TRANSLATION NO. 2294

DATE: 1958

DDC AVAILABILITY NOTICE

This document is subject to special export controls and each transmittal to foreign governments or foreign nationals may be made only with prior approval of Commanding Officer, Fort Detrick, ATTN: SMUFD-AE, Frederick, Md. 21701

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

#2294

This communication was designed to draw the attention of practitioners to the mixed culture agglutination reaction as a valuable supplementary method of laboratory diagnosis, for use in cases of chronic dysentery and on suspected carriers. Where a positive mixed culture agglutination reaction is obtained but bacteriological examination gives negative results it may be time to register the persons concerned and to keep them under observation in intestinal diseases clinics.

CONCLUSIONS

(1) We examined dysentery patients, convalescents, persons suffering from acute intestinal disorders and suspected carriers, using the bacteriological method and the mixed culture agglutination reaction; a total of 2371 examinations were made. Our results indicated that the mixed culture agglutination reaction has no particular advantages when used in cases of acute dysentery. However, this method reveals the dysentery antigen 3-7 times more often than the bacteriological method when used in cases of chronic dysentery, on convalescents, or on suspected carriers. In the case of healthy individuals, cultures from the faeces and the agglutination reaction were negative at all times.

(2) Long-term study showed that a large percentage of the persons who gave a positive mixed culture agglutination reaction, but yielded negative results in bacteriological examinations, were suffering from chronic dysentery.

(3) We recommend that intestinal diseases clinics should keep a record of all those who give a positive agglutination reaction, and should examine them by laboratory and clinical methods (sigmoidoscopy, etc.) over a period. Observations should be continued for not less than two months.

Translated by R. E. HAMMOND

REFERENCES

BERNHOF, F. G., Zh. mikrobiol., èpidemiol. immunobiol. No. 12, 62–64, 1944
BERNHOF, F. G., Zh. mikrobiol., èpidemiol. immunobiol. No. 4, 39–44, 1945
BERNHOF, F. G., and SKRYNNIK, P. I., Zh. mikrobiol., èpidemiol. immunobiol. No. 2, 226–235, 1937
BERNHOF, F. G., and SKRYNNIK, P. I., Zh. mikrobiol., èpidemiol. immunobiol. No. 1, 58–61, 1939
BOTVINNIK, S. A., POPKOVA, N. F., and SMIRDINA, E. A., Zh. mikrobiol. èpidemiol. immunobiol. No. 7, 68–71, 1953

STUDIES OF *BOTULINUM* ANTIGENS AND ANTISERA BY ZONE ELECTROPHORESIS AND AGAR GEL DIFFUSION*

IU. Z. GENDON

Tarasevich State Control Institute for Sera and Vaccines

(*Received* 30 *July* 1957)

ONE of the methods which enables us to obtain crude antigens of high concentration with insignificant traces of inert protein is the method of cultivation in cellophane sacs.

In the preparation of highly active purified preparations it is necessary to know their antigenic structures and their fractions to be able to discriminate between active and

* Zh. mikrobiol., èpidemiol. immunobiol. No. 2, 95–100, 1958 [Reprint Order No.: MIC 824].

inert fractions, as this is of the greatest importance for the selection of the most rational methods of purification and concentration.

In recent years new and easily performed methods of studying the fractional structure of toxins and antitoxins have been devised. Such methods are for instance zone electrophoresis, agar gel diffusion and the combination of these two methods, immuno-electrophoresis.

The possibility of preparing active toxins by cultivation in cellophane sacs and the importance of these methods, which enable us to study in detail the antigenic structure and the fractions of toxins and antitoxins, can be particularly clearly demonstrated if we use *Clostridium botulinum* toxin as a model, as this is the most powerful of all known bacterial toxins.

It was our task above all to prepare *botulinum* toxins and toxoids of type A in cellophane sacs to compare these preparations with the usual toxins and toxoids and to study the antigenic and fractional structure of toxins and antitoxins of type A, in order to detect the most active fractions.

In the beginning of our work we developed the method of cultivation in cellophane sacs and also studied the factors which might have an influence upon the growth and toxin formation of *Cl. botulinum*.

The method of cultivation in cellophane sacs briefly consists of the following: into a glass flask is placed a cellophane tube fixed on to a special glasswool–gauze plug. The tube is filled with saline and the flask is filled with the nutrient medium; after sterilization the organisms are inoculated into the cellophane sac and the whole system is placed into the incubator.

The experiments were performed with *Cl. botulinum* strain No. 98 of type A. In the majority of cases liver broth containing 0·5 per cent glucose (pH 7·2) was used as nutrient medium.

It was found that maximum toxin formation takes place on the fifth–sixth day when the bacteria are cultivated in the usual way and on the eighth–ninth day when the bacteria are grown in cellophane sacs. It was further found that the maximum toxin formation and the autolysis of the bacteria coincided in time. The bulk of the exotoxin enters the surrounding medium apparently only after the death and autolysis of the bacterial cells. During the growth of the bacteria the pH steadily decreases and the lowest level was found at the peak period of toxin formation.

Study of 55 batches of toxin showed that toxins prepared in cellophane sacs were 10–40 times more active in regard to the Dlm (mice) than ordinary toxins and they contained up to 6 million Dlm per ml, compared with 60,000–200,000 Dlm per ml for the usual toxins. The nutrient medium surrounding the cellophane sac contained no toxin. In regard to the number of experimental doses per ml the cellophane toxins were 10–40 times more active than the usual toxins and contained 2000–10,000 experimental doses per ml compared with 100–300 in the usual toxins. The toxins prepared in cellophane sacs had high flocculation activity and flocculated within 25–30 min after the beginning of the experiment, whereas no flocculation could be observed with the usual toxins when the experiments were performed under identical conditions. In the ring precipitin test the cellophane toxins produced a ring even in a dilution of 1 : 14–1 : 16, whereas the usual toxins produced a ring only if they were undiluted. The cellophane toxins contained approximately one-half the amount of amino and total nitrogen of the usual toxins. When the Dlm per mg N was calculated the toxins prepared in cellophane sacs proved to be 37 times purer than the usual toxins.

The cellophane toxins were less stable than the usual toxins during storage for 12 months in a refrigerator without preservative. The lower stability can possibly be related to the higher pH level (6·8 in cellophane toxin and 5·8–6 in the usual toxins).

Comparative study of the concentration of toxins by vacuum drying, dialysis against glycerol, ultrafiltration, salting out with ammonium sulphate and precipitation with trichloroacetic acid showed that approximately equal degrees of concentration were attained. Since the initial cellophane toxins were much more active, their concentration gave preparations containing many more Dlm than preparations obtained by concentration of the usual toxins.

Toxins prepared in cellophane sacs offer many advantages from the economic point of view. This method enables us to obtain twice as many Dlm from the same amount of nutrient medium than by the usual method. Also, the nutrient medium used for the production of cellophane toxin can be used once again for the preparation of toxin by the usual method. In our experiments we obtained from such a medium a *botulinum* toxin of type A containing 30,000 Dlm per ml.

The next part of the work was devoted to the comparative study of the antigenic structure of toxins grown by various methods and the corresponding antisera, using the method of precipitation by diffusion in agar. The method used in these experiments was briefly as follows: 2 ml of 1% agar mixed with antitoxic serum were poured into a tube of 5-6 mm diameter; this layer was covered by 1 ml neutral agar and after the latter had hardened the antigen was poured on to it; the tubes were incubated or left at room temperature and after a certain time rings of precipitate were observed.

It could be shown that in order to detect the maximal number of antigen fractions in the serum the highest possible toxin concentration must be used, and the corresponding antiserum must be employed in the optimal concentration, ensuring the formation of the greatest number of precipitate rings. (In our experiments the optimal concentration was 250-500 A.U. per ml of serum agar.) The number of antigenic fractions found in the toxins depended on the batch of toxin and antiserum as well as on the method used for purification and concentration. The highest number of fractions was found when the toxin was reacted with sera purified and concentrated by the "Diapherm 3" method devised in the Institute of Experimental Medicine, Academy of Medical Sciences of the U.S.S.R.; somewhat fewer fractions were found with sera purified and concentrated by combined dialysis, and much fewer fractions were observed if crude serum was used.

Study of the antigenic structure of the toxins by the agar gel diffusion test showed that the usual toxins contained 15 antigenic fractions, one of which corresponded to the proteins of the nutrient medium; in the toxins prepared in cellophane sacs 14 antigenic fractions could be differentiated and the fraction corresponding to the proteins of the nutrient medium was absent. At least 5 antigenic fractions found in the toxins were of inert nature; these were present in the cellophane toxins in much smaller amount than in the usual toxins.

Study of the changes occurring in the antigenic fractions during purification and concentration showed that precipitation with trichloroacetic acid secured a more complete elimination of inert antigenic fractions from the toxin than salting out with ammonium sulphate.

To compare the fractions present in the proteins of usual and cellophane toxins and in the corresponding antisera and to establish to which of the fractions the bulk of the toxigenic principle was attached, and which fraction of the antiserum carries the antitoxic and precipitated antibodies, we used the method of fractionation by paper electrophoresis.

The apparatus used for the electrophoresis, the method and the results obtained by fractionation of normal sera from various animals and of antitoxic horse antiserum to *Cl. botulinum* type A toxin were set forth in a previous paper (Zh. mikrobiol., èpidemiol. immunobiol. No. 9, 91, 1956).

Study of separate antiserum fractions by preparative paper electrophoresis showed that in crude serum the antitoxin is mainly carried by the T-globulin fraction, and some

antitoxin was also found in the γ- and β-globulins; in sera purified and concentrated by combined dialysis the antitoxin was present in the γ- and to a lesser extent the β-globulin fraction; in sera purified and concentrated by the Diapherm method the bulk of the antitoxin was attached to the γ-globulin fraction.

The content of precipitating antibodies in various antiserum fractions was estimated by immuno-electrophoresis in agar. This method consists in preliminary fractionation of the serum by electrophoresis in agar, followed by diffusion and precipitation in agar with an antigen situated parallel to the axis along which the antiserum had been fractionated. The reaction leads to the formation of precipitate arcs: lines drawn from the top of each arc perpendicularly to the fractionation axis show the location of the particular fraction from which the corresponding precipitating antibody had diffused.

The investigations showed that in horse antisera to type A *botulinum* toxin the precipitating antibodies were attached to the same protein fractions as the antitoxic antibodies.

The subsequent stage of the work was devoted to a study of fractions present in the proteins of usual type A toxins as compared with those found in cellophane toxins and in the nutrient medium from which the toxin was prepared. Four protein fractions were found in the nutrient medium. In crude usual and cellophane toxins we were able to differentiate 5 protein fractions, 2 of which had a positive charge and 3 a negative charge. In toxins prepared in cellophane sacs, fractions with negative electric charge which possess the lowest electrophoretic mobility ($0{\cdot}06 \times 10^{-5}$ cm^2 $volt^{-1}.sec^{-1}$), were predominant in quantity; it was these fractions which carried the bulk of the toxic principle as had been shown by the study of separate fractions by preparative paper electrophoresis. It was found by paper electrophoresis that purification and concentration of the experimental cellophane toxins led to a more complete elimination of inert fractions and to a higher concentration of fractions carrying the bulk of the toxic principle than purification and concentration of the usual toxins.

We further studied the question of the use of cellophane toxoid as antigen for active immunization. In experiments in which toxin was inactivated by formalin we had shown that type A toxins prepared in cellophane sacs were rendered completely non-toxic by the addition of 0·6 per cent of formalin for 14–15 days; if 0·4 per cent of formalin were used the process lasted 25–30 days. Approximately the same times were needed to inactivate the usual toxins, although these contained much less of the specific toxic principle than the cellophane toxins. The toxoids prepared from the latter were 5–11 times (on an average 7 times) more active from an antigenic point of view than the usual toxins and contained 320–350 FU (fixation unit) compared with 40–60 FU in the usual toxins. In regard to their total and amino nitrogen content the cellophane toxoids were approximately twice as pure as the usual toxoids. Calculation of the FU content per mg N showed that their purity was 14 times higher than that of the usual toxoids. They have a high flocculating capacity and flocculate within 90–115 min, whereas the usual toxoids did not flocculate under the same experimental conditions. The immunogenic properties of the toxoids were studied in experiments on the immunization of mice and guinea-pigs. Two immunizing injections with cellophane toxoid produced a higher degree of immunity in mice than the usual toxoid. The guinea-pigs were immunized with 1, 2, 3 and 4 injections with both types of toxoids containing an equal dose of FU per ml. It appeared from these experiments that the cellophane toxoids were 2–4 times more immunogenic than the usual toxoids.

Adsorption of cellophane toxoids onto aluminium phosphate increased their immunogenic activity about 7–8 times.

Our studies thus showed that the production of type A *botulinum* toxins in cellophane sacs is practicable, economical and of great practical importance.

The method of fractionation by paper electrophoresis, preparative electrophoresis, immuno-electrophoresis and precipitation by diffusion in agar applied to a study of the fractions and antigenic structure of toxins, toxoids and antitoxins, and also to establish the role of each separate fraction, will be of assistance in establishing the requirements which preparations of the highest quality should comply with, and in devising the most rational method for the purification and concentration of antigens and antibodies.

CONCLUSIONS

(1) *Cl. botulinum* type A toxins prepared by cultivation in cellophane sacs are of much higher activity than toxins formed under the usual cultural conditions; they contain less inert protein and possess a greater capacity for flocculation.

(2) By precipitation in agar 15 antigenic fractions could be differentiated in the usual type A *botulinum* toxins and 14 in the cellophane toxins. The number of antigenic fractions depends on the batch of antiserum and also on the methods used for purification and concentration.

(3) Fractionation by preparative electrophoresis on filter paper has shown that in crude horse antisera to type A *botulinum* toxin the antitoxic antibodies are mainly carried by the T-globulin fraction, and also a certain part of the antitoxin is found in the β- and γ-globulin fractions; in sera purified and concentrated by combined dialysis the antitoxic antibodies are attached to the γ-globulin and to a lesser extent to the β-globulin fraction; in sera purified and concentrated by the Diapherm method the bulk of the antitoxin is attached to the main fraction. In type A *botulinum* antisera the precipitating antibodies are found in the same fractions as the antitoxic antibodies.

(4) In type A *botulinum* toxins 5 protein fractions can be differentiated, only one of which carries the bulk of the toxic principle; it is this fraction which predominates in toxins prepared in cellophane sacs; purification and concentration of these toxins leads to a more complete elimination of inert fractions and to a higher concentration of the fraction carrying the bulk of the toxic principle than purification and concentration of the usual toxins.

(5) Type A *botulinum* toxins prepared in cellophane sacs are easily inactivated by formalin and the toxoid thus obtained is of higher activity than the usual toxoids in regard to its antigenic as well as its immunogenic properties.

Translated by F. S. FREISINGER

BAITS CONTAINING ORGANOPHOSPHORUS INSECTICIDES FOR USE AGAINST HOUSE-FLIES*

E. V. SCHNEIDER

Central Scientific and Experimental Disinfection Institute

(*Received* 15 *January* 1957)

THE aim of our work was to make a general study of the new Russian insecticides of the organophosphorus group, chlorophos, diazinon and carbophos, which are intended for use against flies in the places where they swarm and breed. We used chlorophos as a liquid solution and diazinon and carbophos as an emulsion; molasses or crude treacle served as the bait.

* Zh. mikrobiol., epidemiol. immunobiol. No. 2, 100-104, 1958 [Reprint Order No.: MIC 828].