

OBSERVATIONS ON THE ACTION OF BOTULIN TOXIN:

With a Note on GRASS DISEASE IN HORSES.

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OBSERVATIONS ON THE ACTION OF BOTULIN TOXIN

With a Note on GRASS DISEASE IN HORSES.

I. INTRODUCTION

The subject of the following research on the action of Bacillus Botulinus toxin was suggested by Professor A.J. Clark.

I am also indebted to Professor Clark for the interest taken in the progress of the work and for suggestions as to various points to be investigated.

That Bacillus Botulinus is the cause of a type of food poisoning giving rise to a definite set of symptoms was first demonstrated by van Ermengem in 1895. In that year an outbreak of food poisoning occurred affecting twenty people who had partaken of a ham. The ham which was available for investigation was found not to be in a decomposed condition, though it had a somewhat rancid smell. Inoculations from this meat into animals produced typical symptoms in rabbits, guinea pigs, apes and pigeons. The symptoms included paralysis and eye symptoms such as ptosis and unequal pupils. Van Ermengem then proceeded on bacteriological lines and as a result/

l.c.

result of his work he isolated from the ham and from the spleen of one of his patients who died an organism which on culture produced a very virulent poison. The symptoms produced corresponded to those of the original disease. This organism van Ermengem named Bacillus botulinus. From that time the disease has been known as Botulism.

Though the cause of this type of food poisoning was thus established, the disease must have been known for a considerable time previous to 1895. The earliest published accounts seem to be those of Justinus Kerner , a Schwabian physician. In 1820 he issued an account of the disease occurring in Württemberg. According to this author the earliest described cases occurred in the year 1793. From this date and until the discovery of Bacillus botulinus the disease was known as Sausage poisoning. Other records of a similar disease occurring prior to 1895 exist, but the literature is only of historical interest.

The more modern literature on botulism during the last fifteen or twenty years has come mainly from work carried out in the United States where a very large number of cases of botulism have occurred. During the last three or four years especially a number of very important contributions have been made/

made to the literature of the subject.

In the following account only those papers will be mentioned which have a more immediate bearing on the subject of this thesis.

Before reviewing the literature which deals with the mode of action of the toxin of *Bacillus botulinus*, it would be well to give a very brief outline of the bacteriology of the organism from 1896, when the organism was first discovered and named by van Ermengem, to the present time.

(22)

Leuchs, in 1910, showed that there are two strains of the bacillus, each of which showed immunological differences. This work was confirmed by Dickson and Burke in 1919, and these authors named the two strains type A and type B. Type A is highly proteolytic and resembles that obtained by van Ermengem. Type B is also proteolytic but is more resistant than type A.

(23)

In 1915 Dickson showed that *Bacillus botulinus* could be grown on a purely vegetable medium and that such a medium would contain all the conditions necessary for potent toxin production.

(16)

Buckley and Shippen in 1917, working with a human strain of *Bacillus botulinus* toxin, showed that it could produce forage poisoning in horses.

(14)

Graham and Bruechner (1919) isolated *Bacillus botulinus* from Indian corn which had caused/

caused forage poisoning in horses and they were able to reproduce an exactly similar disease with toxin which they isolated from the bacillus.

(24) Bengston in 1922 isolated a culture of bacillus botulinus from the larvae of Lucilia Caesar. This strain differed from types A and B and she therefore named it type C.

(18) In the same year Seddon isolated a culture of the bacillus from a case of Midland cattle disease in Tasmania. He proved that it was allied to Bacillus botulinus and that its toxin produced symptoms of botulism in animals. This organism he named Bacillus parobotulinus.

(25) Pfenniger (1924) states that Bacillus parobotulinus is not to be distinguished either morphologically or culturally from type C. He also states that the antitoxin for type C will neutralise the toxin of Bacillus <sup>para</sup>botulinus.

In the literature of this part of the subject there are in addition occasional references to what are known as the Nevin and Boise strains of Bacillus botulinus. The term Nevin refers to the observer who isolated the organism from a case of poisoning as the result of eating home-made cheese in America. The term "Boise" refers to the locality of an outbreak of botulism in 1919, the outbreak taking/

taking place at Boise, Idaho.

The Boise strain belongs to type A, the Nevin strain to type B.

One may therefore summarise the bacteriology of B. Botulinus as follows: It is an organism consisting of several different varieties, all of which have a similar morphological characters and produce toxins which act in the same way though differing in the degree of resistance and in potency.

The strains of Bacillus botulinus used in this work for the production of toxin were Burke 750 (type A) and Burke 751 (type B). They were obtained from the Lister Institute, London.

II. /

## II. HISTORICAL.

Any theory as to the mode of action of Bacillus botulinus toxin depends primarily on the clinical symptoms during life and pathological findings after death from botulism. The clinical symptoms not only in man but in animals experimentally inoculated with toxin point to an affection of the nervous system. This fact has never been disputed but the exact site of action of the toxin is still very doubtful.

It was with the main object of endeavouring to fix the place and mode of action of the toxin that this research was carried out.

Until recent years it may be said that the view held generally as to the mode of action of the toxin was that it exerted a paralytic effect on the central nervous system, the bulbar region of the brain being the chief locus of action.

(1) Marinesco, Kempner and Pollock (2) and others describe in support of this theory changes in the central nervous system. These changes consist in degeneration of many of the cells of the grey matter of the central nervous system. Degeneration of the Nissl granules, chromatolysis, swelling of the axons and development of lacunae inside the motor cells of the nerve nuclei so that they become altered in/

in outline. There is proliferation of the glia  
The nuclei in the bulb are affected and also the  
motor cells in the anterior horn of the spinal cord.  
These are the only important pathological findings  
recorded, though others have been recorded, consisting  
chiefly of fatty degeneration in various organs  
especially the liver, kidney and spleen.

(3)  
Dickson describes congestion of the meninges  
round the base of the brain in the region of the pons  
and medulla, and minute haemorrhages into the substance  
of the cord and at the base of the brain. He also  
records multiple thrombosis in the arteries and  
veins of the central nervous system generally with,  
excepting hypereamia of the lungs, negative  
findings in all other organs.

(4)  
Ophüls and Sernal believe the lesions to be  
due to multiple thrombosis in the veins and arteries  
of the central nervous system followed by ischaemic  
necrosis and later inflammatory changes in the central  
nervous system. There is according to these authors  
no direct effect on the nerve cells, the degenerative  
changes being secondary and due to disturbance of  
the blood supply of the part.

(5) (6)  
In recent years Schübel, Edmunds and Long  
(7)  
and Edmunds and Keiper attribute the toxicity to  
more or less complete paralysis of the motor nerve  
end-plates in striated muscle and in the diaphragm

with some impairment of other nerve endings such as the vagus. These authors attribute the symptoms of botulism to a peripheral motor paralysis. These papers will be discussed more fully subsequently.

(21)  
Schlomer describes two cases of botulism in man and concludes from the clinical appearances that there is a peripheral paralysis of the vagus. It will be shown later that such a conclusion cannot be correct.

(8)  
Coleman was unable to demonstrate any combination of the toxin with brain tissue.

(9)  
Cowdray and Nicholson, in view of the researches of Schübel, Edmunds and Long, carried out a very complete examination of the histology of the central nervous system in botulism. They used mice, guinea pigs and rabbits, injected with botulin toxin and made a very careful examination of the whole central nervous system in animals at various stages of intoxication. In their own words "the results of our observations indicate that except for a slight degree of vascular engorgement all the lesions which we have noted in the brains of mice, guinea pigs and rabbits suffering from botulinus poisoning are readily susceptible of some explanation other than that they are produced by the direct action of the toxin on the central nervous system." They also/

also studied the literature and concluded that the older work on the central nervous system in botulism was very doubtful.

(10)  
Dickson and Shevky investigated first the manner in which *Bacillus botulinus* toxin acts upon the body. Their experiments were carried out on decerebrated cats. They determined the threshold stimulation of the vagus in normal and in animals suffering from botulism for cardiac inhibition. Both vagi were cut high up and the distal ends stimulated. Coincident blood pressure readings were obtained through a cannula in the left carotid artery. In botulism animals it was found that although the threshold stimulus was always high, the secondary or final threshold stimulus in many instances lay within the limits of variation for normal animals. Similar results were obtained on testing the threshold stimulus of the chorda tympani, the vagal nerve supply to the small intestines, the oculomotor nerve for contraction of the pupil, etc. On the blood pressure they found that the toxin had no effect and the function of the splanchnic nerves was not lost. They concluded that the toxin exerted a specific effect on the parasympathetic which results in a blocking of the impulses transmitted along these nerves. They further/

further state that the location of the change was undetermined but not central and not due to organic break in the conducting apparatus.

In a recent paper <sup>(11)</sup> the same authors discuss the effects of the toxin on the voluntary nervous system. Experiments were performed to see if there was any blocking of impulses from the motor areas of the brain. Four rabbits and two cats under ether anaesthesia were used. Results exactly similar to all those obtained from normal control animals were noted.

The threshold stimulus in skeletal motor nerves also did not differ from the normal. Similar results were obtained on determining the threshold stimulus in a spinal reflex and for vasomotor reflexes from stimulation of afferent nerves. Similar results were also obtained on stimulating cranial nerves 3, 7 and 11.

They concluded therefore that the somatic motor fibres of cranial and skeletal nerves are not affected in the manner they had demonstrated in the parasympathetic fibres, but that impulses are transmitted as readily and that initial muscle response to stimulation is as active in botulism animals as in normal animals. Nothing was found which indicated/

indicated that the nerve supply of the voluntary muscles was in any way affected by the botulin toxin and therefore one could not explain such symptoms as ptosis, marked muscular weakness or limberneck in chickens as being due to this. They noted clinically that sometimes a human victim could raise the eyelids or an extremity once or twice but was not able to repeat the act.

They therefore decided to investigate whether fatigue might not play a part in determining the symptom complex of botulism. Experiments to this end were carried out on decerebrated cats. They concluded that in botulism though the initial threshold stimulus is within normal variations, the thresholds for contractions of the muscle at intervals during the course of fatiguing soon increases in value. Rest brings the initial threshold stimulus back to normal but the subsequent rise in threshold values is much more rapid. Fatigue is proportional to the intensity of intoxication.

(7)  
Edmunds and Keiper used frogs and dogs for their experiments. In the dogs they studied salivation and noted that the effect of the toxin is first to stimulate and then to paralyse the chorda tympani. Using frogs (no doses are stated) they stimulated the sciatic nerve after injecting toxin. They/

They conclude that all the essential symptoms of botulism can be explained by an incomplete curare-like paralysis of the endings of the motor nerves of the voluntary muscles including the diaphragm and by a more or less complete paralysis of the parasympathetic nerve endings perhaps preceded by a stage of stimulation. The doses of toxin used were always 3 c.c. or more. In frogs it is interesting to note they obtained no improvement in botulism frogs after injecting physostigmine which is antagonistic to curare.

(6)

Edmunds and Long carried out their experiments on cats and dogs mainly. The toxin they used required 2-5 c.c. to kill a cat and dogs required even more - 10-30 c.c. Their minimal lethal dose for guinea pigs was 0.01 c.c. in 24 hours.

First they investigated substances such as pilocarpine, atropine, pituitary extract, etc. on isolated portions of oesophagus, stomach and intestine of rabbits, cats and chickens, and obtained only normal responses. The only difference was that the tissues remained active for a short time only, generally half an hour. On the circulation, using cats and dogs, the effect of the toxin was to cause a slight reduction in the blood pressure with acceleration of the heart from 180 (normal) to 200 or 210. They obtained depression of the vagus and finally after giving doses of 15

to 20 c.c. toxin, the vagus became inactivated. These experiments were on cats and rabbits and one dog. On stimulating the vagus no response was obtained

From these and other experiments the authors conclude that the essential action of B. botulinus toxin is to cause a more or less complete paralysis of the motor nerve endings in striated muscle and in the diaphragm and also impairment of vagus.

(5)  
Schübel using as experimental animals mainly frogs but also cats, compares the action of B. botulinus toxin to curare and concludes that the toxin acts primarily on the central nervous system and later peripherally on the motor nerve endings. Again large doses, generally 1 c.c. or over, of the toxin are used.

From the above survey of the chief papers dealing with the mode of action of B. botulinus toxin, it will be seen that there is no unanimous view as to the pathology of the toxin.

One other point requires mention before proceeding to give an account of the work done during this investigation. This concerns the relation of certain diseases of animals to the Bacillus botulinus. Thus in recent years the bacillus has been proved to be associated with such diseases as limber-neck in fowls, forage poisoning in horses and/

and Borna disease. Other observers have also sought to prove that grass disease in horses in this country is due to the Bacillus botulinus.

(12)  
J.F. Tocher and his co-workers state that they have proved that a bacillus similar to Bacillus botulinus can be isolated from horses suffering from grass disease and that subsequent subcutaneous injections of toxin from this bacillus produce a disease which these authors say is identical to ~~grass~~ *with* grass disease. The bacteriological methods and technique they used during the course of their investigations are open to criticism, and moreover they did not demonstrate that they could reproduce a picture similar in all respects to grass disease by feeding their toxin to horses by the mouth. This is an important point in connection with the proof of any toxin of bacillus botulinus, for this toxin differs from all other toxins in that it exerts an action when taken by the mouth. Certain animals are much more resistant to the toxin when given by mouth than others, e.g. the sheep is very resistant, whereas the horse is very susceptible.

With the diseases in animals proved to be due to B. botulinus or its toxin, this work does not deal, but the question of whether grass disease in horses is botulism or not received very careful consideration and/

and was made a special study in view of the great importance this disease has acquired in recent years.

On looking through the literature of experimental botulism in horses the results are very unsatisfactory, the reason being that many of the observations were carried out before all the different strains of *B. botulinus* had been differentiated and the recorded symptoms are incomplete and therefore of very little value, while post mortem examinations are infrequent and often carried out a considerable time after the death of the animal. Again the method of administering the toxin is generally by injection and often unfiltered broth culture is used, whilst in other cases no statement at all appears as to whether the toxin had been filtered or not.

About 50 cases of experimental botulism are described in which unfiltered broth was used.

These are recorded by Graham, Himmelberger and Pontius (13) and Graham and Bruechner (14). These latter authors describe a case (14) which serves as an excellent illustration of the inadequacy of their observations. It is therefore given in detail.

15/5/17. /

15/5/17. A horse was allowed to ingest 2 c.c. of broth culture of B. botulinus in a kilo. of oats.

Symptoms:- 19/5/17. Prehended and masticated feed awkwardly accompanied by slight ptyalism.

20/5/17. Drawn appearance of flesh, profuse salivation, paresis of pharynx, muscular weakness and a stupid listless attitude were observed. Became decumbent at 1 p.m. In a decumbent position the animal moved the feet violently and suffered from marked dyspnoea.

21/5/17. Still decumbent. Marked dyspnoea. Tongue pendulous and protruding; feed refused and an audible clicking sound was noted in the pharynx.

22/5/17. Decumbency continued. Salivation, mucous discharge from nostrils. and enuresis were observed

23/5/17. Death occurred at 7 p.m."

No post mortem examination seems to have been carried out.

(15)

Hart and Hayes describe cases in which toxin, type A, was used. Three horses were injected subcutaneously and two were fed with it, one receiving 3 c.c. in a quart of barley, the other 6 c.c. Neither showed any symptoms with these doses and even 45 c.c. only produced death after a considerable interval. Again no clear picture can be drawn from their accounts of the disease.

(16)

Buckley and Shippen have described seven cases in which toxin, type B, was used on horses and/

and donkeys by feeding. The smallest dose which killed a donkey was 0.05 c.c. of broth culture added to oats.

Theiler<sup>(17)</sup>, Seddon<sup>(18)</sup> and others describe cases in which toxin of *Bacillus paratuberculosis* was administered. The accounts of all these authors vary in several details as regards symptoms and no regular observations have been made of the pulse, respiration and temperature of affected animals. The necessity for an accurate study of the clinical course of botulism in horses was thus evident.

The method of preparation of the toxin is given below and thereafter follows a description of the experiments carried out in the course of this investigation.

Mice, guinea pigs, hens and horses were the animals used and a minute study of the results of experimental inoculation of botulin toxin was undertaken. Numerous post mortem examinations, particularly on guinea pigs and horses were also carried out.

III. /

### III. PREPARATION OF TOXIN

Schübel has shown that *Bacillus botulinus* grows best and produces a toxin of maximum potency in a medium containing liver. A modification of the medium used by him was therefore made up as follows: 1 kilogram of butcher's steak was minced up as finely as possible and allowed to stand in 1.5 litres of tap water for about 16 hours. The mixture was then heated to boiling point and kept boiling for half an hour. On cooling, the mixture was again passed through a very fine mincer and returned to the water.

1.5 gms. of sodium bicarbonate, 3 gms. of dry pancreatic extract (dogs) and 20 c.c. of chloroform were added.

At this stage the reaction of the mince broth was tested with litmus and found to be slightly alkaline.

The mince broth was next incubated for two days at 37° C. During each day of incubation the mixture was shaken from time to time to ensure thorough mixing.

After/

After incubation dilute hydrochloric acid was added until the reaction of the mixture to litmus was very faintly acid. The whole was then sterilised in Kochs for one and a half hours. (Höttingerstammlung)

A liver bouillon was also prepared as follows: 3 lbs. of liver were passed through a mincer and minced as finely as possible. The minced liver was then allowed to stand overnight in 3.5 litres of tap water.

As *Bacillus botulinus* grows best and produces most potent toxin in presence of glucose and sodium chloride, 100 gms. of the former and 30 gms. of sodium chloride were added.

The mixture was then made up to 4 litres. This was equivalent to 2.5% glucose.

2 gms. per cent. of di-sodium phosphate were also added.

The broth was sterilised by heating to 100° C. on three successive days. Each time it was cooled quickly.

100 c.c. of the Höttingerstammlung were taken and added to 400 c.c. of liver broth, thus making 500 c.c. of culture medium.

A culture of *Bacillus botulinus*, type A. No.750, was obtained from the Lister Institute, London.

Four/

Four tubes containing Robertson's Bullocks heart medium were inoculated with the culture obtained from the Lister Institute. Two of these tubes were incubated at 25° C. and the other two at 37° C. All the tubes were covered with a layer of sterile paraffin. After two days' incubation all the tubes showed evidence of bacterial growth and Bacillus botulinus was found on microscopic examination. No other organisms were found. By repeated inoculations of petri dishes and agar slopes by both aerobic and anaerobic incubation, it was proved that the culture was a pure one.

The 500 c.c. liver and meat medium were inoculated with this culture of Bacillus botulinus and incubated anaerobically (the medium being covered with sterile paraffin) at 25° C. for seven days.

On the second and third days there was evidence of bacterial growth. The fluid turned cloudy, opaque, and there was marked evolution of gas and a strong butyric odour was liberated.

On the eighth day the broth culture was centrifuged for six hours and a bacterial-free toxin was obtained by filtration through Berkfeldt filters.

The toxin thus obtained was of a golden yellow/

} 2

2

The colour of a toxin is most important

yellow colour, clear and transparent.

0.5% sterile carbolic acid was added and the toxin covered with sterile paraffin and stored in the ice-chest, small quantities being used as required.

This toxin remained undiminished in potency for six months when stored as above.

*Acknowledgments*

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IV. /

IV. STANDARDISATION OF TOXIN.

Experiments on Mice.

Dilutions of the filtered toxin in sterile saline were made.

Doses of 0.1 c.c., 0.01, 0.001, 0.0001, 0.00001 and 0.000001<sup>c.c.</sup> were injected subcutaneously into mice in order to determine the minimum lethal dose for mice.

The average weight of each mouse was from 18-22 grams. For each dose two mice were injected.

Dose	Mouse		Time	Estimated Incubation Period
	A	B		
0.5	+	+	20 hrs.	12 hours
0.1	+	+	17 hrs.	5 days
0.01	+	+	24-48 hrs	12-16 hrs
0.001	+	+	54-60 "	24-30 "
0.0001	s	s	-	46
0.00001	0	0	-	-
0.000001	0	0	-	-

Symptoms /

+ = died.  
s = showed symptoms.  
0 = survived; showed no symptoms.

Symptoms:

Soon after the injection the mouse lies in the corner of the cage and the respiration is very rapid. It quickly recovers, however, and appears to be quite normal in about half an hour. After an incubation period varying according to the dose from 5 to 26 hours, symptoms of botulism appear. These symptoms are essentially the same in all cases though in one case one symptom may be more obvious than another.

The first four mice, i.e. those receiving doses of 0.5 and 0.1, all died through the night. They were injected at 4.30 p.m. on the 23/11/27. At 6 p.m. the same night there were no symptoms and the animals appeared normal. At 9.30 p.m. the same day three of them were showing definite symptoms. Both the mice which had received a dose of 0.5 c.c. and one of those which had received a dose of 0.1 c.c. were lying down at the back of the cage and were unable to move except with very great difficulty. Respirations were very rapid and dyspnoea marked. Movements of the alae nasae obvious. On prodding with the blunt end of a pencil no obvious notice was taken and even after prodding gently with the sharp end of a compass only feeble attempts were made to get out of reach and even these were soon abandoned/

abandoned. On offering bread and milk no attempt was made to take these. It was noticeable that there seemed to be a general weakness of all the muscles of the body and affecting most particularly the muscles of the hind legs. If the affected mouse was placed on the bench, it was unable to move more than a few inches. There was no obvious salivation.

By the next morning (24.11/27) at 9 a.m. these three mice and also the fourth which had not shown any obvious symptoms, were dead.

By this time it was noticed that three other mice were showing symptoms of botulinus intoxication, viz. the two which had received each a dose of 0.01 c.c. and one which had received a dose of 0.001 c.c. The two former showed marked symptoms. The most striking feature was the difficulty in locomotion. There appeared to be a general weakness of the muscles of the body but as in the former cases this was much more marked in the back legs. On movement the mouse was able to move its fore legs and appeared to have considerable strength in them, but the hind quarters were "dragged" along and were more an obstacle than an aid to locomotion. Respiration was also rapid, shallow and in two cases irregular. There were distinct spasmodic respirations and cyanosis of lips and ears.

After/

After 24 hours (25/11/27, 4 p.m.) two of them died (dose 0.01) and death was obviously due to paralysis of respiration. The second died 48 hours (25/11/27, 4 p.m.) after inoculation, death again being due to paralysis of respiration. The third mouse (dose 0.001 c.c.) died after 54 hours (25/11/27, 10 p.m.).

The remaining mouse which had received a dose of 0.001 c.c. began to show symptoms about 30 hours (24/11/27, 9 p.m.) after injection. In this case the symptoms were similar but respirations, although rapid and shallow, were regular. It died through the night 25-26/11/27. None of these above described was able to feed after onset of symptoms.

The two mice which were injected with 0.0001 c.c. did not succumb and only after 46 hours were there any apparent symptoms suggesting an action of the toxin. After this time, it was noticed that their movements were not so quick as they had formerly been. They were able to feed quite well but in the course of a few days they began to lose weight and after two weeks this was quite obvious. There was no paralysis or salivation, but when allowed to run along the top of a bench, each animal moved with a "hunched up" attitude as if it were not/

not able to stretch itself completely. After one month both of these animals had very visibly lost flesh and were practically skin and bone. After two months they were still alive, but a few days later one of them was found dead. The other recovered.

No mouse with a dose of less than 0.0001 c.c. showed any symptoms.

Post mortem examination:

Nothing very characteristic was noted. In three cases the stomach was distended and slightly dilated and always contained food. No congestion of mucous membrane. In every case there was distinct hyperemia and congestion of the intestines. Most marked in the small intestine, less so in the large. Contents in each case fluid. The bladder was usually full of urine but not over-distended.

On injecting some of the urine (1 c.c. and 2 c.c. doses) into other mice no symptoms of botulism developed.

Liver and spleen normal.

Kidneys in 5 cases showed slight congestion and about half the mice had some congestion of brain.

Lungs - no active congestion except in some cases a degree of hypostatic congestion.

On/

On 26/11/27 a white mouse, 20 gms. weight, was given 0.0001 c.c. toxin, type A, subcutaneously.

During the first week no symptoms developed. Then the animal began to lose weight gradually and weakness especially of the back legs was evident. Weight at end of second week, 19 gms.

During the third week weakness and wasting became more marked. Great difficulty on moving though definite paralysis was not present. Feeding normally. No salivation. Weight at end of third week, 18.5 gms.

On the same date (26/11/27) other three mice were injected with doses of 0.00025, 0.0005 and 0.00075 c.c.

The two former showed symptoms as described above. The one which had received a dose of 0.00075 c.c. after showing typical symptoms four days after injection, died on the eighth day.

#### Toxin by Mouth to Mice.

Six mice were given toxin by the mouth by means of a fine pipette.

Two	received	a	dose	of	0.1	c.c.
"	"	"	"	"	0.3	"
"	"	"	"	"	0.5	"

No deaths occurred.

When 1 c.c. of the toxin was given to other two mice by mouth, acute symptoms developed and the mice were dead in 24 hours.

Conclusion: Mice are relatively insusceptible to toxin by the mouth.

Toxicity of Toxin, Type B. to Mice.

2/12/27. 3.30 p.m. Four mice, 20-25 gms. weight were given doses of toxin, type B, subcutaneously as follows:-

Mouse 1	given	0.1 c.c.	toxin, type B.
2		0.01 c.c.	"
3		0.001 c.c.	"
4		0.0001 c.c.	"

3/12/27. 11 a.m. All the mice were found dead.

The experiment was therefore repeated as follows:

Mouse 5	given	0.001 c.c.	toxin, type B, subcutaneously
6		0.0001 c.c.	"
7		0.00001 c.c.	"
8		0.000001 c.c.	"

4/12/27. 11 a.m. No. 5 was found dead.  
No. 6 found dead at 9 p.m.  
(34 hours after injection.  
Nos. 7 and 8 were showing typical symptoms as described in Expt. for toxin, type A.

5/12/27. 1 p.m. Nos. 7 and 8 died 50 hours after injection.

5/12/27. Two mice were now injected subcutaneously as follows:

Nos. 9 and 10	given	0.000001 c.c.	toxin
			type B.
11 and 12		0.0000001 c.c.	"

No. 9 died after 53 hours, showing definite symptoms 24 hours after injection.

No. 10 was also showing effects of the toxin on 8/12/27 but did not die until 8/12/27, 77 hours after injection.

Nos. 11 and 12 never developed symptoms of any kind and survived.

Toxicity of Toxin to Guinea Pigs, Type A.

(1) By subcutaneous injection.

5/12/27. Guinea pigs of 250 gms. average weight were used and were given doses of 0.1 c.c., 0.01, 0.001, 0.0001, and 0.00001 c.c. toxin, Type A subcutaneously at 2.30 p.m.

At 8 p.m. the same day all the animals appeared well, were feeding and showed no symptoms.

The guinea pigs (1 and 2) which had been given doses of 0.1 c.c. and 0.01 c.c. died during the night and therefore no symptoms were observed.

The guinea pig (3) which had received a dose of 0.001 c.c. was showing definite symptoms and died 48 hours after injection.

The 4th animal which had received a dose of 0.0001 c.c. was also showing signs of botulism. It died after 7 days.

The guinea pig which had received a dose of 0.00001 c.c. survived and showed no symptoms.

As the general symptoms which arise as a result of subcutaneous injection of Botulin toxin (type A and B) are well exemplified in the case of guinea pigs 3 and 4 and are similar in all cases, these will be described in detail.

Guinea/

Guinea pig (3).

5/12/27.

- 2.30 p.m. Given 0.001 c.c. toxin B. botulinus, type A subcutaneously in right leg. Weight, 302 gms.
- 3.0 Sitting at back of cage, breathing rapidly, but otherwise no signs of distress.
- 4.0 Moving about cage normally. Feeding. Respiratory movements normal.
- 5.0 No signs of action of toxin. Animal alert and active and observed to be feeding off green cabbage leaves. Cage previously cleaned out and contained a small amount of faeces. Cage again cleaned.
- 6.0 No symptoms apparent.
- 7.0 Cage again contained small quantity of normal looking faeces and also small amount of urine had been passed.
- 8.0 Animal still appears normal.
- 9.0 do.
- 10.0 On being offered a piece of cabbage leaf this was partially eaten. Swallowing.
- 11.0 Moving about cage with usual agility. Bowels active; urine passed. Respirations normal.

(Note:- Guinea pigs 1 and 2 which had received respectively 10 and 100 times the dose received by this animal were also at this time (11 p.m. 5/12/27) showing no symptoms whatever and appeared to be normally active and feeding).

6/12/27. /

6/12/27.

9 a.m.

Guinea pigs 1 and 2 were found dead and had evidently been so for at least 2-3 hours.

Guinea pig 3 was showing definite symptoms now. It was lying down at the back of the cage made very feeble attempts to rise. There was no actual paralysis but there was marked general weakness especially of the hind legs. The animal was only able to move short distances (one or two feet) with difficulty and appeared to become very easily exhausted. On turning it over and laying it on its back, it made strenuous endeavours to right itself and both fore and hind legs were actively moved. These attempts were of comparatively short duration and one had the feeling that in struggling to get on to its feet the guinea pig became very easily exhausted. After trying unsuccessfully for a time to right itself, it eventually did manage to do so and dragged itself to the back of the cage.

There was no obvious salivation or apparent paralysis of the tongue. Respirations were normal.

Faeces and urine had been passed through the night. Appearance of faeces normal.

11 a.m.

Condition much the same. Attempts made to eat. Cabbage leaves were nibbled but swallowing appeared to be impossible. The animal, however, was bright and eager for food.

2 p.m.

Animal lying in cage, head (up to now kept erect) resting on floor of cage. Respirations rapid and alae nasae showed active movement. When laid on back only very feeble and short lived/

6/12/27. (Contd.)

lived efforts made to right itself. These efforts were unsuccessful. Not feeding now and not showing any desire for food. No faeces or urine passed since morning.

- 5 p.m. Condition much the same. No definite paralysis of any extremity. On lifting the animal in both hands, it had a characteristic "limp", "feel."
- 7 p.m. About this time spasms of respiration became apparent. Spasm affecting diaphragm. Otherwise condition as above.
- 10.30 p.m. No further change.

7/12/27.

- 9 a.m. Condition as last night but weaker. Spasm of respiration still present. No definite paralysis of any extremity. No attempts made to take food. Small quantity of urine passed. No movement of bowels. No evidence of salivation.
- 11 a.m. Died.

Symptoms, Guinea Pig No. 4.

5/12/27.

- 2.30 p.m. Given 0.0001 c.c. B. botulinus toxin, type A, subcutaneously right thigh.

Observed at hourly intervals until 11 p.m. No symptoms appeared. Animal bright, active, feeding well during afternoon and evening. Urine and faeces passed.

6/12/27.  
9 a.m.

Animal appears normal. Feeding well and active.  
Throughout/

Throughout the day no change was noticed.

7 p.m. Animal sitting at back of cage. Rather dull movements, not so active as formerly. When prodded with a thin stick, no attention paid for some time, then moves off to another part of the cage but movements are slower than formerly. No evidence of paralysis. When laid on its back, rights itself almost immediately. Respirations normal.

10.30 p.m. No change.

7/12/27.

9 a.m. Sitting at back of cage. Movements slow, evident weakness of back legs but no complete paralysis. When placed on top of flat bench moves about freely but movements slower than usual. Instead of attitude when walking being natural, it moves in a "bunched up" attitude, the fore part being approximated to the hind part as if it were unable to stretch itself completely. Feeding. Bowels active and urine passed. Respirations normal.

5 p.m. Condition unchanged. No further symptoms.

8/12/27.

9 a.m. Slightly weaker. When turned over on its back, correct position regained with some difficulty. Weakness most marked of back legs. Feeding well. Urine and faeces passed.

5 p.m. No further symptoms developed. Condition as above.

9/12/27.

9 a.m. Still able to move about, but movements now distinctly sluggish. Marked weakness of hind legs, almost amounting to paralysis. The animal seems to "drag" itself/

itself along using its fore legs mainly. Not feeding so well but still able to swallow. Respirations normal. No paralysis of bowel or bladder.

5 p.m. Lying in cage, head resting on floor. Very feeble attempts at locomotion. Not feeding now. Respirations rapid but regular.

10 p.m. No further change.

10/12/27.

9 a.m. Dying condition. Respiration rapid but regular. Lying in cage, half on side, unable to hold up head. No urine or faeces passed during night.

12 p.m. Died.

Toxicity of Toxin, Type A, to Guinea Pigs.

(2) By Feeding.

Experiment II - 5/12/27.

Four guinea pigs were given doses of B. botulinus toxin, type A, by mouth at 5 p.m.

The first was given 1 c.c., the second 0.1 c.c. the third 0.01 c.c., and the fourth 0.001 c.c.

Symptoms and Course.

5/12/27.

- 5 p.m. Toxin administered by mouth as above.
- 6 p.m. Animals all apparently normal, bright, running about cage and feeding.
- 7 p.m. No symptoms yet apparent.
- 9 p.m. do.
- 10 p.m. do.
- 11 p.m. All the animals appeared bright and active. Breathing regular; no distress.

6/12/27.

9 a.m. The guinea pig which had received a dose of 1 c.c. was found lying dead in its cage and had apparently been dead for several hours.

The guinea pig which had received a dose of 0.1 c.c. was showing very definite symptoms and it was obvious that it would die in a short time. The symptoms noted were identical with those already described in the case of guinea pig No.3 of the previous experiment.

The/

6/12/27 (Contd.)

The animal was almost completely paralysed and was unable to move. Again the paralysis was most marked in the hind quarters and to a less extent in the fore legs. Slight salivation was noticed and the animal made to attempt at feeding. Its head was resting on the floor of the cage. There were no ocular signs such as ptosis or paralysis and the animal did not appear to be in any pain. Its condition gradually grew worse and at 1 p.m. the same day it was found dead.

The third guinea pig (dose 0.01) and the fourth (dose 0.001) were not showing any symptoms. Both were active and feeding well.

6/12/27.

5 p.m. The third guinea pig was now beginning to show evidence of the action of the toxin.

At this time it was seen sitting at the back of its cage breathing rather more rapidly than before. It also appeared to be somewhat dull and its response to stimuli such as a sharp point was decidedly slow. There was no difficulty in movement and no signs of paralysis. When laid on its back the animal soon regained its proper position. On being offered a green cabbage leaf it took nibbled portions and swallowed them.

7 p.m. Condition unchanged. No additional symptoms.

10.30 p.m. Urine and faeces passed. Condition as above; no additional symptoms.

7/12/27.

9 a.m. There was distinct paresis and the animal had difficulty in moving about. On movement the familiar "bunched up" attitude/

7/12/27(Contd.)

attitude was noted. When laid on its back it took about five minutes before its efforts to turn over were successful. Urine and faeces had been passed during the night. It was observed during the morning to nibble cabbage leaves but had difficulty in swallowing although there was not complete paralysis of deglutition.

5 p.m. About this time although the animal appeared ready to feed and nibbled at leaves placed in an accessible position in the cage, it could not swallow the nibbled portion remaining in the mouth between gums and cheeks. The animal was visibly thinner. Salivation present.

8/12/27.

9 a.m. Animal lying on its side; breathing spasmodic; unable to rise. On lifting, very feeble movements of front and hind legs. Died 11.30 a.m. 66 $\frac{1}{2}$  hours after injection.

The guinea pig which had received a dose of 0.001 c.c. by mouth did not at any time show symptoms.

Pathological Finds in Guinea Pigs.

The following is a summary of the post mortem findings in the case of the seven guinea pigs which died as a result of the above experiments. In each case, except in the three in which death occurred during the night and which had probably been dead for several hours, the post mortem examination was performed within a few minutes of the death of the animal.

Alimentary system:

Mouth and pharynx: usually a small amount of unswallowed food was found between the tongue and gums and between the gums and cheeks.

Oesophagus: mucous membrane healthy.

Stomach: in 3 cases the stomach was dilated and in all cases was full of partially digested food. There was never any signs of inflammation or haemorrhages into the membrane.

Small Intestine: This showed varying degrees of congestion. In some cases very slight but in others quite marked. The congestion was not uniform throughout the whole length of the bowel but varied from place to place. Contents of gut thin and viscid.

Large intestine : Full of semi-solid material.  
No evidence of congestion. Contents normal in colour and/

and consistence. No dilatation.

Liver and Spleen - normal.

Kidneys - generally showed slight congestion.

Lungs - in three cases hypostatic congestion only p.m. finding. In one case slight hypercamia.

The other 3 cases showed no abnormality.

Bladder: generally full but never distended.

Mucous membrane pale and smooth.

Brain and C.N.S.: (3 cases) (recent death) Slight hypercamia of brain and meninges.

Heart: no abnormalities.

These findings, all negative except a varying degree of congestion of small intestines and hypercamia of central nervous system, were also observed in other animals which died as a result of injection of toxin during the later experiments.

Toxicity /

10 p.m. Guinea pig No. I lying at back of cage unable to move. Salivation present. Paralysis of hind legs; fore legs weak. Head resting on floor of cage. Respirations regular; normal rate.

Other guinea pigs not affected.

30/12/27.

9 a.m. Guinea pig No. 1 dead.  
Also No. II (dose 0.001 c.c.).  
No. III, which had received the same dose as No. II, was showing typical symptoms; marked paresis of hind quarters; moves only with great difficulty and only a very short distance. Laid on back, unable to turn over but makes feeble efforts which were not sustained for any length of time. Respirations spasmodic.

5.30 p.m. Animal died.

Guinea pigs Nos. IV and V were also observed to be suffering from the effects of the toxin. They were dull and moved with difficulty about the cage, and became easily exhausted. Salivation was present and swallowing was impossible although green cabbage leaves were nibbled and attempts made throughout the morning to feed. Faeces and urine passed during morning.

Symptoms gradually became worse. Salivation most marked. Guinea pig No. IV was found dead at 8 p.m. and No. V died through the night.

At /

Toxicity of Bacillus botulinus Toxin, Type B. to  
Guinea Pigs.

(A) By Subcutaneous Injection.

29/12/27. 11 a.m.

Guinea pigs of an average weight of 300 gms.  
were used.

1 Guinea pig (No. I) was given a dose of B.botulinus  
toxin, type B. 0.01 c.c.

2 Guinea pigs were given a dose of 0.001 c.c. (Nos.  
II & III)

2 " " " " 0.0001 c.c. (Nos. IV  
& V.)

2 p.m. None were showing any symptoms at this  
time. All were bright, active and feeding.

4 p.m. The guinea pig which had received a dose of  
0.01 c.c. was observed to be somewhat dull  
but had no other definite symptoms. It  
was able to move about fairly actively on  
stimulation, but preferred to sit at the  
back of cage when left alone. The other  
animals appeared normal.

5 p.m. No additional symptoms: condition as above.  
No other animal yet affected.

7 p.m. Still no other animal except the one  
mentioned showing signs of intoxication.  
Guinea pig No. 1 now showing distinct  
weakness and slowness in moving about.  
If laid on back, only turns over with  
difficulty. Not feeding.

10 p.m. /

30/12/27.

at 11.30 a.m. other guinea pigs were injected as follows:-

Guinea pig No. VI	0.00001	toxin, type B.
" VII	0.000001	" "
" VIII	0.0000001	" "

No. VI developed typical symptoms as described in 48 hours and died on the 5th day.

No. VII, after first showing exactly similar symptoms on the 3rd day following injection, died on the 7th day.

No. VIII survived and showed no symptoms.

(B) By Mouth.

29/12/27.

11 a.m. Four guinea pigs were given toxin, type B. by the mouth as follows:-

Guinea pig No. I.	0.01 c.c.
" II.	0.001 c.c.
" III.	0.0001 c.c.
" IV.	0.00001 c.c.

Guinea pig No. I showed no symptoms throughout the day.

10 p.m. Still appeared normal.

30/12/27. /

30/12/27.

9 a.m. Guinea pig No. 1 found dead. No. 2 showing definite though slight symptoms. Weakness especially of hind legs. Slight salivation. Still able to feed but appeared slow.

5 p.m. No. 2 almost completely paralysed posteriorly. Not feeding now. Marked salivation.  
Died about 9 p.m.

Guinea pig No. 3 appeared slightly dull.

31/12/27. This condition passed off; animal bright and feeding well. Did not develop any symptoms subsequently.

Fourth guinea pig (dose 0.00001 c.c.) also survived without showing any symptoms.

Experiments/

Experiments on Hens.

Experiment I.

- 21/2/28. White hen, weight 1.5 kg. given 5 c.c. toxin, type B. per stomach tube.
- 22/2/28. No symptoms; feeding well and active.
- 23/2/28. No symptoms.
- 24/2/28. Animal lying on breast in the cage. Able to walk a short distance when lifted on to its feet. Weak, staggering gait. No apparent weakness of neck muscles. Head kept well up and the animal was able to feed.
- 25/2/28. Would not feed now. Weakness of neck muscles evident. Unable to keep head erect but rests it on the floor of the cage. Unable to stand or to rise. Occasional fluttering movements of wings.
- 26/2/28. Condition as above but getting weaker.
- 27/2/28. Animal found dead.

Post mortem examination.

No pathological findings except slight degree of congestion of the liver. All other organs healthy appearance.

Experiment II.

- 1/3/28. Brown hen, weight 2 kg. given 5 c.c. toxin, type A. per os. at 2.30 p.m.
- 2/3/28. Active and feeding well. No symptoms.
- 3/3/28. /

Experiment II (Contd.)

3/3/28. Animal lying on floor of cage but able to rise and carry out flying movements, wings sometimes rising one foot high in the air. Unable to keep head erect owing to weakness of neck muscles. The head was kept resting on the floor of the cage

The animal gradually became weaker during the day and died during the night of 3rd-4th March.

Post mortem examination:

No pathological findings.

Experiment III.

1/3/28. White hen, weight 1.5 kg. given 1 c.c. toxin, type B. per stomach tube.

2/3/28. No symptoms.

3/3/28. Appears normal. No symptoms of weakness or depression. Feeding well.

Animal survived. No symptoms became apparent.

Effect/

V. Effect of Acid and Alkali on Potency of the Toxin.

Type B.

(19)

Bronfenrenner and Schlesinger in America have carried out a large amount of research on the toxin of Bacillus botulinus. Amongst other points they investigated the effect of digestive juices on the potency of botulinus toxin. This point they investigated because of the fact already noted that the toxin is poisonous whether it be administered parenterally or per os. More particularly they studied the resistance of botulinus toxin to changes in hydrogen ion concentration of the medium.

They used toxin of a known potency and mixed it with equal volumes of hydrochloric acid or sodium hydroxide of different concentrations and incubated for 24 hours at 37° C. At the end of that time the hydrogen ion concentration of each toxin mixture was determined by means of a potentiometer. Each mixture was then diluted with physiological salt solution and 0.3 c.c. of each of these solutions was injected intraperitoneally into three mice. Each mouse received 0.00003 c.c. of the original toxin or 10 minimal lethal doses modified to various degrees by incubation with acid and alkali respectively. The statement that a dose of  $3 \times 10^{-21}$  c.c. of botulinus toxin could produce/

produce death has been criticised as improbable, if not impossible. Their conclusions are based on the time taken to kill a mouse. Thus the original minimal lethal dose of the unaltered toxin for a mouse in 24 hours was  $10^{-6}$ . From these and other experiments they state that acid increases the potency of the toxin. A dose of toxin which will kill a mouse of 20 gms. weight in 48 hours of  $3 \times 10^{-7}$  produces the same effect as a dose of  $3 \times 10^{-18}$  or even  $3 \times 10^{-21}$  if its hydrogen ion concentration is the same as the acidity of the gastric contents pH 4. Alkali reduces the potency of the toxin to less than a tenth.

(20)

Geiger and Gouwens on the other hand also working on the effect of acid on the potency of the toxin found that four strains of *Bacillus botulinus* toxin were not increased in potency by acidification. Using toxin from type A, *Bacillus botulinus*, they found its minimal lethal dose to be 0.00001 c.c. The hydrogen ion concentration of the toxin was 6.4.

They acidified the toxin first with hydrochloric and then with acetic acid to a pH 3.

Controls showed no change in minimal lethal dose of the non-acidified toxin. After storing the acidified/

acidified toxin for periods varying from 1 hour to 34 days in an ice-chest, they tested the minimal lethal dose at varying intervals. The minimal lethal dose of the acidified toxin in all cases remained unchanged.

These statements of Bronfenbrenner and Schlesinger especially as concerns the effect of acid on the potency of the toxin seemed so extraordinary as to require investigation, especially in view of the results obtained by Geiger and Gouwens. I, therefore, in a manner described subsequently, after determining the hydrogen ion concentration of the original toxin obtained for this work, investigated the minimal lethal dose with acidified toxin and with alkalinised toxin. The results obtained are reviewed in the general discussion.

The hydrogen ion concentration of the toxin was determined using Sorensen's method. The toxin was found to have a pH of 6.3. Two 2 c.c. portions of the toxin were now taken. One was acidified to pH 4.3 by adding 0.5 N hydrochloric acid (sterile). This was incubated for 24 hours at 37° C.

Similarly by adding 0.5 N sodium hydroxide the pH of the other 2 c.c. was adjusted to 8.3 and this incubated for 24 hours at 37° C. At the end of the 24 hours an equivalent of 1 c.c. of the/  
the/

the original toxin was in each case diluted with sterile standard saline solution in different dilutions.

Dilutions of the acidified toxin were made and 1 c.c. injected subcutaneously into mice in doses of  $10^{-2}$  to  $10^{-7}$ . For each dose 3 mice were injected.

In the same way, 1 c.c. of the alkaline toxin was injected subcutaneously into mice representing a dose of the original toxin of  $10^{-1}$  to  $10^{-6}$ . Again for each dose 3 mice were used.

The minimal lethal dose of the toxin (unaltered) was retested and the dose killing a mouse in 48 hours was 0.0001 c.c.

Effect of Acid Toxin, pH 4.3.

Dose	Mouse			Time
	A	B	C	
0.01 c.c.	+	+	+	24 hours
0.001 c.c.	+	+	+	36 "
0.0001 "	+	s	s	46 "
0.00001 "	s	0	0	Survived
0.000001 "	0	0	0	-
0.0000001 "	0	0	0	-

Effect/

+ = died.  
 s = showed symptoms  
 0 = survived; showed no symptoms.

Effect of Alkaline Toxin, pH 8.3.

Dose	Mouse			Time
	A	B	C	
0.1 c.c.	+	+	+	16 hours
0.01	+	+	+	24 "
0.001	+	s	s	56 "
0.0001	0	0	0	Survived
0.00001	0	0	0	"
0.000001	0	0	0	"

In view of these results and to note at the same time what effect, if any, 24 hours' incubation at 37° C. would have on potency of the unaltered toxin, the experiment was repeated.

Acid Toxin, pH 4.3.

Dose	Mouse			Time
	A	B	C	
0.01 c.c.	+	+	-	15 hours
0.001	+	+	s	39 "
0.0001	s	s	0	Survived
0.00001	0	0	0	"

Alkaline/

Alakline Toxin, pH 8.3.

Dose	Mouse			Time
	A	B	C	
0.1 c.c.	s	s	s	74 hours
0.01	0	0	0	Survived
0.001	0	0	0	"
0.0001	0	0	0	"

Toxin with unaltered pH. (6.3)

Dose	Mouse			Time
	A	B	C	
0.1 c.c.	+			15 hours
0.01	+	+		18 "
0.001	+	+	+	39-48
0.0001	+	s	s	50 "
0.00001	s	0	0	Survived

These results show that alkali diminishes the potency of the toxin very greatly and acidification to pH 4.3 causes a smaller reduction in potency of the toxin. The minimal lethal dose with the acidified toxin is roughly one tenth of the minimal lethal dose of the original toxin.



Summary:

The results of the experiments carried out on the effect of alkali on the potency of the toxin support the general view held that alkali diminishes the potency greatly (Bronfenbrenner, etc.). Thus the minimal lethal dose of 0.0001 c.c. for the toxin used in our experiments was reduced on alkalisation with 0.5 N sodium hydroxide to a hydrogen concentration of 8.3 to 0.01 c.c. in the first series of experiments, and in the second series 0.1 c.c. failed to produce death within 48 hours.

The effect of acidification is to reduce the potency of the toxin slightly by about one tenth. This is an opposite effect from that obtained by Bronfenbrenner and Schlesinger and confirms the results obtained by Geiger and Gouwens.

VI. ACTION OF BOTULINUS TOXIN ON HORSES.

Experiments on Horses.

Experiment I.

14/12/27. Clydesdale colt, 2 years, given 5 c.c. toxin, type A, per stomach tube, 5 p.m. Weight 831 lbs. Pulse 48/m. Respiration 14/m. Temperature 100.5°.

6 p.m. Appears normal.

15/12/27.

10 a.m. Normal. Feeding and drinking. Bowels active, urine passed. Active.

2 p.m. Would not drink water. Slight difficulty in feeding, oats dropped occasionally, but able to swallow. Appears slightly dull.

4 p.m. Distinctly dull. Unable to drink. Not feeding.

5.30 p.m. Horse lying down on its side. Unable to rise. Breathing regular, not rapid. Unable to raise its head or sit up. Trotting movements of fore and hind legs at intervals. Easily exhausted. Tongue paralysed.

Throughout the rest of the day condition as above. No further movements of bowels or bladder.

Died during the night.

Post Mortem Examination.

Central Nervous System. Brain - numerous small punctate haemorrhages especially in right cerebral hemisphere, not so evident on left. No congestion of meninges. No evidence of thrombosis.

Pons medulla cerebellum - no abnormality.

Alimentary/

Alimentary System.

Stomach - not dilated, full of partially digested fodder, greenish yellow colour. Mucous membrane not congested.

Small intestines- contents only small amount of fluid present. On one or two places along the intestines petechial haemorrhages seen on subperitoneal surface. Numerous submucous haemorrhages varying in size from pin point to size of a sixpence, scattered along length of intestines. Mucous membrane not congested.

Large Intestine - Contents semi-solid, well formed. No congestion or impaction.

Bladder - Ruptured as abdomen was opened but did not contain much urine. No congestion or haemorrhages.

Kidneys - Both kidneys congested. Most marked in cortex. Capsule smooth, strips easily.

Lungs - Slight hypercamia.

Spleen - Healthy.

Liver - One or two infarcts  $\frac{1}{2}$ " to 1" long and  $\frac{1}{2}$ " to 2" deep. Surface smooth.

Experiment II.

Dark brown pony mare, weight 343 lbs.

16/12/27. Given 1 c.c. toxin, type A, per stomach at 5 p.m. Pupil moderately dilated. Pulse 40, respiration 12, temperature 100.5.

17/12/27. Pulse, respirations and temperature normal. Pupil slightly dilated. No symptoms. Feeding well. Bowels active; urine passed.

18/12/27. /

- 18/12/27. Pulse, respirations and temperature normal. Slightly lethargic attitude(?) Feeding well. Gait normal.
- 19/12/27. Pulse, respirations and temperature normal. Definitely lethargic. Dull. Not feeding quite so well. Dropping of food occasionally. Faeces and urine passed.
- 20/12/27. Definite depression evident. Great difficulty in feeding. Unable to take all its food. Chewed for a long time before swallowing and swallowing performed with difficulty. Marked salivation and dropping of food and saliva from mouth. Slight impairment of motion due apparently to weakness of hind quarters. Weight 50l lbs. Weight of faeces passed 55 lbs.
- 21/12/27. Found lying stretched out on right side. Unable to rise. Tongue paralysed. Occasional trotting movements with fore and hind legs but rapidly exhausted. Foul smell from mouth. No passage of urine or faeces during last 24 hours. Temperature 99, pulse 55/min.; respirations 13/min. Slight congestion of conjunctiva and slight redness of mucous membrane of the mouth. Pupils react to light moderately dilated. No ocular paralysis.
- 22/12/27. Still alive. Passed some urine voluntarily last night. No faeces passed. Pulse 80/min. Temperature 105. Respirations 20/min. Dying condition. At 11 p.m. same day still alive but very weak. Died during the night.

Post Mortem Examination.

Central Nervous System: Brain - slight congestion of vessels. No haemorrhages. Membranes not congested. No pathological findings in pons medulla or cerebellum. No evidence of thrombosis.

Alimentary/

Alimentary System: Tongue, no necrosis.

Stomach - not dilated, average amount of contents consisting of partially digested food, greenish-yellow colour. No congestion or haemorrhages in mucous membrane.

Intestines - Small intestines contained a small quantity of fluid. Numerous submucous haemorrhages varying in size from pin point to half-crown. Occasional subperitoneal haemorrhages. No undue congestion of vessels.

Large intestines full; contents semi-solid of normal colour and consistency. No congestion except at diaphragmatic flexure where a small quantity of sand was found.

Spleen - soft consistence.

Liver - soft, friable. 3 white patches seen on capsule and adherent to liver substance, extending about  $\frac{1}{4}$ " into the substance of the liver.  $1\frac{1}{2}$ " to 2" in length and  $\frac{1}{2}$ " in breadth. Consisting of grisel on cutting through them.

Kidneys - Both congested, especially the cortex. Capsule small, easily stripped.

Lungs - Marked degree of systolic congestion.

Bladder - Empty. No congestion of mucous membrane. No haemorrhages.

Blood - Fluid; no signs of clotting or thrombosis.

(See Chart on page 72).

Experiment III.

Black pony gelding, weight 819 lbs.

30/12/27. At 12.30 p.m. given 0.1 c.c. Bacillus botulinus toxin, type B, by stomach tube.  
Temperature 100.5. Pulse 45.  
Respirations accelerated on account of struggling. Pupil moderately dilated.

31/12/27. /

- 31/12/27. Pulse 48/min. Respirations 12/min. Temperature 100. Animal appears normal. Feeding and drinking. Active. Bowels active, motions normal consistence and colour.
- 1/1/28. Pulse 48/min. Respirations 12/min. Temperature 100. Animal still appears normal. No symptoms yet. Feeding well and able to drink. Faeces and urine passed.
- 2/1/28. 11 a.m. Slight difficulty and slowness in feeding now apparent. No paralysis of tongue but some difficulty in swallowing. Able to take oats but not hay. Food dropped from mouth in chewing. Would not drink water. Slightly depressed and slow in moving. No salivation. Faeces passed of normal consistence and volume. Urine also passed. Pulse 46/min. Respirations 16/min. Temperature 99.
- 3 p.m. Condition as above.
- 6 p.m. Animal lying down stretched out on right side and unable to rise. Tongue hanging out of mouth and definitely paralysed. Trotting movements of fore and hind legs. Pupil moderately dilated.
- 3/1/28. Pulse weak, rate 60/min. Respirations accelerated 28/min. Temperature 98. Otherwise animal in same condition as previous night. Trotting movements still present but weaker. Small amount of faeces passed of normal consistence.
- 4/1/28. 10 a.m. Pulse imperceptible. Respirations 36/min. Temperature 103. Animal very weak. Trotting movements at long intervals and very feeble.
- Died 12.30 p.m.

Post/

Post Mortem Examination.

Central Nervous System. Brain and meninges no hypercamia. No haemorrhages.

Pons medulla, cerebellum - no abnormal appearances.

Alimentary System. Stomach - Contents fluid, greenish colour. No dilatation. Petechial haemorrhages scattered over surface of mucous membrane.

Intestines. The small intestines contained a small quantity of thin watery contents. There was no congestion of the intestines and no haemorrhages were present.

The large intestine was full of faeces of normal consistence and colour. No congestion or haemorrhages.

Bladder - Full but not distended. Numerous petechial haemorrhages present.

Lungs - Slight degree of hypostatic congestion.

Liver, spleen and other organs showed no abnormality.

(See Chart on page 73).

Experiment IV.

Brown pony, weight 427 lbs.

- 10/1/28. 11.30 a.m. given 0.08 c.c. B. botulinus toxin, type B. by drenching.  
Pulse 45/min. Respirations 16/min.  
Temperature 101.4. Pupil moderately dilated.
- 11/1/28. Pulse 45. Respiration 16/min. Temperature 100. Appearance normal. Feeding and drinking. Urine and faeces passed.
- 12/1/28. Throughout the morning and afternoon no symptoms were noted. The animal appeared bright and active and was feeding well. Seen at 6 p.m. there was no change in its condition. At 9 p.m. saliva was seen to be dropping from the mouth and it had/

had eaten the oats but no hay. No other symptoms noted. Pupil moderately dilated.

13/1/28. Found lying in decumbent position. Tongue paralysed, hanging out at corner of mouth. Foul odour from mouth. Urine had been passed but no faeces. Periodic trotting movements of legs but animal rapidly exhausted.

Pulse 48/min. Respiration 16/min.  
Temperature 99.

Animal still alive at 11 p.m.

14/1/28. Animal found dead.

#### Post Mortem Examination.

Central Nervous System. No pathological findings.

Alimentary System. Stomach - average size. Contents normal consistency and colour. Slight congestion of glandular epithelium. Other parts not congested. No haemorrhages.

Intestines - Mucous membrane of upper portion of small intestine normal in appearance. Towards the lower half a certain amount of congestion was evident. Approaching the caecum extensive submucous haemorrhages were noted, extending from 4 to 6 inches in length with healthy portions of bowel intervening. Two feet from the ileocaecal valve other smaller foci of haemorrhages were noted varying in size from a threepenny piece to half a crown.

The caecum appeared healthy and of normal size with fluid contents of normal colour.

No haemorrhages or congestion throughout the large intestine.

Kidneys - The left kidney showed marked congestion especially of the lower pole. On section the congestion was most marked in the cortex. The capsule stripped easily and smoothly. The right kidney showed appearances similar to above but in a lesser degree.

Liver /

Liver - The right lobe of the liver was adherent posteriorly to the diaphragm. On section the liver substance was friable. Post mortem congestion present.

Spleen - Soft.

Lungs - Plura smooth, dark red in colour. Both lungs showed hypostatic congestion.

Heart - No pathological findings.

Bladder - Full of urine but not distended. Large number of small haemorrhages scattered all over the mucous surface, bright red in colour; varying in size from pin head to half crown.

(See Chart on page 74).

Experiment V.

Brown pony, weight 680 lbs.

17/1/28. Given 0.001 c.c. B. botulinus toxin, type B. per stomach tube at 3.30 p.m.

Pulse 45/min. Respirations 17. Temperature 99.8. Pupil moderately dilated.

Date.	Pulse per minute.	Respirations per minute.	Temperature
18/1/28.	45	12	100
19/1/28	44	12	99
20/1/28	45	12	99
21/1/28	45	12	100
22/1/28	45	12	99
23/1/28	45	14	98
24/1/28	45	12	99.4
25/1/28	45	12	99
26/1/28	45	12	99.4

No symptoms developed during the whole of this period, 17th to 26th January, and on 3rd February the animal still appeared normal. It took its food well and never had any difficulty in swallowing while its bowels acted regularly and the faeces passed were of normal consistence and colour.

4/2/28. Weight of animal now 656 lbs. It had therefore lost 24 lbs. since 17/1/28.

The pony was not <sup>w</sup> given a second dose of toxin; this time 0.01 c.c. B. botulinus toxin, type B. by stomach tube.

Date.	Pulse per minute.	Respirations per minute.	Temperature
4/2/28.	46	12	99.6
5/2/28.	46	12	99.6
6/2/28.	46	14	100
7/2/28.	46	14	100
8/2/28.	48	14	99
9/2/28.	34	16	100.2
10/2/28	40	20	100.1
11/2/28	44	14 .	100.2
12/2/28.	38	10	100.2
13/2/28.	34	10	100.2
14/2/28.	38	10	100.2
15/2/28.	34	10	100.1
16/2/28.	34	14	100.1
17/2/28.	34	12	100.1

During the whole of this time no symptoms of botulism appeared. The animal was bright, active and feeding well. No bowel or bladder symptoms noted.

It began to be apparent, however, during this period that the animal was losing weight appreciably. It appeared leaner and its coat had lost its original gloss, while the ribs became plainly visible.

Weighed on 21/2/28 its weight was found to be 608 lbs. It had therefore lost an additional 48 lbs. since 4/2/28 or a total of 72 lbs. since 17/1/28.

Date	Weight	Amount of Toxin	Wt. lost
17/1/28	680 lbs.	0.001 c.c.	-
4/2/28	656	0.01	24 lbs.
21/2/28	608	0.05	48 "
		Total =	72 "

On 21/2/28 the horse was given a third dose, 0.05 c.c. B. botulinus toxin, type B. per stomach tube.

21/2/28 Pulse 40/min. Respirations 12/min. Temperature 97.

22/2/28 Pulse 40/min. Respirations 12/min. Temperature 98.5.

During the day of <sup>1st</sup> 27th February the horse showed no symptoms of intoxication. Feeding and drinking well.

The/

The next day, 22nd February, slight slowness in feeding was observed, but the animal was able to swallow. Faeces and urine were passed, the faeces being of normal consistence and colour. No salivation was present and the pupil was moderately dilated.

23/2/28. Animal found dead and had apparently been dead for at least two or three hours.

Post Mortem Examination.

Central Nervous System - Negative findings.

Alimentary System - Stomach - Average size, full of fluid contents but not distended. Congestion of mucous membrane at fundus. Other parts not congested. No haemorrhages.

Intestines - Throughout the whole length of the small intestines haemorrhages were noted in the mucous membrane. These varied in size from pin points to half a crown with occasional larger areas. Contents semi fluid, small in amount.

The large intestines contained semi solid faeces of normal consistency and colour. No congestion of mucous membrane and no haemorrhages.

Lungs - healthy.

Bladder - full of urine but not distended. Numerous petechial haemorrhages scattered all over mucous surface, mostly of the size of a pin head but some larger ones.

Kidneys - Both capsules stripped easily leaving a smooth surface. Both kidneys showed a little congestion, the congestion being more marked in the right kidney.

Liver - Surface smooth. No congestion.

Spleen/

Spleen - Average size, firm consistence. Fair number of petechiae scattered here and there over its surface. On section, no abnormality noted.

Heart - healthy.

(See Chart on page 75).

Experiment VI.

Pony mare, weight 329 lbs.

23/2/28. Given 0.006 c.c. B. botulinus toxin, type B. per stomach tube.

During the subsequent three days the animal appeared quite normal, but on the 28/2/28 it was noted that the horse was eating more slowly, though all its food was taken. The next day this slowness in feeding was still apparent but on 1/3/28 the animal was again feeding well and appeared normal.

6/3/28. No symptoms having appeared a second dose of the same toxin was given, 0.003 c.c. Weight 308 lbs. Animal had therefore lost 21 lbs.

No symptoms developed and on 12/3/28 a further dose of 0.003 c.c. was administered per os. The horse's weight on this date was 314 lbs. and it had therefore after losing 21 lbs. gained 4 lbs. since the last weighing on 6/3/28.

19/3/28. No symptoms having developed during the interval, another dose of 0.003 c.c. toxin was given/

given and the horse again weighed. Its weight was found to be 287 lbs., having lost 27 lbs. during the last week, or a total of 42 lbs.

2/4/28. No symptoms being yet apparent the dose was increased ten times and the animal therefore received 0.03 c.c. B. botulinus toxin, type B. Weight 293 lbs.

Still no symptoms developed and the horse was feeding and drinking normally and therefore on 9/4/28 a dose of 0.3 c.c. of the toxin was administered in the same manner as before. Weight now was 270 lbs.

On 10th and 11th April slight salivation was noticed and the animal was slow in feeding.

12/4/28. Found down unable to rise. Galloping movements as described in previous cases. Died during the following night.

Table/

Table showing gradual loss in weight.

Date	Weight in lbs.	Dose of Toxin given. c.c.	Total Loss in Weight. lbs.
23/2/28	329	0.006	-
6/3/28	308	0.003	21
12/3/28	314	0.003	17
19/3/28	287	0.003	42
2/4/28	293	0.03	36
9/4/28	270	0.3	59

This case illustrates the difficulty in producing a picture of "chronic" botulism which was also found in Experiments III and IV and especially in Experiment V.

In Experiments V and VI, gradual loss of weight is the main outstanding symptom. Experiment VI shows also that repeated injections of small doses of toxin appear to produce a degree of immunity. Thus in Experiments VI and VII we have two horses practically the same weight, viz. 293 lbs. and 295 lbs. respectively; yet horse VI which receives a dose three times as large as horse VII shows no symptoms, whereas horse VII dies as a result of administration of one third (0.01) of the dose which horse VI received. The former required a dose thirty/

thirty times larger, viz. 0.3 c.c. to produce death.

It is further brought out that when a horse does receive an effective dose death occurs in a few days, i.e. it is not possible to produce "chronic" botulism. Botulism is either acute or subacute.

Experiment VII.

Horse, weight 295 lbs.

- 2/4/28. Given 0.01 c.c. B. botulinus toxin, type B. per stomach tube.
- 3/4/28. Pulse 56. Respirations 14. Temperature 100. Appearance normal, feeding and drinking. Active.
- 4/4/28. Found dead.

Experiment VIII.

Horse, weight 340 lbs.

- 9/4/28. Given 0.02 c.c. B. botulinus toxin, type B. per stomach tube. Pupils moderately dilated.

No symptoms appeared until the 14th April. Up to this time the pulse, temperature and respiration were all normal.

- 14/4/28. It was noticed that salivation was present and that the animal was swallowing with difficulty. Pulse 42/min. Respiration 20. Temperature 100.
- 15/4/28. Marked difficulty in eating and drinking. Dull. Salivation present but no definite paralysis of the tongue. Slight nasal discharge. Moves about stall slowly. Pulse 38/min. Respiration 20/min. Temperature 100.2.

16/4/28 /

- 16/4/28. Condition much the same. No additional symptoms. Pulse, respiration and temperature still normal. Pupils moderately. Urine and faeces passed. Faeces normal consistence and colour.
- 17/4/28. Lying on side, unable to rise. Trotting movements with fore and hind legs. No urine or faeces passed. Tongue not definitely paralysed. Pulse, respirations and temperature still normal.
- 18/4/28. Appears weaker. Movements of legs still present but fatigue easily induced. Small amount of faeces passed, normal in consistence. Pupils moderately dilated. No paralysis of tongue present. Respirations rapid, 34/min. Temperature 101. Pulse 58 in morning and 74/min. at night.
- 19/4/28. Much weaker, otherwise condition as on previous day. Still no paralysis of tongue. Respirations 34/min. Pulse 86/min. Temperature 100. No paralysis of tongue.

Died 5 p.m.

(See Chart on page 76).

#### Post Mortem Examinations on Horses VI, VII and VIII

There were as before no definite pathological findings except in the case of horse VIII which showed a degree of hypostatic congestion of the lungs. Occasional minute haemorrhages in the small intestines were also noted. All other organs appeared healthy.

Charts/

Summary of the Action of the Toxin on Horses.

Horse	1	2	3	4
Dose and Type of toxin	5 c.c. A	1 c.c. A	0.1 c.c. B	0.08 c.c. B
Mode of administration	Stomach tube	Stomach tube	Stomach tube	drenching
Latent period	21 hrs.	72 hrs.	72 hrs.	55 hrs.
Time reqd. to produce death from time of administration	36-48 hrs.	7 days	6 days	5 days
Effect on wt. of animal	-	Lost 42 lbs. in 4 days	-	-
Effect on pulse	Remained normal	Normal until just before death	Normal throughout	Normal throughout
Bowels	Normal	Slight constipation towards end	Constipation	Constipation
Tongue	Paralysed	Paralysed	Paralysed	Paralysed
Feeding	Unable to drink or swallow food	Swallowing impossible	Swallowing impossible	Swallowing impossible
Attitude	Prostrate	Prostrate	Prostrate	Prostrate
<u>Post Mortem Findings in</u>				
Brain	Haemorrhages	Negative	Negative	Negative
Stomach	Half full	Not dilated	Not dilated	Not dilated
Colon	No impaction	No impaction	No impaction	No impaction
Liver	No infarcts	Negative	Negative	Post mortem

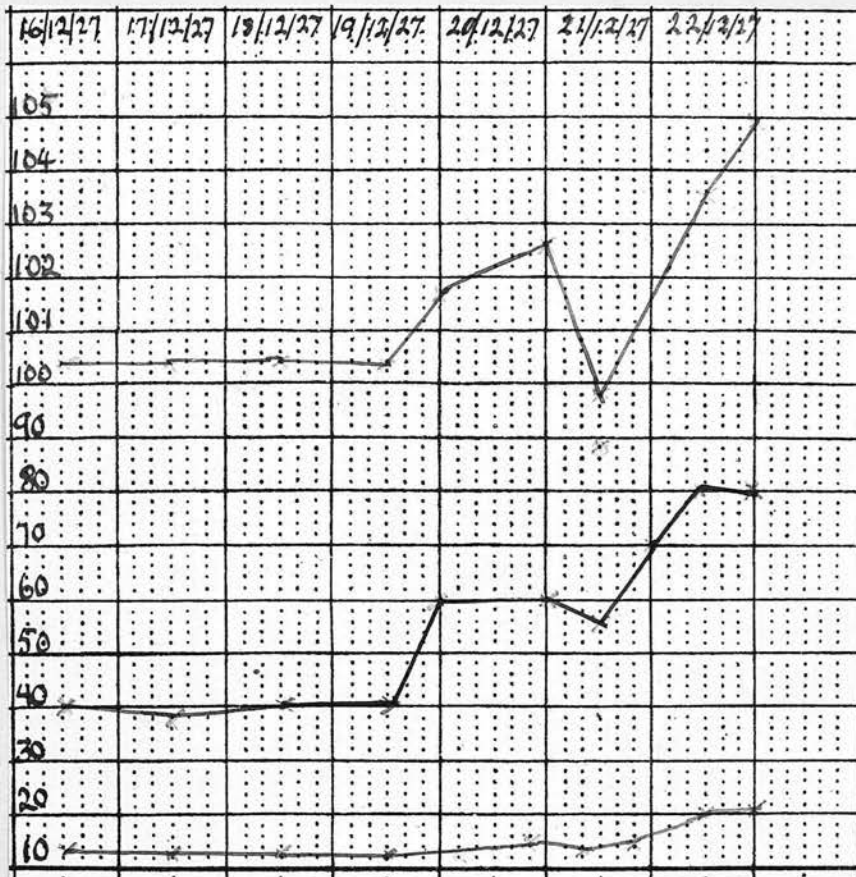
Horse	5	6	7	8
Dose and Type of toxin	(1) 0.001 c.c. B. (2) 0.01 c.c. B. (3) 0.05 c.c. B.	(1) 0.006 c.c. B. (2) 0.003 c.c. B. (3) 0.003 c.c. B. (4) 0.003c.c. B. (5) 0.03 c.c. B. (6) 0.3 c.c. B.	0.01 c.c. B	0.02 c.c. B
Mode of administration	Stomach tube	Stomach tube	Stomach tube	Stomach tube
Latent period	24 hrs. after 3rd dose	24 hrs. after 5th dose	No symptoms observed	6days
Time reqd. to produce death from time of administration	48 hrs. after 3rd dose	3 days after 6th dose	72 hrs.	10 days
Effect on wt.	Total loss of 72 lbs.	Total loss of 59 lbs.	-	-
Effect on pulse	Normal throughout	Normal throughout	Normal	Normal until 2 days prior to death when animal decumbent
Bowels	Active	Active	Active	Constipation
Tongue	Pareisis	No definite paralysis	-	Not paralysed
Feeding	Slow	Slow	-	Slowness
Attitude	Died during night	Prostrate	Died during night	Prostrate

Post Mortem/

Horse	5	6	7	8
<u>Post Mortem Findings</u> <u>in</u>				
Brain	Negative	Negative	Negative	Negative
Stomach	Not dilated	Not dilated	Not dilated	Not dilated
Colon	No impaction	No impaction	No impaction	No impaction
Liver	Negative	Negative	Negative	Negative

Chart for Horse No. II.

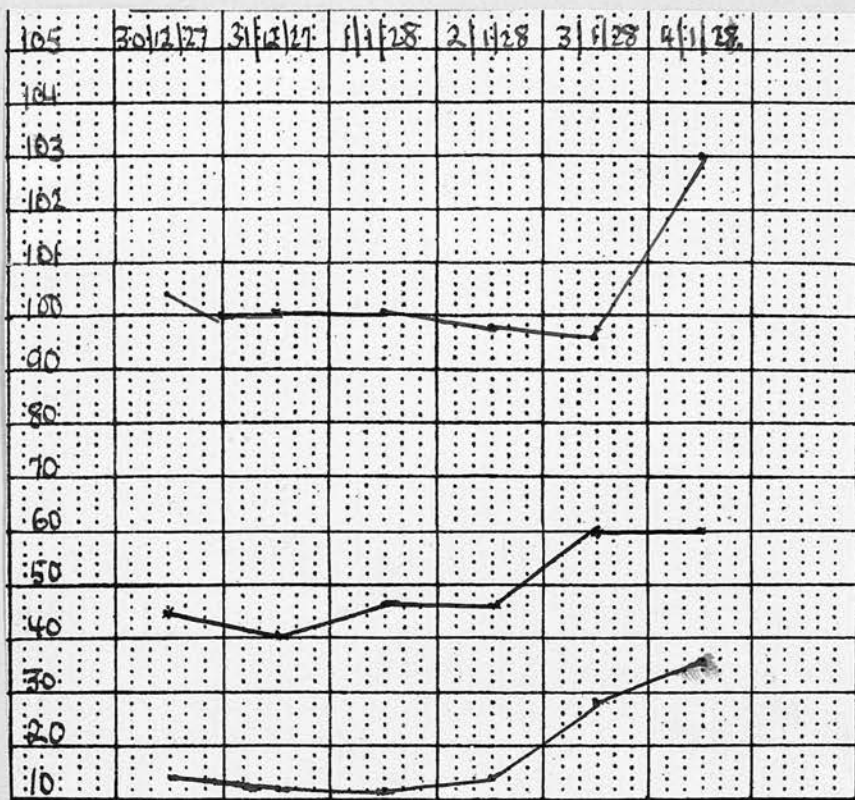
Given 1 c.c. Bacillus botulinus toxin, type A.



Red Line = Temperature.  
Blue Line = Pulse.  
Green Line = Respirations.

Chart for Horse No. III.

Given 0.1 c.c. toxin, type B.

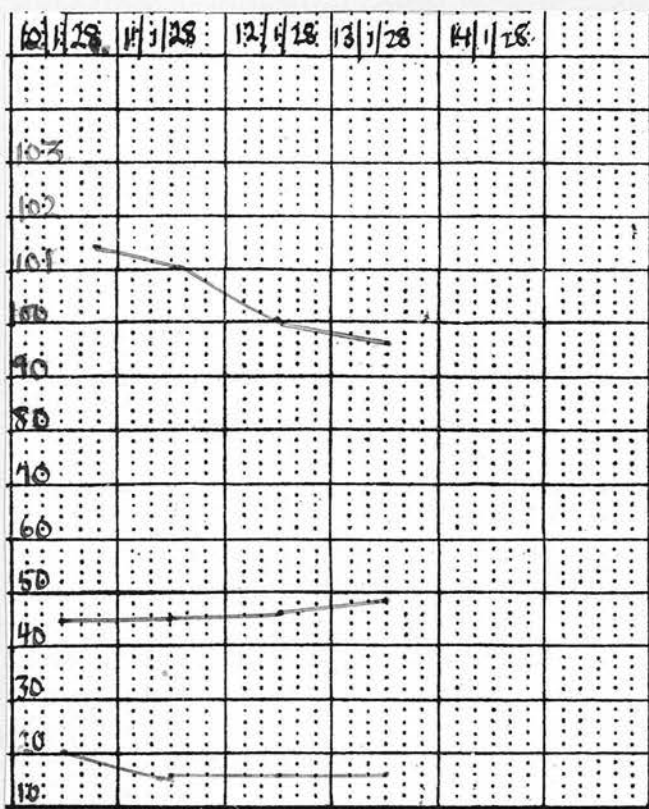


Red Line = Temperature.  
Blue Line = Pulse.  
Green Line = Respirations.

Chart for Horse No. IV.

Given 0.08 c.c. Bacillus botulinus toxin,

Type B.



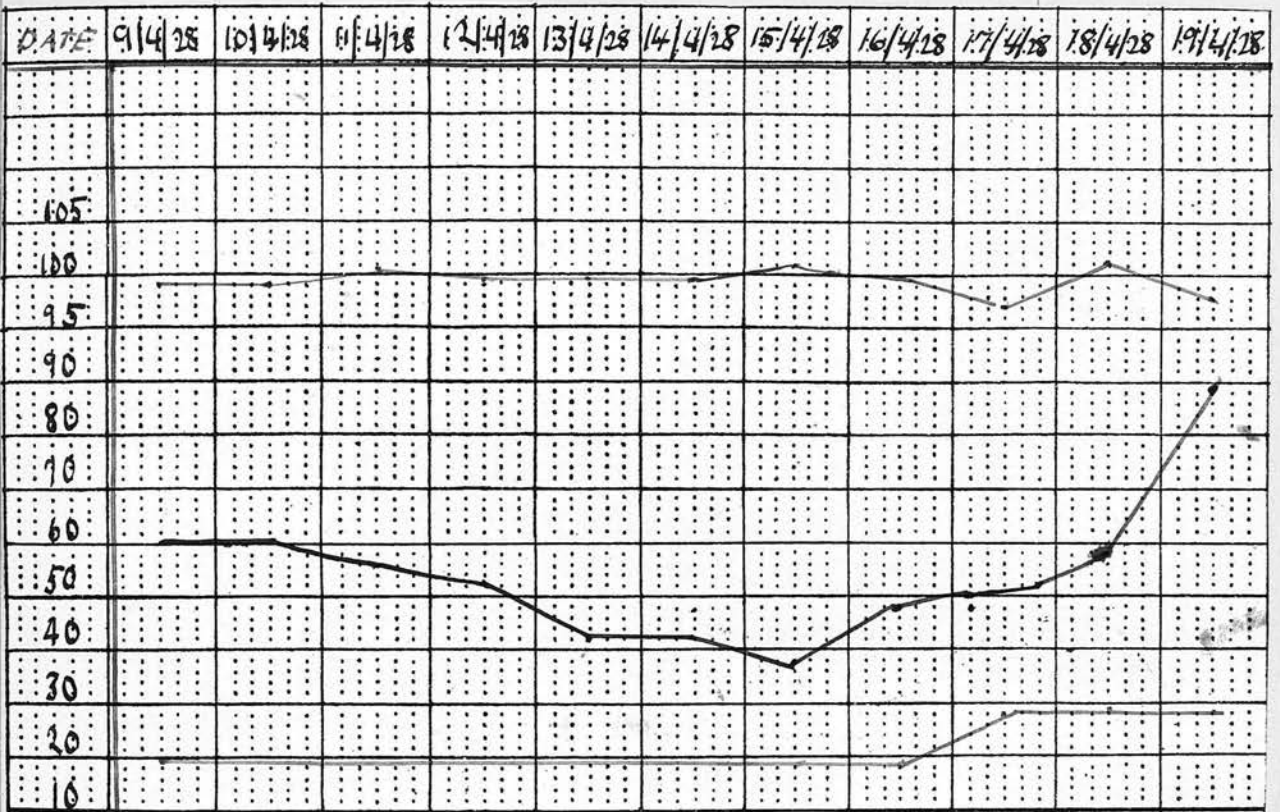
Red Line = Temperature.  
Blue Line = Pulse.  
Green Line = Respirations.



Chart for Horse No. VIII.

Given 0.02 c.c. Bacillus botulinus toxin,

Type B.



Red Line = Temperature.  
Blue Line = Pulse.  
Green Line = Respirations.

VII. DISCUSSION and CONCLUSIONS

(1) Site and Mode of Action of Botulinus Toxin.

In determining the site and mode of action of any poison or toxin, chief reliance is placed primarily on the symptoms and clinical appearances of the disease taken in conjunction with any pathological, anatomical changes found post mortem after death of a person suffering from the bacterial disease or of an animal in which the disease has been experimentally produced. By physiological experiments confirmation is frequently forthcoming.

The pathological changes noted in the large number of experiments carried out during the course of this investigation in mice, guinea pigs and especially in horses, did not reveal any characteristic that could be considered to be specific or pathognomonic for botulism. The most constant alterations were varying degrees of congestion of the small and large intestines and occasional presence in the small intestines or bladder of submucous haemorrhages. Beyond a slight degree of congestion of the meninges in some cases no morbid appearances were noted in the brain or central nervous/

? Shomlois

nervous system. The presence of such lesions moreover could not be ascribed to the direct action of the toxin and were probably connected indirectly with the disease being a secondary

To explain the pathogenesis of the disease we have to rely on the symptoms during life, which as has been noted point to an affection of the nervous system generally, and on the evidence adduced by other researchers.

It has been shown in the literary review of the main work done in this connection that there are two or perhaps three main views as to the mode of action of the toxin. First there is what might be called the old view that the toxin affects the central nervous system. The main evidence for this view consists in the degenerative changes described previously by various workers, in the region of the medulla and of the anterior horn portion of the spinal cord.

Secondly, there is the view held (Edmunds, Long and Keiper) that the toxin acts mainly peripherally producing partial or complete paralysis of the terminations of the motor nerves to the voluntary muscles and of the vagus. This view is also held by Cowdray and Nicholson whose work has been mentioned.

There/

There is a third possible view, viz. that the toxin acts mainly on the central nervous system but also peripherally. This is suggested by the work done by Schübel.

As regards the first view there can be no doubt that definite changes of the nature described previously do occur in botulism though possibly in certain cases these are not very marked and may be so slight as to escape notice altogether.

Cowdray and Nicholson in supporting the second theory and after a full investigation of the central nervous system in botulism give as their view that any changes observed "are susceptible of some explanation other than that they are produced by the direct action of the toxin". This is a matter of opinion, but even admitting their contention for argument's sake, might it not be possible for a toxin to affect the central nervous system and yet leave no microscopical traces of its action? Their work cannot be accepted as conclusive proof that the toxin has no central action.

The work of Dickson and Shewky is also open to criticism on some points. Their conclusion was that the toxin affects specifically the parasympathetic and causes blocking of impulses transmitted/

transmitted along these nerves and that fatigue played a part in producing the symptoms of botulism. They used rabbits and decerebrated cats for their experiments. These authors do not record the doses received by the animals on which they made their experiments. The animals are merely described as "Botulism animals", or "an amount of toxin which was estimated to be sufficient to cause the death of the animals in about 48 hours" was administered, the animals being of approximately the same weight. It should be considered essential, however, that any experiments of the nature carried out by these workers should be carried out on susceptible animals or animals in which botulism in some form is known naturally to occur. Cats are relatively resistant to the toxin by inoculation and are insusceptible to the toxin by the mouth. Moreover, it has been demonstrated that in horses loss of weight is a constant symptom. Depression of the parasympathetic and muscular paralysis cannot explain this loss in weight; but the loss of weight due to the toxin acting centrally would account for the great muscular weakness. In this respect the action of botulinus toxin might be compared to diphtheria toxin. In diphtheria there is often a similar/

similar loss of weight and in this case the toxin undoubtedly has a central action. In the experiments on horses described in this investigation where pulse readings were regularly taken the pulse rate never (except as a terminal phenomenon which was usually due to hypostatic congestion of the lungs) rose above normal, i.e. there is no clinical evidence of depression of the parasympathetic.

The experiments of Edmunds and Keiper as regards choice of animals (frogs and dogs were used) is open to the same criticism as noted above. The fact has also been noted that they obtained no improvement in botulism frogs after injecting physostigmine.

Edmunds and Long, using cats and dogs, the latter being very resistant to the toxin, obtained normal results on isolated portions of stomach intestines, etc. contracting with atropine, pilocarpine and other drugs except that the activity of the muscle was soon exhausted. This suggests that diminished function of muscle is not confined to striated muscle and is a general effect of the toxin, not necessarily specific.

Conclusion: From a survey of the literature and a detailed study of the symptoms in mice, guinea pigs and/  
and/

and horses suffering from experimental botulism, it is concluded that the symptoms are due to a toxic bulbar paralysis, the toxin acting centrally on the nuclei of medulla and grey matter of spinal cord. There is no conclusive evidence that botulin toxin acts specifically in paralysing the parasympathetic and the nerve endings in voluntary muscles. Any observed effects in inducing fatigue are secondary and affect unstriated as well as striated muscle.

(2) Relationship of Bacillus botulinus to Grass Disease in Horses.

The first outbreak of grass disease probably occurred at Bury in Forfarshire in 1907 though no doubt isolated cases must have occurred previous to this date. Within the last fifteen or twenty years the disease has become much more common and at the present time the economic aspect of the problem is a very serious one indeed. The disease usually occurs amongst horses in the late spring or early summer when they are turned out to feed on the grass, though occasional cases do occur in horses fed on forage in stables.

The/

The original assumption as to the causation of the disease was that it was due to feeding on a poisonous clover, but no evidence has been obtained in support of this hypothesis.

In recent years many investigators have endeavoured, working on bacteriological lines, to isolate an organism. Many organisms, especially streptococci, have been found associated with the disease but none has been found which is constantly present and which invariably reproduces the disease when the organism or its toxin is administered to animals by the mouth.

The similarity in some respects of grass disease to such diseases as Borna disease in Germany and to forage poisoning in America suggested the possibility that grass disease might also be due to the *Bacillus botulinus* or its toxin. This view was specially investigated by J.F. Tocher and co-workers who found an antitoxin in the blood of grass disease horses which they stated had an antagonistic action to the toxin of *Bacillus botulinus*. They also found in all cases examined an organism similar to *Bacillus botulinus*. This is not the result obtained by other workers who isolated different organisms and Tocher did not prove that his/

his bacillus gave all the reactions of *Bacillus botulinus*, though it appeared morphologically similar.

The detailed experiments on horses and post mortem examinations performed in our own work prove conclusively that botulism in horses and grass disease are entirely dissimilar in clinical symptoms and post mortem appearances.

As regards the morbid appearances of the two diseases two stand out with prominence. Acute grass disease is invariably associated with dilatation of the stomach and congestion of the colon, the colonic contents being of a hard cement like consistence, black in colour. Often the impaction is so marked and the resultant weight of the colon so great that two men have difficulty in raising the rigid impacted colon from the ground. As is seen from the accounts given here, botulism in horses never reproduces this. On the other hand though there may be constipation during the last day or two of life, the contents of the colon are always of normal colour and consistence and there is no dilatation of the stomach. The constipation is the result of the general weakness. A detailed comparison is given in Table II (see page 88).

The liver in experimental botulism never shows/

shows apart from a little passive congestion occasionally, any abnormality. In grass disease on the other hand jaundice is common and marked changes are often noted in the liver, the nature of which are being investigated.

As regards the symptoms in horses, these are contrasted in the table given on page 86 , and it will be noted that clinically the two diseases are entirely separate entities.

In Table II a comparison is made of the morbid appearances found in grass disease and those found in experimental botulism in horses.

Table I.

Comparison of Symptoms of Grass Disease and Botulism in Horses.

<u>Grass Disease</u>		<u>Botulism</u>
Standing generally but often lying down and rising at intervals. Lethargic. Listless.	Posture	Active during incubation period. Then sudden onset. Lies down unable to rise. Trotting movements with fore and hind legs. Mind appears bright.
?	Conjunctiva	Normal
Normal	Pupil	Not affected
Average rate 60 to 120/min.	Pulse	Never accelerated, average rate 40-50.
Average 15/min.	Respirations	Average 15-20/min.
Never subnormal generally 101-104	Temperature	Often subnormal; never raised.
Generally dry but may be copious - almost constant.	Salivation	Not constant. Generally present when tongue shows degree of paralysis. Most marked when completely paralysed, probably due to difficulty in swallowing.
Frequently present often patchy in type	Sweating	Rarely present; never patchy.
Generally present in muscles of shoulders and quarters	Muscular tremors	Noticed in one case only
Constipation marked Faeces solid.	Faeces	Constipation not so marked. Faeces always normal consistency and colour.
	Urine/	

Table I. Contd.

<u>Grass Disease</u>		<u>Botulism</u>
Often retention noted and pain and straining during act.  Frequent watery discharge  Acute, subacute and chronic	Urine  Nostrils  Course of Disease	Retention inconstant No pain on micturition.  No discharge  Acute and subacute only.

Table II.

Comparison of Morbid Appearances in Grass Disease  
and Botulism in Horses.

<u>Grass Disease</u>		<u>Botulism</u>
Never paralysed.	Tongue	Very frequently paralysed.
Often distended.	Stomach	Never distended.
Constriction.	Pyloric sphincter	Normal.
Constriction.	Ileo-caecal sphincter	Normal.
Slight cerebral congestion generally found but not constant	Central nervous system	Occasional slight congestion of cerebrum and meninges
Often slightly distended. Fluid contents.	Small intestines	Patchy congestion frequent. Fluid contents
Patchy congestion of mucous membrane frequent. Contents often impacted and hard.	Large intestines and contents	Congestion of mucous membrane occasionally noted. Contents always normal consistency.
Toxic changes generally marked.	Liver	Generally healthy. Occasionally degree of passive congestion present.
Normal	Spleen	Normal.
Normal	Kidneys	Often congested.

The/

The typical picture in the two diseases towards the end of an acute attack may be summarised as follows:-

Botulism - The animal is lying down and unable to stand. Saliva is seen dripping from the mouth and the tongue is hanging out. There is no absolute paralysis as the animal can move all limbs. There is no jaundice and the pulse is always normal.

Post mortem, the stomach and intestines with their contents are all normal, as also is the liver.

Grass Disease - The animal is able to stand. Tongue is not paralysed. The stomach is distended and there is congestion of the colon. Pulse is rapid. Tremors of quarters are seen and sweating is evident. Jaundice is present.

Post mortem, the stomach is distended and the colon impacted and solid. The liver shows toxic changes.

While it is thus seen that the two diseases are quite different in symptoms and post mortem findings, yet there are one or two similarities. In both diseases muscular weakness is prominent and there is no marked rise in temperature. There is/

is inability to swallow food or water. The inability to swallow is probably an accidental resemblance being due in grass disease to gastric distention and in botulism to pharyngeal and labial paralysis.

Conclusion:- Grass disease in horses and botulism in these animals are two clearly defined and separate diseases. This is evident after detailed investigation of the symptoms during life and morbid appearances after death from botulism in horses as compared to the corresponding findings in grass disease.

It is also shown that botulism in horses is either acute or subacute. Numerous attempts to produce a "chronic" type of botulism which could be compared to the chronic type of grass disease failed.

VIII. SUMMARY.

(1) The literature on the subject is reviewed and discussed.

(2) From two strains of *Bacillus botulinus* (Burke 750 and 751) cultures were grown on a special meat medium containing liver and the toxin filtered off after incubation for seven days at 35° C.

(3) The minimal lethal dose of the toxins for mice and guinea pigs was worked out.

(4) Experimental botulism in mice, guinea pigs and horses was induced by injection or oral administration of the toxins.

A detailed account of the symptoms in these animals and of the post mortem findings in guinea pigs and horses is given.

(5) The effect of acid and alkali on the potency of the toxin was determined.

It is concluded:-

(1) That the symptoms of botulism are due to a toxic bulbar paralysis, the toxin acting on the grey matter of medulla and spinal cord.

There is no conclusive evidence that in animals susceptible to botulism (e.g. horses and guinea pigs) the toxin has any direct action in depressing the parasympathetic or the nerve endings in voluntary muscles.

(2) Grass disease is not due to the toxin of *Bacillus botulinus*. Botulism in horses and grass disease are two separate clinical entities.

The most mortem findings also differ absolutely in the two diseases.

(3) Botulism in susceptible animals occurs in an acute and subacute form, but not in a chronic form.

(4) Acid does not increase the potency of the toxin but rather tends to diminish it.

Alkali decreases the potency of the toxin very greatly.

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