THE PRODUCTION AND TITRATION OF POTENT HORSE ANTIPNEUMOTOXIN

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In a previous communication[1] it was shown that certain anaerobically produced autolysates of pneumococci contain a poison which produces marked lung lesions when injected intratracheally into small guinea pigs. In a later paper,[2] this poison was shown to be neutralized by the serum of rabbits immunized thereto. These rabbit serums were of relatively low potency, containing approximately 50 neutralizing units per cubic centimeter (0.1 cc. of serum neutralized 5 lethal doses of the autolysate). In the present paper, we wish to report on the production of high titre anti-autolysate serums in the horse[3] and give in detail the method used for their titration.

For the sake of clearness, in this and subsequent papers, the lung-toxic poison will be called "pneumotoxin" and the neutralizing serums for this poison, "antipneumotoxin." We have as yet no absolute proof that the lung-toxic poison is antitoxinogenic, but apparently it is, as it fulfils most of the accepted characteristics of this class of poisons.

EXPERIMENTAL

For the last 8 months, two horses,—Nos. 725 and 726,—have been undergoing treatment with increasing doses of sterile filtrates of the pneumotoxin from Types I, II and III. The first injections of these filtrates were in doses of 0.5 cc. to 1 cc. and produced a marked rise in temperature. For the last 3 months, each of these horses has been

[1] Parker, J. T., and Pappenheimer, A. M., *J. Exp. Med.*, 1928, **48**, 695.

[2] Parker, J. T., *J. Exp. Med.*, 1929, **49**, 695.

[3] These horses were immunized to the autolysate filtrates at the Eli Lilly Company in Indianapolis, under the supervision of Mr. W. A. Jamieson.

receiving 400 cc. of the toxic autolysates every 3 or 4 days, without obvious reactions of any kind. Horse 787 has been receiving increasing doses of the pneumotoxin from pneumococcus Type I alone. Horse 382 had been receiving a variety of pneumococcus products, consisting of vaccines, broth cultures of the live pneumococcus, etc., since December 1925, but in November 1928, it was started on the pneumotoxin from Types I, II and III. Horse 685 had been on treatment with pneumococcus vaccine alone since December 1927, but in January 1929 these were discontinued and it was started on inoculations of pneumotoxin from pneumococcus Types I, II and III.

Method of Titration of the Serums for Neutralizing Antibodies to the Lung-toxic Poison

The tests for the presence of neutralizing antibodies were carried out as follows:—Dilutions of the serums in broth were first prepared. 0.1 cc. of the various serum dilutions, or broth instead of the serum dilutions, were pipetted into separate precipitin tubes which were kept in ice water. To each tube 0.9 cc. of chilled pneumotoxin was added and the contents immediately mixed. Heavy vaseline seals were then added to all the tubes, care being taken to avoid the formation of bubbles between the surface of the mixture and the vaseline. The tubes were then left at room temperature in the dark for 2 hours. After this, they were again placed in ice water, the vaseline seals removed and the contents of each tube pipetted into a chilled Wassermann tube. One or two guinea pigs were then injected intratracheally with 0.2 cc. of each preparation. To avoid the danger of oxidation by exposure to air, the vaseline seals were not removed until immediately before the inoculation of the guinea pigs. Guinea pigs weighing from 190 to 210 gm. were used in all the experiments.

Pneumotoxin from Types I, II or III were employed, autolysates from one type only being used in each experiment. These toxins were of such strength, that 0.2 cc. of mixtures of 0.9 cc. of toxin and 0.1 cc. of broth (0.18 cc. of toxin) invariably killed guinea pigs of 200 to 210 gm. in less than 24 hours with typical symptoms and autopsy findings.

One unit of toxin is the amount which, when injected intratracheally, will kill a guinea pig weighing 200 to 210 gm. in from 4 to 24 hours,

with typical symptoms and autopsy findings; while one unit of antitoxin represents the smallest amount of serum which is necessary to protect a guinea pig of the same weight against one unit of toxin when the mixture is injected intratracheally.

Results from These Neutralization Experiments

The strength of the horse serums in neutralizing antibodies for the lung-toxic poison appears to depend quite regularly on the amount of pneumococcus toxic autolysate the horses have received. The serums of Horses 725 and 726, the horses most highly immunized to the toxic autolysates, have shown a progressive and rapid increase in their content of neutralizing antibodies during the last 3 months (an increase of from 5000 to 40,000 units).

On the other hand, serums from several bleedings from Horse 787, which had recently been started on pneumococcus autolysate injections, and from Horses 382 and 685, which had been treated with a variety of pneumococcus products for several years and only recently with injections of toxic autolysates, all showed relatively small amounts of neutralizing antibodies. However, recent tests carried out with a concentrated preparation from the latest bleedings from Horses 382 and 685, show an increase in these antibodies. (See Table I.)

Effect of Normal Horse Serum, Antipneumococcus Horse Serum or Antipneumococcus Horse Serum Concentrated by the Felton Method[4] on the Lung-Toxic Autolysates

Neither normal horse serum nor unconcentrated antipneumococcus horse serum containing 500 protective units per cubic centimeter to Pneumococcus I had any detoxifying effect on the toxin when used in 1–10 dilution—0.1 cc. of serum added to 0.9 cc. of toxin—and set up under the same conditions as the other mixtures. The antipneumococcus serum concentrated by the Felton method and containing 1000 protective units per cubic centimeter to Pneumococcus I, detoxified the toxin when used in 1–20 dilution, but had no effect on the toxin when used in 1–50 dilution.

[4] We are indebted to Dr. William Park of the New York City Board of Health for these serums.

Experiment I. Table I

Serum Tested:

787. 4th bleeding.
725. 7th bleeding.
31222. Concentrated preparation from 6th bleeding of horses 725 and 726.
31221. Concentrated preparation from the 86th bleeding of Horse 382 and the 13th bleeding of Horse 685.

The in Vitro Neutralization of Lung-Toxic Autolysates with Autolysate Horse Serums

Pig No.	Weight	Serum tested	Final dilution of serum	Symptoms	Died or survived	Amt. consolidation in lungs	Calculated units of antitoxin per cc.
350	192	787	1–50	0	S.		
312	194	787	1–100	++	D <18 hrs.	+++	250
327	208	787	1–200	+++	D <18 hrs.	+++	
317	192	725	1–1000	0	S.		
308	206	725	1–2000	0	S.		20,000
346	190	725	1–4000	0	S.		
315	206	725	1–8000	+++	D <18 hrs.	++	
322	190	31222	1–2000	0	S.		
311	192	31222	1–4000	0	S.		20,000
314	204	31222	1–8000	++	D <48 hrs.	++	
321	206	31222	1–8000	+++	D <18 hrs.	+++	
325	190	31221	1–200	0	S.		
323	202	31221	1–400	0	S.		2,000
349	202	31221	1–1000	+++	D <40 hrs.	+++	
324	204	31221	1–2000	++	S.		
307	210	—	—	+++	D <18 hrs.	++	
309	208	—	—	+++	D <18 hrs.	++	
358	206	—	—	+++	D <18 hrs.	+++	

This table is self explanatory.

Table I shows the results of a typical experiment made to determine the strength of these neutralizing substances in the serum of immunized horses. There is one discrepancy in this experiment, in that Guinea pig 349, which received twice as much of Serum 31221 in its toxin-serum mixture as did Guinea pig 324, died; while the latter pig, although extremely sick for several days, survived. In tests carried out with the greater serum dilutions, discrepancies of this sort occa-

sionally occur. We believe that these discrepancies are due to the greater susceptibility to the toxin of a small percentage of pigs (approximately 10%), in which instances the lethal dose of toxin may be $\frac{1}{2}$ to $\frac{1}{4}$ the usual lethal dose. Therefore, for accurate results in the titration of a serum, it is necessary to use several pigs for each of the final dilutions of serum.

In a previous paper, it was shown that the pneumotoxin for Types I and II were antigenically similar. This experiment, Table I, brings out a point which we have recently confirmed several times, *viz.*, the pneumotoxin from Pn. Type III is also antigenically similar to toxins from Types I or II. (See Pig 350 in the table.)

Toxin used was a toxic autolysate from Pn. Type III. 0.9 cc. of the toxin was added to 0.1 cc. of the diluted serum, or to 0.1 cc. of broth. After standing for 2 hours at room temperature under vaseline seal, guinea pigs were injected intratracheally with 0.2 cc. of the mixtures.

CONCLUSIONS

1. The serum of horses immunized with increasing doses of certain anaerobically produced autolysates of pneumococci contain potent neutralizing antibodies for the pneumotoxin.

2. The method for the *in vitro* titration of these horse antipneumotoxic serums is given.