Photo]

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SIR MALCOLM CAMPBELL

AT THE WHEEL OF HIS "BLUE BIRD"

AT DAYTONA

WEARING HAMBLIN'S "FULL-FIELD" GOGGLES

FITTED WITH

"SALVOC" SAFETY LENSES.

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COMMUNICATIONS

THE OPHTHALMIC LESIONS OF BOTULISM: ADDITIONAL NOTES AND RESEARCH

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FOR many years physicians have known that debilitating ocular symptoms, serious prostrating disorders of the gastro-intestinal tract and even death itself occasionally followed the ingestion of poisoned food. Since van Ermengem's¹ discovery of the bacillus botulinus in 1895, this anaerobic organism or its toxin is always found present in cases of food poisoning accompanied by the usual ocular syndrome. Although our present day knowledge of botulism goes but little beyond that of van Ermengem's time, there is a much greater diffusion of the information. Through the attention and efforts of house-wives, farmers, commercial canners, food chemists, bacteriologists and epidemiologists, botulism has become a rare disease. *The Jl. of the Amer. Med. Assoc.*² stated that in the United States and Canada from 1899 to 1928, 165 outbreaks were recorded, with a total of 529 cases. While many of these cases lack authentication by bacteriological and toxicological tests, they are, no doubt, off-set by the unreported typical cases and the undiagnosed atypical ones.

The clinical picture of botulinus intoxication has been set forth by van Ermengem as follows: "Secretory disorders of the gastrointestinal tract; symmetrical motor paralysis, complete or partial, depending very likely on lesions of the medulla, especially of the nuclei of various cerebral nerves and of the anterior horns of the spinal cord. It is characterized:

1. By an arrest of secretion or by a hypersecretion of saliva and bucco-pharyngeal mucus;
2. By more or less complete external and internal ophthalmoplegia (ptosis, mydriasis, paralysis of accommodation, diplopia, internal strabismus);
3. By dysphagia, aphonia, obstinate constipation and retention or urine;
4. By a general weakening of all voluntary muscles;
5. By absence of fever, but with no disturbance either of general sensibilities or of the intelligence;
6. To this syndrome are added frequent respiratory and circulatory troubles, which can lead to a more or less rapid death through bulbar paralysis;
7. Finally, the characteristic manifestations do not appear earlier than 12 or 24 hours after the ingestion of the food and are often preceded by gastro-intestinal troubles that appear gradually and persist for weeks."

The importance of the ocular symptoms was emphasized by Lancaster³ who said that "usually the eyes are the first organs to be affected, and in mild cases the paralysis may be limited to the eyes." From the paucity of reports by ophthalmologists it is only reasonable to assume that in most cases the patients were attended by their family physicians. In fact, most of the data pertinent to the ocular symptoms have been furnished by others than ophthalmologists.

van Ermengem's tabulation of the ocular lesions is generally accepted, although others have suggested additional symptoms. Ruge⁴ reported one case in which he made a diagnosis of papillo-retinitis. Uhthoff⁵ stated that internal ophthalmoplegia is the most frequent cause of the amblyopia or amaurosis occurring in botulism, but that involvement of the optic nerve may figure in the visual reduction in some cases. He added, however, that there is no positive ophthalmoscopic or anatomical proof of lesions of the optic nerve. Dickson⁶ said that there may be initial scintillations and dimness of vision, but changes in the retina are rarely found. de Saint-Martin⁷ reported four cases of involvement of the optic nerve and retina that persisted for months after other symptoms of the intoxication had subsided.

Soon after the discovery of the bacillus botulinus, Marinesco⁸ made histological studies of animals that had been fatally poisoned

with botulinus toxin. He concluded that botulism was a disease of the central nervous system wherein the chief lesions were situated in the ganglion cells of the motor nuclei of the cranial nerves. Essentially similar conclusions were announced by Kempner and Pollack⁹ in 1897, by Ossipoff¹⁰ in 1900, and by Römer and Stein¹¹ in 1904. van Ermengem's summary of the work of these experimenters is briefly as follows: It produces a cloudy, fatty degeneration of endothelium; of the secretory cells of certain glands; of striated muscle fibres; of the anterior horn cells of the cord, pons and medulla; and of the motor ganglion cells in the midbrain. There is an increase of neuroglia and a degeneration of the Nissl bodies.

In 1914, Wilbur and Ophüls¹² in reporting the pathology of a human case, declared that "the nuclei of the ganglion cells are perfectly normal." Since they observed marked thrombosis of the cerebral vessels, they were of opinion that the symptoms of botulism depended upon vascular changes. After extensive experimentation on animals, Dickson and Shevsky¹³ held that the lesions of botulism were not of central distribution but were peripheral. Edmunds and Keiper¹⁴ concluded from their studies that all essential symptoms of botulism can be explained by a more or less curare-like paralysis of the endings of the motor nerves to the voluntary muscles, including the diaphragm, and by a more or less complete paralysis of the parasympathetic nerve endings.

From the lack of unanimity of opinion regarding the cause of the eye symptoms, one of us outlined a series of experiments on animals with a view to deciding whether or not lesions of the visual pathway result from the toxin of *Clostridium botulinum*. Some of the details of these studies have been published¹⁵ elsewhere, therefore they will be merely summarized in this report. So many different problems presented themselves as the work progressed that it was necessary to restrict the experiments in order to develop a few special features. As one of us had a particular interest in the toxicological aspect of the toxin produced by the bacillus botulinus, a great deal of work was devoted to this phase.

In the experiments the material used was as follows: seven dogs, six cats, nine rabbits, three guinea-pigs, five albino rats, three cocks, and between thirty and forty frogs. The rats, rabbits, guinea-pigs and dogs were injected subcutaneously over the abdomen with botulinus toxin; the cocks were injected in the breast; the cats were used for conjunctival instillation and injection into the anterior chamber; the frogs were turned to account in furnishing nerve-muscle preparations for pharmacological experiments. Other animals not included in these figures were two rabbits, two cats and one dog utilized for the purpose of obtaining

nerve-muscle preparations of the extra-ocular muscles and motor nerves. By using the homologue of the external rectus muscle of a cat this preparation was obtained once but it was not well suited to the purpose. The toxin used in these experiments was supplied through the courtesy of Dr. K. F. Meyer, director of the George Williams Hooper Foundation for Medical Research, University of California, and Professor Robert Graham, Department of Animal Pathology, University of Illinois. The three specific strains of botulinus toxin were provided, namely, types A, B and C. The experiments were conducted in the department of Ophthalmic Research and Pathology of the Medical School of the University of Colorado and the Departments of Histology, Pathology and Pharmacology of the Creighton Medical College.

In the case of the dogs, rabbits, white rats and guinea-pigs, the intoxication was carried to the point of death, either by the primary injection of the toxin or by repeated non-lethal doses. The latter procedure was tried in most instances in order to subject the animals to the intoxication over a longer period of time. In all experimental animals that were studied for histological changes in the eye and brain, the autopsies were performed immediately after death to rule out possible post-mortem degeneration. In many cases where death was imminent, the eyes were enucleated under ether. Even small amounts of the anaesthetic caused death and facilitated the immediate removal of the brain.

As rabbits were found to be more satisfactory than other animals for these detailed studies, a brief résumé of the experiments of this group will be given. In none of the rabbits was excessive lacrimation noted. There was no salivation. Dysphagia was present in all instances in advanced stages of the intoxication. This note was entered in the protocols when mucous râles were heard in the throat of the animal. Dryness of the corneae was noted in all registered members of this group except one, which had no eye symptoms, although it lived for six hours after paralysis of the legs and neck had set in. Three of the rabbits were not considered with the group. Two of them had died during the night and were not fit for histological specimens. The other survived two average lethal doses of the toxin without symptoms and was merely kept for observation of later symptoms, of which none was noted. Of the six rabbits counted in this group, only one failed to show pupillary widening of at least some notable degree.

Chinchilla rabbits of approximately five pounds (2·3 kg.) in weight were used and were checked against normal rabbits of the same age and weight for pupillary measurements. It may be added that the rabbits were all kept in the same degree of illumination prior to and during the studies. One rabbit, or 16 per cent., developed nystagmus and a temporary convergence. Two members

of this group had episcleritis at a late stage. One rabbit showed marked sympathetic irritability during the height of the intoxication. This was manifested by a pronounced mydriasis when the animal was agitated. As soon as it became quiet, the pupils contracted to their previously normal size. There was no instance in which miosis could not be induced by strong light, although the reflex was not visible as such in several cases.

For more than two decades it was generally accepted that all of the symptoms which occur in botulism were chiefly dependent upon destructive lesions in the brain tissue. Although this proposition did not explain all of the symptoms that were noted in certain cases, yet it remained unchallenged for a considerable period. Apparently, but little attention was devoted to the subject for a number of years, at least in regard to investigative work.

Among the first to report upon the pharmacological aspect of botulinus toxin was Schübel¹⁶. He recorded that a poisoning of the nerve does not occur in from four to eight hours; that the nerve absorbs toxin, if at all, only very slowly. At a later date, however, he stated that he had been able to demonstrate a curare-like action of the toxin in frogs and also degenerative changes in the spinal cord of these animals. Dickson and Shevsky¹³ observed a marked susceptibility to fatigue in the endings of the motor nerves. In the analysis of their experiments with frogs Edmunds and Keiper¹⁴ concluded that the mechanism between the nerve and muscle was affected; in other words, there was a lesion of the nerve endings.

In our work with this phase of the problem it was surmised that at least some hint of the toxicological action of the toxin of *Clostridium botulinum* might be gained through the agency of the classic nerve-muscle preparations. Accordingly the sciatic-gastrocnemius combination was employed in all these experiments. Toxin of different dilutions with normal salt solution was used for immersion for intervals of 15 minutes; after which the nerve (or muscle) was tested by an electrical stimulus. By means of the usual inductorium apparatus and a writing lever to record responses upon a smoked drum, the preparations could be quickly set up, tried for reaction, and again be immersed in the toxin. A control specimen soaked in normal salt solution was utilized in the same manner for each experiment. The inductorium was set at eight centimetres for nerve stimulation and at three centimetres for direct muscle stimulation. Single shocks of the make-and-break current were used.

The first experiments were made with a ten per cent. dilution of the toxin at a temperature of 15° C. As no effect was produced by this technique, we then repeated the studies at a temperature of 25° C. and with a 35 per cent. solution of the toxin. The effect was striking in that all nerve responses failed after 45 minutes,

even when the strongest current was applied. After repeating this experiment several times and obtaining identical results we were assured that the sciatic nerve in these preparations absorbed toxin very readily when the temperature was kept at 25°C . We then continued the experiment by using dilutions of 10, 15 and 25 per cent. of the toxin, controlling the temperature of the solution by means of a water bath. It was of much interest that the period

FIG. 1.

Action of toxin on frog nerve-muscle preparation. Upper, saline control; lower, 35 per cent. toxin. Fifteen minute soakings at 25°C . Muscle contraction by electrical stimulation of nerve; single shocks until no response.

of viability of the nerve could be well nigh mathematically calculated, depending upon the strength of the toxin that was used. In the case of the preparation which was soaked in 25 per cent. toxin it was observed that the nerve was not responsive after one hour. Those that had been immersed in 15 per cent. toxin showed no response from nerve stimulation after one and one-fourth hours, while those soaked in 10 per cent. toxin gave no nerve response to the strongest electrical stimulus after 105 minutes.

At the time of lost nerve response it was seen that the muscle would still respond to direct stimulation. By continuing the treatment of the preparation in the toxin, however, a final failure of muscle response was observed. This was almost as striking in its failure to respond as the nerve failure had been, apparently depending upon the dilution of the toxin. Where the preparation was in 25 per cent. toxin the failure of muscle response to the usual stimulus was noted after one and three-quarter hours.

FIG. 2.

Upper figure, 15 per cent. toxin; lower figure, 25 per cent. toxin. Fifteen minute soakings at 25°C. First, contraction of muscle by electrical stimulation of nerve until no response, then electrical stimulation of muscle until no response.

When 15 per cent. toxin was used, the loss of muscle response occurred in two and three-quarter hours. However, in increasing the strength of the electrical stimulus, that is, by setting the inductorium at zero, the muscle gave a weak reaction for some time before total failure could be demonstrated. In most instances the controls were unaffected by the time element and did not exhibit fatigue after the stimulus was increased. There were a few occasions when fatigue was recognized as a factor in the results; namely, when the frogs had been kept so long that they had become inactive.

It was assumed from the frog experiments that the toxin of

clostridium botulinum is a protoplasmic poison as regards peripheral nerve and striated muscle tissues, being apparently more selective in its effect upon the former. Upon arriving at this assumption we endeavoured to anticipate and dispose of the objections to the protoplasmic poison theory of the toxin. The toxin

FIG. 3.

Ten per cent. toxin, 15 minute soakings at 25°C. N.: contraction of muscle by electrical stimulation. M.: contraction of muscle by direct electrical stimulation.

FIG. 4.

Upper figure, casein broth media; lower figure, saline control. Fifteen minute soakings at 25°C. Contraction of muscle by electrical stimulation of nerve.

used in the studies with frogs was type A, which had been furnished for the purpose by Dr. K. F. Meyer. It was prepared in casein digest media and contained no preservative. When used in these experiments the toxin possessed a minimal lethal dose (for a 250 g. guinea-pig) of 0.1 c.c. in 36 hours. The hydrogen-ion concentration of the toxin was seven as compared with eight of normal salt solution. Although there was slight fluctuation of the pH, as is to be expected, this factor was reasonably constant throughout the

FIG. 5.

Upper, 25 per cent. toxin heated at 100°C. for one hour. Fifteen minute soakings at 5°C. Muscle contraction by electrical stimulation of nerve: lower figure, saline control.

studies, as frequent determinations showed. In order to assure accuracy upon this point, casein digest medium was also obtained from Dr. Meyer. Then by using this broth in normal salt solution corresponding to the dilutions of the toxin that had been employed, it was determined that even in the stronger solutions the nerve-muscle preparations gave adequate response to the usual stimulation after two and one-half hours. This experiment removed the possible objection that the previously recorded results had been caused by the protein in the media. Since the hydrogen-ion concentration of the toxin and that of the media alone were identical, the last experiment also disposed of the objection that the results were dependent upon that factor.

The studies of numerous investigators who have worked upon this phase of the question reveal a marked variation in the heat resistance of the toxin of clostridium botulinum. van Ermengem¹⁷ reported that a temperature of 58° C. for three hours materially weakened the toxin, while a temperature of 80° C. for one-half hour was sufficient to render it almost completely inert. He goes on to state that at 100° C. the toxin was completely destroyed in ten minutes. Shippen¹⁸ working with the Nevin, type B, strain of toxin found that it was destroyed by a temperature of 65° to 70° C. in 20 minutes. Thom, Edmondson and Giltner¹⁹ reported type B. toxin destroyed in ten minutes at 70° to 73° C. Orr²⁰ investigated ten strains of botulinus toxin which were rendered inert in five minutes at 80° C.

For our experimental studies of the heat resistance of the toxin we endeavoured to make the destruction complete. Accordingly, the toxin was heated to a temperature of 100° C. and this was maintained for one hour. White mice which were injected with one-half c.c. of this mixture showed no effect in four days whereas mice of the same size had been killed in from two to three days with 0.001 c.c. of the toxin before it was treated by heat. This test with living animals was considered as fair evidence of destruction of the toxin by heat. (It seems deserving of comment that Dr. K. F. Meyer²¹ had written that the toxin, which we employed for these studies, showed the greatest resistance to heat of any strain that he had been privileged to examine.) The heated toxin was then tested with frog nerve-muscle preparations by the technique previously detailed. A 25 per cent. solution of the heat treated toxin in normal salt solution was used and was checked against a control in normal salt solution. This study showed that while the toxicity was greatly lowered, it did not seem to be entirely lost. There was a diminution of nerve response in two hours, while the control was normal. Repetitions of this experiment gave almost identical results. If there were any break in our technique which might explain the presence of any toxic substance in these boiled solutions, we have failed to detect it. We offer no explanation of this phenomenon as we ourselves are not entirely satisfied with this result. We are continuing to study this problem with a view to reporting our final opinion at a later date.

The histological changes in the nuclei of the third and fourth cranial nerves were: round cell infiltration; lymphoid cells packed into the parenchyma; extravasation of red blood cells; distention of capillaries with erythrocytes; stagnation of blood; migration of lymphoid cells; thickening of capillary endothelium; neuronophagia, chromatolysis, satellitosis, necrobiosis, nuclear displacement, nuclear shrinking, vacuolation, powdery granulation of Nissl bodies and complete disintegration of the ganglion cells;

FIG. 1.

Low power, massive interrupted round-cell infiltration in the choroid ($\times 95$).

FIG. 2.

Perivascular infiltration of the iris ($\times 200$).

FIG. 3.

High-power view of degenerating ganglion cells of the retina ($\times 1,750$).

FIG. 4.

High-power view of degenerating nerve cells in the midbrain ($\times 500$).

FIG. 5.
Haemorrhagic lesion in the midbrain ($\times 300$).

FIG. 6.
Disintegration of the retina ($\times 400$).

FIG. 7.

Capillary dilatation in the ganglion cell layer of the retina ($\times 300$).

FIG. 8.

Perivascular infiltration in the meninges of the midbrain ($\times 90$).

increase of neuroglia. Similar changes were observed in other parts of the midbrain. Besides a diffuse small round cell infiltration beneath the ependymal lining of the third ventricle, there were diffuse round cell infiltration and massive extravasation of erythrocytes in the meninges. The meningeal vessels were distended with red corpuscles. Thrombosis was not frequent in the midbrain. The changes in the optic nerves were: focal infiltration in the parenchyma, diffuse increase of neuroglia, and round cell infiltration of the pial and arachnoidal sheaths. The changes in the optic tracts were: round cell infiltration, extravasation of erythrocytes, emigration of lymphoid cells and stagnation of blood. The chiasmal changes were maximal infiltration, excessive packing of lymphoid cells in the parenchyma and extensive extravasation of red cells. The retinal changes were: fat formation in the ganglion cell layer; pyknosis, chromatolysis and vacuolation of the ganglion cells; a powder-like reduction of the pigment granules; engorgement of the vessels with red corpuscles and stagnation of blood. In the choroid there was maximal infiltration involving all layers. There was round cell infiltration of the corneo-scleral junction. In the ciliary body there was round cell infiltration together with an increase in the connective tissue element.

The exudative lesions consisted chiefly of lymphocytes and monocytes, some of which had differentiated into polyblasts while others had become transformed into plasma cells. The exudate occurred for the most part about the vessels, but in many instances there was a tendency to migration into the parenchyma. Where maximal infiltration was noted as many as 15 rows of lymphoid cells were present around the vessels.

Findings

Though it is not deemed expedient to draw conclusions that are not fully supported by the present research, as some of the problems mentioned have not received sufficient consideration, the multiplicity of the lesions, together with the severe character of cell destruction and the round-cell infiltration, speaks for the overwhelming virulence of the toxin of *Clostridium botulinum*.

This paper may be presumed to support the work of the European physicians in regard to their histological findings in the nuclei of the cranial motor nerves.

Botulinus toxin is a protoplasmic poison to peripheral nerve and striated muscle tissues, although more selective in its action upon the former.

Since the toxin of *Clostridium botulinum* is a protoplasmic poison to peripheral nerve and striated muscle and since it produces marked degenerative changes in the central nervous system and

also in all the tunics of the eye, it is possible and even probable that it is a general protoplasmic poison.

The theory of botulinus toxin acting as a general protoplasmic poison would explain all of the lesions herein and heretofore recorded as findings in established botulinus intoxication.

That botulinus toxin may contain a substance of exceptional heat resistance, when judged in the main by delicate frog nerve-muscle experiments, is apparent from this study. More detailed work will have to be done in this field however, before the above statement can be advanced with any degree of assurance or finality.

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