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**A note on the preparation of anti-colon streptococcus serum.**

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About two years ago, Dr. H. A. Cotton requested that I should undertake for him the preparation of a combined Anti-Colon Streptococcus Serum prepared from horses treated with cultures of *B. Coli* and *Streptococci* isolated from cases under treatment at the New Jersey State Hospital, Trenton, N. J.

A certain number of cases under treatment at that institution have been found to yield pure cultures of streptococci of diverse types and also strains of *B. Coli*. These organisms are obtained from material removed at operation such as tonsils, extracted teeth, lymph glands, especially those found in the mesentery of the colon, and other material from the abdominal cavity.

The treatment of the horse was with eleven strains of *B. Coli* and eight strains of streptococci, most of which belonged to the hemolytic group.

The immunization was begun with small doses of the killed organisms, but after several weeks the use of live cultures was adopted. It was found that the injections of the mixed antigens of the two bacteria were followed in some instances by severe reactions in the horse and that when the antigens were given on different days that the colon antigens were not tolerated as well as the streptococcus antigens. The injection of the colon bacilli induced such violent reactions in some instances that recourse was finally had to the method of preparing the antigen suggested by the author for the preparation of anti-meningococcic and anti-pneumococcic sera.

The plan of treatment as finally decided upon was the injection of washed broth cultures of streptococci and washed agar cultures of colon bacilli. The two antigens were suspended in salt solution and given intravenously for four injections on successive days followed by a rest period of three days when the injections with gradually increasing amounts were repeated.

After the horse had been under treatment for about 8 weeks, a trial bleeding was made and the titre of the serum determined by the use of the agglutination test as used by the Hygienic Laboratory for the testing of Anti-Meningococcic Serum. Based on the results of the tests of the trial bleedings, an adjustment was made in the proportion of the individual components of the mixed antigen in order that the serum should be as well balanced as possible.

It was found, however, that in spite of all efforts to promote the formation of antibodies to each strain of bacteria used in the injection of the horse that agglutinins, at least, were never produced in measurable amounts to a few strains. This of course is not unique as many have found that some strains of bacteria do not induce antibody formation.

A few weeks after the trial bleeding the horse was bled for production by taking 6 liters of blood six days after the last injection and 6 liters 48 hours later. The serum from the two bleedings was combined, four tenths of one per cent. tricresol added, filtered through Berkefeld filters, tested for sterility and for certain antibodies.

It was found that the serum agglutinated all of the strains of *B. Coli* except two in dilutions from 1-100 to 1-400 and that all of the strains of streptococci were agglutinated in dilutions from 1 to 200 to 1 to 400.

In addition complement fixation tests with the serum gave fixation but not to a high degree with both the hemolytic and non-hemolytic groups of streptococci.

Protection tests in animals have not been made but it is believed that sufficient data has been accumulated in the literature to justify the advance of the opinion that a properly prepared anti-streptococcic serum will afford a considerable measure of protection against streptococci if given 12 to 18 hours before the bacteria are injected. There is not the same data for an anti-colon serum as but few attempts have been made to make and use such a serum.

While most are agreed as to the possibility of giving protection to animals by the previous administration of an anti-streptococcic serum, there is considerable difference of opinion as to the curative value of such a serum, although the weight of opinion is in its favor.

Some of the failures of anti-streptococcus serum may have been due to the fact that the particular strain of streptococcus causing the infection was not among those groups used for the immunization of the horse. The importance of this was not realized until comparatively recently and it is possible that further work may show that a potent anti-streptococcic serum can be prepared for each strain.

The pathogenicity of the colon bacillus for laboratory animals shows much variation as the intravenous administration of some strains in small amounts sometimes quickly results in death, while large amounts of other strains are well borne.

The symptoms and fatal results are probably due to the action of toxins or poisons contained in the body of the bacteria.

The colon bacillus has been claimed to be the cause of a variety of conditions in various parts of the body, particularly in the region of the abdominal cavity, but some observers have thought it questionable if the colon bacillus was the primary cause of the lesions due chiefly to the observation that it is not always found in pure culture.

The growth of the colon bacillus is not attended with the production of a soluble toxin or poison of a high degree and therefore the injection of filtrates into animals of broth cultures is not followed by marked antibody formation, but if animals such as the rabbit or horse are given gradually increasing doses of killed or live cultures there quickly appear in their blood antibodies such as bacteriolysins, agglutinins, precipitins, and complement-fixing substances.

The fact that antibodies are produced in high degree in immunized animals gives support to the opinion of Dr. Cotton that a combined Anti-Colon Streptococcus Serum is of value in preventing post-operation infections due to colon bacilli or streptococci and perhaps may be of value in the treatment of infections by those organisms already developed.