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PRODUCTION OF MONOVALENT BOTULINUS ANTITOXIC SERUM TYPES A AND B

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Monovalent botulinus antitoxic horse serums types A and B have been prepared in this laboratory for prophylactic and therapeutic purposes. Although only a small number of animals, five horses, were used in this work, a record of the results of the methods employed is of interest and illustrates how differently horses may respond to immunization with these toxins.

Kempner (1), Forssman and Lundstrom (2), Graham and Brueckner, (3), and Dickson and Howitt (4) have reported on the production of fairly potent monovalent antitoxic goat serums. Kempner lost one goat out of three with chronic botulism. Dickson and Howitt found that the three goats used varied so in their reactions to the toxins that no stated method of immunization could be followed.

Leuchs (5) and Hart and Hayes, (6) produced monovalent horse serums, but methods of testing the toxins and serums vary so much that a comparison of the results is difficult.

Bengston (7) made tests on a limited number of polyvalent antitoxic serums according to the standard of the United States Hygienic Laboratory and concluded that it is apparently difficult to produce satisfactory polyvalent serums, antitoxin of one or the other type usually predominating. The serums tested contained from 2 to 40 units type A antitoxin, the type B antitoxin being much stronger. The highest titer obtained was 450 units.

TOXIN PRODUCTION

The type A toxin used was prepared with strain A 16 received from Prof. M. J. Rosenau of Harvard University.

Two type B strains were used for producing toxins: one the Nevin strain, isolated by M. Nevin in this laboratory in 1914, the other a strain received from Professor Rosenau.

The regular van Ermengen broth was used with the following slight modification. In preparing the medium, the dextrose was omitted and the broth adjusted to pH 8.4 before sterilization, thus avoiding the increase in acidity which occurs during the sterilization of an alkaline dextrose medium. Sufficient 20 per cent dextrose solution to make a final 2 per cent was added at the time of inoculation.

Liter flasks of the broth inoculated with twenty-four to forty-eight hour broth cultures of the organisms were incubated at 33° to 35°C. for five weeks. The toxins were then preserved with 0.5 per cent phenol and filtered through pulp and finally through a filter candle to insure sterility. The potency of the toxins was determined by the subcutaneous method, according to the standard of the United States Hygienic Laboratory, the minimal fatal dose being the amount of toxin which, when injected subcutaneously, will kill a guinea-pig weighing 250 grams in about ninety-six hours.

ANTITOXIN PRODUCTION

The monovalent antitoxic serums were produced by subcutaneous inoculation of horses with the homologous toxin. All but one of the five horses used had at times during the previous three or four years been under immunization with other toxins or bacterial antigens but had been allowed to rest for several months and were in good physical condition when immunization with botulinus toxins was begun. The titer of the serums was determined by testing against a standard toxin according to the method prescribed by the United States Hygienic Laboratory in which one-tenth unit of antitoxin protects against one test dose of toxin (about 100 M.F.D.'s). Bleedings of eight to nine liters were taken when the titer was sufficiently high. The serums were preserved with 0.3 per cent cresol and filtered, after three or four weeks, through a filter candle to insure sterility.

During the preparation of these serums, three methods of immunization varying slightly from one another were employed.

Method I. Frequent injections of toxin with rapid increase in dosage: In this method, used with type B toxin of one strain, subcutaneous injections of toxin were given at three-day intervals starting with an initial dose of $\frac{1}{20}$ M.F.D. of toxin and increasing the amount 40 to 50 per cent at each succeeding injection until the dose was 50 cc. Increases of 20 to 50 cc. of toxin were then made till the maximum dose of 500 cc. was reached. For the small doses, toxins of low potency M.F.D. 0.005 to 0.001 cc. were used. Later, toxins of higher potency were substituted. The first ten doses given by this method were $\frac{1}{20}$, $\frac{1}{5}$, 1, 2, 5, 10, 20, 50, 75, 100 M.F.D. of toxin. Three months after immunization was started the titer of the serum was about one unit of antitoxin per cubic centimeter. The maximum dose of toxin given thus far was 60,000 M.F.D. (60 cc. of toxin M.F.D. 0.001 cc.). At the end of five months when bleedings were taken, the titer of the serum was 150 units of antitoxin per cubic centimeter. At this time 500 cc. of toxin M.F.D. 0.0001 cc. were being given.

This same method was used in beginning the immunization of two horses with type B toxins of the other strain. The animals died with typical symptoms of botulism after the tenth and twelfth doses of toxin (100 to 140 M.F.D.). Another horse was then given repeated injections of 25 M.F.D. of toxin at three-day intervals to determine the cumulative effect of sublethal doses. Two days after the sixth dose, the horse developed typical symptoms of botulism and was killed.

Since apparently some horses would not respond readily to rapidly increasing doses of botulinus toxin, the method of immunization was modified.

Method II. Slow increase of dosage with intervals of rest: This method was used for immunizing one horse with type A toxin. A series of two doses of toxin was given on successive days followed by a rest of seven days. After the first two series of doses of $\frac{1}{20}$ and $\frac{1}{5}$ M.F.D. and 1 and 2 M.F.D. of toxin, the increase in the amount of toxin given in any one series was very gradual, not more than 5 to 10 M.F.D. Four months after immunization was started the serum had a slight protective action but the titer was still less than $\frac{1}{10}$ unit of antitoxin per cubic centimeter. At the end of five months the total amount of toxin given in the two doses of a series was 300 M.F.D. or 0.3 cc. of toxin M.F.D. 0.001 cc. The titer of the serum was between 1 and 10 units per cubic centimeter.

The doses of toxin were then increased more rapidly. At the end of seven months the titer of the serum was less than 50 units of antitoxin per cubic centimeter. The horse was in poor physical condition and was given a rest during which time he died but there was no evidence of botulism.

Method III. Frequent injections of toxin with slow increase in dosage: This method was tried with one horse immunized with type A toxin. Doses of toxin were given every third or fourth day as in the first method, but the amount of toxin given at each injection was increased very gradually. Thus, starting with an initial dose of $\frac{1}{20}$ M.F.D., the first ten doses of toxin were $\frac{1}{20}$, $\frac{1}{10}$, $\frac{1}{5}$, 1, 2, 4, 6, 8, 12 M.F.D. This gradual increase in the doses of toxin was continued for the first three months at which time the maximum dose given was 390 M.F.D. or 0.39 cc. of toxin M.F.D. 0.001 cc. The titer of the serum at this time was a little less than 1 unit of antitoxin per cubic centimeter. The doses of toxin were then increased rapidly, increases of 40 to 50 per cent being given as in method I, till the dose of toxin reached 50 cc., then increases of 20 to 50 cc. up to the maximum dose of 500 cc. The titer of the serum six months after immunization was started, when bleedings were taken, was 1400 units of antitoxin per cubic centimeter. Toxin of higher titer, M.F.D. 0.0002 cc., was substituted for the 0.001 cc. toxin but there was no marked increase in the titer of the serum up to the time immunization was discontinued two months later.

While the number of horses immunized by any of the above methods was so small that no definite conclusions can be drawn as to the relative value of any one method, the results suggest that the third method, of frequent injections of toxin with slow increase of dosage, may have certain advantages over the other two, since very potent serums can be produced by this method and there is less danger of animals succumbing during treatment.

SUMMARY AND CONCLUSIONS

Potent monovalent botulinus antitoxic serums, types A and B, were produced by immunization of horses with homologous botulinus toxins. The maximum titer of 1400 units per cubic centimeter was obtained with type A toxin.

Not all horses respond to such rapid increases in the doses of botulinus toxin as were given in the first method of immuniza-

tion described. Two of the three horses immunized by this method succumbed after the tenth or twelfth dose with typical symptoms of botulism.

Antitoxin production with the one horse immunized by the second method (slow increase of dosage with intervals of rest) was slower than with the horses immunized by the other methods.

The small doses of toxin given at the beginning in the third method of immunization (frequent injections of toxin with slow increase in dosage) apparently stimulated antitoxin production as readily as the larger doses used at the beginning in the first method (frequent injections of toxin with rapid increase in dosage) and with less danger to the animal.

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