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# An Electrophoretic Study of Tetanus Antitoxin Sera.

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In a previous paper[1] we described an electrophoretic analysis of several antipneumococcal horse sera that showed antibody activity in the γ-component. Since then 2 of the horses have been used to produce tetanal antitoxin. Subsequent electrophoretic analyses detected the appearance of a new component apparently associated with the freshly developed antitoxic activity.

Early in September, 1939, horses No. 9514 and No. 6225 began to receive tetanal toxin instead of Types 1 and 2 pneumococcal antigens. Three months later they had high antitoxin-titers. Electrophoretic analyses of sera taken then, and before immunization with toxin had begun, are reproduced in Figs. 1 to 4. Only traces of antipneumo-

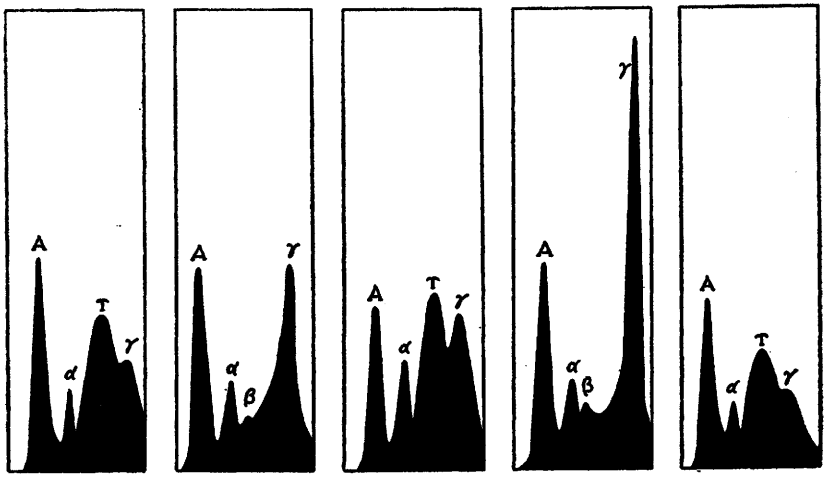


FIG. 1. FIG. 2. FIG. 3. FIG. 4. FIG. 5.

FIG. 1. Electrophoretic diagram of serum tetanal antitoxin from horse No. 9514. All sera were diluted 1:3 and dialyzed to equilibrium with a pH 7.6, 0.02M phosphate buffer that was also 0.15M with respect to NaCl.

FIG. 2. Diagram of antipneumococcal serum from No. 9514 before antitoxic immunization commenced.

FIG. 3. Diagram of serum tetanal antitoxin from horse No. 6225.

FIG. 4. Diagram of antipneumococcal serum from No. 6225 taken before antitoxic immunization began.

FIG. 5. Diagram of antitoxic serum from horse No. 7686 which had been undergoing hyperimmunization for 2½ years.

[1] Moore, D. H., van der Scheer, J., and Wyckoff, R. W. G., *Science*, 1939, **90**, 357.

coccal precipitin could be found in the sera taken in December and areas underlying the γ-peaks had obviously decreased greatly. With the development of antitoxin there had appeared, however, a strong new component (labeled T) with a mobility, $-2.2 \times 10^{-5}$ cm$^2$ sec$^{-1}$ volts$^{-1}$, midway between β and γ. The same component was present in the strongly antitoxic serum of horse No. 756, which had never been used before for antibody-production, and in the sera of other horses producing tetanal antitoxin.

An antipneumococcal component of the same mobility was found by Tiselius and Kabat[2] in an antipneumococcal horse serum. We[1] did not observe this component in our antipneumococcal sera but inasmuch as our sera came from horses long subject to hyperimmunization we suggested that their new component might possibly be a characteristic of serum from freshly immunized horses. This, however, cannot explain the presence of the new component in the sera containing tetanal antitoxin because the serum from horse No. 7686, which has been on antitoxin production for 2½ years, also showed T as its principal component (Fig. 5). We have recently examined serum from horse No. 9514, which had been on antipneumococcal serum-production for one year; like the sera from long-immunized animals its antibody was in the γ-component (Fig. 2).

2 Tiselius, A., and Kabat, E. A., *Science*, 1939, **87**, 416; *J. Exp. Med.*, 1939, **69**, 119.