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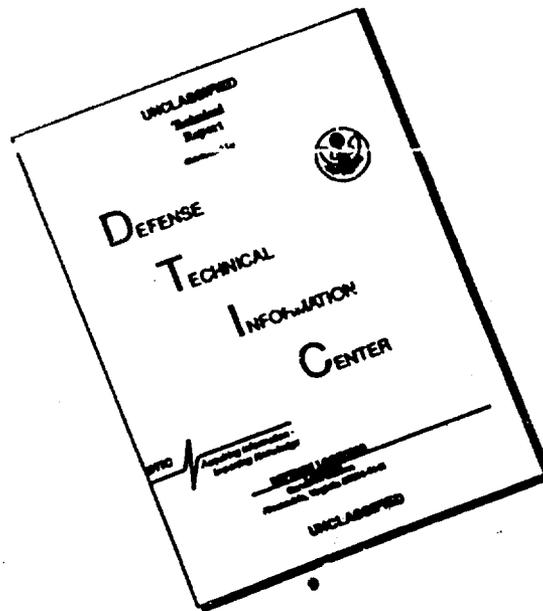
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Distribution of a Shewan Preparation with Botulinal Toxin in the
Organism of White Mice

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Up to now the distribution of botulinal toxin in organism has been studied with the aid of the biological test (I.N. MORGUNOV, 1941; L.A. PEISAKHIS, 1953). Usually, the extractions of poisoned animals' organs are administered in various dilutions to white mice and then, according to the rapidity of resulting death, the quantitative capacity of the toxin is estimated in examined tissues.

But, the method of quantitative determination of toxin by way of the biological test has a number of disadvantages; it does not permit to determine nonfatal concentrations of toxin. It has been established that tissues of some organs expose the toxin to a lasting peris and this hinders the extraction of toxin. In such cases the results depend largely on the method of a prior prepa-

ration of tissues (K.I. MATVEV and T.I. BULATOVA, 1948).

We used in our current research a sulfur tracer (S^{35}) preparation with added botulinum toxin type B to study its distribution in the organism of white mice. The preparation is commonly used for exploratory research and in the production of antitoxin.

The tracer preparation with toxin was formed with admixture of S^{35} -methionine to a medium selected for growing *Cl. botulinum* culture.

Radioactive methionine was selected for tracing as the starting product in accordance with the idea that sulfur is a component of the botulinum toxin's molecule. Also, this amino acid is always available in culture media used for growing botulinum bacteria.

Experimental Method

1. Production of the tracer preparation with toxin. We added to 500 ml of casein-fungous medium 0.5% of glucose and 37 mc of S^{35} -methionine, supplementing it with 10 ml of freshly grown culture of *Cl. botulinum* type B, strain No.175, and then we placed this under thermostatic control at 37°C. After 5 days of germination, we filtered the culture fluid through a cotton-gauze filter and centrifuged it at 3,000 revolutions per minute for 20 minutes in order to liberate it from microorganisms. Following this, proteic substances containing botulinum toxin precipitated on the bottom after a 60% saturation with ammonium sulfate.

The film resulting from precipitation was diluted with 100 ml of distilled water and then dialyzed for several days in tap

water and in distilled water at 16°C in order to purify it from low molecular tracer combinations. The radioactivity of the preparation decreased by 30% after dialysis.

The second precipitation of the fluid toxin was accomplished after a 40% saturation with ammonium sulfate. The subsequently formed film that contained botulinum toxin was passed through a filter paper and left to dry at room temperature in a vacuum of a microanalyzer. Then, the dry preparation of the toxin was pulverized in a mortar placed in a special manually operated box-like receptacle and after this we determined the toxin's titer and its radioactivity. Thus, we obtained 30 mg of a dry preparation of the tracer toxin with the activity approximating 1 mc/mg. The toxicity of the preparation equalled 5,000 Dlm (minimum lethal doses).

2. Measurement of radioactivity in tissues. The experiments were conducted on 30 white mice, each weighing 15 to 18 gm. Altogether we resolved 246 determinations. The toxin diluted as a suspension in 0.5 ml of physiological solution was administered intravenously in a quantity of 1,000 Dlm per animal. This dose corresponded to 71,250 pulses per minute according to radioactivity.

The animals were decapitated at various time intervals in order to measure the radioactivity in their tissues. The first group (11 mice) was killed after 20 minutes, the second (10 mice) - after 60 minutes and the third group (9 mice) - after 150 minutes. The latter time interval coincided with a development of

poor conditions in poisoned animals, which was manifested by infrequent breathing, paralysis of extremities and relaxation of all muscles. We examined the following tissues: blood, brain, muscles, liver, kidneys, lungs, heart and intestines.

The suspensions of organs (50 to 100 mg) were triturated in glass homogenizers with 0.5 ml of distilled water, then 0.2 ml of homogenate were applied on a target made of aluminum foil. Following the drying of targets under thermostatic control, the ^{unations}determinations of radioactivity in B setting were accomplished with the aid of the end-type counter; they disclosed the effectiveness of S³⁵ at approximately 15 to 17%. The weight of dry substance on the target surface of 2.27 cm² did not exceed 10 mg. Consequently, the measurements were ascertained in a "thin layer of the substance", where, in actuality, a self-absorption of radioactivity seemed very unlikely. The activity was recalculated per 1 gm of raw tissue.

Results

The results of the investigations were presented in charts as mean exponents (see figures A and B). Each point on the chart's curve represented the arithmetical mean of 8 to 11 determinations.

The experimental data indicate that, 20 minutes after administration of the tracer preparation with toxin, the investigated tissues effectuated the radioactivity level expressed in pulses/minute per gram in the following decreasing order: lungs - 2,690; liver - 2,230; heart - 2,107; kidneys - 1,586; intestines - 1,362; muscles - 449; brain - 429; blood - 287.

A decrease in radioactivity was noted after 60 minutes in the liver, heart, intestines, brain and blood. On the other hand, the radioactivity level advanced in kidneys and in muscles (see figure A).

a - activity of tissues in pulse/minute per gram; b and d - time in minutes; c - concentration rate of tissue/blood.

Figure A and B - Radioactivity changes in tissues, also concentration rate of $\frac{\text{blood}}{\text{tissue}}$ in the organism of white mice after intravenous administration of tracer preparation with botulin toxin. A and B: 1 - blood; 2 - brain; 3 - muscles; 4 - kidneys; 5 - liver; 6 - intestines; 7 - heart; 8 - lungs.

Prior to death, animals showed an increased concentration of radioactivity traces in some tissues (blood, heart, lungs, intestines) and a decrease in other (liver, muscles, kidneys) tissues.

Thus, a redistribution of the tracer preparation with toxin takes place in an organism during intoxication time along with a

redistribution of any tracer products that might result from the toxin's conversion between various tissues.

The dynamics of accumulation of radioactive tracer in some tissues (blood, intestines, heart and lungs) represent per se a curve with the lowest level after 1 hour. But, in other organs (kidneys and muscles), we observed the highest level of radioactivity after 1 hour. The concentration of tracer gradually decreased in the liver (see figure A).

Unlike in other tissues, the capacity of tracer products in the brain actually did not change after 150 minutes. But, a very low concentration of the tracer preparation with toxin was observed in the blood. It was found that some positive dependence existed between the radioactive level in the blood and that in other investigated tissues. In most instances the concentration rate of radioactive tracer in $\frac{\text{tissue}}{\text{blood}}$ occurred again with changes in the dynamics up to absolute concentration (see figures A and B).

Evaluation of the Results

The measurements of radioactivity in tissues proved that, following the administration of botulinical toxin and up to the moment of animals' death, the contents of radioactivity in all organs were considerably higher than in the blood, namely the concentration rate exceeded one unit. This correlation implied a quick penetration of the toxin from a blood stream into various tissues in organism. And this, in turn, indicates that botulinical toxin passes freely through cellular membranes.

It is difficult to state to what extent a high penetrability can be specific with relation to the toxin in question, which, by its nature, happens to be a protein. As we know, cells of many organs are generally highly penetrable by homologous and heterologous proteins.

The latter are detected in the cytoplasm and in cellular nuclei of the liver, also in nuclei of the central nervous system and in other organs, even in 10 minutes after their intravenous administration (COONS, LEDUS and KAPLAN, 1951), (HURCOWITZ and CRAMPTON, 1952).

The detected by us redistribution of the toxin's preparation in the organism of mice showed uneven trend in various tissues. A concentration decrease in tissues of heart, lungs, intestines and blood was accompanied by an increase in the level of toxin in muscles and in kidneys. Thus, simultaneously with the elimination of toxin from some organs, its accumulation occurred in other organs. Hence, a possibility is not excluded that the redistribution of toxin is conditioned by its chemical conversion in structures of the organism.

The changes in the dynamics of toxemia that manifest themselves by an increase, or by decrease and again by an increase in the concentration of botulinical toxin, are reported in the literature (L.A. PRISAKHIS, 1953). The author used biological method to study botulinical toxemia in white mice.

The presence of a rather steady level of toxic contents in the brain conforms with the report of T.I. BULATOVA and K.I. MAT-

VREY (1948), who explained this by the properties of brain tissues that are capable to bind botulinal toxin relatively firmly.

It should be mentioned that our findings as to the dynamics of distribution of a tracer preparation with botulinal toxin in the organism of white mice differ considerably from the distribution of homologous and heterologous proteins in the organism (GITTLIN and WHIPPLE, 1953; HUROWITZ and CRAMPTON, 1952) after inclusion of proteins of bacterial origin (A.I.GRISHENKOV, 1960), and also from the distribution of S^{35} -methionine (I.E.MALAKHOV, 1955 and L.P.PANCHENKO, 1956).

This fact may indicate indirectly that, in our experiments, after administration of the tracer preparation with botulinal toxin, the distribution of radioactive tracer was specific to some extent and, apparently, the position of the toxin's molecule in the organism was indicated, especially when the essence of the distribution of the tracer preparation with botulinal toxin agreed in many respects with the distribution evidenced by biological method (I.N.MORGUNOV, 1941; L.A.PEISAKHIS, 1953).

The study of distribution of the tracer preparation with botulinal toxin offered an objective idea about the quantitative contents of the toxin in animal organs.

Yet, on the basis of this alone, it is impossible to conclude any preferential spot of the toxin's action, because the severity of the toxin's invasion may depend not only on its quantity, but also on the sensitivity of tissues to the toxin.

The utilisation of a tracer toxin does not exclude the use

of biological tests for toxin's indicants.

Moreover, the radioactive method of indication is more promising for exposing the dynamics of botulinal toxin's distribution in an organism in the presence of nonfatal concentrations, and also in problems connected with the toxin's interrelation with the microstructures in tissues.

Conclusions

1. The cultivation of Cl. botulinum in a nutrient medium that contained 3^{35} -methionine enabled us to obtain a tracer preparation with botulinal toxin type B that maintained a stable recording of the radioactive tracing.

2. After intravenous administration of the tracer preparation with toxin, our study of its distribution in the organism of white mice proved that the tracer readily penetrated into tissues of the lungs, liver, heart, muscles, brain and intestines.

The highest concentration of radioactive tracing was found in the lungs. The lowest level of radioactivity was recorded in the blood during all periods of research.

3. During 150 minutes after intravenous administration of the tracer preparation with botulinal toxin, we observed in the organism of white mice a redistribution of radioactivity with a periodic decrease of the tracing concentration in some organs and then with an increase in other organs.

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Summary (copy)

The labelled preparation of the "B" type botulismotoxin preparation was obtained from the cultural fluid after growing "Cl. botulinum" on a medium containing S³⁵-methionine. The preparation was purified by a two-fold precipitation with ammonium sulfate and subsequent dialysis in the running fresh and distilled water. The obtained preparation of dry tagged botulismotoxin was radioactive in the range of 1 mc/mg, while its toxicity equalled 5,000 Dlm/mg. The distribution of the labelled preparation was studied on white mice every 20, 60 and 150 minutes after an intravenous injection of 1,000 Dlm. The investigation covered: blood, brain, muscles, liver, kidneys, lungs, heart and intestines. The toxin

preparation was found to rapidly find its way from the blood flow into body tissues. The highest radioactivity rate at all periods of the intoxication was recorded in the lungs, and the lowest - in the blood. The radiotag concentration in the examined tissues changes along with the developing intoxication, except for the brain, where the radio-lab. level remains fairly stable.