AD-781 011

PRODUCING A STABLE TOXIN CLOSTRIDIUM SEPTICUM FROM A HORSEMEAT CULTURE

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15 October 1972

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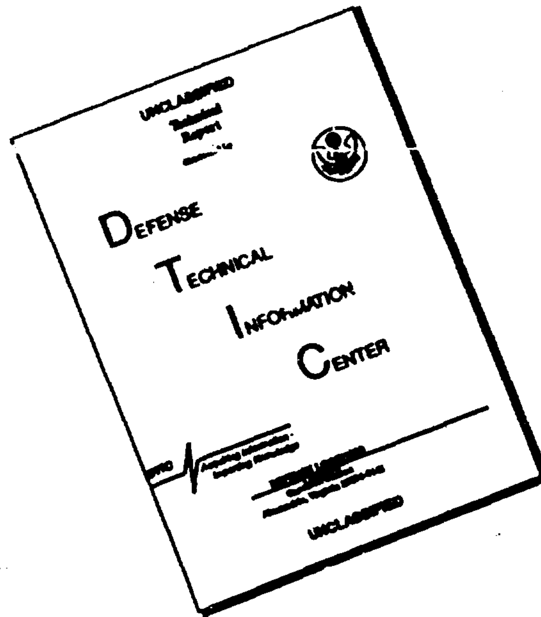

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In Reply Refer to:
FSTC-HT-23- 2302-72
DIA Task No. T70-23-01

Date: October 15, 1972

TRANSLATION

ENGLISH TITLE: PRODUCING A STABLE TOXIN CLOSTRIDIUM SEPTICUM FROM A HORSEMEAT CULTURE

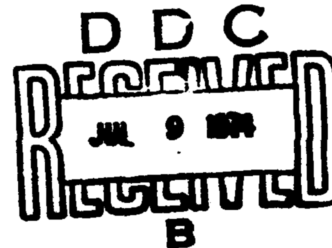
FOREIGN TITLE: POLUCHENIYE STAVIL'NOGO TOKSINA CLOSTRIDIUM SEPTICUM V PITATEL'NOY SREDE IZ KONSKOGO MYACA

AUTHOR: L. BERGOL'TSEVA, et al. LANGUAGE: RUSSIAN

SOURCE: Voprosy proizvodstva vaktsin i syvorotok, vypusk 1, "Zdorov'ya", Kiev, 1968, pp 62-66

TRANSLATOR: Universal Language Services, Inc.

REQUESTOR: SMUEA-PO-F



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Producing a Stable Toxin Clostridium Septicum from a Horsemeat Culture

L. A. Bergol'tseva et al.

The quality of a toxin, its activity, and the full value of its antigen structure as well as other properties determine to a large extent the effectiveness of hyper-immunization of horses, the producers of antitoxin serums.

Often the preparation of the toxin Cl. septicum in commonly used cultures is difficult, and in addition they are quite expensive, require the use of food products, and the process of their preparation is cumbersome, labor- and time-consuming.

These factors have prompted us to search for a culture medium, the use of which would provide constant supply of quality toxin and would not require great expenditures. As raw material for our culture medium we chose horsemeat which is waste from serum production.

In preliminary experiments, the possibility of preparing the toxin Cl. septicum from a culture medium of horsemeat had been established, after which research was carried out on preparing the optimal culture medium.

We prepared different variants of nutritive media from acid hydrolysates of cooked and uncooked horsement; a method of processing the meat before hydrolysis was worked out; and the conditions for preparing the hydrlysates were changed. Hydrolysis was carried on in an open pot and at different pressures.

The media that were prepared from hydrolysates differed in their proportions of the quantity of hydrolysates and the amount of beef and horsemeat broth that was added.

In short, a technique was worked out for preparing a nutritive medium consisting of an acid hydrolysate and horsemeat broth.

We studied the influence of the length of growth, temperature, source of carbon food, and uterine culture on the toxigenesis of Cl. septicum, as well as the anitgen structure and other properties of the toxin we obtained.

We tested the hydrolysates prepared from the meat of nine horses. 42 series of bouillon were made and 28 tests. All research was done on Cl. septicum, strain 59.

The toxigenesis of Cl. septicum was studied at temperatures of 34° and 37°C. The best results were obtained at 34°C. The maximum toxin production at this temperature took place in 19 to 22 hours. The optimal source of carbon in the substances we used (dextrines prepared by various means; maltos, glugose) as well as in other nutritive media, turned out to be glucose in concentrations of 0.25 to 0.5 percent (measured in dry state). As far as uterine culture is concerned, and its age, physical state and also the culture in which it was obtained, it was established that the activity of the toxin was practically identical whether dry or liquid culture was used,

and it did not depend on the age or the initial culture medium. During observation of the established optimal rate of breeding cultures of Cl. Septicum, we obtained toxin with an activity of 400 to 700 Dlm/ml. Since we were unable to find any information on antigen structure research in toxin Cl. septicum grown in a liquid nutritive medium on horsemeat, we carried out a study of the antigen composition of ten series of the toxin by the double immunization method of Ouchterloni (1953). We studied centrifuges and filtrations of bouillon of culture (400 to 450 Dlm/ml), and also the toxin concentrated by the salting out using ammonium sulphur oxide (900 to 1500 Dlm/ml.) The reaction was started with native monovalence anti-toxin horse serums of Cl. septicum, containing some 400 to 600 ME/ml. Immunodiffusion reaction occurred at a temperature of 37°C.

The initial precipitates were detected in 24 to 48 hours; the maximum development of the precipitation spectrum reached 4 to 5 days. After the expiration of this period the plates were washed, dried and colored with amidoschwarz or nitrogen carmine, as described by Uriel and Scheidegger (1955).

It was established that the clearest and best developed immunogram could be obtained by using concentrated toxins and also under the condition of marking or sketching of the serum on patterns or stencils of heavy filter paper: these markings were placed on the surface of an agar plate.

In our experiment various series of native toxin in reaction with homologous serum formed one or two lines of precipitation; in reaction with the concentrate up to eight lines of precipitation (see the table). A. A. Bornyakova (1959), using the ring precipitation method to study the antigen structure of 46 series of toxin Cl. septicum prepared in Pope culture and in casein bouillon , detected one ring each.

The results of our experiments provide a basis for assuming that during the reaction of the immunal immunoprecipitation under determined optimal conditions for toxins of Cl. septicum, prepared in a medium of horsemeat, its possible to detect a rather complex antigen composition, consisting of up to 8 components.

In order to clarify the question about the serological specificity of Cl. septicum, the reaction of immunoprecipitation was placed not only with homologous but also with heterologous serums. It was then established that the toxin we are studying forms one line of precipitation with the serum against Cl. perfringens, and Cl. botulinum of type B.

The problem of the antigen relationship of Cl. septicum with toxins with other stimulants of gas gangrene is of definite interest and demands further study.

One of the basic disadvantages of using Cl. septicum is its great lability. Usually not having Cl. septicum in sufficient activity and as a consequence of its great lability, we are forced to employ various methods of concentrating it which causes a great loss of active beginnings. It should also be noted that concentrated Cl. septicum does not possess stable properties either. We addressed ourselves to the problem of studying the properties of the toxin we prepared and determining the conditions of its stability. The

Antigen Composition of Cl. septicum Toxin by Immunodiffusion

Toxin		No. of lines of precipitation in reaction to horse serums	
Series	Dlm/ml	Serum 143	Serum 295
3 (2)	450	1-2	1-2
11 (1)	400	3-4	1-2
15 (C)	300	1-2	2
15 (F)	300	1-2	2
11 (2)	400	3-4	1-2
3 (4) Con.	900	7-8	6-7
17 (1) Con.	1500	5-6	5-6
Native bouillon		-	-
Concentrated bouillon			

Abbreviations: C - centrifuge; F - filtration; Con.- toxin concentrated by ammonium sulfate.

use of native toxin is preferred for immunization since the antigen is put into a natural unchanged state. From the economic point of view this is also greatly desirable.

It is widely known that native Cl. septicum is very unstable in the process of being worked with in bouillon cultures as well as in storage, shaking or jarring and filtrations.

I. A. Larina and Z. M. Volkova (1958), note that the toxin, prepared in casein and meat cultures, is highly unstable: it breaks down during storage and processing, making it very difficult to obtain high quality preparations. The same is confirmed by other researchers.

For this reason it is necessary to give horses freshly prepared toxin, before the expiration of the date of titration and sterility, which necessitates large expenditures for the nutritive medium.

In the process of trying to obtain native Cl. septicum, we tried various methods, using solutions of peptone, glycocoll, saccharose, and also a culture medium. The best results were obtained with the culture medium. To preserve the initial activity, it is necessary to make a previous washing of the sterilizing plate in seitz filters of the nutritive medium.

A stable native Cl. perfringens can also be obtained in this same way. The methods for obtaining stable concentrations of Cl. septicum and Cl. perfringens are different. To obtain a stable Cl. septicum concentrated by ammonium sulphate one must dissolve the precipitation in a filtration of the culture, and then filter through sterilized plates, washed beforehand with the nutritive medium. Sterile Cl. septicum, obtained in this way, retains its activity for half a year and longer (the period of our experiment).

Using stable toxin with high activity, we were able to introduce into horses a specific antigen in small volumes (10 to 80 ml.). As a result of the hyper-immunization of horses, we obtained mono- and also poly-valence serums (Cl. perfringens, Cl. oedematiens, and Cl. septicum) with high antitoxin titrations.

The basic advantage of the culture we propose is the possibility of obtaining stable Cl. septigum toxin which in activity not only is not inferior but in a series of cases exceeds the activity of the toxin prepared in other nutritive media (400 to 700 Dlm/ml) if the raw materials were available and sufficiently cheap.

Conclusions

1. As a result of the research carried out, a technique has been devised for producing a culture medium for the toxin Cl. septicum from horse meat (the basis of serum production): the optimal conditions for toxigenesis for Cl. Septicum in the proposed culture medium have been established. The activity of the toxin reaches 400 to 700 Dlm/ml.

2. The antigen structure of native and concentrated Cl. septicum was studied by the double immunodiffusion method in agar gel following Oukhterloni's method in a modification we undertook.

The results we obtained verified the full value of antigen composition of the toxin, grown in a culture medium of horse meat and prepared by the technique we worked out.

3. The properties of the condition for stabilizing native and concentrated Cl. septicum were studied and the conditions for creating it, thanks to which the activity of the toxin can be preserved for six months and longer (the period of our observation).

4. Due to this method of perparing a stable toxin of high activity, it is possible to prepare it in quantity for storage and to inject horses with specific antigen in small volumes (10 to 80 ml). This is of special value for immunizing with a polyvalant antigen.