

THE INFLUENCE OF CONCENTRATION (GIBSON'S
METHOD) ON THE PRESENCE OF TETANUS
TOXIN IN BLOOD SERUM.¹

By JOHN F. ANDERSON.

Passed Assistant Surgeon, and Assistant Director, Hygienic Laboratory, United States Public Health and Marine-Hospital Service, Washington, D. C.

A number of workers have observed the very interesting fact that during the period of incubation of tetanus, and in some cases during the early symptoms, in certain animals tetanus toxin is present in the circulating blood.

Perhaps the most notable of these observations was that recorded by Bolton, Firch and Walden² in their report on the cases of tetanus, including seven deaths therefrom, following the use of diphtheria antitoxin, in St. Louis, in 1901.

These observers found that the serum from a certain horse which was bled for diphtheria antitoxin three days before it was killed, on account of having tetanus, contained tetanus toxin. They found that 0.1 c.c. of this serum contained sufficient toxin to cause the death of guinea-pigs and that 10 c.c. had caused the death of children.

The great importance of this danger has been fully recognized and guarded against by all producers of antitoxic sera since that time. It is now as much a routine practice of antitoxin producers to make what is known as safety tests as to make potency tests. A safety test is made by injecting about 5 c.c. of the serum into guinea-pigs and observing the animal for some days, particularly for symptoms of tetanus. Moreover, as one of the causes of the St. Louis accident was the use of serum a few days after being collected and before tests had been made, most producers rarely market serum under six months old.

¹Received for Publication, May 27, 1909.

²Bolton, B. Meade, Fisch, C., and Walden, C. C., Report of the commission to investigate the cases of tetanus in St. Louis following the administration of diphtheria antitoxin, *St. Louis Med. Rev.*, 1901, xlv, 361-368.

A repetition of the St. Louis occurrence is most improbable, for all producers of antitoxin have thrown every possible safe-guard around the production and testing of sera, both for their own protection and on account of the stringent requirements of the Public Health and Marine-Hospital Service.

Since the concentration of antitoxin by the Gibson process or Banzhaf's modification has come into such general use there has been a feeling on the part of some that the process removed all the toxic impurities, among them tetanus toxin, which might be in the untreated serum as a result of accidental infection of the animal which furnished the serum.

I have (lacking experimental proof in its favor) never been convinced that this belief was correct and have insisted that horses with infections, such as severe abscesses due to cocci, should not be used to furnish antitoxin, even if the serum was to be concentrated.

A short time ago one of the sheep at the Hygienic Laboratory developed tetanus from an accidental wound and I had it bled to death from the jugular vein on the beginning of the second day of the disease. Tests of the toxicity of the sheep's serum showed that 2 c.c. would kill a 250-gram guinea-pig with tetanus in about four days, and that amounts less than 1 c.c. would cause severe symptoms of tetanus in guinea-pigs.

In order to determine if the concentration of the serum by precipitation with ammonium sulphate and acetic acid would destroy the tetanus toxin in it, about 60 c.c. of the serum was put through the Gibson process. After the dialysis was completed there remained about 10 c.c. of the globulin solution. This was tested for the presence of tetanus toxin by subcutaneous injection into guinea-pigs as follows:

Table Showing Toxicity of the Concentrated Serum.

Number of guinea-pig.	Weight of pig.	Amount of concentrated serum.	Equivalent in native serum.	Result.
1	330	1.0 c.c.	6.0 c.c.	Slight symptoms of tetanus sixth day.
2	325	1.0 c.c.	6.0 c.c.	Slight symptoms of tetanus sixth day.
3	340	2.0 c.c.	12.0 c.c.	Marked symptoms of tetanus sixth day.
4	325	2.0 c.c.	12.0 c.c.	Marked symptoms of tetanus sixth day.
5	350	4.0 c.c.	24.0 c.c.	Marked symptoms of tetanus third day.

From the table it will be seen that while a part of the tetanus toxin in the serum had been removed during the concentration and dialysis, there was sufficient left to cause marked symptoms in a dose of 2 c.c.

This shows very plainly that concentration of serum by precipitation with ammonium sulphate and acetic acid, followed by dialysis, does not remove all of the toxic impurities that may be in serum, and that the foreign toxin is probably carried with the anti-toxin in the globulin fraction.

The sheep serum with which I worked did not contain a very large amount of tetanus toxin, as it required 2 c.c. to kill a 350-gram guinea-pig; this was probably due to the fact that the serum was collected after the onset of the symptoms of tetanus and Bolton and Fisch³ have shown that the maximum amount of tetanus toxin is present during the period of incubation, and before the onset of the symptoms. I now have another lot of serum containing more tetanus toxin for further work, and am engaged in a study of the behavior of the toxin in serum when concentrated by the Banzhaf modification of the Gibson process. In the Banzhaf method the serum is heated at from 55° to 56° C. for a long period and the toxin in the serum is probably destroyed thereby.

I am also studying the relation of the toxin in the serum to the different fractions removed by ammonium sulphate.

This paper is presented in order that producers of antitoxic sera may be informed that concentration of serum by precipitation with ammonium sulphate and acetic acid does not necessarily remove toxic impurities present in the untreated serum, and that they may be warned against the use of serum, even for concentration, except from perfectly healthy animals.

³ Bolton, B. Meade, and Fisch, Carl, *Trans. of the Ass. of American Phys.*, 1902, xvii, 462.