the two animals. The rabbit antiserum showed no evidence of a new protein component, but there was an increase in the amount of γ-globulin to 56 per cent. of the total protein, as compared with 17 per cent. in normal serum (determinations by the scale method). Comparison with the electrophoretic diagrams obtained from the absorbed serum showed that specific precipitation removed 35 per cent. of the γ-globulin, whereas the other components were not markedly affected. 19.6 per cent. of the total protein concentration was thus accounted for as antibody, in excellent agreement with 18.6 per cent., the value obtained by direct analysis for antibody nitrogen.

The horse antiserum, however, showed a very strong new component, migrating between the β- and γ-globulins, and this was no longer present in the sample from which antibody had been removed (Fig. 1). The

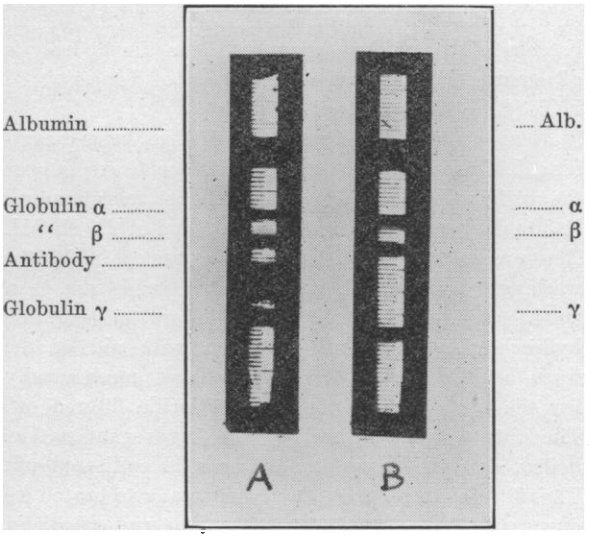

FIG. 1. Electrophoretic diagrams of unabsorbed (A) and absorbed (B) antipneumococcus horse serum.

mobilities of the other components in the absorbed and unabsorbed sera were the same within experimental error as were the mobilities of normal sera in the same salt medium.

TABLE 1

MOBILITIES IN $cm^2\ sec^{-1}\ volt^{-1} \times 10^5$ OF MAIN PROTEIN COMPONENTS OF IMMUNE HORSE AND RABBIT ANTISERA. TEMP. 0° C. BUFFER 0.15M NaCl, 0.05M TOTAL PHOSPHATE

	Albumin	Glob. α	Glob. β	Antibody	Glob. γ
Horse serum pH 7.71 ..	5.5	3.7	3.0	2.1	0.9
Rabbit serum pH 7.50 ..	5.9	4.3	3.3	..	1.2

The data in Table 1 are consistent with the observations of Heidelberger and Pedersen[2] and indicate that pneumococcus anticarbohydrate produced by the horse exists as a new component, while the same antibody as produced by the rabbit is an addition to the normal γ-globulin component of serum.

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AGGLUTININS FOR HUMAN ERYTHROCYTES IN TYPE XIV ANTI-PNEUMOCOCCIC HORSE SERUMS[1]

IN the course of clinical trials with therapeutic antipneumococcic horse and rabbit serums, unusual reactions sometimes leading to death were encountered in occasional patients receiving anti-pneumococcus type XIV horse serums. In one patient who recovered, hemoglobinuria occurred soon after an intravenous injection of type XIV horse serum. Bullowa[2] also has encountered fatal reactions with therapeutic type XIV serums. Experiments carried out in an attempt to elucidate the mechanism of these reactions revealed that the serums of horses immunized against type XIV pneumococci have agglutinins in high titers for human erythrocytes of all four groups. Agglutinins in low titers for human erythrocytes of each of the blood groups have been observed in normal horse serums,[3] and agglutinins for group AB and A cells have been noted in rabbit serums.[4] The latter have been shown to be in the nature of Forssman's antibodies.

The results of some of the observations made thus far are summarized briefly.

(1) Every one of nineteen different specimens of type XIV anti-pneumococcic horse serum agglutinated human red blood cells of all four blood groups in dilutions of 1:80 to 1:2560 of the serums. These serums were obtained from three different laboratories and were produced by immunization with at least three different strains of type XIV pneumococci. They included monovalent and bivalent (some with type VI and others with type XIX), concentrated and unconcentrated serums. In the concentrated serums the titers of agglutinins for the blood cells of each group were higher than the corresponding titers in the unconcentrated serums from which they were prepared. Erythrocytes from different individuals of the same blood group were agglutinated to the same titer in the same type XIV horse serum. Hemolysins

[1] From the Thorndike Memorial Laboratory, Second and Fourth Medical Services (Harvard), Boston City Hospital, and the Department of Medicine, Harvard Medical School, Boston, Mass.

[2] J. G. M. Bullowa, "The Management of the Pneumonias." New York, Oxford University Press, 1937, p. 316.

[3] V. A. Herman, *Jour. Immun.*, 31: 347, 1936.

[4] G. H. Bailey and N. S. Shorb, *Am. Jour. Hyg.*, 13: 831, 1931; 17: 329 and 358, 1933; C. A. Stuart, *et al.*, Jour. Immunol., 31: 25, 1936; O. Thomsen, *Ztschr. Immunitatsforsch.*, 87: 335, 1936; M. Eisler, *ibid.*, 88: 240, 1936.

for human erythrocytes could not be demonstrated in these serums by the usual methods.

(2) Among 41 samples of anti-pneumococcic horse serums of types other than type XIV, only two agglutinated human erythrocytes in a dilution of 1:20 or higher. One concentrated bivalent type V and VII horse serum and one unconcentrated type IX horse serum agglutinated human group B erythrocytes in dilutions of 1:40 and 1:20, respectively. These forty-one serums included at least one specimen of serum for each of the twenty-nine other available specific pneumococcus types. Sixteen of them were concentrated preparations.

(3) Ten samples of different type XIV anti-pneumococcic rabbit serums were obtained from the same three laboratories as supplied the horse serum. These rabbit serums were prepared with the same strains of type XIV pneumococci as were used in making the type XIV horse serum. None of these rabbit serums agglutinated human group B or group O cells (1:5 dilution was the lowest tested), but four agglutinated both AB and A human erythrocytes in dilutions of 1:20 to 1:80. Among twenty rabbit anti-pneumococcic serums of eleven types other than type XIV, eight showed agglutinins for human AB and A in titers of 1:10 to 1:160, but none agglutinated either group B or group O cells. The agglutinins for groups AB and A human erythrocytes in the various anti-pneumococcic rabbit serums, both type XIV and those of other types, were associated with agglutinins and hemolysins for sheep erythrocytes and could be absorbed with sheep red blood cells.

(4) After sufficient absorption with type XIV pneumococci to remove the homologous pneumococcus agglutinins, no agglutinins could be demonstrated, in the type XIV anti-pneumococcus horse serums, for human erythrocytes of any of the four blood groups. Absorption with human erythrocytes of each of the four groups completely removed the agglutinins for human red blood cells of the homologous and of the three heterologous blood groups, but left the type XIV pneumococcus agglutinins essentially unchanged. Large amounts of erythrocytes were necessary to carry out these absorptions.

The details of these observations and of further experimental studies will be reported elsewhere.

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PREPARATION OF PURE D-ARGININE

ALTHOUGH the best method for preparation of pure arginine derivatives appears to be through the flavianate isolation, as introduced by Kossel and Gross,[1] and subsequently improved[2, 3] to give a high-purity arginine hydrochloride, none of these papers presents details for the obtainment of free base d-arginine. Another article[4] has described an unsuccessfull attempt to convert the hydrochloride into pure arginine base.

The essential precautions for obtaining pure d-arginine from the hydrochloride now appear to be the choice of a satisfactory protein source, and the removal of arginine-silver complex from the final free arginine solution, since the arginine-silver complex is soluble in solutions of arginine.[5] Satisfactory protein sources include salmine and gelatine of U. S. P. grade or better. Casein, hog's blood and defatted canned sardine spermatic tissue were found to be unsatisfactory.

For the preparation of d-argininium chloride, the classical method of Brand and Sandberg[3] was followed, except that it was imperative to dilute 39 ml of 5 N HCl in 300 ml of water in hydrolyzing 50 gm batches of benzylidene arginine. For the hydrolysis of commercial salmine, seven parts of concentrated HCl (S.G. 1.19) sufficed.

In an exemplary preparation of free base d-arginine, 8.03 gm of silver nitrate (3 per cent. excess over the arginine hydrochloride requirement) was dissolved in 50 ml of water and treated with 50 ml of 2N NaOH. The precipitated silver oxide was washed until the washings were neutral, and the silver oxide was then transferred to a solution of 9.66 gm of arginine hydrochloride in 50 ml of water, with aid of the wash-bottle. The mixture was stirred mechanically for ten minutes, and the silver chloride filtered off. The filtrate was saturated with hydrogen sulfide, boiled, and the coagulated silver sulfide filtered off. The filtrate from this operation was evaporated to dryness under reduced pressure on the water bath in a stream of carbon dioxide-free air. The residue was dissolved in 20 ml of boiling water, and placed in a desiccator over sodium hydroxide. When the solution had cooled, the desiccator was evacuated. The yield was 7.84 gm of arginine, melting at 228° (corr.) with dec. This corresponds to 98 per cent. recovery from the hydrochloride. Recrystallization from 16 ml of hot water and 40 ml of freshly boiled absolute ethanol gave a 96 per cent. recovery.

No difficulty was experienced in crystallizing arginine prepared from salmine or gelatine. One crystal of 3 cm length was obtained by slow evaporation of the solvent during two weeks. Arginine solutions

[1] A. Kossel and R. E. Gross, *Zeit. Physiol. Chem.*, 135: 167, 1924.

[2] P. Brigl, R. Held and K. Hartung, *Zeit. Physiol. Chem.*, 173: 151, 1928.

[3] E. Brand and M. Sandberg in F. C. Whitmore, "Organic Syntheses," 12: 4, 1932.

[4] E. Schulze and E. Steiger, *Zeit. Physiol. Chem.*, 11: 43, 1887.

[5] W. Gulewitsch, *Zeit. Physiol. Chem.*, 27: 178, 1899.