CHANGES IN THE COMPOSITION OF THE BACTERIAL MASS OF Clostridium perfringens TYPE A DURING DEVELOPMENT OF CULTURES

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Strains of Clostridium perfringens of low toxicity, isolated like toxic strains from patients with gas gangrene, produce hardly any toxin on artificial nutrient media.

The object of the present investigation was to study the composition of the bacterial mass of Cl. perfringens obtained from cultures of strains possessing high and low toxicity. The results of a comparative study of the chemical composition of the bacterial mass of strains of different toxicity may be important in connection with the further study of the special features of the metabolism of Cl. perfringens essential to toxin formation.

EXPERIMENTAL METHOD

Experiments were carried out with toxic strains of Cl. perfringens BP6K (No. 28) and No. 235, accumulating toxin to the extent of between 100 and 400 MLD/ml, and strains Nos. 60, 180, and 2910 of low toxicity, producing not more than 5 MLD of toxin per ml. All the strains were kept on Tarozzi medium. To prepare the bacterial mass, 10 ml of fresh culture was seeded into 2 liters of casein-fungus medium [1]. Samples of the cultures were taken at the beginning of the logarithmic phase (5 h) and at its end (9 h), in the stationary phase (13 and 20 h), and from 24-h cultures.

The bacterial mass was separated from the medium by centrifugation at 6000 rpm for 30 min and washed three times with water. Acid-soluble compounds were removed from the fresh bacterial mass by extraction twice with 5% trichloroacetic acid solution in the cold for 30 min each time, and the residue was washed with 2% trichloroacetic acid solution and twice with water. The bacterial residue was dried with acetone and ether, and the total nitrogen and phosphorus in the dry powder were determined after mineralization with sulfuric acid.

RNA and DNA were determined by the method of Tsanev and Markov [2] in a type SF-4 spectrophotometer, phosphorus by the method of Taussky and Shore, and nitrogen by the micro-Kjeldahl method in a colorimetric modification with Nessler's reagent. The results of the determinations were expressed per 100 mg of dried bacterial powder. The strength of the toxins of the 20-24-h cultures was determined in minimal lethal doses (MLD) for albino mice weighing 12-14 g.

EXPERIMENTAL RESULTS

The content and the dynamics of the changes of the nitrogenous substances of the bacterial mass in the process of development of the cultures of strains with high and low toxicity are shown in Fig. 1. The total level of macromolecular nitrogenous substances at all periods of development of the toxic strains was much higher than in the strains of low toxicity.

The strains of low toxicity were characterized by a comparatively low content of macromolecular nitrogenous substances at the beginning of the logarithmic phase of growth and a considerable increase in their relative proportion in the dry substance obtained from the 9-h culture. In the stationary phase the content of nitrogenous substances continued to rise, although less intensively.

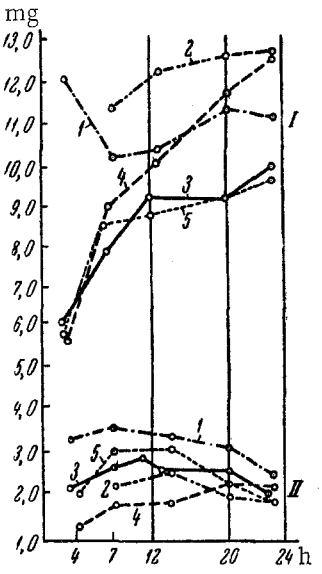

Fig. 1

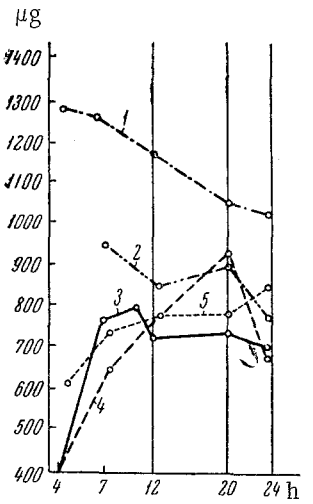

Fig. 2

Fig. 1. Content of total nitrogen and total phosphorus in bacterial mass of Cl. perfringens. I) Nitrogen; II) phosphorus; 1) strain No. 28; 2) strain No. 235; 3) strain No. 2910; 4) strain No. 60; 5) strain No. 180.

Fig. 2. RNA content in bacterial mass of Cl. perfringens. Legend as in Fig. 1.

A different picture was found in cultures of the toxic strain BP6K, yielding toxin in concentrations of 200 and 400 MLD/ml. During the logarithmic phase, not only was their content of macromolecular nitrogen not increased, but on the other hand, the breakdown of complex nitrogen compounds not passing into the extract fell. With the ending of proliferation in the stationary phase, in strains of both high and low toxicity, synthesis predominated and macromolecular nitrogen compounds accumulated.

Hence, the phase of multiplication of the toxic microorganisms was accompanied by a lowering of the level of the macromolecular nitrogen compounds.

In strain No. 235, forming only 50 MLD of toxin, in the course of development the nitrogen content increased progressively, as in the strains of low toxicity.

The total phosphorus content in the dry bacterial powder (see Fig. 1) of the strains of low toxicity gradually increased until the end of the logarithmic (strains Nos. 180, 2910) or stationary (strain No. 60) phase. In the 24-h cultures the content of all the phosphorus compounds fell.

In the dried bacterial cells of the toxic strains the total phosphorus content at the beginning of development of the cultures increased, but it reached its maximum much sooner than in the nontoxic strains, in fact in the middle (strain BP6K No. 28) or at the end of the logarithmic phase (strain No. 235), after which the total content of phosphorus compounds began to fall.

The following changes were observed in the RNA content (Fig. 2) in the process of development of the cultures.

In the logarithmic phase of growth of the strains of low toxicity accumulation of RNA took place until the stationary phase.

The RNA content in the toxic strains was much greater and it reached its maximum in the first hours of the logarithmic phase. Later, in the course of the logarithmic phase, the RNA level fell sharply. It is interesting that toxic strain No. 235 occupied an intermediate position between the least and most toxic strains—No. 28, on the one hand, and Nos. 2910 and 180 on the other. As in the case of the strains of low toxicity, the RNA level in strain No. 235 was low at the beginning of the logarithmic phase, but later it did not rise, as in strains of low toxicity, but it fell still lower, as in toxic strain No. 28. When proliferation of the cells and the production of toxin ceased in the stationary phase, the RNA content in the bacterial powder of strain No. 235 rose as in the case of the strains of low toxicity.

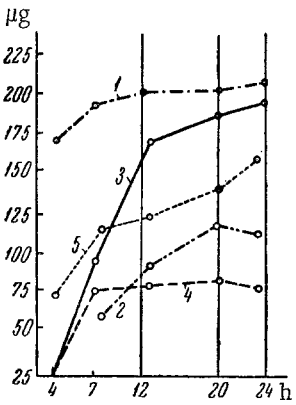

Fig. 3. DNA content in bacterial mass of Cl. perfringens. Legend as in Fig. 1.

Accumulation of Bacterial Mass during Parallel Growth of Toxic and Nontoxic Cultures

Strain	Toxin production (in MLD/ml)	Wt. of bacterial mass (in mg/30 ml)		
		4 h	7 h	12 h
BP6K	200	40	32	31
No. 2910	5	11	38	39
No. 235	50	94	105	69
No. 60	5	51	67	33
BP6K	100	42	30	15
BP6K	5	30	46	41
BP6K	200	49	59	57
BP6K	50	46	89	86

Hence, the higher level of RNA of the toxic strains correspond to the higher content of macromolecular nitrogen compounds.

The DNA level (Fig. 3) showed more or less of an increase in content in both the toxic and the nontoxic strains. However, analysis of the course of the curves in parallel experiments with toxic and nontoxic strains revealed a slight, but definite, inhibition of accumulation of DNA during the development of the toxic strain. In the nontoxic strains, and also in strain No. 235, the accumulation of DNA took place with less inhibition.

During the first hours of development of the cultures of the toxic strains considerable amounts of macromolecular nitrogen and phosphorus compounds accumulated, and later these were utilized in the logarithmic phase. In the nontoxic strain the accumulation of these substances gradually increased as the culture developed.

Against the background of the metabolic behavior observed, it was interesting to compare the accumulation of bacterial mass during the development of the investigated strains. It was found (see the table) that the largest bacterial mass was usually obtained from cultures at the beginning of the logarithmic phase of development. Later the weight of the bacterial mass from equal volumes of culture not only did not increase, despite the multiplication of the microorganisms, but on the contrary fell, and if in some cultures the weight of the bacterial mass of a toxic strain increased in the logarithmic phase, it did so to a much smaller degree than in the parallel culture of the nontoxic strain.

During cultivation of the toxic strain on slightly modified media and the formation of different amounts of toxin, it was found that the weight of the bacterial mass in the culture yielding more toxin was higher at the beginning of the logarithmic phase than in the nontoxic culture, but later the weight of the bacterial mass in the less toxic culture increased more intensively than in the parallel toxic culture.

It may be postulated that in the phase of proliferation of the toxic cultures the breakdown of RNA and protein to compounds of lower molecular weight predominates over their synthesis.

The ability to perform intensive synthesis of RNA and protein was retained in the bacterial cells of the nontoxic cultures in the phase of proliferation.

LITERATURE CITED

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