THE APPARENT ALTERATION OF TETANUS TOXIN WITHIN THE SPINAL CORD OF DOGS*

WARFIELD M. FIROR, M.D., AND AUSTIN LAMONT, M.D.

BALTIMORE, MD.

FROM THE DEPARTMENT OF SURGERY OF THE JOHNS HOPKINS MEDICAL SCHOOL, BALTIMORE, MD.

IN 1935 and 1937, a series of experiments was carried out which showed that pure reflex motor tetanus (clonic spasms) can be produced without the accompaniment of the slightest degree of muscular rigidity. To demonstrate the separation of these two cardinal manifestations of tetanus, it is necessary to inject only minute quantities of tetanus toxin into an anterior horn of the dog's spinal cord. Previously Abel[1] and his coworkers had succeeded in bringing about a state of unyieldnig rigidity in the limbs of dogs by multiple intramuscular injections of tetanus toxin. They have shown that by the deposition of 1/8,000 of the ordinary lethal dose of this toxin at each of 40 different sites one can render the hind limbs of a dog rigid for three months. This observation added weight to their contention that the cause of muscular rigidity, as seen in both local and general tetanus is to be found in the action of the toxin on the voluntary muscles, and not in its action on any part of the central nervous system. To substantiate this belief we attempted the experiments referred to above. The demonstration that the effect of tetanus toxin on centers in an anterior horn of the spinal cord is the production of pure reflex motor tetanus was made in 11 dogs. The protocols of these experiments were the basis of an article which appeared in February, 1938.[2] The observations on these 11 dogs have been fully and accurately confirmed in 14 additional experiments. The present communication is not concerned with the confirmation of the preceding report, but deals with the study of an unexpected and unexplained phenomenon which occurred consistently. We refer to the observation noted in a preceding communication,[2] that every dog receiving an intraspinal injection of tetanus toxin died, although the quantity employed was a tiny fraction of the lethal dose given by any other route. In an effort to understand the cause of death following the intraspinal injection of tetanus toxin we have carried out a series of experiments that are herein reported. This study has led to the formulation of a new theory of the pathogeny of tetanus.

Before recording the actual experiments, however, it is necessary to describe the technic that has been devised for accurately depositing as little as 1/2,000 cc. at any given site. It is also desirable to make some statements concerning the dosage and the materials used in the experiments.

In all of our experimental work we have used batches of tetanus toxin prepared by Dr. Bettylee Hampil of the Sharp and Dohme laboratories. The

* These researches were aided by a grant from the John and Mary Markle Foundation.

details of the method of preparation will be furnished upon request. The toxin has been kept in sterile rubber-stoppered vials at 2° to 4° C. Not only has Doctor Hampil run preliminary assays of each shipment of toxin, but Dr. William Chalian, working in Dr. John J. Abel's laboratory, has repeated these preliminary assays and from time to time has reassayed each vial to detect any loss in potency. Bacteriologic studies have been made to guarantee the continued sterility of the toxins. Various dilutions of toxin, Batch No. 678, were made by Dr. John Brewer in the Department of Bacteriology. The dilutions were made with beef infusion broth, and the resulting mixtures were placed in sealed glass vials under nitrogen.

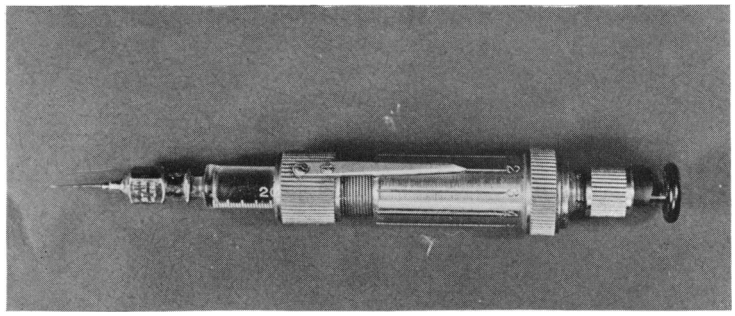

FIG. 1.—Photograph of syringe constructed in order to inject minute quantities of toxin.

Throughout our work we have adopted the dog unit described by Doctor Abel as follows: "We have found in the course of numerous experiments extending over a period of five years that injection of 480 guinea-pig LD 50's per kilogram invariably induces fatal tetanus in dogs of medium weight and age, and have arbitrarily chosen this figure as our lethal dose for dogs. This dose kills dogs taken at random in from five to nine days (when given intravenously, intramuscularly, or subcutaneously).* Neither a median nor a minimal lethal dose, but certainly larger than a statistically determined median lethal dose would be, it was adopted as a matter of convenience. We have always stated the dosage of the injected toxin in terms of standard guinea-pig units, so that our work can readily be checked by others without reference to our arbitrary dog unit."[3]

The technic for injecting minute quantities of toxin which was described in connection with our earlier spinal cord experiments was not infallible and was limited to a maximum of 0.003 cc. per injection; consequently, the apparatus illustrated in Figure 1 was devised and has been used in all animals whose number is over 104. This device† consists of a tuberculin syringe the barrel and plunger of which are held tightly by jackets of stainless steel. These jackets are threaded upon each other so that one complete turn either

* Parentheses ours.

† The authors wish to thank Dr. George Gey for his supervision of the design and construction of this instrument.

aspirates or expels 1/100 cc. Fractions of this amount are easily registered by the arm on the upper jacket which clicks at every tenth of a turn. It is entirely possible to turn the plunger accurately 1/20 of a turn, thereby delivering 1/2,000 cc. If a tuberculin syringe breaks, it is a simple matter to replace it. With this injection apparatus there may be an error of 0.0005 per 0.01 cc. because of the variations in the bore of tuberculin syringes.

EXPERIMENT 1: *Method of Procedure.*—This experiment was devised to ascertain whether or not the clonic spasms of the muscles which follow the injection of tetanus toxin into an anterior horn of a dog's spinal cord are responsible for the animal's death. To gain this knowledge a series of dogs was prepared in which toxin was placed in the distal end of the lumbar cord a few minutes after the conus and all adjacent spinal roots had been severed. By this procedure the usual evidences of reflex motor tetanus were eliminated. Postoperatively the animals had a flaccid paralysis of the hind limbs. From a study of Table I it is apparent that elimination of clonic spasms of the voluntary muscles does not prevent death following the intraspinal injection of minute quantities of tetanus toxin. Although the range of dosage given in Table I is from 1/12 to 1/50 of the ordinary lethal dose, these figures represent the maximum amount that could have been given. They do not allow for leakage and for loss of potency from exposure to the air. That this loss of potency can be as great as 50 per cent was accurately determined by assays.

Protocol of Typical Experiment.—Dog No. 15: Male, mongrel, weight 8.3 Kg. *Operation* in June 3, 1937: Under ether anesthesia, the operative field was shaved and prepared with a double application of iodine. The fifth, sixth, and seventh lumbar vertebrae were exposed and their dorsal spines and laminae removed. The operation was remarkable for the slight amount of bleeding. The conus and overlying dura were divided with scissors and the motor and sensory roots of the last three lumbar nerves on both sides were cut. The mobilized end of the cord with its surrounding dura was lifted from the spinal canal, and after all bleeding had been stopped by packing with moist gauze, a small opening was made in the ventral surface of the dura. A No. 25-gauge hypodermic needle was inserted into the central portion of the cord through this opening, and 0.003 cc. of tetanus toxin, Batch No. 670, was injected. There was no obvious leakage upon withdrawal of the needle. The wound was closed in layers with silk. The amount of toxin injected represented at the most 1/50 of a lethal dose if given intravenously.

Following the operation the dog had a flaccid paralysis of the hind limbs. There were no clonic movements of any of the muscles. The dog died at 9.30 A.M. July 5, 1937. *Autopsy* showed a few intraperitoneal adhesions. The viscera were entirely normal. The wound was clean. Specimens were taken for microscopic study.

EXPERIMENT 2: *Method of Procedure.*—The question arose whether or not injections of tetanus toxin into the white matter of the cord would be as lethal as those into the gray substance; accordingly, a group of dogs were given toxin in the region of one crossed pyramidal tract. All of the operations were performed in the lumbar region; the white fibrous band connecting the various denticulate ligaments served as a landmark for these injections. Following the laminectomy the dura was incised in the midline and the dural flap away from the operator was raised so as to expose a denticulate ligament.

This was cut to permit the cord to fall away from the dura and to facilitate rotation of the cord. Gentle upward traction on the dural flap served to bring the white line referred to above into clear view. The operator then inserted the tip of a No. 26-gauge hypodermic needle tangentially under this line. The depth of the puncture was easily controlled and in no instance was it more

TABLE I

CAUDAL PREPARATIONS

Date	Dog No.	Toxin No.	Dosage in Dog Units	Site of Injection	Symptoms and Remarks All the animals had flaccid paralysis of the hind limbs	Survival in Days	Autopsy
5/18/37....	10	30A	1/12 or less	Anterior midline	No clonic spasms	5	Grossly normal
5/27/37....	12	670	1/20 or less	Anterior right	Cord traumatized at operation	5	Hemorrhagic cystitis. Otherwise normal
6/3/37.....	15	670	1/48 or less	Anterior midline	No spasms observed	2	Grossly normal. Microscopic of cord
6/ 8/37....	16	670	1/40 or less	Anterior right	No spasms observed	2	Bladder distended with bloody urine. Slight consolidation in lungs
7/ 7/37....	22	670	1/24 or less	Anterior right	No spasms observed	2	Grossly normal
7/ 7/37....	23	670	1/33 or less	Anterior right	No spasms observed	1	Grossly normal
9/ 8/37....	40	31A	1/50 or less	Anterior midline	Injections not satisfactory. Moderate hemorrhage from puncture wound	8	Grossly normal
9/18/37....	42	31A	1/14 or less	Anterior midline	No spasms observed	2	Microscopic sections of cord. Grossly normal

An additional animal was operated upon by a student assistant. The dog became emaciated and developed ulcers over both thighs, and died on the seventeenth day. It seems fair to assume that the injection was faulty.

CONTROLS

6/ 9/37....	17	0	0	0	Operative control	30	Emaciation. Considerable vomiting before death. Autopsy negative
9/20/37....	45	31A boiled	1/13	Anterior midline	Foot injured and infected on eleventh P.O. day. Animal sacrificed on twelfth day.	12+	Infected leg and thigh.
9/21/37....	48	31A boiled	1/14	Anterior midline	Developed ulcers on thighs from pressure necrosis	16	Infected ulcers. Lungs consolidated.
9/22/37....	50	31A boiled	1/7	Anterior midline	Distemper	20	Massive consolidation of lungs. Bloody fluid in peritoneal cavity
9/30/37....	44	31A boiled	1/13	Anterior midline		5	Grossly normal

than 2 Mm. Frequently the point of the needle was so superficial that one could see a slight bulge following the deposition of the toxin solution. These injections were certainly made with greater accuracy than those aimed at the region of the anterior horn. This fact may account for the greater consistency in the results.

Eighteen animals were operated upon in this series. The amount of toxin injected ranged from 1/15 to 1/1,000 of an intravenous lethal dose. The eight dogs receiving smaller doses will be described in Experiment 6. In ten instances the dosage was approximately 1/20 of the calculated lethal dose. The physiologic alterations that ensued were the same in each of these ten animals. Death occurred with great regularity between 48 and 60 hours following the injection. No animal showed neurologic signs on the day of operation. The animals that were observed between 18 and 24 hours after the injection showed a little weakness in the right hind limb. Between 24 and 36 hours postoperatively, all of the animals showed hyperactive cutaneous and tendon reflexes in the right hind limb. In one instance gentle blowing on the foot pads would initiate a series of clonic contractions of the right hind limb but not of the left. Later all the animals showed constant muscular tremors or spontaneous clonic movements. These grew more severe until the animals were down. For a few hours before death the spasms were diminished. Not a single instance of rigidity (Starre) was observed, although in three dogs the contralateral limb was held in extension; nevertheless, in these three dogs the limbs were actively and passively flexed at all their joints. In two dogs there was a tendency to bite at the base of the tail as if the area annoyed the animal. There was not, however, any evidence of real tetanus dolorosus. One cannot draw any conclusion from this experiment because miscroscopic sections show that some of the toxin reached the anterior horn. The only advantage that white matter injections have over anterior horn injections lies in the greater accuracy with which the toxin can be placed.

EXPERIMENT 3: *Method of Procedure.*—The possibility of the upward passage of tetanus toxin in the spinal cord was commented on in the earlier report on the experimental production of reflex motor tetanus. It was pointed out that after the introduction of tetanus toxin into the lumbar cord the resultant clonic spasms are strictly limited to the site of injection. This has been found true in all subsequent lumbar injections. Surely, if this exceedingly potent toxin passed *as such* to the medullary centers by way of the spinal cord, there would be some clinical manifestation en route. Furthermore, the absence of lymphatics in the cord lessens the possibility of such a passage. The amazingly rich network of blood capillaries makes it highly improbable that any readily absorbable molecule could migrate within a cord from the lumbar region to the medulla. In order, however, to prove beyond doubt that the molecule of tetanus never reaches the vital medullary centers by moving upward in the cord, we carried out a series of experiments with transected cords, the results of which are summarized in Table II. From a study of the data assembled in this table it is clear that the introduction of

TABLE II

TOXIN INJECTED INTO DISTAL SEGMENT OF TRANSECTED CORD

Dog No.	Date of Transection	Site of Transection	Interval between Transection and Injection in Hours	Toxin No.	Dosage in Dog Units	Site of Injection	Survival Period in Days	Autopsy
33	8/ 2/37	Dorsal 7	3/4	LYO-2	1/5-	Lumbar 4—right anterior horn	7	Infection of lower incision extending down to cord
34	8/ 5/37	Dorsal 8	3/4	LYO-2	1/3-?	Lumbar 4—right posterior root	6	Infection of lower incision and of cord, which is almost severed
111	12/ 6/37	Dorsal 10	120	31A	1/4	Lumbar 2—right anterior horn	14	Grossly normal
113	12/ 7/37	Dorsal 9	96	31A	1/3	Lumbar 2—right anterior horn	7	Grossly normal
114	12/ 7/37	Dorsal 9	144	31A	1/6	Lumbar 2—linea alba	12	Pus in bladder. Otherwise normal
115	12/ 8/37	Dorsal 9	120	31A	1/5	Lumbar 2—linea alba	12	Grossly normal
116	12/ 8/37	Dorsal 9	144	31A	1/3-	Lumbar 2—linea alba	5	Bloody fluid in peritoneal cavity but no injection of peritoneal surfaces. Bladder distended with bloody fluid
117	12/ 8/37	Dorsal 9	144	31A	1/4+	Lumbar 2—linea alba	7	Grossly normal
164	1/29/38	Dorsal 6	72	678	1/20	Lumbar 3—right linea alba	9	Second incision infected. Cord slightly edematous
165	1/29/38	Dorsal 3	72	678	1/20	Lumbar 3—right linea alba	6-	Grossly normal

CONTROLS

Three controls with their cords divided in the middorsal region lived for one, two, and three months, respectively. They showed no loss of weight and were finally sacrificed. An additional animal which received one-sixth of a lethal dose of toxin showed no neurologic effects and died on the twenty-ninth day after the second operation.

tetanus toxin into the distal segment of a divided cord produces somewhat different effects from those that follow similar injections into an intact cord. The hind limbs of dogs with spinal cords divided in the middorsal region begin to jerk spontaneously within 20 hours after the introduction of tetanus toxin within the lumbar cord. At this time there is definite tactile, reflex tetanus; the slightest cutaneous stimulation serves to precipitate a series of clonic spasms of the hind limbs. The tendon reflexes are obtainable, and the effort to elicit them brings on a series of jerking, convulsive movements of the lower

half of the body. When these animals are held up by a firm grip around the thorax the hind limbs may become quiet, but when the dogs are lying down there is some spontaneous jerking. During the second 48 hours the severity and frequency of these clonic movements are increased. At the same time some stiffness is noticed in the paralyzed limbs. This stiffness is bilateral and increases in severity, occasionally giving rise to an unyielding rigidity. In most instances, however, the stiffness can be overcome by steady pressure and the limb completely flexed. Stiffness or rigidity of the affected limbs has occurred in every dog with a transected cord. Incidentally, these animals afford confirmation of the observations recorded in a previous paper.[2]

All of the animals in this experimental group were given a diet of raw beef, milk, and biscuits. In most cases this diet was eaten until the last 48 hours of life, but despite the fact that it was well taken, all of the animals lost a great deal of weight. This can be easily accounted for by the constant activity of the dogs. In one instance the loss amounted to one-fourth of the body weight in ten days. Female dogs were used so as to facilitate the passive emptying of the urinary bladder. This was done regularly.

All of the dogs with transected cords lived several days longer than those with intact cords; nevertheless, every animal died, despite careful feeding and nursing. Dogs with noninjected, transected cords have lived in this laboratory under similar conditions for many weeks without the slightest weight loss.

Autopsy of these experimental animals showed no gross changes which would explain death. It is not surprising that the action of the toxin in the distal end of a severed cord should be distinctly different from that in an intact cord. The physiologic reactions of a severed cord differ greatly from those of the intact cord. The significant fact is that all of the animals died without any apparent cause. The same observation was made on three dogs in which the dorsal cord was divided four hours after the deposition of toxin into the lumbar cord. One of these animals lived for 19 days. The frequency and severity of the clonic movements greatly diminished during the last few days of life, and it seemed as if the animal would recover. At autopsy there was evidence of a large hemorrhage into the cord at the site of injection, and this may have accounted for the diminished symptoms. The animal lost so much weight during the 19 days that it was impossible to draw any conclusions as to the cause of death.

Protocol of Typical Experiment.—Dog No. 165: Adult, female, mongrel, weight 7.2 Kg.

First Operation.—January 29, 1938, 11 A.M.: Under anesthesia, the operative field was shaved and prepared with iodine and alcohol. A laminectomy was performed on the third dorsal vertebra. The dura was not opened, but a segment of dura and spinal cord measuring 1 cm. in length was excised. The bleeding was controlled with packing. There was a gap of almost 2 cm. between the severed ends of the cord. The wound was closed with silk throughout.

January 30: There was flaccid paralysis of the hind limbs.

Second Operation.—February 1, 10 A.M.: Under ether anesthesia, the operative field was prepared as before. A laminectomy on the third lumbar vertebra was carried out.

The dura was incised in the midline and a denticulate ligament on the right side was divided. The cord was rotated so that the operator could have easy access to the lateral columns. With the special injection apparatus, 0.0026 cc. of tetanus toxin, Batch No. 678, was placed under the linea alba. There was slight oozing following the puncture. The wound was closed with silk throughout.

February 2: There is beginning jerking of the hind limbs. Tactile, reflex tetanus is present. The bladder was emptied. The dog is eating well.

February 3: Both hind limbs jerk while the animal is lying down. They are held extended but can be passively flexed; the right is stiffer than the left. The tendon reflexes can be elicited on the right but not on the left because of the constant clonic spasms. The animal feels hot. Rectal temperature 105° F. The bladder was emptied. The dog is eating and drinking.

February 4 and 5: There is no change in the animal's condition.

February 6: The animal seems sicker than yesterday.

February 7: The animal was found dead. Autopsy showed the lungs clear; no gross pathologic changes in the abdominal viscera. Both wounds were well healed. There was no sign of wound or urinary tract infection. The cord was not edematous at the point of injection.

EXPERIMENT 4: *Method of Procedure.*—Many of the animals that are included in the preceding experiments were unobserved at the time of death. In several instances, however, an effort was made to watch the manner of death, and every time that this was done it was seen that the animal would give several deep terminal gasps. The heart beat was palpable for two or more minutes after all respirations had ceased. In fact, on five occasions the chest was opened and the heart was observed to continue beating from three to five and one-half minutes after the last visible respiratory effort. This observation brought up the question whether or not paralysis of the respiratory centers accounted for the death of the animals in these experiments; accordingly, it was decided to inject minute quantities of the toxin into these centers. Fortunately, Gesell, Bricker, and Magee[4] had studied the location of the inspiratory and expiratory centers of the dog. The numerous experiments carried out by these investigators prove that the mere introduction of a sharp needle or electrode into the dog's medulla is not followed by any untoward symptoms; nevertheless, we repeated and confirmed this observation. As an added control for the deposition of tetanus toxin into so vital an organ as the medulla, we placed from 0.001 to 0.006 cc. of diphtheria toxin, boiled water, toxic serum, or filtrates from colon bacilli, meningococci, Staphylococci (H. A. strain), and typhoid bacilli (Table IV). Only with the first named substance were there any detectable effects upon the well-being or length of life of the animals. Four of the five animals receiving diphtheria toxin into the medulla died within 40 hours. The fifth, which received only 0.0005 cc. in a single injection, lived 13 days. The autopsies of the four dogs that received larger amounts of diphtheria toxin showed gross and extensive areas of hemorrhagic necrosis about the site of injection. On the fifth day there was an area of softening 2 to 3 Mm. in diameter at this site. This animal died from an acute hemorrhagic pancreatitis. It is interesting to note that diph-

theria toxin placed in the lumbar cord produces flaccid paralysis but does not cause death.

In all of the animals receiving tetanus toxin in the medulla, there was a latent period during which the dog seemed perfectly well. Even when such an enormous amount of toxin as 1/12 of a lethal dose was put into the medulla (Dog No. 175), the animal remained symptomless for seven hours. Following some of our medullary injections, the latent period has been as long as five days. It is clear that this latent period, or incubation period as it is sometimes called, is proportional to the amount of toxin injected.

The first symptom that is noticeable is difficulty in swallowing. This is soon followed by clonic spasms of the pharyngeal muscles whenever the dog attempts to eat or drink. Later these spasms occur spontaneously. The picture simulates that condition which has been described clinically as tetanohydrophobia. The spasms are not painful and between them the animals remain quiet. In no instance was there any evidence of involvement of the muscles of the trunk or limbs. The time of death varied with the quantity of toxin placed in the brain. When 1/10 of a lethal dose was injected, the animal died in about 24 hours, whereas, when 1/1,100 of a lethal amount was used, the dog lived 17 days. On the whole, it seems that death occurs a little sooner following the deposition of toxin into the medulla than into the lumbar cord, but the difference, if any, is too slight to be significant. It is certainly true that the manner of death is the same in both instances, and forms a very great contrast to the manner of death following injections of diphtheria toxin into the medulla, for in the latter group of animals there is no gasping, and the animals are comatose during the last few hours of life and die quietly.

Because of the interference with swallowing we gave glucose solutions and saline parenterally to all the animals that lived longer than 36 hours, but despite these treatments there was always great loss of weight. In the case of dogs with pharyngeal spasms living more than a week, it is impossible to say whether death is due to exhaustion, inanition, or some specific action of the toxin. The data of this experiment are summarized in Tables III and IV.

Protocol of Typical Experiment.—Dog No. 136: White and brown fox terrier, female, age nine months, weight, 5.3 Kg.

Operation.—January 5, 1938, 2.30 A.M.: Under ether anesthesia, the operative field was shaved and prepared with applications of iodine and alcohol. A dorsal midline incision was made, beginning at the inion and extending for 6 cm. The fascia was divided in the midline and the muscles were retracted. The anesthetist then flexed the animal's head to the greatest possible degree, and the dura was opened from the edge of the foramen magnum to the atlas. Great care was taken when enlarging this incision to avoid tearing into the adjacent venous sinus. After absorbing the excess cerebrospinal fluid with gauze, the operator rongeured away a small piece of the occipital bone and thus obtained good exposure of the medulla. One injection of tetanus toxin was made 2 Mm. to the right of the obex and a similar one on the left side. Each injection amounted to 0.0025 cc. of a 1/50 dilution of toxin, Batch No. 678, and was equivalent to 1/80 of an intravenous lethal dose. There was no bleeding. The wound was filled with warm normal saline and closed throughout with silk.

TABLE III

INJECTIONS OF TETANUS TOXIN INTO THE MEDULLA

Dog No.	Date	Toxin No.	Total Dosage in Dog Units	Site of Injection	Remarks	Symptoms	Survival Period in Hours	Autopsy
136.....	1/ 5/38	678	1/40	Right and left	Given fluids intraperitoneally	Pharyngeal spasms	33	Grossly normal
137.....	1/ 5/38	678	1/42	Right and left	Only fair exposure. Given fluids	Pharyngeal spasms	27	Grossly normal
138.....	1/ 6/38	678	1/42	Right and left	Delayed appearance of pharyngeal spasms	Pharyngeal spasms	60	Grossly normal
140.....	1/11/38	678	1/500	Right only	Rough dilution. Poor exposure. Given fluids intraperitoneally	Typical spasms	216	Grossly normal
142.....	1/12/38	678	1/450	Right and left	Rough dilution. Given fluids	Typical spasms	72	Grossly normal
145.....	1/12/38	678	1/10	Right and left	Fair exposure	Typical spasms	20	Sacrificed, but was moribund. Grossly normal
146.....	1/14/38	678	1/10–	Right and left	Poor exposure	Typical spasms	24	Grossly normal
148.....	1/18/38	678	1/12	Right and left	Could drink	Slight pharyngeal spasms, not typical	48	Liver large and granular
149.....	1/18/38	678	1/12	Right and left	Good exposure	Typical spasms	24	Grossly normal
166.....	2/ 2/38	678	1/1,000	Right only	? Needle came out. Could not pass stomach tube	Spasms after one week	9 days	Grossly normal
167.....	2/ 2/38	678	1/1,100	Right only	Ate beef and milk for 4 days	Spasms after 5 days	8 days	Grossly normal
175.....	2/ 7/38	678	1/12	Right and left	Poor exposure	Typical spasms. Vomiting	27	Hemorrhage in substance of medulla along needle track
176	2/ 9/38	678	1/20	Right only	Copious bleeding	Typical spasms. Turns to right	22	Grossly normal
178.....	2/10/38	678	1/23	Left only	Poor exposure	Typical spasms. Turns to left	24	Grossly normal

TABLE III (*Continued*)

Dog No.	Date	Toxin No.	Total Dosage in Dog Units	Site of Injection	Remarks	Symptoms	Survival Period in Hours	Autopsy
188.....	2/23/38	678	1/1,000	Right and left	Received fluids intraperitoneally	Typical spasms. Barking and growling	140	Liver cirrhotic. 70cc. bloody fluid in peritoneal cavity. Nothing else
189.....	2/23/38	678	1/1,100	Right and left	Eats well	Barking and growling. No symptoms	7 weeks	Sacrificed
190.....	2/23/38	678	1/2,000	Right and left	Eating until sixth day. Wants to but cannot thereafter	Occasional typical spasms	240	Questionable consolidation of lungs, probably insufficient to cause death
196.....	3/18/38	678	1/1,200	Right and left	Got distemper. Received fluids intraperitoneally	Could not eat. Had spasms after sixth day	17 days	Consolidation of lungs. Lost 30 per cent of weight
197.....	3/18/38	678	1/2,000	Right and left	Received fluids intraperitoneally	Did not eat. Had typical spasms just before death	148	Died in spasm. Some consolidation of lungs

January 5, 10 P.M.: The animal appeared quite normal.

January 6, 9 A.M.: When first seen, the animal was quiet, alert, walking about, wagging its tail. There was no trismus, no tetanus dolorosus. It gave an occasional quick jerk of the head. Frequent attempts to drink produced violent clonic spasms of the pharyngeal muscles which lasted 30 seconds. Auditory and tactile stimuli did not provoke spasms. The knee kicks were present and active, and testing them did not produce a seizure.

Twelve noon: Temperature, 107.6° F. per rectum. The dog was given 300 cc. of saline and 5 per cent glucose solution intraperitoneally. It was observed closely thereafter, until the time of death at 11.30 P.M. For the first four hours of this period, spontaneous clonic spasms of the pharyngeal muscles occurred at intervals of two to 12 minutes. The duration of each was from 15 to 30 seconds. Between the attacks the dog was quiet and in no apparent pain. At times it would shake its head and had a tendency to incline the head to the right side. It frequently scratched its right ear. The respirations became increasingly rapid. After 4 P.M. the pharyngeal spasms were interspersed with spells of jerking of the head.

At 5 P.M. another smaller injection of glucose solution was given intraperitoneally. Rectal temperature, 105° F.; pulse, 160; respirations, 84. The spasms seemed to occur at greater intervals but to last longer. The dog died at 11.30 P.M. The autopsy was negative.

EXPERIMENT 5: *Method of Procedure.*—The death of dogs under the conditions of the preceding experiments might conceivably result from a multiplication of the toxic molecule within the spinal cord and the subsequent

absorption of a fatal amount of the blood stream. Such a happening is, however, highly improbable because none of the animals showed any peripheral signs of general tetanus. To exclude the possibility of such a mechanism being at work, a number of dogs were given varying amounts of tetanus antitoxin at varying intervals after the spinal injections. It was evident from this experiment that both the amount given and the interval influence the result. A determination of the exact relationships that exist between the proportion of toxin-antitoxin and the interval between injections was not necessary for our purpose. A simpler and more exact experiment was to have the antitoxin circulating in the blood *before* the toxin was placed in the spinal cord. The results of this experiment show clearly that as much as 100 times the neutralizing dose of antitoxin can be circulating in the blood stream at the time of the spinal injection without affecting the symptoms or the time of death. This experiment shows that the death of the animals is not due to multiplication of the tetanus toxin within the spinal cord.

TABLE IV

CONTROLS FOR INTRAMEDULLARY INJECTIONS

Dog No.	Date	Material Injected	Amount Injected	Site of Injection	Remarks	Symptoms	Survival Period
118	1/ 5/38	Boiled water	0.004 cc.	Right and left		None	Indefinite
122	1/ 5/38	Boiled water	0.004 cc.	Right and left	Slight trauma to cord	None	Indefinite
124	1/ 6/38	0	0	Right and left	Inserted needle	None	Indefinite
154	1/21/38	B. *coli* toxin	0.004 cc.	Right and left	Good exposure. Distemper	None	Indefinite
155	1/21/38	Meningococcus toxin	0.004 cc.	Right and left	Good exposure. Distemper	None	Indefinite
159	1/25/38	H.A. Staphylococcus toxin	0.004 cc.	Right and left	Good exposure	None	Indefinite
162	1/27/38	B. *typhosus* toxin	0.006 cc.	Right and left	Good exposure	None	Indefinite
163	1/27/38	Meningococcus toxin	0.006 cc.	Right and left	Good exposure	None	Indefinite
179	1/12/38	Diphtheria toxin	0.006 cc.	Right and left		Not seen	20 hours
180	2/15/38	Diphtheria toxin	0.002 cc.	Right only		Cannot stand. No jerks, *etc.*	27 hours
181	2/15/38	Diphtheria toxin	0.003 cc.	Right only		Turned somersaults in cage for short period	40 hours
186	2/17/38	Diphtheria toxin	0.001 cc.	Right only		Not seen	23 hours
187	2/17/38	Diphtheria toxin	0.005 cc.	Right only		None	13 days
194	2/28/38	Toxiferous blood	0.006 cc.	Right and left		None	Indefinite

EXPERIMENT 6: *Method of Procedure.*—After establishing the fact that intraspinal injections of minute amounts of tetanus toxin cause death, we attempted to study the effects of introducing still greater dilutions of the toxin. An abstract of the data on six dogs which received intramedullary

injections of 1/1,000 or 1/2,000 of an ordinary lethal dose is given in Table IV. These dogs had clonic pharyngeal spasms and lived from six to 17 days. Because of the interference with swallowing, it is impossible to attribute their death to the action of any secondary substance. There were eight other dogs in which the toxin was put into the white matter of the lumbar cord. The first four of these dogs were given approximately 1/1,000 of a lethal dose, and except for a slight transient weakness in gait, probably due to trauma, they showed no effects from the injections. There were no significant changes in the superficial or deep reflexes, and no evidence of clonic spasms. In this respect the injections of toxin into the white matter of the lumbar cord differ from those into the medulla. This difference is probably due to the toxin reaching a larger number of motor cells following medullary injections than after lumbar injections. This experiment will have to be repeated on a larger series of animals and be combined with careful histologic studies before it can be used as an argument that tetanus toxin has no effect on the white matter of the cord. The four remaining dogs were given 1/500 of a lethal dose in the lumbar cord. Two of these showed no symptoms attributable to the toxin. The third died with typical spasms on the fourth postoperative day. The autopsy was negative. The fourth dog was most interesting. It was given two injections of 0.0025 cc. each of a 1/50 dilution of toxin, Batch No. 678, into the right side of the lumbar cord. During the next four days there were no significant symptoms. On the fifth day the right hind leg jerked constantly when the animal was standing. There was difficulty in walking. The knee kick on the left was normal but could not be tested on the right because of the constant clonic movements. The cutaneous reflexes on the right were increased. There was no stiffness. This state of tactile, reflex tetanus continued for three days, at which time the jerking gave place to tremors, which lasted four days. Thereafter the dog remained entirely well.

This animal is the only one that recovered after having unmistakable clonic spasms. It corresponds, we think, to dogs receiving from one-half to three-quarters of a lethal dose intravenously, for in these animals signs of descending tetanus develop but the dogs recover. When less than one-half of a lethal dose is given intravenously (or into the spinal fluid), no symptoms occur. It seems that the corresponding ineffective dose for lumbar injections is less than 1/500 of the intravenous dose.

Pathology.—The report of these experiments would be incomplete without some word concerning the pathologic alterations that result from the introduction of tetanus toxin into the medulla and spinal cord of dogs. Grossly, one sees only a thin blood clot along the puncture wound made by the needle. No edema has been observed nor any visible inflammatory reaction. Histologically there are definite cytologic alterations. These are best seen in the medullary specimens but are essentially the same in other parts of the cord. The alterations are surprisingly well localized to the site of injection. The adjacent cranial nerve nuclei are apparently unaffected. There is a slight polymorphonuclear infiltration about the lesion and around the neighboring

blood vessels. The needle tract is outlined by cells which seem to be microglia. The endothelium of the blood vessels is intact and there are no evidences of thrombosis. Another alteration is the presence of large numbers of phagocytic cells, apparently macrophages. The third change is in the nerve cells. Here one finds changes in the Nissl substance, which seems pale and granular. This change can be best described as chromatolysis. The nuclei of some of the cells are irregular, swollen, and distorted. A few of the nerve cells are dead; others appear viable but altered. With fiber stains one finds great alteration in the myelin, but this observation has to be interpreted with caution since the specimens were not fixed immediately upon the death of the animals. In order to really trace the sequence of cytologic alterations, a special study with the vital staining technic is now being carried out. In this an effort is being made not only to eliminate changes resulting from delayed fixation, but also to determine how much of the pathologic picture is due to trauma, how much to the presence of a foreign substance, and how much may be looked upon as the specific action of the toxin.

Discussion.—From the foregoing experiments it seems clear that tetanus toxin causes death when placed in the dog's spinal cord. This result ensues after the deposition of as little as 1/500 of the ordinary lethal dose. In several experiments we placed fractions of a lethal dose in the sciatic nerve, an anterior and a posterior nerve root, the adrenal, and the brain without any noticeable effect. This last organ was injected in six dogs. Twice the motor cortex was identified by electrical stimulation before placing the toxin in it. In not a single dog could one detect the slightest visible reaction to the toxin. This observation is in keeping with the clinical picture of tetanus, which is singularly free from cerebral symptoms. The fact that death occurs despite the introduction of the toxin into a nonvital center and despite the severance of the cord above the point of injection, points to the conclusion that tetanus toxin is altered in the spinal cord to form a new substance that is absorbed by the blood stream and causes the death of the animal. That this new substance is not susceptible to the neutralizing action of tetanus antitoxin is shown by Experiment 5, in which the presence of 100 neutralizing doses of antitoxin in the blood stream failed to prolong life.

The concept that tetanus toxin is altered in the spinal cord is not a new one. Forty years ago, Courmont and Doyon[5] suggested that central nervous system symptoms of tetanus do not appear until the toxin has been changed into a strychnine-like body. The concept that this secondary substance is a cause of death is new. In addition to the experimental data presented in this paper, there are two other observations that lend weight to the correctness of this concept. The first is the fact, well known to all investigators of the disease, that it is impossible to demonstrate the presence of tetanus toxin in the spinal cord of animals dying from tetanus, despite the preponderance of spinal cord symptoms. In commenting upon the fixation of toxin by the spinal cord after intravenous injections, Doctor Abel has recently expressed this fact by saying that "neither the bio-assay nor any other method now at

our disposal enables us to detect and assay this fixed fraction of the injected toxin."[3] It seems that the inability to detect the toxin can well be explained by its alteration.

The second fact is that tetanus antitoxin is of no avail in experimental animals receiving one or more lethal doses of toxin intravenously if it is given after central nervous symptoms appear. Abel and Chalian[6] have recently studied the length of time in which antitoxin is effective after varying amounts of toxin given intravenously. They have shown, for instance, that following three intravenous lethal doses, one can save the dog with antitoxin up until the appearance of central reflex symptoms. With larger doses there is a shorter period in which antitoxin is effective. This insusceptibility of toxin to antitoxin, once fixation and incubation have taken place, can be looked upon as additional evidence of its alteration.

SUMMARY AND CONCLUSIONS

A technic has been devised for the accurate injection of minute amounts of tetanus toxin into various parts of the dog's spinal cord. By this procedure it is possible to produce pure reflex motor tetanus without the slightest evidence of muscular rigidity. As little as 1/2,000 of an intravenous lethal dose placed in the medulla suffices to bring on reflex motor spasms of the pharyngeal muscles. The intraspinal injection of 1/400 or more of the usual intravenous lethal dose of tetanus toxin has always been followed by the death of the animal, despite the fact that the toxin was placed in a nonvital center such as the lumbar cord. The explanation that death results from an upward passage of the toxin is untenable because transection of the cord above the site of injection does not prevent death. Similarly, division of all sensory and motor pathways below the lesion is without effect. The death of the animal cannot be caused by a multiplication of the tetanus molecule and subsequent reabsorption because the presence in the circulating blood of 100 neutralizing doses of antitoxin does not prevent a fatal outcome. The tentative explanation put forward to account for the results obtained in the foregoing experiments is that tetanus toxin is altered in the spinal cord to form a secondary substance that is responsible for the dog's death.

REFERENCES

[1] Abel, John J., Hampil, B., and Jonas, A. F., Jr.: Researches on Tetanus. III. Bull. Johns Hopkins Hosp., **56**, 317–336, 1935.

[2] Firor, W. M., and Jonas, A. F., Jr.: Researches on Tetanus. VI. Bull. Johns Hopkins Hosp., **62**, 91–109, 1938.

[3] Abel, John J., Hampil, B., Jonas, A. F., Jr., and Chalian, W.: Researches on Tetanus. VII. Bull. Johns Hopkins Hosp., **62**, 522–563, 1938.

[4] Gesell, R., Bricker, J., and Magee, C.: Amer. Jour. Physiol., **117**, 423–452, 1936.

[5] Courmont, J., et Doyon, M.: Le Tétanos. Paris, p. 47, 1899.

[6] Abel, John J., and Chalian, W.: Researches on Tetanus. VIII. Bull. Johns Hopkins Hosp., **62**, 610–633, 1938.

DISCUSSION.—DR. PETER HEINBECKER (St. Louis, Mo.): The authors, by using minute amounts of tetanus toxin injected directly into the cord, have apparently been able to separate local from clonic tetanus. This is an unusual physiologic feat. Apparently, the dosage was just adequate to bring about a facilitation for those reflexes initiated by the afferent fibers concerned in clonic reflexes, but not sufficient to facilitate appreciably the mechanism for proprioceptive reflexes concerned in local tetanus.

I am unable to accept the interpretation that local tetanus is a manifestation of alteration in the muscle itself. I adhere to the view that both local tonic and clonic tetanus are an expression of influence of the toxin on the central nervous system.

The problem of the potentiation of tetanus toxin on injection into the spinal cord is a difficult one to analyze. In 1933, Condrea and Poenaru produced evidence that tetanus toxin was modified by the diluent into which it was placed prior to injection. They found that the toxic effect increased remarkably when the toxin was united with peptone. Recently, a report has been issued by Zuger and Friedemann, in which they stated that there was a potentiation of tetanus toxin on mixing it with muscle in vitro. It is possible that such a potentiation was here also due to admixture with a substance like peptone, which increased the rapidity with which the toxin reached the vital centers. There was no evidence in any of their experiments to indicate that the amount of toxin was altered, because, in vitro, no additional antitoxin was required to neutralize the toxin mixed with peptone or muscle.

Rivers mixed tetanus toxin with spinal cord tissue and found that the amount of antitoxin required to neutralize it, in vitro, was even less than that required to neutralize the toxin alone. However, Doctor Firor informs me that after incubation, such a mixture of toxin with cord does result in a potentiation of the mixture. It is possible that in his experiments in which injections were made into the cord, slight injury resulted, and the body, acting as an incubator, permitted the development of a substance which when mixed with the toxin permitted a more rapid penetration into the vital areas than when the toxin alone was present. However, I am not certain that this is the explanation, and I feel that Doctor Firor will have to present very excellent evidence to show that a new substance has been formed, a new toxin, before the idea can be accepted.

I consider the method of action of tetanus toxin to be similar to that of strychnine on the central and peripheral nervous system. Strychnine acts by lowering the threshold for nervous excitation and it also acts by altering accommodation of the nervous system to stimuli. By that, I mean that ordinarily nerves, and presumably cells, tend to fail to respond to a prolonged stimulus after a certain period of time. We have found in our laboratory that in the presence of strychnine such a failure to respond does not occur, and I consider the activity of tetanus toxin to be very similar to that of strychnine.

I think the manner of death by tetanus to be one of exhaustion, exhaustion of the muscles from continued stimulation arising in the central nervous system. There is no evidence of a failure of the central nervous system. The muscles just tire and fail to respond. Realization of this point will guide us in our therapy. We can organize a rational form of therapy.

I have one graph which I would like to show. Some weeks ago, Doctor Firor talked to me about this subject and I told him that I could present an illustration indicating how strychnine acted upon the central nervous system, and possibly reveal how tetanus toxin acts.

Figure 1, 3A is a record of the activity of the phrenic nerve in a curarized animal. You see, there is a series of impulses coming down, then there is a

pause, and during the next respiratory effort, another series of impulses will descend. After strychnine, we get the type of activity shown in Figure 1, 3B. The central nervous system fails to accommodate and one gets long-continued activity.

When, as shown in Figure 1, 4, you record the activity in the sciatic nerve of such a curarized animal, it will be found that on very slight stimulation of the saphenous nerve, a series of responses is obtained. This would represent ordinary tactile stimulation. When the animal is stimulated a little harder, a long-continued series of responses results. It is the effect of such a continued action of the nervous system on muscles which leads to fatigue and is, in my mind, the cause of death.

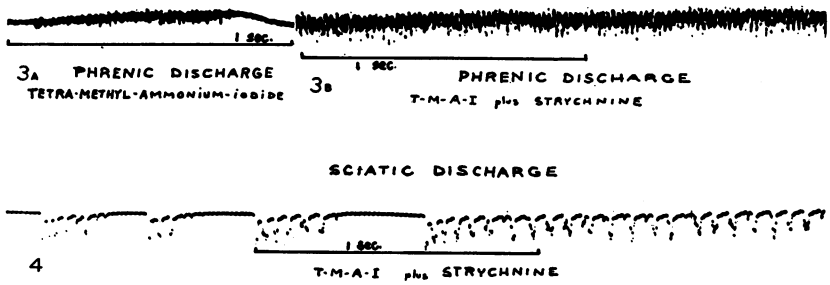

FIG. 1.—(3A) Graph showing phrenic discharge in a curarized animal. (3B) Result on phrenic discharge after administration of strychnine. (4) Result on sciatic discharge after administration of strychnine.

One other interesting point here—because this animal is curarized, we do not get proprioceptive impulses from the periphery, so that we have absolutely smooth intervals in our record. Such an animal, being entirely without tone, does not show the electrical evidence of proprioceptor reflexes which one obtains in the ordinary animal.

DR. WARFIELD M. FIROR (Baltimore, Md.) closing: I want to thank Doctor Heinbecker for his discussion and for his healthy incredulity. I think he is absolutely right in saying that one must offer excellent evidence before he can assume that a second toxin is formed, because this is a very unphysiologic concept—the body steps up the potency of a lethal agent for its own destruction.

I disagree, however, with Doctor Heinbecker, that fatigue is the cause of death, because in the series of animals in which we divided the conus and the lumbar nerves, there were no muscular contractions, there was no muscular activity. Nevertheless, these animals died in exactly the same manner and the same time as those that suffered from very violent muscular activity.

I am glad also that he brought up the point about the spinal cord experiments of Doctor Rivers. I may say in passing that you will note in the abstract a mention of four dogs in which tetanus toxin was injected into the lumbar cord and then, just before death, that segment of the cord was excised and injected into healthy dogs, and caused their death. We have not included that experiment in this particular communication because we have been able to obtain that result in only five out of 20 experiments, and do not think that such a proportion is statistically significant. Nevertheless, one has to explain the death of those five animals; and the experiments which we are now conducting will, I think, shed light on that point and will also offer the indisputable proof that Doctor Heinbecker wants.