ISOLATION OF PROPERDIN PREPARATION FROM BOVINE BLOOD SERUM AND STUDY OF ITS PROPERTIES

(UDC 612.124:612.118.24-019:636.2]-08)

G. Ya. Rozenberg, V. Ya. Vissarionova, Yu. M. Mikhailova, N. D. Papush, and V. Ya. Chernyak

Laboratory of Fractionation of Blood Proteins (Head—Professor G. Ya. Rozenberg), Central Order of Lenin Institute of Hematology and Blood Transfusion (Director —Docent, A. E. Kiselev) and Chair of Infectious Diseases (Head—Professor K. V. Bunin) of I. M. Sechenov First Moscow Order of Lenin Medical Institute (Presented by Member of the Academy of Medical Sciences USSR, L. A. Zil'ber)

Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 60, No. 11, pp. 45-48, November, 1965
Original article submitted October 11, 1963

In 1954 Pillemer and associates isolated a new protein from blood serum—properdin. The authors demonstrated that the properdin system, consisting of the protein properdin, four components of the complement, and magnesium ions, plays an important role in the bactericidal activity of the blood and in the natural immunity of the organism. According to the data of other authors [4], the properdin system is of vital importance in the elimination of toxic phenomena which develop as a result of radiation injury. The accumulated scientific data sometimes are contradictory concerning the nature of properdin and its biological properties. It is now believed that Pillemer and his students overestimated the significance of the properdin system in natural immunity. At the same time, numerous studies published in recent years indicate that the properdin system is an important indicator of the physiological state of the organism.

The purpose of this work was to isolate a purified active properdin preparation and to study its properties.

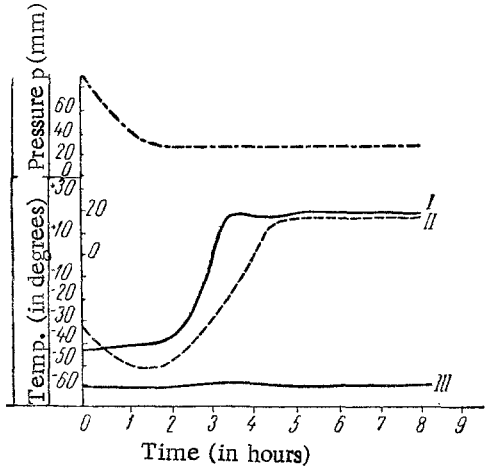

Fig. 1. Graph of the system of drying of a properdin preparation. I) Temperature of cassette; II) temperature of product; III) temperature of condenser.

PROCEDURE AND RESULTS

It is known [6] that a precipitate (A) can be isolated from serum with the aid of ethanol in the cold, and after dispersion in barbiturate buffer gives a suspension containing properdin with an activity of 100 units/ml. The administration of this suspension to mice leads to their rapid death. We modified this method and developed a method of purifying properdin to obtain a nontoxic preparation. The original isolation of properdin together with the admixture of inert proteins was performed by the gradual creation of a 20% ethanol concentration in serum cooled to 0°. The precipitate A obtained was subsequently subjected to special purification.

Isolation of Purified Nontoxic Properdin (P)

Precipitate A (properdin and inert proteins), after suspension in a physiologic solution of NaCl, was mixed for 60 min at 18° for complete solution of the properdin. The solution was centrifuged at 18° to remove the precipitate (impurities). Solution B containing the bulk of the properdin, was cooled to 0°, and then cooled ethanol was added to it to an 8% concentration in the

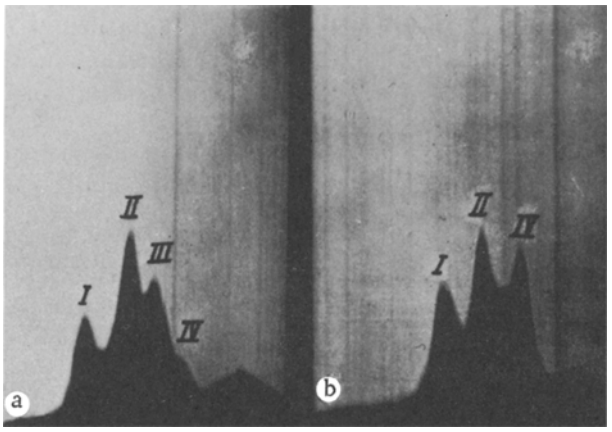

Fig. 2. Electrophoretograms of purified preparation. Explanations in text.

Fig. 3. Sedimentation diagram of purified active properdin preparation. Notations of fractions and their sedimentation constants: 1) S_1 (16 S); 2) S_2 (12 S); 3) S_3 (8.8 S); 4) S_4 (6.2 S).

mixture. After centrifuging of the mixture at 0°, the precipitate with properdin obtained was dissolved in physiological saline. Intravenous injection into mice of the preparation thus prepared in a dose of 0.5 ml (3000 units/kg) did not induce any toxic phenomena of death of the animals, while the mice died as a result of the injection of 1 ml (6000 units/kg). In view of this, we performed a supplementary purification of the properdin by repeated fractionation of a solution of the preparation, cooled to 0°, with ethanol at an 8% concentration in the mixture. After centrifuging at 0°, the properdin preparation was dissolved in physiological saline with 8% glucose, added to stabilize the protein. The solution was recentrifuged at 20° for 7 min at 17,000-18,000 rpm. The purified properdin solution obtained contains an average of 27% of the total amount of this protein in the initial serum. Upon intravenous injection of the preparation into white mice in a dose of 1 ml (6000-12,000 units/kg), no reactogenicity or toxicity was detected.

The method described permits the production of purified properdin preparations with activity up to 240 units/ml.

The properdin preparation was titrated according to the method of Pillemer in the modification of R. A. Rutberg [2].

A series of experiments was conducted using the isolated properdin.

1. The corresponding system of lyophilization of properdin (under vacuum from the frozen state) in the vacuum drying apparatus produced by Yuzif-rua was developed. The basic process of drying was conducted at a condenser temperature of −60°, initial temperature of cassette −43°, temperature of product −35-50°, with gradual raising to 20° and at a residual rarefaction of 25-30μ. The preparation was dried at a 20° temperature of the cassette and product. Duration of the drying 8 h (Fig. 1). The dried preparation possesses the activity of the initial properdin solution, is readily soluble and nontoxic when administered to white mice. Drying of the properdin permits its storage at room temperature for 4 months without any decrease in the initial activity, while a solution of the preparation is active under these conditions only for 6-12 days. At low temperatures (−14 to −20°), the dry preparation, just like its frozen solution, retains its initial activity for more than 3 years.

2. An electrophoretic investigation was made of a solution of properdin in veronal buffer (pH 8.6, $\mu = 0.1$). Figure 2a and b presents electrophoretograms of two solutions of the same properdin preparation: a) active (180 units/ml), b) inactivated during prolonged dialysis (4 days) under temperature conditions (6°) unsuitable for conservation of activity. On the electrophoretogram of the active properdin solution, 4 peaks are visible: I) α-globulin (mobility $\approx 4.0 \cdot 10^{-5}$ cm^2/sec); II) β-globulin (mobility $\approx 2.9 \cdot 10^{-5}$ cm^2/sec); III) properdin (mobility $\approx 2.1 \cdot 10^{-5}$ cm^2/sec) and IV) γ-globulin (mobility $\approx 1.5 \cdot 10^{-5}$ cm^2/sec); V) "salt anomaly" (nonmobile in electric field).

In the inactivated preparation, the peak III, to which the properdin activity is related, is absent. In addition, the relative content of γ-globulin (more accurately, of proteins with close mobility) was significantly increased here. This evidently indicates a coupling of the inactivation of properdin with its transformation to proteins with large electrophoretic mobility.

3. We investigated the active properdin preparation in the "Five" analytical air ultracentrifuge. Figure 3 shows one of the sedimentation diagrams. The experiment was conducted in the usual 12 millimeter cell at 50,000 rpm and 22°. The photograph was taken 61 min after the set velocity had been reached. The direction of sedimentation in the cell is indicated by the arrow. The initial protein concentration was 10 mg/ml in phosphate-salt buffer, prepared according to the prescription indicated in the work of Todd et al. [9]. As was shown by an investigation of the precipitate and supernatant liquid for various durations of the experiments, almost all the initial properdin activity (88%) is associated with the 16 S and 12 S fractions. A total of 11% of the activity is already found on the bottom of the cell after 7 min of the experiment.

Fractions with sedimentation coefficients similar to those that we isolated were found in a preparation of human properdin, produced by Spicer et al. [8] also by alcohol precipitation. In an experiment with a multicomponent system, with incomplete separation of the peaks, the relative content of these fractions may be estimated only approximately. Such an estimate shows that the 16 S accounts for about 3-4% (in Spicer's experiments the 18 S fraction comprised 4%); the relative weight of the 6.2 S is about 45% (in Spicer's experiments the 5 S and 6 S fractions occupied 70%). Lepow et al. [5] also detected four fractions in an ultracentrifuge analysis of a separation of human properdin, and their sedimentation diagram qualitatively coincides with that which we obtained.

4. For a study of the bactericidal activity of a sterile properdin preparation, we verified its action on microbes of the Salmonella group in vitro and in vivo. Salmonellas were used as a result of the fact that in food toxicoinfections of Salmonella etiology, great significance is attributed to the natural immunity factors, since the use of antibiotics is insufficiently effective [1].

As the microbes we selected 8 h agar cultures of Salmonellas from specially isolated strains from patients with food poisoning. The work was conducted according to our modification of the procedure of Wardlaw and Pillemer [10] in two steps—first step: verification of the bactericidal properties of RPb (serum entirely devoid of properdin) with respect to Salmonellas; second setup: investigation of the bactericidal properties of a properdin preparation using RPb and strains resistant to it. Controls: 1) verification of the statics of the biological properties of RPb; b) barbital buffer as control medium for comparison with the action of bactericidal agents (RPb, RPb and properdin); 3) control of RPb with an activated complement; 4) control of inactivated properdin; 5) control of dilution of bacteria. The bactericidal activity of properdin was determined according to the formula (in %):

$$100 \cdot \left(1 - \frac{\text{final amount of viable bacteria}}{\text{original amount of viable bacteria}}\right).$$

In an investigation of 47 strains of Salmonellas of groups B, C, D, and E (according to the classification of Kaufman and White), 11 proved sensitive to RPb. Of the remaining 36 strains, 17 were resistant and 19 were properdin-sensitive. In each group of Salmonellas, both properdin-resistant and properdin-sensitive strains were encountered.

5. In experiments in vivo we used 11 day chick embryos (150). They were infected with a 24 h culture of a properdin-sensitive strain of Salmonella typhimurium, from a suspension of which dilutions with 10, 100, and 1000 microbial cells per ml were prepared. All the doses of the culture in a volume of 0.1 ml were mixed with 0.4 ml of properdin of various activities, and the mixture was administered to the embryo. Control: embryos infected with a culture with physiological saline, as well as a culture with inactivated properdin. The experimental results were determined 48 h after infection and incubation at 37°. The degree of protective action of properdin was evaluated according to the arbitrarily adopted index of effectiveness (IE): the ratio of LD_{50} of the culture administered together with properdin to LD_{50} of the culture without properdin.

The data obtained indicated that properdin exerts a pronounced protective action in experimental Salmonella infection. The IE of the action of properdin, administered in a dose of four units per g of weight of the embryo, was equal to 269.4. When the properdin dose was increased to 8 units, the degree of protective action was somewhat reduced and was equal to 222.1.

LITERATURE CITED

1. Yu. M. Mikhailova and E. G. Mel'nik, Sov. Med., 7 (1961), p. 131.
2. R. A. Rutberg, Dokl. AN SSSR, 125, 4 (1959), p. 931.
3. V. Ya. Vissarionova, Probl. Gematol., 3 (1964), pp. 53-55.
4. G. DelBuono and D. Fumarola, Radiobiol. Radioter. Fis. med., 16 (1961), p. 33.
5. I. H. Lepow, L. Pillemer, and M. Schoenberg et al., J. Immunol., 83 (1959), p. 428.
6. F. Miya and S. Marcus, Proc. Soc. exp. Biol. (New York), 99 (1958), p. 247.
7. L. Pillemer, L. Blum, and I. H. Lepow et al., J. Exp. Med., 103 (1956), p. 1.
8. D. S. Spicer, L. I. Priester, and E. V. Smith et al., J. Biol. Chem., 234 (1959), p. 838.
9. E. Todd, L. Pillemer, and I. H. Lepow, J. Immunol., 83 (1959), p. 418.
10. A. Wardlaw and L. Pillemer, J. Exp. Med., 103 (1956), p. 553.

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
