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In the last few years we observed an increase of sickness due to food poisoning, in which the pathogenetic factors were enterotoxic staphylococci. This became the subject of a number of publications: Bergdoll (6), Burbińska and associates (9), Feig (13), Kozariwa and associates (18), Flisska (25), Sedlak (28). At the same time, studies were made to obtain enterotoxin in a pure form and its compositions with amino acids. Hilnick and Bergdoll (16), who described the properties of purified enterotoxin (molecular weight 24,000 - 3,000), identified 18 amino acids in its composition. Studies carried out in subsequent years showed that enterotoxins are non-homogeneous in terms of their antigenic property. Casmann and associates (10) confirmed the existence of at least two toxic proteins which they designated as A and B.

Studies of the mechanism of the effects of staphylococcal enterotoxin on the organism (Bayliss [4], Anderson and associates [1], Richmond and associates [27], Lavergne and associates [19]) encountered difficulties so far both because of a lack of a preparation which would be sufficiently pure as well as because of the shortage of appropriate indices which would provide information about the distribution of the enterotoxin in the organism. Nor were there any studies made of the degree to which enterotoxins are absorbed orally, and of the speed and the way in which they are eliminated from the organism.

The marking of staphylococcal enterotoxin by radioactive iodine was designed to create more favorable conditions in the actual research to clarify the problems under discussion.
As a type of control, we also carried out studies to determine the distribution and excretion of human \(^{131}\)I-albumin in rabbits.

**Materials and Methods**

1. Marking of Staphylococcal Enterotoxin by Radioactive Iodine

In order to mark the enterotoxin by radioactive iodine, we used an enterotoxin of the B type which was obtained from Dr. Bergdoll of the Food Research Institute at the University of Chicago. The marking of the enterotoxin by radioactive iodine was done by means of a modified method of Masouredis (20, 21):

a) 1.7 mg of enterotoxin was dissolved in 1 ml of glycol buffer.

b) Solution of isotope: 1 ml of Na\(^{131}\)I with total activity of 650 mCi were added to 0.12 ml of the carrier (consisting of 100 ml of a solution which included 55 mg of KI and 25 mg KIO\(_3\)). Then we mixed both solutions and left them at the room temperature for a period of 30 minutes, and the resulting preparation was subjected to a dialysis in the presence of 0.1 M of phosphate buffer. The resulting marked enterotoxin had the following characteristics: characteristic activity 60 mC/mg of enterotoxin, activity of the solution 59.09 ± 1.20 mCi/ml, measured by a scintillation chamber counter, concentration of protein 1.36 mg/ml, the amount of \(^131\)I not combined with the protein was 2%, number of atoms of I corresponding to a molecule of enterotoxin — 1.1.

The marking of staphylococcal enterotoxin by radioactive iodine was done at the Department of Radiobiology and Health Protection of the Nuclear Research Institute in Warsaw.

2. Human Albumin Marked by Radioactive Iodine

Albumin obtained from the Nuclear Research Institute at Swierk was a 5% solution of human albumin marked by \(^{131}\)I in a physiological solution of salt. The isotonic preparation contained 27 mg/ml of albumin. The content of non-combined iodine was 1.5%.

3. Measurement of Distribution of Enterotoxin Marked by Iodine in Rads

Staphylococcal enterotoxin marked by radioactive iodine was administered orally or intravenously to animals (always by the vein on the rim of the earlobe). Then the animals were placed separately in metabolic cages which made it possible to collect separately the urine and the excreta. After a period of time which was determined for individual experimental groups, the rabbits were put to sleep, and samples were taken for studies from the following material: blood, intestines, lungs, kidneys, spleen, brain, skin of the thin and large intestines. The amount of the material was measured exactly and placed in appropriate containers which were filled with a 30% solution of NaOH to 3 ml for purposes of homogenisation. The
Activity of gamma radiation of the samples under study was marked by means of a set which counted in the scintillating counter for gamma radiation with the application of the following: 1) scintillating sound with photoduplicator RCA 6655, 2) scintillating wall crystal "2x" of the 8DS type, 3) electronic set of thetracerlab. SC-57 type, 4) electronic computer of the PEL-5 type.

Progress of Experiments and Results

In each of the groups under study we used 6 rabbits which received staphylococcal 131I-enterotoxin in doses of 13.5 C/kg. The radioactivity of the organs, blood, urine, and excreta was noted after 6 and 18 hours and after the 2nd, 4th, 6th, and 8th day after the administration of the labeled enterotoxin.

Distribution and excretion of 131I-labeled staphylococcal enterotoxin administered orally to rabbits.

After oral administration we found in the blood (1 ml of the material under study) 0.009% of the administered dose after 6 hours. The radioactivity of blood continued to increase in the following days, and on the 4th day of the test it amounted to 0.03% of the administered dose.

The radioactivity of urine amounted to 0.015% of the administered dose as early as after 6 hours, and after 18 hours it increased to 0.209%. In the course of the following days we observed a decrease of radioactivity, and on the 8th day the urine contained 0.014% of the dose.

Kidneys showed the highest degree of radioactivity after 18 hours, the lining of stomach showed the maximum radioactivity in the course of the first 18 hours (0.065%–0.061% of the administered dose). The thin intestine showed a similar, but lower value after 6 hours from the administration of staphylococcal 131I-enterotoxin (0.036%), and the same applies to the large intestine. The degree of radioactivity of the spleen was low (up to 0.007%). The brain tissue also showed a low value of radioactivity after 6 hours (0.004% of the administered dose), and it increased only in the course of the following days.

The radioactivity of the excreta on the 2nd day of the study amounted to 0.162% of the administered dose, and on the 4th day it increased to 0.219%. In the course of the following days the radioactivity gradually decreased.

Figure 1. Degree of Radioactivity of Material After Oral Administration of 131I-labeled Staphylococcal Enterotoxin to Rabbits. Doses: 131I-labeled staphylococcal enterotoxin in doses of 13.5 C/kg administered orally.

Key: 131I - % of administered dose. b - Day of administration of 131I-labeled staphylococcal enterotoxin. 1 - Excreta. 2 - Large intestine. 3 - Liver. 4 - Small intestine. 5 - Lungs. 6 - Kidney. 7 - Stomach. 8 - Bile. 9 - Urine. 10 - Blood. 11 - Brain. 12 - Spleen.
Figure 1 gives a graphic presentation of the results mentioned above. The results are computed in percent of the dose.

Distribution and excretion of $^{131}$I-labeled staphylococcal enterotoxin administered intravenously to rabbits.

After intravenous administration of labeled enterotoxin, it was found that blood contained 0.068% of the administered dose after 6 hours. During the subsequent period the radioactivity of the material decreased, and on the 8th day it amounted to 0.028% of the administered dose in 1 ml of blood.

The radioactivity of the brain amounted to 0.187% of the administered dose as early as after 6 hours, and after 18 hours it increased to 0.352%. In the course of the following day the radioactivity of urine gradually decreased. Liver showed the highest degree of radioactivity 18 hours after intravenous administration of labeled enterotoxin.

Figure 2. Degree of Radioactivity of Material After Intravenous Administration of $^{131}$I-labeled Staphylococcal Enterotoxin to Rabbits. Doses: $^{131}$I-labeled staphylococcal enterotoxin.
in doses of 13.5 C/kg administered intravenously.

Key: 131I% of administered dose. b -- Day of administration of 
I-labeled staphylococcal enterotoxin. l -- Excreta. 2 -- 
Large intestine. 3 -- Liver. 4 -- Small intestine. 5 -- 
Lungs. 6 -- Kidney. 7 -- Stomach. 8 -- Bile. 9 -- Urine. 
10 -- Blood. 11 -- Brain. 12 -- Spleen.

Stomach showed a high degree of radioactivity -- 0.077% as early as 
after 6 hours, and after 18 hours the radioactivity of the stomach wall in-
creased even higher up to 0.159%. The radioactivity remained within those 
limits on the second day of the study. The small and large intestines 
showed similar values.

A low degree of radioactivity was found in the spleen. No increase 
of radioactivity was observed in the brain tissue, which after 6 hours con-
tained 0.013% of the administered dose, and the maximum radioactivity oc-
curred after 18 hours with 0.016% of the dose.
Radioactivity of the excreta after 18 hours contained 0.06% of the dose, it continued to increase during the following days, and the maximum value was reached on the 6th day of the study, namely 0.396% of the dose.

The results given above, computed in terms of percent of the dose, are given in the form of a graphic presentation in Figure 2.

Distribution and excretion of $^{131}$I-labeled albumen in rabbits.

Human albumin was administered intravenously and also in doses of 13.5 C/kg. After administration of the labeled albumin, the radioactivity of blood amounted to 0.351% of the dose after 18 hours, and in the course of the following days it gradually decreased. On the 8th day of the study the radioactivity of 1 ml of blood under study still amounted to 0.144% of the administered dose.

Radioactivity of urine after 18 hours amounted to 0.017%, but on the following day it increased to as much as 0.197% of the dose and it continued approximately at the same level in the course of the following 2 days.

The radioactivity of the liver reached its maximum on the 2nd day of the study.

The degree of radioactivity of the stomach wall, small and large intestines took approximately the same course and showed a relatively low value.

Low activity was also found in the spleen and in the brain tissue. Excreta reached maximum radioactivity on the 6th day after administration of $^{131}$I-labeled albumen (0.087% of the administered dose).

A graphic presentation of the above results is given in Figure 3, which gives figures computed in percent of the dose.

Figure 3. Degree of Radioactivity of Material After Intravenous Administration of $^{131}$I-Albumen administered to Rabbits. Doses: $^{131}$I-labeled staphylococcal enterotoxin in doses of 13.5 C/kg administered intravenously.

Key: a = % of administered dose. b = Day of administration of $^{131}$I-albumen. 1 = Excreta. 2 = Large intestine. 3 = Liver. 4 = Small intestine. 5 = Lung. 6 = Kidney. 7 = Stomach. 8 = Bile. 9 = Urine. 10 = Blood. 11 = Brain. 12 = Spleen.
Discussion

When we use staphylococcal enterotoxin labeled by radioactive iodine, we must discuss the influence of iodization on the preservation of biological characteristics. This problem has not been studied before and can be examined only on the basis of literature which dealt with the influence of radioactive iodine on other bacteria toxins. Long assumes on the basis of his studies that toxins marked by radioactive iodine do not change their biological character, even though they may change physically or chemically. Hausouredis emphasizes the fact that when we introduce one atom of $^{131}$I in one molecule of diphtherial toxin and carry out that reaction at pH 7.5, he did not notice any loss of toxicity and there were no major immunochecmical changes of the labeled albumin. For that reason we should not try to get labeled preparations with high activity of their own (Belcher [5], Chagas [11], Grobansky and associates [14], Danowski [12]).

$^{131}$I, which was released from albumin combinations in catabolic processes was found exclusively in the form of free iodine (Barnaby and associates...
In the actual studies the process of iodizing of staphylococcal enterotoxin was carried out at pH 7.5. On the other hand, the number of atoms of I which corresponded to the molecule of the enterotoxin was 1.1.

As a result of the above data we can assume that the physical-chemical properties of enterotoxin were not subjected to any major changes and the labeled albumin corresponded to the physiological conditions.

Radioactive iodine is built during the iodization process into thyroxine, which is a component of staphylococcal enterotoxin (Broda [8], Rapoport [25], Karlson [17]).

Studies concerning the degree of absorption of $^{131}$I-enterotoxin through the mucous membrane of the digestive tract and its distribution in the rabbits when administered orally shows that its concentration is relatively low in the blood. There was also a low degree of radioactivity in the lungs, liver, spleen, and brain tissue. On the other hand, a higher degree of radioactivity was found in the stomach wall and in the walls of the small and large intestine. This may indicate that $^{131}$I-enterotoxin has a considerable affinity to those tissues. If we use the T test by Student, we find that the difference in the distribution of radioactive combinations in those organs are statistically significant ($p (t_1 > t_0) > 0.01$).

Excretion of staphylococcal enterotoxin marked by radioactive iodine through urine started as early as after 6 hours and reached the maximum degree after 18 hours.

In the case of intravenous administration of $^{131}$I-enterotoxin to rabbits, it was found that the blood contained a higher degree of radioactivity than in the case of oral administration. Increased radioactivity was also found in the stomach wall and in the walls of the small and large intestine. Excretion of marked enterotoxin through the feces started as early as after the 2nd day.

The mechanism of the effects of staphylococcal enterotoxin on an organ have not been explained as yet, and the results of the studies are not always in agreement.

Anderson (1) and Anderson and associates (2) found that isolated sections of intestines were more contractable under the influence of the enterotoxin.

Richmond and associates (27) believe that the increase of contractability of an isolated intestine of rabbits under the influence of enterotoxin may correspond to the contraction of intestines in humans in case of poisoning by enterotoxin.

Lavergne and associates (19) came to the conclusion on the basis of
their studies that pathological phenomena in cases of staphylococcal food poisoning do not reflect a direct influence of the enterotoxin on the digestive tract.

Bayliss (4) and Moser and associates (23) believe that the sudden vomiting during that stage of food poisoning is related to the effects of the enterotoxin to the vomitive center. That reaction is supposed to be a reflex.

In the opinion of Bayliss (4), enterotoxin affects these terminal cells of the sensory nerves, primarily in the small intestine. The irritation is supposed to be transferred through the sensory fibers of the poisoned nerve to the vomitive center, and then through the mobile fiber of the sensory system to the muscles of the esophagus, stomach, and diaphragm.

In our own studies we found that there is a low and even degree of radioactivity of the brain tissue in rabbits which receive labeled enterotoxin. These results suggest that enterotoxin does not show any tropism to the brain tissue, even though we cannot exclude the possibility that the brain tissue is more sensitive even to a low concentration of enterotoxin. It may be useful to determine the threshold of sensitivity of the brain tissue to enterotoxin in order to explain this problem more accurately.

Control studies involving intravenous administration of $^{131}$I-albumin to rabbits have shown that such albumin appears in the blood in a relatively low concentration. Among the internal organs, the following showed the highest radioactivity: kidneys, liver, and lungs. On the other hand, a low degree of radioactivity was found in the walls of stomach, small and large intestines, and brain.

As demonstrated by Myant and associates (24), Helmkamp and associates (15) and Birke and associates (7), $^{131}$I-gammaglobulin in human beings acts in a similar way to albumin.

**Conclusions**

1. $^{131}$I-labeled staphylococcal enterotoxin administered to rabbits either orally or intravenously shows an affinity to the wall of stomach, small and large intestines, as shown by the high degree of radioactivity of these tissues.

2. Labeled enterotoxin appears in a relatively small concentration in blood, and it is eliminated from the organism primarily through the urine.

3. Studies which have been carried out did not indicate any increased tropism of the enterotoxin to the brain tissue.

4. Control studies involving the use of labeled human albumin in rabbits showed a high degree of concentration of such albumin in the blood, lungs, liver, and kidneys. On the other hand, the tests did not show any
increased affinity of $^{131}I$-albumin to the walls of the stomach, small and large intestines.

Bibliography

