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DISTRIBUTION OF A LABELLED PREPARATION OF BOTULISM TOXIN IN THE BODY OF WHITE MICE

/Following is a translation of an article by Z.P. Pak and T.I. Bulatova, in Farmakologiya i Toksikologiya (Pharmacology and Toxicology), Vol XXV, No 4, Moscow, July/August 1962, pages 478-482./

Chair of Pharmacology (Head -- Prof V.V. Vasil'yeva) of the 2nd Moscow State Medical Institute imeni N.I. Pirogov and Laboratory of Indication (Head -- Prof K.I. Matveyev) of the Institute of Epidemiology and Microbiology imeni N.F. Gamaleya

Up to the present time the distribution of botulism toxin in the organism has been studied by the biological method (I.N. Morgunov, 1941; Feysakhis, 1953). For this purpose, extracts from the organs of the poisoned animal were injected in various dilutions to white mice and, by the rate of the onset of their death, the quantitative content of the toxin in investigated tissues was evaluated.

However, the biological method of determination of the quantity of toxin has a number of drawbacks. It does not permit the determination of non-lethal concentrations of the toxin. In addition, it has been established that the toxin is stably fixed by the tissue of certain organs, thus hampering its excretion. In such cases the results depend to a large extent on the method of preliminary processing of the tissues (K.I. Matveyev and T.I. Bulatova, 1948).

In the present work we investigated the distribution in the organism of white mice of a sulfur (S^{35})-labelled preparation of the B-type botulism toxin which is widely used in research practice, as well as for the preparation of an anatoxin.

The labelled toxin preparation was obtained by the addition of S^{35}-methionine to the nutritive medium employed in the growth of Clostridium botulinum culture.

Radioactive methionine was selected as an initial labelling product for the reason that sulfur is an ingredient of the composition of the molecule of borulism toxin. Besides, this aminoacid is always present in the nutritive media employed in the growth of Clostridium botulinum.

Methods

1. Obtaining of labelled toxin preparation. To 500 ml of a casein-fungal medium were added 0.5 percent glucose and 37 mc of S^{35}-methionine; in it were seeded 10 ml of freshly-grown culture of Type B No 175 Cl. botulinum, and the mixture put in a thermostat at 37°. After five days of growth, the cultural fluid was filtered through a cotton-gauze filter and centrifuged at 3,000 revolutions per minutes in order to separate it from microbial bodies. After that, the protein products containing the botulism toxin were precipitated by means of ammonium sulfate at 60 percent saturation.

The film, formed after precipitation, was dissolved in 100 ml of distilled water and dialyzed for 24 hours in running fresh and distilled water at 16°, in order to purify it from the low-molecular labelled compounds. Following dialysis, the radioactivity of the preparation decreased by 30 percent.

The repeated precipitation of the fluid toxin was carried out with ammonium sulfate at 40 percent saturation. The resulting film, containing toxin, was pressed out with filter paper and dried at room temperature in a microanaerostat under vacuum conditions. The dry toxin preparation was triturated in a mortar which had been placed in a special manually-operated box, and the titer and radioactivity of the preparation was then determined. As a result, 30 mg of a dry preparation of labelled toxin were obtained of an activity of about one mc/mg. The toxicity of the preparation comprised 5000 DLM (minimal lethal doses).

2. Determination of the radioactivity of the tissues. Experiments were staged on 30 white mice weighing 15 to 18 mg. A total of 246 determinations were carried out. The diluted toxin in the form of a suspension in 0.5 ml of a physiological solution was injected intravenously in the amount of 1000 DLM per animal. In its radioactivity this dose corresponded to 71,250 pulses per minute.

In order to determine the radioactivity of tissues, the animals were beheaded at various time intervals. The 1st group (11 mice) -- within 20 minutes, the 2nd (10 mice) -- within 60 minutes, and the 3rd (nine mice) -- within 150 minutes. The last period coincided with the onset in the poi-

soned animals of a grave condition, accompanied by rare respiration, paralysis of the extremities, and weakening of the entire musculature. The following tissues were examined: blood, brain, muscles, liver, kidneys, lungs, heart, and intestines.

Weighted portions of tissues (50-100 mg) were triturated in glass homogenizers with 0.5 ml of distilled water; 0.2 mg of the homogenate were then placed on an aluminum foil target. After drying the targets in a thermostat, the radioacitvity determination was carried out on a B device by means of a surface counter of about 15 to 17 percent of S^{35} effectiveness. The weight of dry substance on the target of the size of 2.27 cm^2 did not exceed 10 mg. Thus, the measurements were carried out in a "thin layer" of the substance, where the self-absorption of radioactivity is practically negligible. The activity was recounted per gram of fresh tissue.

Results

The results of investigations are shown in diagrams in the form of mean indexes (see Fig. a and b). Each point on the diagram curve represents the arithmetical mean of 8 to 11 measurements.

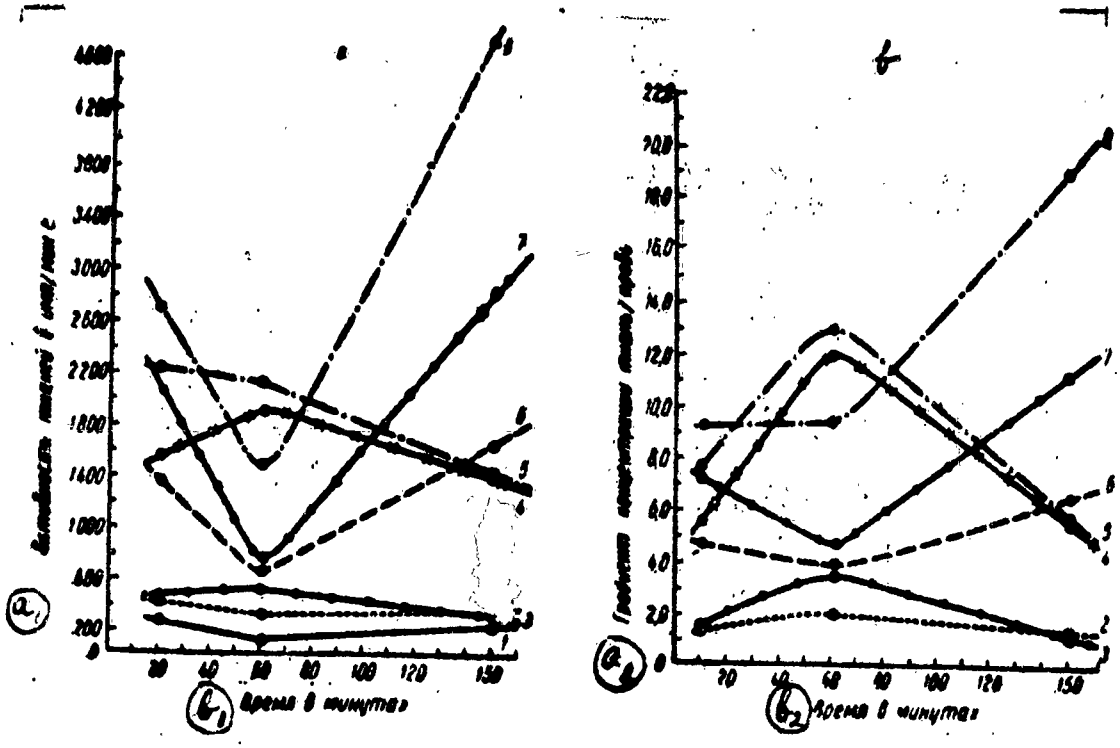
The experimental data show that, within 20 minutes following the injection of the labelled toxin preparation, the investigated tissues were arranged in the following decreasing order in regard to the radioactivity level expressed in pulses per minute per gram: lungs -- 2,690, liver -- 2,230, heart -- 2,107, kidneys -- 1,586, intestine -- 1,362, muscle -- 449, brain -- 429, blood -- 287.

Within 60 minutes a reduction of radioactivity was noted in the liver, lungs, heart, intestines, brain, and blood. In the kidneys and muscles, on the contrary, the radioactivity level increased (see Fig, a).

Prior to the death of the animals, in some tissues (blood, heart, lungs, intestine) a rise in the concentration of the radiotag was observed, in others (liver, muscles, kidneys -- reduction)

Thus, during intoxication a redistribution between various tissues takes place in the organism of the labelled toxin preparation, or of some labelled products of its transformation.

In some tissues (blood, intestine, heart, and lungs), the dynamics of radiotag accumulation represents a curve with the lowest level reached within an hour. In other organs (kidneys and muscles), the highest radioactivity level was noted within an hour. In the liver the tag concentration gradually decreased (see Fig, a).



Changes in the radioactivity of tissues and the $\frac{\text{blood}}{\text{tissue}}$ concentration gradient in the organism of white mice following intravenous injection of a labelled preparation of botulism toxin.

a and b: 1 -- blood; 2 -- brain; 3 -- muscles; 4 -- kidneys; 5 -- liver; 6 -- intestine; 7 -- heart; 8 -- lungs.

a_1 -- Activity of tissues in puls/min gm
b_1 -- Time in minutes
a_2 -- Concentration gradient: tissue/blood

In the brain, in contrast to other tissues, the content of labelled products remained virtually unchanged during the period of 150 minutes. The lowest concentration of the labelled toxin preparation was observed in the blood. It turned out that between the radioactivity level of the blood and other investigated tissues there is a definite relationship. The $\frac{\text{tissue}}{\text{blood}}$ concentration gradient of the radiotag repeats in the majority of cases the dynamics of changes of an absolute concentration (see Fig., a and b).

Discussion of results

Determination of the radioactivity of tissues showed that, following injection of the botulism toxin and up to the moment of the animal's death, its content in all organs is considerably higher than in the blood, i.e., the concentration gradient is higher than 1. This correlation indicates a rapid penetration of the toxin from the blood stream into various organic tissues. This, in its turn, attests to the fact that the botulism toxin easily passes through the cellular membranes.

It is difficult to say as to what extent the high penetrating property is specific for the given toxin which is of protein nature. It is known that cells of many organs are generally easily penetrable to homologous and heterologous proteins.

The latter are detected in the cytoplasm and cellular nuclei of the liver, central nervous system, and other organs as early as within 10 minutes following their intravenous administration (Coons, Leduc, Kaplan, 1951; Coons, Kaplan, 1950; Gittlin and Whipple, 1953; Hurowitz and Crampton, 1952).

The redistribution of the toxin preparation in the organism of mice, which we have elicited, is of non-uniform nature in various tissues. The reduction of concentration in the tissues of the heart, lungs, intestines, and blood is accompanied by a rise of the toxin level in the muscles and kidneys. Hence, simultaneously with the toxin excretion from some organs, its acumulation takes place in other organs. The possibility is not excluded that the toxin redistribution is caused by its chemical transformations within the structures of the organism.

Changes in toxemia dynamics which are characterized by a rise, fall, and another rise of the botulism toxin concentration were described in the literature (L.A. Peysakhis, 1953). This author employed the biological method in his study of botulism toxemia in white mice.

The presence in the brain of a fairly constant level of the toxin content conforms with the report of T.I. Bulatova and K.I. Matveyev (1948), who point out the property of cerebral tissue of incorporating relatively firmly the botulism toxin.

It should be noted that our data on the dynamics of distribution in the organism of white mice of the labelled botulism toxin preparation differ considerably from the distribution of homologous and heterologous proteins in the organism (Gittlin and Whipple, 1953; Hurowitz and Crampton, 1952), including also the proteins of bacterial origin (A.I. Grishenkov, 1960), as well as from the S^{35}-methionine distri-

bution (I.Ye. Malakhov, 1955; L.F. Panchenko, 1956).

This may indirectly attest to the fact that in our experiments the radiotag distribution, following administration of the labelled botulism toxin preparation, is specific to some extent and apparently reflects the location of toxin molecule in the organism, especially in view of the fact that the distribution character of the labelled preparation of the botulism toxin corresponds in many respects to its determination by means of the biological method (I.N. Morgunov; 1941; L.A. Peysakhis, 1953).

The study of the distribution of a labelled botulism toxin preparation offers an objective idea of its quantitative content in the organs of animals.

However, one cannot come to a conclusion, based on this fact alone, as to the principal location of the toxin effect, since the gravity of toxin-induced affections may depend not only on its quantity, but also on the sensitivity of tissues to it.

The employment of labelled toxin does not exclude the biological methods of its determination.

At the same time, in the elicitation of the distribution dynamics of the botulism toxin in the organism in its non-fatal concentrations, as well as in regard to the problems of its interrelations with tissue microstructures, the method of radioactive indication offers greater prospects.

Conclusions

1. Growing of Cl. botulinum on a nutritive medium containing S^{35}-methionine makes possible the obtainment of a labelled preparation of type B botulsim toxin with a firm fixation of the radiotag.

2. Investigation of the distribution in the organism of white mice of the labelled toxin preparation following its intravenous administration, showed that it penetrates rapidly into the tissues of the lungs, liver, heart, muscles, brain, and intestines.

The highest concentration of the radiotag takes place in the lungs. The lowest radioactivity level is found in the blood at all investigation periods.

3. During 150 minutes, following intravenous administration of the labelled botulism toxin preparation, a redistribution of radioactivity takes place in the organism of white mice with a periodic reduction of the radiotag concentration in some organs and its increase in others.

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