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# FOREIGN TECHNOLOGY DIVISION

EPIDEMIOLOGY REVIEW

(SELECTED ARTICLES)

STATEMENT #2 UNCLASSIFIED

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# EDITED TRANSLATION

EPIDEMIOLOGY REVIEW (SELECTED ARTICLES)

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PREPARED BY:

TRANSLATION DIVISION
FOREIGN TECHNOLOGY DIVISION
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**ABSTRACT**

Of 282 patients, incubation time was less than 24 hours in 42, 1-3 days in 158 and 4 days in 82. Nausea was noted in 156, emesis in 142 and diarrhea in 87 of 321 hospitalized patients. Ophthalmologic studies revealed accommodation disturbances in all of the patients noted, other ocular disturbances in many, including diplopia, e. g., in 22.6%. Duration of symptoms, diagnostic aspects, treatment and various other clinical details are reported and discussed. Presented at the 3rd Scientific Assembly of Polish Epidemiologists and Infectologists.

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**ABSTRACT**

The latex method for detecting botulin types A, B and E in either blood sera or food items, utilizing only materials produced in Poland, is described in detail. It permits the detection of as little as 0.1 of the subcutaneous MLD of the toxin for a 20-Gm mouse.


AFSC FORM AUG 66 4 (Test form under revision)

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# OBSERVATIONS ON BOTULISM IN POZNAN PROVINCE
# REPORT 3. THE CLINICAL PICTURE

Kazimierz Neyman[1]

This report like report 2 is based on the case histories of 321 patients in the Poznan and Zielonogórsk Provinces. Incubation period of the illness in the 282 cases in which we were able to confirm it was: up to 24 hours in 42 cases (14.9%); 1 to 3 days in 158 cases (56.0%); 4 days in 82 cases (29.1%).

At the beginning of the illness the following symptoms were noted: nausea in 156 patients (48.6%), vomiting in 142 patients (44.2%), and diarrhea in 87 patients (26.5%).

At the clinically-developed stage the following objective and subjective symptoms were observed according to frequence of occurrence: dryness of the mouth (94.4%), "disturbances within the eyeballs" (84.4%), constipation (81.0%), difficulty in swallowing (17.1%), hoarseness (16.2%), double vision (14.3%), drooping eyelids (10.0%), muscular debility of trunk and extremities (7.8%), and urination disturbances (5.9%).

For elucidating individual interpreations of the various "disturbances within the eyeballs", 65 patients from the Contagious Diseases Division in Poznan were selected for further evaluation. The following was observed: deficiency in accomodation, 100%; disturbances in reaction of the pupils to light, 72.3%; dilated pupils, not wholly reacting to light, 27.8%; unequal size in pupils, 6.2%; drooping of both eyelids, 12.4%; drooping of one eyelid, 6.2%; deviation of the eyeballs, 4.6%; double vision, 26.2%.

After an initial period of vomiting, which occurred in approximately one-half the cases (exactly 44.2%), and diarrhea, the toxin affecting the smooth muscles of the intestines led to obstruction. Such obstruction occurred in 81% of the patients and continued in spite of medication, together with deficiency in accomodation for a fairly long time (average 7 to 10 days). We observed, however, single incidents of diarrhea lasting up to several days.

Among other motion disturbances we must mention involuntary movement of the facial muscles (18.5%) and "debility" of the muscles of the trunk and extremities (10.7%).

Among the complications observed in patients in our Division, the following were noted: 4 instances of salivary gland inflammation near the ear, 3 cases of bronchial inflammation of the lungs, 2 cases of extensive mouth thrush, and one case of ulcers of the palate.

The remaining patients on the average left the hospital after 16 days in a satisfactory general condition, but approximately 30% of them still had some disturbance in accomodation.

For 256 cases in the Province the average dose of serum was 110 ml, but at the same time in our Division it was 180 ml, and that was administered mainly into the muscles. Antibiotics were prescribed only where complications existed or where complications threatened. Confirmation of the clinical diagnosis by additional biolog-

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[1]See page 5.

ical tests on specimens collected from 65 patients in Poznan was obtained for only 5 patients. Confirmation of botulism in food products suspected of contamination was obtained for 6 patients. Finally in 3 patients there was indisputable epidemiological evidence. For diagnosing the remaining 51 patients (78%) exclusively clinical criteria were used. In the data collected special attention must be called to the low death rate; it was only 1.2%.

# THE LATEX REACTION IN SERODIAGNOSIS OF BOTULISM AND DETECTION OF THIS TOXIN IN FOOD PRODUCTS

Tadeusz Rycaj[2]

A point of departure for developing the reaction were the observations of Singer and Plotz (1958) that the gamma-globulin of human or animal blood serum is absorbed on particles of latex, sensitizing it just as hemolysin for ram corpuscles (amboceptor) sensitizes ram corpuscles, or Coombs type antibodies sensitize Rh corpuscles.

Commercial latex type LBS--301 2 (polymer of n-butadiene) is diluted with 5 times its volume of double-distilled water, poured into Petri dishes and evaporated at room temperature to its original volume. This operation must be repeated 5 times. After the last evaporation, the latex is passed through ordinary filter paper. Botulinus anatoxin effective for type A + B, -- both purified and concentrated, a product of Warszawska Wytwornia Surowic i Szczepionek (Warsaw Producers of Serum and Vaccines) -- is diluted 1.2 with Sörenson's glycine buffer at pH 8.3. To 10 ml anatoxin diluted with 0.5 ml, the latex is added very slowly by drops and continuously stirred. After addition of the latex, the antigen must be placed into a water bath for 2 hours at a temperature of 56°, then for 24 hours at a temperature of +1°.

After this time particles of latex sensitized by botulinus anatoxin settle to the bottom of the vessel; the nonsensitized latex remains above the deposit.

The suspension of nonsensitized latex must be carefully removed with a pipette and the sediment must be suspended in 9 ml Sörenson's glycine buffer, pH 8.3, with an addition of methiolate.

In this way a unique, effective antigen for detection of botulism type A and B is produced.

It is more usual, however, to prepare an antigen separately for type A, type B, and type E.

A control antigen must be prepared at the same time, sensitizing latex with euglobulins of horse serum. For this purpose 90 ml 0.0027/N HCL -- made freshly each time from 0.1/N HCL -- is added by drops and continuously mixed with 10 ml normal, fresh horse serum. The euglobulin in centrifuged out after 90 minutes and then suspended in 9 ml Sörenson's glycine buffer, pH 8.3, to which 0.5 ml latex is added. The remaining steps are the same as those in the preparation of antigens with antitoxins.

## Carrying out the Reaction

Half the pipettes are filled with latex antigen specific for types A, B, and E, and confirmed to be fresh and not inactive by the serum of the person under investigation, or by clear extract of the articles of food in which the presence of botulinus is suspected.

Food items under investigation must first be chopped, then covered with two parts by weight of Sörenson's glycine buffer, pH 8.3, and when this is adequately

[2]See page 5.

pulped, stirred and left at room temperature for one hour. Next the suspension must be centrifuged and the clear liquid inspected for botulinus.

The pipettes are set vertically in plasticine and placed in a controlled temperature box at 37°C for one to two hours. At the end of this time the results are read. At the same time control tubes are set up with the control antigen and with antigens from previous absorption of possible toxin from the material being studied by antitoxins which were previously added to this material (blood serum of the patient).

For this purpose the extract of food under investigation, or blood serum, is transferred to 3 centrifuge tubes, 1 ml per tube, then antitoxin for type A is added to the first tube, antitoxin for type B to the second, and antitoxin for type E to the third.

The antitoxin is added at the rate of 1 ml to each tube; care is exercised that this quantity should contain at least 10 new antitoxin units. The tubes are shaken, and then left for 30 minutes in a water bath at a temperature of 37°, and controls are centrifuged and prepared as necessary.

For carrying out the above reaction we used pipettes with a diameter of 0.8 mm and length of 90 mm suitable for detecting "sharp phase albumin" (CRP).

Testing the sensitivity of this reaction with the designated botulinus toxin type A and B obtained from the Warszawska Twornia Surowic and Szczepionek we confirmed that this method can detect as little as 0.1 MLD of toxin injected subcutaneously into a 20-g white mouse.

## Footnotes

1. to p. 1. Division of Contagious Diseases, J. Strus City Hospital, Poznan.

2. to p. 3. Microbiological Laboratory, General Hospital No. 1, Bydgoszcz.