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WITH BOTULIN TOXIN

-USSR-

By M. K. Markaryan, et al

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FOREWORD

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SANITATION OF WATER CONTAMINATED
WITH BOTULIN TOXIN

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copy [Following is the translation of an article by M. K. Markaryan, N. V. Ryshov, and J.(Ya.?) V. Stannikov of the Academy (imeni?) S. M. Kirov, Leningrad, in Journal of Hygiene, Epidemiology, Microbiology and Immunology, Vol IV, No 4, Prague, 1960, pages 385-389.]

Neither in foreign nor in indigenous literature can one find reports concerning the sanitation of water contaminated with botulin toxin.

In this article we present material based on experimental studies carried out in order to find a method of sanitation for water contaminated with botulin.

For the above purpose we used preparations containing chlorine (chloride of lime and pantocide). Moreover, we filtered the contaminated water over active charcoal, ionexes and carboferrogel-M and exposed it to bactericide ultraviolet radiation (PRK-2) and boiled it as well.

The botulin toxin we derived in liquid form from botulin of culture No 98, type A, and culture No 255, type B. The determination of the minimal lethal dose for white mice showed in the case of culture 98 and with intra-peritoneal inoculation at a dilution of 1:100 an amount of 0.25 cm³, and in the case of culture 255 the same amount at a dilution of 1:10,000 under the same experimental conditions. The toxin was produced as follows: 1) 48-hour cultivation on mastic tarocci culture medium; 2) Keeping cultures for 10-25 days at room temperatures; 3) Filtering over Seitz filter and control of sterility.

After six breedings in the mastic tarocci culture medium the minimal lethal dose of both cultures remained unchanged; we therefore limited our further tests to culture 255 as it was the more toxic one (1:100,000).

In order to find out up to which degree the water is toxin-free, we conducted experiments with white mice inoculating these intra-peritoneally with 0.25 ml of the contaminated water samples. Samples of this water were applied to the test animals after the water had been purified by means of a number of methods.

Since, in the case of intra-peritoneal application, any effects of the remaining chloride on the animals had to be excluded, the water was completely purified of chloride by filtering it over active charcoal, and in some cases by adding a suitable amount of sodium sulphate.

The control animals were also inoculated with toxin-contaminated water. Survival of the animals was considered proof that the toxin had been inactivated, their death however--with its characteristic clinical picture--pointed at the presence of active toxin in the water.

For each test usually six test and six control animals were used. Altogether we used for our 115 tests 1,200 animals (white mice).

The water examined had mostly a pH of 6.9-7.4 and a temperature of 16-19° Celsius for the duration of the entire test.

From the theoretical as well as from the practical point of view, of utmost importance is the question of how the botulinum toxin is acted upon by the chlorine preparations used for water sanitation in communal practice. In order to answer this question, we used chloride of lime and pantocide (sodium salts of parasulphur dichloramide benzoic acid), which is very constant, preserving the original amount of active chloride for a long time.

We used the following method for this part of the test: a certain amount of clarified 1%-chloride solution (25-30%) or pantocide pellets were added to one liter of botulin-contaminated water contained in a tubulated flask. After a certain time the water was dechlorified and intraperitoneally injected into the test animals.

In doing so, it was important to establish the effectiveness of those active chloride doses (1-2 mg per liter), which most often are used in practice for the sanitation of water. The purification of this water with these chloride amounts (at a residual chloride content of 0.7 and 1.65 per liter) remained without effect if the chloride was permitted to act upon the water for 15-30 minutes, proven convincingly by the death of the white mice with its characteristic picture. Not even a chloride effectiveness twice as long than before--prolonged to one hour--could eliminate the toxin.

In five further tests the amount of active chloride added for water sanitation was increased by several times the earlier amount, in the first case to 4 mg per liter and in the second to 6 mg per liter. As in the earlier tests the chlorination of water with the dose mentioned (at a residual chloride content of 3.8 and 5.54 mg per liter) proved ineffective after the chloride had been present in the water for 15 to 30 minutes.

The purpose of further tests was to establish the effectiveness of water sanitation when the chloramine preparation pantocide was used. Each pellet of pantocide contains on the average about 2.7 mg of active chloride. On the basis of these five tests it was established that two pellets of pantocide per liter of contaminated water at 15-30 minutes of exposure (residual chloride) 4.9-5 mg/liter) do not eliminate the toxin. Nor did a prolongation of the effective exposure to 45 minutes and even to a whole hour yield any results. Only if the time of effectiveness of the pellets is increased to 1-1/2 hours, with an unchanged content of residual chloride, the desired effect is achieved. This was proven by the survival of the test animals.

Another series of 19 tests showed that the toxin can be rendered ineffective more quickly by a considerable increase of the active chloride doses. If the water is cleaned with active chloride doses of 10-14 mg per liter (residual chloride content of 9.6 to 13.7 mg per liter) sanitation of water will be achieved after 30-40 minutes. It can therefore be cleaned only with a considerable overdosis of chloride. It was also necessary to find out which effect boiling had with regard to sanitation of the water contaminated with botulin toxin.

In literature is also mentioned a relatively large resistibility of the botulin toxin in biological media. According to V.I. Tec [Tek?] (2) the botulinum toxin withstands a temperature of 80° Celsius for 30 minutes and boiling for 10 minutes in media containing albumen.

In this series we conducted 25 tests. The experiments verify mostly the great effectiveness of 10 to 15 minutes of boiling for the sanitation of the contaminated water.

It is known that in recent years water cleaned by ultraviolet radiation has been used. Therefore, we also wanted to determine if our water could be cleansed with this method.

For our tests we used PRK-2 quartz mercury lamps with the following spectrum characteristics: rays A--19.13%, rays B--44%, bactericide group C--36.15%. The effect of the ultraviolet radiation depends on the distance of the exposed object. According to Galanin and Markarjan (1) it amounts to 550 mkal/cm² min. at a distance of 1 m.

The tests in this series were conducted as follows: The botulin-toxin contaminated water diluted at a ratio of 1:1.000 was poured into a Petri bowl in a layer about 0.5 to 0.6 cm thin. The bowls were exposed to radiation with a PRK-2 lamp from various distances for various periods.

Already the first few tests showed that exposure of 5-10 minutes (at a lamp distance from the water surface of 0.5 m) is not adequate to result in sanitation of the water. Therefore, reirradiation was prolonged. On the basis of 15 tests of this kind it was established that radiation of the contaminated water from a distance of 1 m at an exposure time of 15 minutes was equally negative. Not even a reduction of the distance between Petri bowl and lamp by about one half and a doubled reduction of the layer of water to 0.15 to 0.2 cm were sufficient.

There were more tests conducted in order to investigate the possibility of achieving sanitation by means of filtration over various preparations (active charcoal, carboferrogel, ionex). It had to be established what kind of a role these preparations played in rendering the toxin ineffective--on the one hand during filtration, i.e., under dynamic conditions; and on the other hand under static effects, i.e., when the contaminated water was thoroughly shaken after the preparation had been added. The series of tests conducted under static conditions was done as follows: To the water contaminated with botulin toxin (concentration of 1:5,000) a certain amount of the preparation was added. The suspension was shaken thoroughly for five minutes and then was permitted to settle for 30 minutes. As soon as the water had come to a rest it was carefully decanted from the preparation deposited on the bottom. The samples of the water poured off were intra-peritoneally injected into the test animals, the amount being 0.25 ml.

When adding active charcoal to the contaminated water (8%-suspension) the great absorption capacity of the preparation became evident: All inoculated test animals stayed alive while the control animals died after one or two days with the typical clinical symptoms of poisoning.

An equally large absorption capacity as active charcoal (1) i.e., with regard to the effects of botulin toxin in water, is also characteristic for carboferrogel, if added to water in approximately the same amounts. Also in the tests with ferrogel-cleaned water the white mice inoculated with this water remained alive.

During the tests aimed at the determination of the role played by these preparations in the neutralization of the botulin toxin through filtering we proceeded as follows: One of the preparations (active charcoal, carboferrogel) was placed into a tubular glass which had a faucet and a glass grate at the bottom. The height of the filter was usually 20-25 cm. The water was slowly filtered over this filter stratum. The results of 33 tests proved that active charcoal possesses a good degree of absorption with regard to

the toxin under conditions shown⁽¹⁾ as well as under static conditions.

With regard to the carboferrogel it was established that it also cleans water well under analogous conditions. It could be assumed that in view of the high dispersion degree of the particles the carboferrogel would also be effective with a thinner filter. We succeeded indeed in eliminating the toxin in the water by filtration of the water over a filter stratum of only 10 cm, that is half the filter height of the earlier test.

We were also interested in examining the possibility of cleansing botulin-contaminated water with modern preparations (Ionexes). In recent times, as is known, these have frequently been used in order to improve water quality. For this purpose we used Katex resin of the type Espatit I and the Anex EDE-10. Both resins were added to the water with a toxin concentration of 1:5,000. Then the 10% watery suspension was shaken vigorously for five minutes and permitted to settle for about 30 minutes. The ten tests conducted showed that ionexes cannot eliminate toxin in the water under static conditions.

If ionexes are applied under dynamic conditions, i.e., in gradual filtration of the water over a layer of these preparations 30-40 cm thick at a speed of 5-6 m per hour, they are ineffective: which was proven by the death of all test animals during the first or the second day.

Summary

1) Chlorination of water containing botulin toxin with doses of chlorine generally used for communal water supplies, i.e., 1-2 mg/l (residual chlorine 0.7-1.6 mg/l) is ineffective.

2) Water infected with botulin toxin can be decontaminated only by using large doses of chlorine (residual chlorine 9.6-13.7 mg/l), i.e., under conditions of considerable overchlorination, with 30 to 40 minutes of exposure.

3) Water containing botulin toxin can be effectively decontaminated by boiling for 10-15 minutes.

4) Botulin toxin can be removed from water by adding active charcoal or carboferrogel, shaking for five minutes and leaving to stand for 30 minutes, and by slowly filtering the water through these substances.

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