(1)

TRANSLATION NO. 2296

DATE: Feb 1966

DDC AVAILABILITY NOTICE

This document has been approved for public release and sale; its distribution is unlimited.

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

Med. Klin. No.41, Oct. 1962, pp.1735-1740

Botulism Caused by Inhalation

By: E. HOLZER

Senior Physician Dr. E. HOLZER, Medical Ward of the Municipal München-Schwabing Hospital (head physician: prof. med. Dr. H. BEGEMANN), München 23, Kölner Platz 1.

(Translated by: Edward Lachowicz, Maryland, Medical-Legal Foundation, Inc., 700 Fleet Street, Baltimore, Maryland, 21001)

In this report are discussed three cases of laboratory intoxication caused by the inhalation of dust that contained botulinal toxin. We describe here the possibilities of resorption by way of the pharyngonasal cavity and by the respiratory tract. We also report here a rare occurrence of the toxic infection involving botulism. The botulinal poisoning has inhibitive effect on the acetylcholine synthesis, or on acetylcholine secretion of the cholinergic nerve fibers and leads to disturbances of the neuromuscular transference at the end plates. Hence, a treatment with acetylcholine seems to have a particular justification in addition to the doses of antiserum, "Kollidon"*) and general therapeutics. We shall also explain the necessity of having other antisera available besides a horse serum.

The botulism, a poisoning with the toxin of Clostridium botulinum, is quite a rare illness that is distinguished by a high mortality. Generally, all persons get sick after eating food that

*) - Translator's Note: polyvinylpyrrolidone.

contains this toxin. Since the toxin is regarded as one of the most powerful known poisons, thus, notwithstanding careful precautions, even the smallest amounts of it taken with spoiled food can cause a fatal disease. Actually, the number of poisonings that occurred in America is considered higher than that in Europe (MEYER[15]). Even during the last war botulinal diseases were infrequent in Germany (KOEPPE[10]). But, during the years of occupation from 1940 to 1944, LEGROUX, LEVADITI and JERAMEC[13] determined in France more than 1,000 poisonings out of 500 foci. While in America the poisonings with Cl. botulinum were predominantly detected by way of cans with vegetables, or cans with fruits as vehicles, the cases examined in Europe revealed the presence of the toxin mostly in poorly preserved canned meats and in dried meats. Recently reported poisonings were caused mostly by contaminated cheese spreads ([16]).

As early as in 1897 van ERMENGEM[6] described the clinical symptoms as follows:

1. A decrease or increase of saliva and secretions of mucus in the mouth, pharynx, etc.

2. More or less pronounced external and internal ophthalmoplegia.

3. Dysphagia to aphagia, aphonia, severe obstipation.

4. Absence of fever, of sensation disturbances and cerebral disorders.

5. Respiratory disorders and cardiac disturbances are frequently associated with this combination of symptoms and can lead, on an

average, to a sudden death with the indication of bulbar paralysis and asphyctic collapse symptoms.

If we add to this description that a considerable and general muscular weakness occurs, while most reflexes remain intact (18) and that objective symptoms may be preceded by subjective disorders, such as numbness, or a feeling of intoxication, one obtains complete features of the clinical picture. Doubtless, mydriasis is not inevitable (19a,b). Various toxins that are produced with the human pathogenesis by Cl. botulinum are the types A, B, C and E (20) and they develop identical symptoms of the disease; they are differentiated only in connection with the type-specific neutralization process of the toxin. According to BINGOLD[1], a differential diagnosis should be made antithetic to encephalitis epidemica, the initial stage of poliomyelitis, diphtheric incapacitation, methyl alcohol poisoning, paratyphoid fever, atropine poisoning, mushroom poisoning, lues cerebrospinalis, bulbar paralysis, myasthenia and acute bulbar myelitis.

Unlike the familiar cases of botulism produced after eating contaminated foodstuffs, we report here three poisonings developed simultaneously as laboratory infections by way of the inhalation of highly purified toxins. Three patients, V.R.♂, K.M.♂ and G.G.♀ autopsied rabbits and guinea pigs, which the day before were exposed to aerosol of a highly purified botulinal toxin type A that was released in a hermetically closed container. The patients V.K. and G.G. were present all the time at the autopsy of five animals, including the skinning of hides and dissection of organs,

while the patient K.M. assisted for a short time only. The three participants wore a complete protective clothing during actual aerosol experiments, but the subsequent observations of animals were carried out in other cages and in another section without protective clothing, but merely protective gloves were used. Moreover, the moistening of the skin of experimental animals (to prevent stirring of dust during removal of skins) was applied to one animal only, but not to the other four. By addition of colloids the toxin became stable.

V.R., whose previous history of allergic reaction to tetanic horse antitoxin is of importance, noticed three days later a "feeling of mucous plug in the throat", which he was unable to relieve by cough, or by hawks. The next day he felt like incurring a beginning of cold, but no fever. He had in the mouth a great quantity of mucus that he could not swallow; he felt slightly benumbed the entire day. In the evening he experienced, for the first time, difficulties in swallowing: he could swallow solid foods only with a liquid. Now, he perceived spontaneously the first suspicion of having botulism. The next day his numbness became more intense, also the difficulties in swallowing increased and a feeling of retarded ocular motions appeared. Standard checking on admittance that day brought the following findings: EZ good, AZ distinctly reduced. Increased motility of mucus. Indistinctness of speech. Pupils round, alike on both sides, moderately wide, react to light and to close focus. Slight rotatory nystagmus. Thoracic organs clinically and roentgenogically correct. Pulse 92/min. RR 135/90

mm Hg. Abdomen inconspicuous, tonicity, trophicity, sheer strength and motility of arms and legs all inconspicuous; release of tendons' reflexes and periosteum reflexes unobstructed. Superficial sensation and deep sensation undisturbed; gait somewhat uncertain. BKS 2/6 mm, blood picture: hb. 17.3 g%, ery. 5.22 mill., hb/E 33.2 γγ, leuko. 9,500: 1% stab., 71% segm., 20% lympho., 8% mono; total albumin 6.8 g%; electrophoresis: albumins 58.0 rel.%, alpha-1- 3.5 rel.%, alpha -2- 11.0 rel.%, beta - 15.0 rel.%, gamma-globulins 12.5 rel%. Electrocardiogram: inconspicuous. Urine condition: sporadic leukocytes and epithelia. - Since, in spite of all efforts, we could obtain only horse antitoxic serum, thus, after previous administration of probatory small doses that were well endured, we administered 100 ml of antitoxic serum after a prior injection of prednisolone on the day of arrival. The next day we administered all together 100 ml of serum again, fractionally. Moreover, the patient received infusion of periston-N, according to recommendations of SCHUBERT (21), and also repeated intramuscular injections of acetylcholine during the first four days. On the second day of his hospitalization, he still had considerable difficulties in swallowing, but only light subjective visual disturbances, and also light headaches, but no numbness. On the third day the difficulties in swallowing decreased considerably, however a slight numbness reappeared. On the fourth day: the condition remained the same. On the fifth day: still slight accommodation disturbances. Noted additional decrease in difficulties of swallowing, but a slight numbness still remained; the patient felt essentially better, in general. On the sixth day: the condition remained the same, however urticaria began to appear

in places of the injections of serum. The patient received antihistaminic injections. He was discharged on the 9th day. Few days later a typical serum illness appeared. After that, a slow convalescence followed.

The animal experiment with the patient's serum administered to a mouse (25) showed a positive result as to botulinal toxin.

G.G. perceived, at first, a sensation of dizziness and intoxication in the evening on the third day after the autopsy of animals; later, indistinctness of speech and difficulties in swallowing appeared. The next day, as slight dizziness continued, a chocking feeling in the throat appeared and this made the eating of noon meal almost impossible. The patient went to bed again after her noon meal, perceiving that ocular motions increased dizziness. In the evening, with difficulties in swallowing, a great quantity of mucus appeared in the mouth and, as it could not be swallowed, the patient experienced speech difficulties. She was so weak on the next day that she was unable to cut a piece of bread. Anyhow, she arrived at her place of work like intoxicated and experiencing severe difficulties in swallowing. Consultation. - Findings on admittance: EZ good, AZ distinctly reduced. Face slightly swollen, swallowing considerably aggravated, pupils moderately wide reacted to light and to close focus. Absence of clearly visible paralysis of the eye muscle. Slight rotatory nystagmus, reading too exertive, letters are blurred. Speech inconspicuous. Thoracic organs clinically and roentgenogically correct. Pulse 72/min. RR 130/70 mm Hg. Abdomen inconspicuous. Tonicity, trophicity, sheer strength and

motility of extremities all unaffected. Superficial sensation and deep sensation normal. Release of tendons' reflexes and periosteum reflexes unobstructed; gait distinctly uncertain. BKS 4/10 mm, blood picture: hb. 14.3 g%, ery. 4.27 mill., hb./E 33.5 γγ, leuko. 11,700, from this 1% Eo, 1% stab., 86% segm., 5 lympho., 7 mono. Electrophoresis: 61% albumins, 4% alpha-1-, 9% alpha-2-, 13% beta and 13% gamma globulins. Electrocardiogram: inconspicuous. Urine condition: normal. - On the day of admittance we administered 100 ml of antitoxic horse serum intravenously and additionally 50 ml (intramuscularly) infusion of periston-N; then, repeated intramuscular doses of acetylcholine (for 4 days). On the second day again 50 ml of serum were administered intramuscularly, whereupon a short-lasting fever of 38.6°C appeared. We still noticed on the second day of treatment a considerable numbness like intoxication, severe difficulties in swallowing, blurred vision and headaches. The difficulties in swallowing continued on the third day, while the headaches and visual disturbances diminished. Headaches disappeared on the 4th day, but some difficulties in swallowing still remained. On the 5th day: no visual disturbances, the patient could read, but slight difficulties in swallowing still remained. On the 6th day: the condition has not changed. On the 9th day: slight general weakness, otherwise no complaints; discharged. The animal experiment on a mouse also positive.

K.M., who assisted for a short time at the autopsies of animals also became ill on the 3rd day having moderate headaches and a feeling of some numbness. On the next days he felt a severe tightness

and a chocking feeling in the throat in addition to ⊃ a slight feeling of difficulties in swallowing. He was admitted as a bed patient with the other two already hospitalised. Findings on admittance: EZ good, AZ slightly reduced; slight difficulties in swallowing. Pupils moderately wide, round and well reacting to light; contraction of pupils to convergence - good. Eyes' motility normal, speech unaffected. Thoracic organs clinically and roentgenologically correct. Pulse 96/min; RR 125/80 mm Hg. Abdomen inconspicuous, tonicity, trophicity, sheer strength and motility of extremities all undisturbed. Superficial sensation and deep sensation normal. Release of tendons' reflexes and periosteum reflexes unaffected. BKS 2/4 mm, blood picture: hb. 16.94 g%, ery. 5.27 mill., hb./E 32.2, leuko. 8,000, from this 1% Eo, 72% segm., 20% lympho. and 7% mono. Electrophoresis: albumins 54.0 rel.%; globulins: alpha-1- 5%, alpha-2- 9%, beta - 13%, gamma - 19%. Electrocardiogram: inconspicuous. Urine condition: normal.

His previous history for the last 7 years indicated two passive preventive inoculations for tetanus, of which one was with a horse serum. Since we could only obtain horse antitoxic serum, thus, notwithstanding the previous history, we attempted to produce a prerequisite for the serum therapy by way of a careful desensitisation act. But, regardless of the doses of prednisolone, Novocain and "Dolantin"*), the patient reacted even to the smallest doses of serum by evoking generalized urticaria eruptions on the skin, consequently additional doses had to be discontinued after a total

---

*) - Translator's Note: Demerol.

of 5 ml. Hence, the treatment followed with infusions of poriston-M, also with repeated blood transfusions and with consecutive doses of acetylcholine (for 5 days). We could control well with antihistaminic drugs numerous urticaria (with hematic eosinophilia) eruptions that appeared particularly on the 6th day. The patient, being exposed only for a short time to the inhalation of the toxin, faced a considerably easier recovery; the symptoms of intoxication were limited to headaches, slight dizziness, moderate difficulties in swallowing and no visual disturbances. Consequently, without a serum therapy, he was also discharged on the 9th day. Of course, the animal experiment, like with the serum of other patients, brought positive results.

The well-defined clinical findings, also positive experiments with animals and good results obtained from the antitoxic serum administered to two seriously ill patients, proved that, in connection with the described diseases, we were dealing with the botulinal poison intoxication. Since the poisoning revealed itself as oral, the mode of intoxication was none other than the inhalation of finely divided "toxic dust" that remained suspended in the fur of animals after aerosolization. In general, botulism is an intoxication, not infection, since all its symptoms can be released by the toxin (8). Findings of recent years indicate however that, contrary to previous opinions, an infection with botulinal bacilli can also occur. K.F.MEYER (15) determined already in 1928 that, no doubt, nontoxic spores of A and B types can germinate in the bodies of animals, then they can develop poisons and produce botulism

In 1948 an experimental and absolutely typical botulism was developed in a muscle of guinea pig by injection of washed germs or cultures of the type C botulism (20). Also, observations were reported that involved three fatal cases of botulinum-genic wound infections (9, 22, 5). Botulism can also appear as a toxic infection like tetanus and not solely as an intoxication. We should mention here occasional reports about postmortal detections of botulinal bacilli in the spleen, in liver and gall, as well as in the intestines (19). Thus, in cases of enteral poisonings, one has to consider that, in addition to the toxin present in foodstuffs, additional production of toxin takes place in the intestines of the host. Hence, of obvious importance is a careful elimination of the gastrointestinal tract immediately in cases of detected obstipation.

The toxin discovered by van ERMENGEM in 1896 was known previously as the "neurotoxin". According to a recent conception, the toxin is a component part of bacterial cells (20) and diffuses progressively in the surrounding medium. The importance of the toxin in the life of bacilli is unknown. Penicillin stops the germs most effectively (24). Subsequently to the doses of chloramphenicol and penicillin, BONVENTRE and KEMPE (3) could not determine any increase of cells, but a quite considerable increase of toxicity in the source of nourishment caused by the lysis of bacillar bodies. Therefore, VIERLING (24) justifiably refers to a possibility that a treatment with antibiotics in the presence of a botulinum-genic infection may strengthen the intoxication. The toxins as such are simple proteins of a globulin type and are solely composed of amino

motor nerves and, perhaps, on the parasympathetic fibers. The adrenergic and the sensory fibers appear to be fully refractory opposite the poison. Unlike the curare effect, the muscle remains sensitive to exogenously applied acetylcholine; the poisoned muscle reacts also to direct stimulation. Consequently, Prostigmin is not an antidote. Often a question arises, whether the mechanism of poisoning leads to a decreased synthesis of acetylcholine, as TORDA and WOLFF (23) believe on the basis of their experiments with frogs' brains, or whether the secretion alone is obstructed. Thus, strictly speaking, botulinal toxins may not be cellular poisons; they supposedly affect biochemical substrate (11). The toxin does not influence cholinesterase. This interpretation of the toxin's effect, secured experimentally, contradicts the clinical observations of MOELLER (18) pertinently to the favorable action from intralumbar administration of antisera versus the intramuscular doses of serum after they proved ineffective: this can only be explained by a neutralization of the toxin that became combined when acting on the central nervous system. One should also refer to the experiments of DAVIES et al. (4) who failed to see any local effect after injections of the toxin into medulla oblongata and into the nervus ischiadicus.

As to the absorption of the toxin in the gastrointestinal tract, the place and the method of resorption have so far not been clarified distinctly. Certain detoxication takes place on the action of the proteolytic enzyme trypsin and chymotrypsin, no doubt. Pertinently to pepsin, this is questionable. But, in view of the

tremendous toxicity, even very minute amounts of the toxin are sufficient for resorption. The assault of proteolytic ferments clarifies the fact well in that in animal experiments more than 100,000-fold larger doses may be necessary for peroral lethal poisoning than for a parenteral dose. The resorption of poison can even result from the mucous membrane in the mouth. Thus, among others, GEIGER (7) points out that the toxin can be picked up via injuries in the gums, also with skin abrasions (burns) and it can be reabsorbed from mucosa. Consequently, even experimental tasting of spoiled foodstuffs without swallowing a sample can lead to a fatal intoxication (15). The scope of information about the described cases that involved possibilities of resorption through pharyngonasal cavity and respiratory tract is important in connection with this report. The incidents were proven experimentally. With the intranasal instillation, the toxicity reveals itself at once in greater poisonous amounts and is substantially higher than in peroral dose. The toxin is readily resorbed from the respiratory tract and the same relationship applies here between particle sizes and resorption, as e.g. during inhalation of medicaments. The fastest resorption takes place in deeply situated parts of the bronchial system, i.e. according to the decrease in the thickness of the epithelium. In principle, the intrapulmonary resorption corresponds to that of intravenous, with the exception that the entire absorption lasts longer. LEGROUX, LEVADITI and JERAMEC (14) determined by experiments on animals the time of death using equal amounts of botulinal toxin type B, namely: intravenous

acids. Out of the five different types of toxins, type A can be prepared in a crystallized form. It was found by means of the electrophoresis and ultracentrifuge that the A type is a homogenous protein and its mol. weight was determined about 900,000; it is composed of 19 amino acids, according to the chemical analysis. How large is the toxic unit itself, it could not be clarified so far. The botulinal toxin is not a pure nerve poison. The entire molecule has also hemagglutinating properties (11). The hemagglutinin cannot be separated from the neurotoxin by chemical means, but it can be adsorbed on erythrocytes, whereupon the diffusion ability of the neurotoxin could be increased. Thus, the actual neurotoxin may be smaller than one corresponding to the determined mol. weight of 900,000. According to LAMANNA (11), the botulism toxin is "unquestionably the strongest of the known poisons"; less than 0.1 (once) of 10^{-3} microgram can kill a mouse. Only tetanus and Shigella neurotoxins seem to have a similarly strong toxic effect. Among the poisons with protein characteristics is, e.g. diphtheria toxin less strong essentially and, among the nonprotein toxins, the dangerous aconitine is with its toxicity far below the level of botulinal toxins. Long discussions have been presented on the subject of the toxin's point of assault. It continues to be a well-founded opinion today that, in the first place, if not in a general manner, only the cholinergic fibers become involved. BISHOP and BRONFENBRENNER (2) proved in 1936 the inhibition of the neuromuscular transference as the essential effect of poisoning. The toxin is supposed to act preponderatingly on the end plates of the

dose - 2 hours, 25 minutes; intrabronchial administration - 5 hours and subcutaneous injection - 7 hours, 45 minutes.

No preliminary findings are available relevant to sporadic effects of the toxin on the respiratory tract of man, because such occurrence cannot take place under normal conditions. Only such poisonings like a laboratory intoxication assume their theoretical importance and, in our cases, also a medical significance. Besides, one must take under consideration a possibility in discussing a war that toxic aerosols can be used as a weapon (12). As we proved, very minute amounts of pure toxin, like those suspended in furs of animals used for aerosol intoxication, were sufficient to trigger the classical desease. The toxin can be sufficiently stabilized by colloidal solvent also against the mechanical assault during atomization (17). According to the previously reported findings of MEYER (15) in connection with the increase of toxicity by serum, it seems that a sure activation of the toxin can be produced with the addition of colloidal solvents.

Considering the therapy, the earliest possible and sufficient doses of antiserum are recommended in order to try to confine the toxin, and also a lavage with "Kollidon" is suggested. Conformably with new discoveries pertinent to the mechanism of activity, also repeated administrations of parenteral doses of acetylcholine are desirable in spite of possibilities of causing a lameness of the neuromuscular synapses with relation to contractions of the muscles. In our circumstances we experienced the inability of obtaining sera of other animals, except a horse serum, i.e. not

only in Germany, but also in other European countries, even in the USA; we considered this a considerable obstacle in the way of treatment. Even, as we hope, the botulinal intoxication will remain scarce, yet, with a high mortality of the disease, therapeutic resources would be perfectible, if we had prepared and available antitoxic sera of other animals as well.

Literature Cited

1. BINGOLD, K.: Botulism. Handbuch der inneren Medizin, 4th edition, vol.1/1:1520. Berlin-Göttingen-Heidelberg, 1952.

2. BISHOP, G.H. and J.J. BRONFENBRENNER. Amer. Journ. Physiol. 117 (1936), p.393.

3. BONVENTRE, P.F. and L.L. KEMPE. Journ. Bacter., 79, (1960), pp. 18 and 24.

4. DAVIES, J.R., R.S.MORGAN, E.A.WRIGHT and G.P.WRIGHT. Journ. Physiol., quotation of PREVOT (20).

5. DAVIS, J.B., L.H.MATTMANN and M.WILEY. J.A.M.A. 146 (1951), p.646.

6. van ERMENGEM: Quotations of MEYER (15).

7. GEIGER, J.C.: Amer. Journ.Publ.Health 14 (1924), quotations of MEYER (15).

8. GRUMBACH, A.: Botulism, in: GRUMBACH, A. and KIKUTH, W.: Die Infektionskrankheiten der Menschen und ihre Erreger. Vol.II, 973, Stuttgart, 1958.

9. HAMPSON, C.R.: Journ. Bacter. 61 (1951), p. 647.

10. KOEPPE, H.W. Botulism, in: COBET, R., GUTZEIT, K. and BOCK, H.E. Klinik der Gegenwart, Munchen-Berlin, 1955.

11. LAMANNA,C.: Science 130 (1959), p.763.

12. LAMANNA,C. Bacter. Rev., 25 (1961), p.323.

13. LEGROUX,R., J.C.LEVADITI and C.JERAMEC. Presse Med. 55, (1947), p.109.

14. LEGROUX,R., J.C.LEVADITI and C.JERAMEC. Ann. Inst. Pasteur 71 (1945), p.490, quotations of LAMANNA (12).

15. MEYER,K.F. Botulism, in: KOLLE,W., KRAUS,R. und UHLENHUT, R.: Handbuch der pathogenen Mikroorganismen. Vol. IV/2:1269. Jena-Berlin - Wien, 1928.

16. MEYER,K.F. and B.EDDIE. Zschr. Hyg. 133 (1951), p.255.

17. MEYN,A. and R.VIERLING. Zbl. Bakter.183 (1961), p.167.

18. MOELLER,J. Dtsch. Med. Wschr. 74 (1949), p.1538.

19a. MOESCHLIN,S. and H.ZOLLINGER. Dtsch. Arch. Klin. Med. 190 (1942), p.62.

19b. MOESCHLIN,S. Klinik und Therapie der Vergiftungen, 3rd edition, Stuttgart 1959.

20. PREVOT,A.R. Botulism, in: Biologie des maladies dues aux anaerobies. Collection de l'Institut Pasteur, Paris, 1955.

21. SCHUBERT,R.: Dtsch. Med. Wschr. 73 (1948), p.551.

22. THOMAS,C.G.Jr., M.F.KELCHER and A.P.McKEE: Arch. Path., 51 (1951), p.623, quotations of MEYER and EDDIE (16).

23. TORDA,C. and H.G.WOLFF. Journ. Pharm. Exper. Ther., 89 (1947), p.320, quotations of GRUMBACH (8).

24. VIERLING,R. Zbl. Bakter. 185 (19 p.195.

25. WINKLE,H. Mikrobiol. und Serolog. Diagnostik, 2nd edition, Stuttgart, 1955.