THE DISTRIBUTION OF THE IMMUNE BODIES OCCURRING IN ANTIPNEUMOCOCCUS SERUM.*

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By a systematic study of the specific antigenic properties of the pneumococcus, Dochez and Gillespie[1] have demonstrated four distinct types of this organism occurring in disease. Such a biologic classification of pneumococci affords the only rational basis for the clinical application of serum therapy in lobar pneumonia. The immunologic and clinical studies of Cole[2] have shown that the protective and curative value of antipneumococcus serum is dependent upon this group specificity, that a serum to be efficacious must be one produced in response to an organism of the same type as that causing the infection. The treatment by specific antisera of lobar pneumonia of pneumococcus origin is at present possible only in infections due to organisms of types I and II. Immune serum produced by the third group, *Pneumococcus mucosus,* fails to confer passive immunity, and the heterogeneous nature of organisms of the fourth variety demands a specific serum for each individual strain, making serum therapy in infections of this type impracticable. However, since approximately 70 per cent. of the cases of this disease is due to organisms of the first two groups, the serum treatment of pneumonia is applicable in the majority of instances. Experimental evidence and clinical experience have demonstrated the necessity of administering relatively large doses of the appropriate serum in combating these infections. The present study was undertaken, therefore, with the hope of determining a method of concentrating and purifying antipneumococcus serum, by which its antibacterial potency might be conserved with a minimum of foreign protein. It seems reasonable to assume that such a process, by concentration of antibody content, might enhance the efficacy of

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[1] Dochez, A. R., and Gillespie, L. J., *Jour. Am. Med. Assn.,* 1913, lxi, 727.

[2] Cole, R., *Arch. Int. Med.,* 1914, xiv, 56.

serum treatment, and, by the elimination of certain irrelevant protein constituents, lessen the incidence, or at least ameliorate the symptoms of serum disease.

Although much is known concerning the immunological reactions of various immune bodies, comparatively little is understood of their true chemical nature. Whether they are themselves protein in character, or merely associated in some obscure chemical combination with the globulin or albumin of immune sera is not definitely known. The fact that they may be thrown out of solution by certain protein precipitants has been utilized in the concentration of antitoxic sera. Most investigators now agree that antibody precipitation by chemical agents is not merely a mechanical or adsorption phenomenon, but that these immune substances partake of the nature of the protein with which they are precipitated.

In the present study it was first necessary, therefore, to determine with which fraction of the serum protein the pneumococcus immune bodies are associated. The serum used in these experiments was obtained by the intensive immunization of horses to virulent cultures of pneumococcus of types I and II. The various protein fractions isolated were tested for antibacterial action against virulent cultures of pneumococcus by protection experiments on white mice, and the results compared with the similar power of the whole serum. This method is less accurate than the more exact titration of antitoxic sera. The potency of antipneumococcus serum can be measured only by its ability to protect highly susceptible animals against actual infection, and the comparative antibody content of any given serum fraction can be interpreted only in terms of the death or survival of such animals. This reaction between antibody and bacterium is biological and not chemical, as in the neutral balance of antitoxin and toxin mixtures. Under these experimental conditions, however, some information has been gained concerning the distribution of the immune bodies occurring in antipneumococcus serum.

Experiment 1.—To determine the protective value of the total globulins precipitated by half saturation with ammonium sulphate.

300 c.c. of antipneumococcus serum I were diluted with an equal volume of water, and 600 c.c. of a saturated solution of ammonium sulphate were added.

The mixture was allowed to stand at room temperature over night, filtered, the precipitate collected, pressed, and dialyzed for eight days against running tap water. To the filtrate crystals of ammonium sulphate were added to full saturation. The precipitate consisting of albumin was filtered off, pressed, and dialyzed as above.

The volume of globulin solution was 100 c.c., so that the globulins were three times as concentrated as in the whole serum. The volume of albumin solution was 58 c.c., being 0.2 per cent. that of the original serum.

Protective Power of Globulins, Albumin, and Serum I.
Pneumococcus I.

Amount of culture I.	Amount of serum fractions.	Serum I.	Globulins.	Albumin.	Controls. Culture alone.
0.05 c.c.	0.1 c.c.	S.	S.	D. 18	.....
0.05 c.c.	0.08 c.c.	S.	S.	D. 18	.....
0.05 c.c.	0.05 c.c.	S.	S.	D. 18	.....
0.05 c.c.	0.03 c.c.	D. 72	S.	D. 18	.....
0.05 c.c.	0.01 c.c.	D. 48	D. 72	D. 18	.....
0.0001 c.c.	.....	.....	.....	.....	D. 36
0.00001 c.c.	.....	.....	.....	.....	D. 36
0.000001 c.c.	.....	.....	.....	.....	D. 36

In the tables D. = died; S. = survived. The figures represent the number of hours before the death of the animal.

Experiment 2.—Antipneumococcus serum II was used, diluted with an equal volume of water, and the globulins were precipitated by half saturation with ammonium sulphate. The albumin was precipitated from the filtrate by complete saturation with crystals of ammonium sulphate. Both fractions were dialyzed for four days against running tap water, and made up to the original volume of serum with 0.85 per cent. salt solution.

Comparative Protective Value of Globulins, Albumin, and Serum II.
Pneumococcus II.

Amount of culture II.	Amount of serum fractions.	Serum II.	Globulins.	Albumin.	Controls. Culture alone.
0.1 c.c.	0.2 c.c.	D. 66	D. 66	D. 18	.....
0.01 c.c.	0.2 c.c.	S.	S.	D. 19	.....
0.001 c.c.	0.2 c.c.	S.	S.	D. 19	.....
0.0001 c.c.	0.2 c.c.	S.	S.	D. 24	D. 19
0.00001 c.c.	0.2 c.c.	S.	S.	D. 24	D. 24
0.000001 c.c.	0.2 c.c.	S.	S.	D. 36	D. 24

Agglutination.

Dilution.	Serum II.	Globulins.	Albumin.	Controls.
1 : 1	++	++	−	−
1 : 10	++	++	−	−
1 : 20	+	±	−	−
1 : 40	−	−	−	−

Experiment 3.—The agglutination test was made by mixing equal volumes of an eighteen hour broth culture of pneumococcus I and varying dilutions of serum fractions.

The antigen used in the precipitin reaction was prepared from the washed bacterial residue of liter cultures of pneumococcus I frozen and desiccated in vacuum over sulphuric acid. The dry powder was dissolved in salt solution, with 1 mg. per c.c., and a 1 : 10 dilution of this was used in the test. Equal parts of antigen and serum dilutions were mixed and incubated in the water bath at 37° C. for two hours.

Agglutination and Precipitation.

Agglutination.				Precipitation.		
Serum dilution.	Serum I.	Globulins.	Albumin.	Serum dilution.	Serum I.	Globulins.
1 : 1	++	++	−	1 : 10	++	++
1 : 10	++	++	−	1 : 15	++	++
1 : 100	−	−	−	1 : 20	++	+
				1 : 25	+	±
				1 : 30	±	−

From experiments I, II, and III it may be concluded that the immune bodies of antipneumococcus serum are not dialyzable, that they are precipitated with the globulins by half saturation with ammonium sulphate, and that the demonstrable antibodies, such as agglutinins, precipitins, and protective substances, are combined or associated with the globulins and not with the albumin of the immune serum.

Experiment 4.—To determine the protective value of the euglobulin and pseudoglobulin.

300 c.c. of antipneumococcus serum I were diluted with an equal volume of water and 600 c.c. of a saturated solution of ammonium sulphate added, allowed to stand over night, and filtered, precipitate (a), filtrate (a). The precipitate (a) containing both globulins was taken up in 600 c.c. of water and saturated with crystals of sodium chloride (210 gm.) and filtered, precipitate (b), filtrate (b). The precipitate (b) was again taken up in water and resaturated with salt, the final precipitate pressed and dialyzed against running tap water until salt-free. The dialysate was made slightly alkaline to effect solution, used undiluted, and called "euglobulin." To the filtrate (b) about 0.2 per cent. of acetic acid was added to precipitate the globulin remaining in solution. This precipitate was pressed and after dialyzing for twenty-four hours was neutralized to litmus by sodium carbonate, and dialysis continued for four days. The dialysate was used undiluted and called "pseudoglobulin." The original filtrate (a) was saturated with crystals of ammonium sulphate; the precipitate containing the albumin was collected by filtration, pressed, and dialyzed.

Comparative Protective Value.

Amount of culture I.	Amount of serum fractions.	Serum I.	Euglobulin.	Pseudoglobulin.	Albumin.	Controls.
0.1 c.c.	0.2 c.c.	D. 72	D. 17	D. 20	D. 17	.....
0.01 c.c.	0.2 c.c.	S.	S.	D. 84	D. 17	.....
0.001 c.c.	0.2 c.c.	S.	S.	S.	D. 20	.....
0.0001 c.c.	0.2 c.c.	S.	S.	S.	D. 24	D. 22
0.00001 c.c.	.....	.....	.....	.....	.....	D. 24
0.000001 c.c.	.....	.....	.....	.....	.....	D. 36

Amount of culture I.	Amount of serum fractions.	Serum I.	Euglobulin.	Pseudoglobulin.	Albumin.	Controls.
0.01 c.c.	0.4 c.c.	S.	S.	S.	D. 48	.....
0.01 c.c.	0.3 c.c.	S.	S.	S.	D. 36	.....
0.01 c.c.	0.2 c.c.	S.	S.	S.	D. 18	.....
0.01 c.c.	0.1 c.c.	S.	S.	S.	D. 28	.....
0.01 c.c.	0.01 c.c.	D. 40	D. 40	D. 40	D. 18	.....
0.01 c.c.	0.005 c.c.	D.	D. 18	D. 48	D. 18	.....
0.0001 c.c.	.....	.....	.....	.....	.....	D. 18
0.00001 c.c.	.....	.....	.....	.....	.....	D. 28
0.000001 c.c.	.....	.....	.....	.....	.....	D. 36

Experiment 4 shows that by the technique employed the immune bodies of antipneumococcus serum are not confined to the so called euglobulin or pseudoglobulin, but occur in both these fractions. Their absence in the albumin of the serum confirms the previous experiment.

Experiment 5.—Fractional precipitation of heated serum by ammonium sulphate.

50 c.c. of antipneumococcus serum I were diluted with a half volume of distilled water and 1 per cent. sodium chloride, and 38.6 c.c. of a saturated solution of ammonium sulphate were added, making the total 34 per cent. saturation. The serum-sulphate mixture was heated in a water bath at 56° C. for four hours, 58° C. for one hour, and 60° C. for five minutes. Filtered hot, the precipitate was pressed and dialyzed for four days against running water. To the filtrate, a saturated solution of ammonium sulphate was added up to 54 per cent. saturation. After standing at room temperature over night the precipitate was filtered off, pressed, and dialyzed for four days. To the final filtrate ammonium sulphate crystals were added to complete saturation, and the filtrate was pressed and dialyzed. The various fractions isolated were designated 34 per cent. globulin, 54 per cent. globulin, and albumin, respectively. After dialysis these fractions were each made up with 0.85 per cent. salt solution to the volume of the original serum, and in the case of the 34 per cent. globulin sufficient sodium carbonate was added to effect solution.

Comparative Protective Value of Globulin Fractions Precipitated by Ammonium Sulphate from Heated Serum.

Amount of culture I.	Amount of serum fractions.	0–34% globulin.	34–54% globulin.	Albumin.	Whole globulins, experiment 1.	Serum I.	Controls. Culture alone.
0.1 c.c.	0.2 c.c.	D. 20	D. 20	D. 20	S.	S.	.....
0.01 c.c.	0.2 c.c.	S.	S.	D. 20	S.	S.	.....
0.001 c.c.	0.2 c.c.	S.	S.	D. 20	S.	S.	.....
0.0001 c.c.	0.2 c.c.	S.	S.	D. 20	S.	S.	D. 18
0.00001 c.c.	0.2 c.c.	S.	S.	D. 24	S.	S.	D. 18
0.000001 c.c.	0.2 c.c.	S.	S.	D. 24	S.	S.	D. 18

Agglutination tests showed the presence of these antibodies in both fractions, and their absence in the albumin.

The method followed in this experiment is essentially that devised by Banzhaf[3] for the concentration of diphtheria antitoxin. In diphtheria immune serum the antitoxin is precipitated with the pseudoglobulin. The addition of salt and the heating of serum-sulphate mixture converts some of the pseudoglobulin into the inactive euglobulin without material loss of its antitoxic potency. By the application of this method to antipneumococcus serum it was hoped that the residue of protective antibodies occurring in the pseudoglobulin might be changed over with the converted euglobulin and the total potency of the serum be thus concentrated in the latter fraction. This, however, did not occur; both fractions still showed protection. Heating antipneumococcus serum to the temperature used in this experiment does not seem materially to affect the activity of the agglutinins and protective antibodies, although some loss of potency is suffered by the latter.

Experiment 6.—To determine the protective value of the globulin fraction precipitated by passage of carbon dioxide through diluted serum.

50 c.c. of antipneumococcus serum II were diluted with ten volumes of distilled water. Carbon dioxide was allowed to bubble through slowly for two hours, the diluted serum being kept at a temperature of 2° C. during the process; it was then placed in the ice box over night, and a sharp separation of precipitate and supernatant fluid was effected by centrifugalization. The carbon dioxide precipitate was dissolved in 50 c.c. of 0.85 per cent. salt solution. The supernatant fluid was half saturated with ammonium sulphate by the addition of an equal volume of a saturated solution of this salt. This carried down the residual globulin remaining after removal of the carbon dioxide-insoluble

[3] Banzhaf, E. J., *Studies from the Research Laboratory, Department of Health,* New York, 1912–13, vii, 114.

fraction. After standing at room temperature over night the precipitate was filtered, pressed, and dialyzed for four days and made up to a volume of 50 c.c. with 0.85 per cent. salt solution.

Protective Value of Carbon Dioxide Globulins.

Amount of culture II.	Amount of serum fractions.	Original serum II.	Carbon dioxide-insoluble globulin.	Carbon dioxide-soluble globulin.	Controls. Culture alone.
0.1 c.c.	0.2 c.c.	D. 36	D. 36	D. 18	.....
0.01 c.c.	0.2 c.c.	S.	S.	S.	.....
0.001 c.c.	0.2 c.c.	S.	S.	S.	.....
0.0001 c.c.	0.2 c.c.	S.	S.	S.	D. 20
0.00001 c.c.	0.2 c.c.	S.	S.	S.	D. 18
0.000001 c.c.	0.2 c.c.	S.	S.	S.	D. 18

Agglutination.

Dilution of serum fractions.	Original serum II.	Carbon dioxide-insoluble globulin.	Carbon dioxide-soluble globulin.
1 : 1	++	++	++
1 : 5	++	++	+
1 : 10	+	+	−
1 : 15	±	−	−
1 : 25	−	−	−

Precipitation.

Antigen 1:1,000.

Dilution of serum fractions.	Original serum II.	Carbon dioxide-insoluble globulin.	Carbon dioxide-soluble globulin.
1 : 1	++	++	+±
1 : 5	+	+	−
1 : 10	±	± (?)	−

From experiment 6 it appears that from diluted antipneumococcus serum there is precipitated by saturation with carbon dioxide a globulin possessing high protective value, and that by the method of titration employed a slightly less potent globulin fraction remains in solution, and can be separated from the albumin by ammonium sulphate. The somewhat anomalous fact, that each fraction of the carbon dioxide globulin apparently possesses protective value equal to the whole serum, may be explained in part by the method of titration. The immunity unit, 0.2 of a cubic centimeter of serum, which has been found most suitable for protection tests, apparently contains an excess of antibody and the zone of carbon

dioxide precipitation may so lie within the limits of this excess that, in the cleavage effected by acidification with carbon dioxide, sufficient antibody is carried over with each fraction of the globulin to afford an equivalent protection. The carbon dioxide-insoluble globulin, although representing approximately only one-fifteenth of the total protein, apparently contains more than half the antibodies.

Experiment 7.—To determine the potency of the serum globulins soluble and insoluble in saturated sodium chloride.

50 c.c. of antipneumococcus serum II diluted with an equal volume of water were saturated with crystals of sodium chloride, and the salt-serum mixture was allowed to extract over night at room temperature. The portion of the globulin insoluble in saturated sodium chloride was filtered off, pressed, and dialyzed for four days against running water. To the filtrate containing the salt-soluble fraction was added an equal volume of a saturated solution of ammonium sulphate. The precipitate containing the salt-soluble globulin was collected, pressed, and dialyzed for four days. The fractions were all diluted with 0.85 per cent. salt solution to the volume of the original serum.

Protective Value of Sodium Chloride Globulins.

Amount of culture II.	Amount of serum fractions.	Original serum II.	Sodium chloride-insoluble globulin.	Sodium chloride-soluble globulin.	Controls. Culture alone.
0.01 c.c.	0.2 c.c.	S.	D. 20	S.	.....
0.001 c.c.	0.2 c.c.	S.	S.	S.	.....
0.0001 c.c.	0.2 c.c.	S.	S.	S.	D. 20
0.00001 c.c.	0.2 c.c.	S.	S.	S.	D. 24
0.000001 c.c.	0.2 c.c.	S.	S.	S.	D. 36

Agglutination.

Serum II ..... ++
Sodium chloride-insoluble ..... ++
Sodium chloride-soluble ..... ++

Experiment 7 shows that the protective substances and the agglutinins of antipneumococcus serum are not confined to either of the globulin fractions separated by saturation with sodium chloride. The salt-insoluble globulin, according to Freund and Joachim,[4] represents the euglobulin, but includes only a part of the similar fraction precipitated by one-third saturation with ammonium sulphate. Banzhaf and Gibson[5] consider that this method yields as

[4] Freund, E., and Joachim, J., *Ztschr. f. physiol. Chem.*, 1902, xxxvi, 407.

[5] Banzhaf, E. J., and Gibson, R. B., *Studies from the Research Laboratory, Department of Health*, New York, 1908–10, iv–v, 202.

sharp a differentiation as possible between the two globulin fractions, and that the sodium chloride separation probably represents more truly the common conception of euglobulin and pseudoglobulin than the more usual fractioning by ammonium sulphate. The results obtained in this experiment are similar to and comparable with those of experiment 4, in which the globulins were first precipitated with ammonium sulphate, and then separated by precipitation first with sodium chloride and then with acetic acid.

Experiment 8.—To determine the potency of the water-soluble and -insoluble serum globulin obtained by dialysis.

100 c.c. of antipneumococcus serum II, undiluted, were placed in a parchment bag and dialyzed for six days against running tap water. The dialyzing bag was then washed out with distilled water, the precipitate collected by filtration, washed with water, taken up in 100 c.c. of physiological salt solution, and made slightly alkaline with sodium carbonate to effect complete solution. To the filtrate containing the water-soluble globulin, an equal volume of a saturated solution of ammonium sulphate was added, the filtrate collected, pressed, and dialyzed for four days, and then made up to volume (100 c.c.) with 0.85 per cent. salt solution.

Protective Value of Globulin Fractions Separated by Dialysis.

Amount of culture II.	Amount of serum fractions.	Water-soluble globulin.	Water-insoluble globulin.	Original serum II.	Controls.
0.01 c.c.	0.2 c.c.	D. 24	S.	S.	.....
0.001 c.c.	0.2 c.c.	S.	S.	S.	.....
0.0001 c.c.	0.2 c.c.	S.	S.	S.	D. 20
0.00001 c.c.	0.2 c.c.	S.	S.	S.	D. 24
0.000001 c.c.	0.2 c.c.	S.	S.	S.	D. 40

Agglutination.

Pneumococcus II.	Serum II.	Water-insoluble globulin.	Water-soluble globulin.
	++	++	++

Seng[6] showed that by dialysis of diphtheria antitoxin only a small part (1/23 to 1/11) of the total globulin was insoluble and that this fraction possessed no antitoxic value. These results were later confirmed by Brieger and Krause.[7] Freund and Joachim do not consider the globulins precipitated by fractionation with ammonium sulphate identical with the two groups obtained by dialysis.

[6] Seng, W., *Ztschr. f. Hyg. u. Infektionskrankh.*, 1899, xxxi, 513.

[7] Brieger, L., and Krause, M., *Berl. klin. Wchnschr.*, 1907, xliv, 946.

Experiment 8 demonstrates the distribution of the antibacterial substances of antipneumococcus serum in both fractions of the globulins separated by dialysis. These results indicate that the globulin precipitated by dialysis (water-insoluble), like that precipitated by carbon dioxide, contains a part, but not all of the immune bodies.

Experiment 9.—To determine the relative potency of the globulin fractions obtained by the addition of progressive amounts of ammonium sulphate.

50 c.c. of antipneumococcus serum II were diluted with a half volume of water and the whole was precipitated by 34 per cent. saturation with ammonium sulphate, 38.6 c.c. of saturated solution. From the filtrate the residual globulin was precipitated by adding sufficient saturated solution of ammonium sulphate to cause 54 per cent. saturation, allowing for the amount of this salt already present in the filtrate. The precipitates of both fractions were collected, pressed, and dialyzed for four days against running tap water. The dialysates were made up to the original volume of the serum with 0.85 per cent. salt solution, and were designated 0 to 34 per cent. globulin and 34 to 54 per cent. globulin, respectively. By the same technique a similar quantity of the same lot of serum was fractioned by salting out at 38 and 42 per cent. saturation, the residual globulin in each case being separated from the albumin in the filtrate by precipitation at 54 per cent. saturation ammonium sulphate.

Relative Potency of the Globulins Obtained by Salting Out with Progressive Amounts of Ammonium Sulphate.

Amount of culture II.	Amount of serum fractions.	Controls. Culture alone.	Globulins precipitated by ammonium sulphate saturation within the limits						
			0–34%	34–54%	0–38%	38–54%	0–42%	42–54%	Original serum.
0.01 c.c.	0.2 c.c.	.....	S.	D. 20	S.	D. 22	S.	D. 18	S.
0.001 c.c.	0.2 c.c.	.....	S.	D. 72	S.	D. 24	S.	D. 24	S.
0.0001 c.c.	0.2 c.c.	D. 36	S.	D. 18	S.	D. 36	S.	D. 36	S.
0.00001 c.c.	0.2 c.c.	D. 36	S.	S.	S.	D. 36	S.	D. 36	S.
0.000001 c.c.	0.2 c.c.	D. 36	S.	S.	S.	D. 36	S.	D. 36	S.

Agglutination with Various Globulin Fractions.

Serum II, lot 3	++
0–34 per cent. globulin	++
34–54 per cent. globulin	±
0–38 per cent. globulin	++
38–54 per cent. globulin	—
0–42 per cent. globulin	++
42–54 per cent. globulin	—

Experiments 1 to 8 show that the immune bodies occurring in antipneumococcus sera I and II are associated or combined with the globulins, but are not confined solely to any one of the globulin

fractions obtained by the various methods of separation employed. Since certain of the experiments seemed to indicate that the major portion of the antibacterial substances are crowded toward the true or euglobulin end of the protein spectrum, an attempt was made in experiment 9 to determine, by fractional salting out with progressive amounts of ammonium sulphate, a point where the zones of globulin and antibody precipitation might coincide and the total potency of the serum be conserved with a minimum of serum globulin. Experiment 9 indicates that this zone of joint precipitation of total antibody with minimal globulin is reached at about 38 per cent. saturation with ammonium sulphate. At this point a fraction of the serum globulin is precipitated which is as active in antibacterial potency as the original serum, while the residual globulin is inactive and apparently unessential.

DISCUSSION.

The chemistry of the proteins of immune serum and their relation to various antibodies have been studied thoroughly in the antitoxic sera. Numerous investigators have shown that diphtheria and tetanus antitoxin are precipitated with the serum globulins. More exact study of these globulins has demonstrated that they may be further subdivided according to their solubility and precipitation by certain chemical reagents. Seng showed that the globulins of immune serum, and according to Marcus[8] those of normal serum as well, are of two kinds: an insoluble globulin precipitated by acetic acid, carbon dioxide, dilution with water, or dialysis; and a soluble globulin unaffected by these reagents but precipitated by the neutral salts of ammonium and magnesium sulphate. Seng showed, and many investigators have since confirmed, the association of antitoxin with the soluble globulin. Fractional precipitation with the neutral salts of the heavy metals yields a similar, though not altogether comparable, separation of the globulins into a soluble and insoluble fraction, the so called euglobulin and pseudoglobulin. It is with the latter of these, the pseudoglobulin, that antitoxin is combined. By the application of this principle Gibson and Banzhaf have devised an efficient and economic method for the concentration of diphtheria antitoxin.

[8] Marcus, E., *Ztschr. f. physiol. Chem.*, 1899, xxviii, 559.

It is now generally accepted that the antibodies occurring in immune serum are protein in character and are not readily dissociated from the protein of the serum in which they occur. Pröscher's[9] attempt to produce a non-protein antitoxin by digestion of antidiphtheria serum with trypsin has not been confirmed. Banzhaf[10] and Mellanby found that the ratio of protein digestion and antibody destruction are approximately the same. Seng, Joachim,[11] Atkinson,[12] Ledingham,[13] and Banzhaf found that certain quantitative changes occur in the serum proteins of animals immunized to bacterial toxins, and that the increase in the globulin content bears a marked relation to the increase in antitoxic potency of the serum. Landsteiner[14] has shown that the antitryptic action of normal serum is associated with the albumin fraction, and Opie and Barker[15] have found antileucoprotease in the same fraction.

The distribution of immune bodies in the blood serum of different animal species varies. Pick[16] found that in immune horse serum diphtheria antitoxin was associated with the pseudoglobulin, while in goat serum it is present in the euglobulin. The lysins and agglutinins of cholera occur in the euglobulin fraction of the serum of immunized goats and horses. Typhoid agglutinins in the immune serum of the horse are found in the pseudoglobulin, while in goats, rabbits, and guinea pigs they occur only in the euglobulin. Hartley[17] has recently shown that the immune bodies of antirinderpest serum are present in the euglobulin fraction of bovine serum.

The present work on the distribution of the immune bodies of antipneumococcus serum is confined to a study of their occurrence in the blood serum of horses immunized to types I and II of the pneumococcus. The sera were produced by repeated intravenous injections of these organisms, beginning with dead bacteria and

[9] Pröscher, *München. med. Wchnschr.*, 1902, xlix, 1176.
[10] Banzhaf, E. J., *Bull. Johns Hopkins Hosp.*, 1911, xxii, 106.
[11] Joachim, J., *Arch. f. d. ges. Physiol.*, 1903, xciii, 558.
[12] Atkinson, J. P., *Jour. Exper. Med.*, 1900–01, v, 67.
[13] Ledingham, J. C. G., *Jour. Hyg.*, 1907, vii, 65.
[14] Landsteiner, K., *Centralbl. f. Bakteriol., 1te Abt., Orig.*, 1900, xxvii, 357.
[15] Opie, E. L., and Barker, B. I., *Jour. Exper. Med.*, 1907, ix, 207.
[16] Pick, E. P., *Beitr. z. chem. Phys. u. Path.*, 1902, i, 351.
[17] Hartley, P., *Memoirs of the Department of Agriculture*, India, 1913–14, i, 177.

progressively increasing the doses, until the animals were able to tolerate the bacterial residue of a liter of live virulent pneumococcus injected directly into the blood stream. The serum was obtained from horses which had been immunized over periods of one to two years. The method employed in obtaining antipneumococcus serum differs entirely from that used in the production of antitoxins. In the former, the immunizing response is provoked by a live virulent organism, while in the latter the stimulus is in the nature of a bacteria-free toxin. The immunity mechanism involved in the elaboration of specific antipneumococcus substances may or may not differ physiologically from that concerned in the production of antitoxins, but the types of antibody evoked in each instance are distinct, and their distribution in the serum globulins is different.

SUMMARY.

The immune bodies of antipneumococcus serum are completely precipitated by 38 to 42 per cent. saturation with ammonium sulphate.

They are incompletely precipitated by (a) ammonium sulphate in less than 38 per cent. saturation, (b) saturation with sodium chloride, (c) dilution and saturation with carbon dioxide, (d) removal of crystalloids by dialysis.

The immune bodies of antipneumococcus serum are, therefore, associated or combined with that fraction of the globulins precipitated by 38 to 42 per cent. saturation with ammonium sulphate. The immune body fraction does not correspond exactly with the ordinary euglobulin (one-third saturation with ammonium sulphate or complete saturation with sodium chloride) or with the insoluble globulins precipitated by carbon dioxide or dialysis. These fractions carry with them only a part of the immune bodies.

Neither the albumin nor that fraction of the globulin not precipitated by 38 to 42 per cent. saturation of ammonium sulphate contain any of the demonstrable antibodies.

The most promising method for the practical purification of the immune bodies occurring in antipneumococcus serum appears to be precipitation by 38 to 42 per cent. saturation with ammonium sulphate.