TECHNIQUE OF PREPARATION OF PYROGENIC POLYSACCHARIDE FROM PROTEUS VULGARIS

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Reports in the foreign press have been regularly published in recent years on the special effect of pyrogenic polysaccharides of bacteria on the course of pathological processes. Polysaccharide preparations of Proteus vulgaris and Bacillus pyocyaneus, released under the name Pyromen, have been particularly studied.

According to these reports, injections of pyrogenic polysaccharides accelerate regeneration of injured nerve fibers [1], promote regeneration of nerve fibers in sectioned spinal cord [8] and partial restoration of conduction [5], normalize blood circulation in injured tissues and prevent necrosis of the frostbitten rabbit ear [6], promote repair processes, lessen scarring and even aid in resolution of newly formed cartilage in the burned ear [2, 7]. Wexler and Tryzynski [6, 7] and Randolph and Rollins [4] reported successful use of Pyromen in treating allergic diseases.

These data as well as many years of clinical experience in fever therapy indicate that the attention of both clinicians and experimenters should be directed to study of the action of pyrogenic polysaccharides on various pathological processes. However, obtaining pure nontoxic preparations involves a number of difficulties. Hence, we decided to report our work on obtaining such a preparation from Proteus vulgaris.

The method we used does not involve original procedures. But after a series of unsuccessful attempts we succeeded in selecting the conditions and a combination of successive procedures permitting preparation of pyrogenic polysaccharide nontoxic in doses exceeding the pyrogenic dose hundred of times.

From a multitude of proposed methods we chose the precipitation by acid acetone and subsequent treatment with phenol according to Palmer and Gerlow [3]. In order to work with minimal volumes of the initial bacterial suspension, washings from an agar culture were used and not a culture in liquid medium.

In order to avoid admixture of endotoxin, which it is difficult later to get rid of, it was decided to avoid use of the ordinarily recommended procedures, such as shaking the bacterial suspension and acid hydrolysis. We observed all necessary precautions in the work for preventing contamination of the preparation by extraneous pyrogenic substances.

The sequence of work was as follows.

1. Wash off a 48-hour culture of Proteus with physiological solution. Use each portion of the latter over again to wash the culture into several small vessels.

2. Centrifuge. Filter centrifugate through a bacteriological candle.

3. Pour filtrate into 10 volumes of acid acetone (in all cases the acetone contains 0.4% by volume of glacial acetic acid).

4. The following day pour off acetone and dissolve precipitate in 360 ml water (in all cases use twice-distilled water distilled with potassium permanganate and sterilized in the autoclave immediately after distillation).

5. Precipitate again with 10 volumes of acid acetone.

6. After 24 hours dissolve precipitate in 150 ml water. Dialyze in colloidal sacs at about 0° against frequently changed distilled water for 5 days (to almost complete decolorization of solution and cessation of coloring of water).

7. Precipitate with an equal volume of 95% ethyl alcohol. Centrifuge.

8. Precipitate the clear supernatant with 10 volumes of acid acetone. Centrifuge.

9. Twice dissolve precipitate in a small amount of hot water and treat with shaking for a day with 6 volumes of crystalline phenol.

10. Decant phenol. Wash precipitate thrice with acetone and dry under vacuum.

11. Dissolve in a small amount of hot water. Centrifuge to remove insoluble particles. Precipitate supernatant with 10 volumes of acid acetone.

12. Centrifuge. Wash precipitate thrice with small portions of 95° ethyl alcohol and dry under vacuum.

A white powder of light gray tint is obtained, which gives a sharp Molisch reaction and no biuret reaction. From washings from two hundred 0.7-1 liter flasks of square section 0.3258 g of the preparation was obtained. Rabbit tests showed that the preparation in amounts of 1-500 γ per kilogram is not toxic on parenteral injection.

It produces a sharp temperature reaction in a dose of 1 γ per kilogram.

Results of testing pyrogenic activity of the preparation are given in the table.

Results of Testing Pyrogenic Activity of Preparation

Date of experiment	Rabbit No.	Rabbit wt. (in g)	Dose (γ/kg)	Temper. before injection of prepn.	Temperature after injection of prepn.				
					after 30 min	after 1 hour	after 3 hours	after 5 hours	after 7 hrs.
13/III 1956	227	2 870	Physiological soln.	38.3°	38.5°	38 6°	38.7°	38 8°	38.2°
	224	2 860	"	38.7°	38.5°	38.7°	38.8°	38 6°	39.4°
	225	2 860	5 0	38.5°	39.0°	40.5°	41.4°	40.9°	40.5°
	222	3 130	5 0	38.7°	39.4°	40.4°	40.9°	39.8°	38.8°
	164	3 200	20 0	38 6°	39.3°	40.3°	41.2°	40.0°	40.0°
	201	2 630	20 0	38.9°	39.6°	40.6°	40,7°	39.7°	40.0°
	211	2 950	100 0	38 8°	39.0°	39.8°	40 5°	40.1°	39.4°
	170	2 680	100.0	39 0°	39.9	40.2°	40.4°	40.3	39.9°
8/IV 1956	206	2 920	Physiological soln.	38 0°	38 2°	38.0°	38.0°	38.1°	—
	214	3 150		38.8°	38.9°	38.8°	38.9°	38.8°	—
	234	3 130	0.1	39 1°	39.0°	39.5°	39.1°	39.0°	—
	220	2 030	0.1	38.4°	38.6°	39.3°	39.0°	38.8°	—
	219	2 940	1.0	38.8°	39.3°	40.3°	40.2°	38.7°	—
	210	3 030	1.0	38.3°	38.6°	39.4°	39.3°	38.7°	—
	232	2 940	200.0	38.2°	39.4°	39.4°	40 7°	40.1°	39.0°
	276	2 900	200.0	38.7°	39 8°	39.9°	39.7°	39.7°	39.5°
	277	2 860	500.0	38.5°	39.3°	39.6°	38 8°	39.0°	—
	258	2 790	500.0	38.2°	39.4°	38.9°	38.1°	38.1°	—

Rabbits that received the preparation in amounts of 200 and 500 γ per kilogram were given a second parenteral injection of the preparation in the same amount after 2 weeks. No symptoms of anaphylactic shock were observed.

Thus, by means of simple technique available to practically every bacteriological laboratory, a Proteus vulgaris polysaccharide preparation can be obtained, which is nontoxic and nonanaphylactogenic in amounts exceeding the pyrogenic dose hundreds of times.

SUMMARY

A method of preparation of pyrogenic polysaccharide from B. proteus vulgaris is presented. At the basis of this method lies the precipitation of the washings of agar culture by acidified acetone from the noncellular filtrate with the following treatment by phenol (Palmer and Gerlow method). The preparation which is thus obtained is nontoxic and nonanaphylactogenic for rabbits in the doses up to 500 γ per kilogram of body weight. This method is simple and may be used in every bacteriological laboratory.

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