no further precipitation occurred. A purified antibody solution was then prepared according to Felton.[4] As shown in Table II, the resulting solution contained 1.68 mg. of precipitin nitrogen per cc., as determined with the acetyl polysac-

TABLE II.
Removal of Agglutinin or Precipitin from Pneumococcus I Antibody Solution B75.

| Vol. antibody solution | Agglutinin N removed by Pn I suspension | Precipitin N found in supernatant | Total antibody N | Total antibody N per cc. | Precipitin N removed | Agglutinin N found in supernatant with Pn I suspension | Total antibody N | Total antibody N per cc. |
|---|---|---|---|---|---|---|---|---|
| cc. | mg. | mg. | mg. | mg. | mg. | mg. | mg. | mg. |
| 0.5 | .93 | 0 | .93 | 1.86 | | | | |
| 1.0 | | | | | 1.68 | .22 | 1.90 | 1.90 |
| 0.5 | .64 | .27 | .91 | 1.82 | | | | |
| 1.0 | .73 | 1.02 | 1.75 | 1.75 | 1.35 | .50 | 1.85 | 1.85 |

charide of Type I pneumococcus,[6] while the amount of agglutinin nitrogen per cc. was 1.86 mg. The discrepancy was found to be due to group specific antiprotein (or anti-C) which it had not been possible to remove by absorption of the serum, since the antibody solution yielded 0.22 mg. of agglutinin N per cc. with Pneumococcus II R suspension; an amount identical with that taken out by Pneumococcus I S suspension from the supernatants of the precipitin analyses. If this interpretation be accepted, the amount of anticarbohydrate agglutinin N was 1.86—0.22, or 1.64 mg. per cc.; in excellent agreement with the amount of anticarbohydrate N found by the precipitin method.

It will be noticed that not only does the total agglutinin N correspond within a few hundredths of a mg. per cc. with total precipitin + agglutinin in the supernatant, but that partial agglutinin + residual precipitin, or partial precipitin + residual agglutinin determinations add to approximately the same total, regardless of the order in which the partial quantitative agglutinin or precipitin determinations are carried out. That the difference in the last instance in the table is as great as 0.1 mg. is undoubtedly due to

[6] Avery, O. T., and Goebel, W. F., *J. Exp. Med.*, 1933, **58**, 731.

antiprotein (or anti-C) remaining in the final supernatant, since only a little over one-third of the total antibody was removed as agglutinin.

## 7236 C

## Glutathione and Methylene Blue in Carbon Monoxide Poisoning.

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The experiments of Brooks[1] based upon theory derived from the investigations of Warburg *et al.*,[2] Meyerhof,[3] Heymans and Heymans,[4] Barron,[5] and others, aroused interest in methylene blue as an antidote for carbon monoxide poisoning. Many case reports (Geiger,[6] Bell,[7] Nass,[8] Christoferson,[9] Brown[10]) and some experimental reports (Brooks,[11] Draize,[12] Haggard and Greenberg[13]) have increased the literature upon this subject. Even the contradictory conclusions of Haggard and Greenberg and the concise summary of Henderson[14] have not sufficed to alter the popular conception of methylene blue as an antidote for carbon monoxide poisoning.

In the following experiments, the effect of glutathione upon carbon monoxide poisoning was first investigated because of Brooks' results with a similar oxidation-reduction catalyst, methylene blue. However, the discouragingly negative results soon prompted an investigation of methylene blue.

White rats (100 to 200 gm.) were placed one at a time in a special

1 Brooks, M. M., *Am. J. Physiol.*, 1932, **102**, 145.

2 Warburg, O., *Z. physiol. Chem.*, 1910, **66**, 305; *Biochem. Z.*, 1923, **162**, 518; 1926, **177**, 471; 1930, **227**, 245.

3 Meyerhof, O., *Pflüger's Arch.*, 1912, **149**, 250.

4 Heymans, J. F., and Heymans, C., *Arch. intern. Pharmacodynamie*, 1922, **26**, 443.

5 Barron, E. S. G., *J. Biol. Chem.*, 1929, **81**, 445.

6 Geiger, J. C., *J. Am. Med. Assn.*, 1933, **100**, 1103.

7 Bell, M. A., *J. Am. Med. Assn.*, 1933, **100**, 1402.

8 Nass, J., *J. Am. Med. Assn.*, 1933, **100**, 1862.

9 Christoferson, A. W., *J. Am. Med. Assn.*, 1933, **100**, 2008.

10 Brown, T. C., *Colorado Med.*, 1933, **30**, 302.

11 Brooks, M. M., *Am. J. Physiol.*, 1933, **104**, 139.

12 Draize, J. H., *Science*, 1933, **78**, 145.

13 Haggard, H. W., and Greenberg, L. A., *J. Am. Med. Assn.*, 1933, **100**, 2001.

14 Henderson, Y., *Science*, 1933, **78**, 408.