IMMUNOCHEMICAL STUDY OF THE PRODUCTS OF THE BIOLOGICAL ACTIVITY OF PROTEIN NATURE IN THE MEDIUM AFTER THE CULTIVATION OF *Yersinia pseudotuberculosis* AS A FUNCTION OF THE TEMPERATURE

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An immunochemical investigation has been made of the culture liquid after the cultivation of ten strains of the microorganisms Yersinia pseudotuberculosis. It has been shown that in the process of vital activity all the strains investigated, to a greater or smaller degree, produce into the culture medium products of catabolic breakdown consisting of fragments of receptor proteins which are possibly, regulators of metabolic processes taking place under the influence of the constantly changing conditions of the growth medium.

In recent years, a number of publications have appeared which describe the previously completely unknown mechanism for the maintenance of biological equilibrium under conditions of a constantly changing external medium with the participation of receptors and antireceptors — products of the catabolic breakdown of membrane and cell proteins of very diverse specificities [1]. These investigations are determining the pathways for the discovery of the homeostatic regulation of biological systems of different natures and the development of practical methods for controlling it.

Fragments of receptor proteins have been obtained in model experiments [2], in the medium after the cultivation of macrophages [3] and of lymphocytes [4], and in human blood serum [3, 4]. Their properties and functions and also their role in the vital activities of the cells in the norm and in the pathology of the organism have been studied. The same investigations are being carried out at the present time with viruses. The investigations performed in this field show that the recombinant protein HIV-I possesses the characteristics of human receptor proteins (P-proteins) [5]. The functional similarity of the hemagglutinin of influenza virus to human P-proteins has also been shown [5].

The present investigation was devoted to the functional and biochemical characterization of the water-soluble products of vital activity of yersinias of protein nature as a function of the temperature of cultivation of the microorganisms.

The culture liquid obtained as a result of the vital activity of the microorganisms, as also physiological fluids of Man and animals, contains the products of the catabolism of this microbe, probably including products of the breakdown of membrane and cell proteins — receptors and antireceptors.

In order to confirm this we have carried out an immunochemical study of the culture liquid after the cultivation of ten strains of *Yersinia pseudotuberculosis* of different degrees of pathogenicity at 37 and 6°C. As a result, it has been shown that the culture liquid after the cultivation of each of the strains investigated at 6 and 37°C agglutinated rhesus-positive erythrocytes of the human O(I) group to a greater or smaller degree, depending on the time of cultivation and on the strains selected. The results are given for *Y. pseudotuberculosis* strain 2781 as the best producer of bacterial hemagglutinin (Fig. 1).

The agglutination properties of the culture liquid in this case are not the result of an interaction of the antibodies contained in them with the antigens of the erythrocytes themselves and with blood group substances, since in the experiment we used erythrocytes of group O(I) containing none of these substances. These erythrocytes represent a pool of human receptors of extremely diverse specificities [6]. Moreover, in the hemagglutination reaction the culture liquids of the strains investigated interacted with the P-proteins from human blood serum; in other words, the P-proteins from human blood serum inhibited the activity of the bacterial hemagglutinin in all cases (Fig. 1, B).

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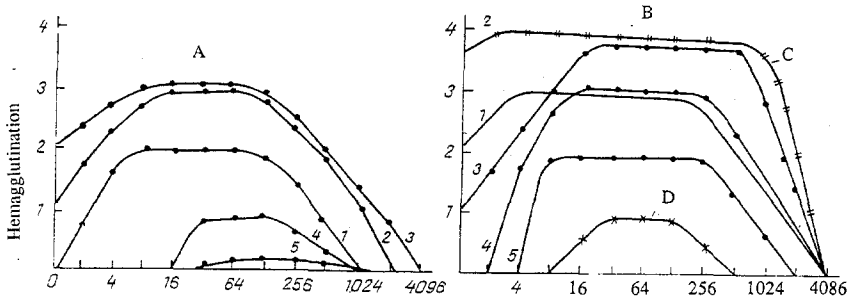

Fig. 1. Hemagglutination reaction of O(I)Rh$^+$ human erythrocytes with the culture liquid after the cultivation of *Y. pseudotuberculosis* str. 2781 at 37°C (*A*) and 6°C (*B*). Times of cultivation: 6 h (1); 10 h (2); 24 h (3); 10 days (4); 30 days (5). *C*) Hemagglutination of erythrocytes by the culture liquid after the cultivation of the microorganism at 6°C for 10 h. *D*) Inhibition of the hemagglutination of erythrocytes by the culture liquid (6°C, 10 h) after the addition to it of a preparation containing human P-proteins (10 mg/ml).

To reveal P-proteins in the culture liquid under investigation we used a recently developed immunochemical method employing rabbit antisera against human P-proteins. From the inhibition of the hemagglutination reaction between the anti-P serum and O(I)Rh$^+$ erythrocytes we showed that P-proteins were present in the culture liquid under investigation, i.e., the culture liquid of the strain investigated also interacted with rabbit anti-P serum.

It must be mentioned that P-proteins were detected in the culture liquid of almost all the strains investigated in the greatest degree after their cultivation at 37°C for 25-30 days (at a dilution of the culture liquid down to 1:512). At 37°C, they were also detected at earlier stages of cultivation but in lower titers. At 6°C, practically no P-proteins were detected in the culture liquid. An only insignificant amount of them was recorded after 28-30 days. In these cases, their titer reached 1:16.

Below, we give figures showing the dependence of the level of P-proteins in the culture liquid after the cultivation of *Y. pseudotuberculosis* str. 2781 at 37°C on the time of cultivation:

Time of cultivation	Titer of P-proteins
6 h	–
10 h	–
24 h	1:8
10 days	1:128
25 days	1:512
30 days	1:512

Thus, it has been found that during its vital activity the microorganism *Y. pseudotuberculosis* produces into the culture medium a bacterial hemagglutinin the predominant amount of which is detected in the phase of exponential growth when the microorganism is cultivated both at 37°C and at 6°C (Fig. 1). This hemagglutinin reacts with human P-proteins, i.e., it exhibits the properties of an antireceptor. P-proteins are also found in the total bacterial water-soluble proteins.

These experiments provide evidence in favor of the assumption that in the process of vital activity microorganisms, just like human and animal organisms, produce into the respective biological fluid the products of catabolic breakdown consisting of fragments of receptor proteins.

EXPERIMENTAL

The microorganism *Y. pseudotuberculosis* was cultivated in meat-peptone broth containing 0.1% of glucose at 6°C and at 37°C for 30 days. After predetermined intervals of time, 5-ml samples of the culture medium were taken. In each aliquot the weight of microbes was determined by centrifugation at 6000 rpm. The culture liquid obtained was used for biochemical investigations.

The hemagglutination reaction of the culture was carried out in the usual way [7] with rhesus-positive human erythrocytes of group O(I).

P-proteins were determined from the inhibition of the hemagglutination reaction between rabbit anti-P serum and rhesus-positive human erythrocytes of group O(I) [8].

REFERENCES

1. A. Ya. Kul'berg, *Priroda,* No. 6, 60-67 (1986); A. Ya. Kul'berg, *Priroda,* No. 6, 3-11 (1987); A. Ya. Kul'berg, N. D. Ivanovska, and I. A. Tarkhanova, *Dokl. Akad. Nauk SSSR,* **287,** No. 2, 486-489; A. Ya. Kul'berg, *Receptors of Cell Membranes* [in Russian], Vysshaya Shkola, Moscow (1987).
2. A. Ya. Kul'berg, N. N. Kulagina, I. A. Tarkhanova, et al., *Immunologiya,* No. 6, 37-41 (1985).
3. A. Ya. Kul'berg, I. A. Elistratova, I. A. Tarkhanova, *Immunologiya,* No. 2, 14-19 (1986).
4. L. A. Rubin, G. Jay, and D. L. Nelson, *J. Immunol.,* **137,** 3841-3844 (1986).
5. A. Ya. Kul'berg, *New Methods for the Prognosis of a Pathological Process,* in: Abstracts of Lectures at an All-Union Symposium [in Russian], Kursk (1991), pp. 6-7.
6. L. M. Bartova and N. N. Kulagina, in: *Regular P-Proteins in Infectious and Other Diseases: A Collection of Scientific Papers* [in Russian], Moscow (1990), pp. 9-13.
7. H. Friemel, *Grundlagen der Immunologie, VEB.* Akademie-Verlag, Berlin, 4th edn. [Russian translation], Meditsina, Moscow (1987), p. 215.
8. L. M. Bartova, N. N. Kulagina, G. U. Margulis, et al., *Methodological Recommendations [for the Determination of (?)]* P-Proteins in Human Blood Serum (Plasma)* [in Russian], Moscow (1989).

*Material omitted from the original [translator].