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BIOCHEMICAL AND IMMUNOLOGIC CHARACTERISTICS OF SERUM PROTEINS AFTER BONE TRAUMA IN RATS

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The role of the blood proteins in mechanisms of posttraumatic regeneration of bone tissue has been inadequately studied. It has been suggested that serum proteins and blood mucopolysaccharides, which are detectable in callus, promote regeneration of bone tissue [2, 3, 5-7] by stimulating proliferation of bone cells [8, 9]. Nevertheless, the particular features of posttraumatic dysproteinemia and the role of the protein–carbohydrate complexes of the blood in the formation of the organic matrix of regenerating bone still remain inadequately studied.

The object of this investigation was a quantitative study of the protein, lipoprotein, and protein–carbohydrate components of blood and callus in rats in the acute stage of bone trauma.

EXPERIMENTAL METHOD

Experiments were carried out on 145 Wistar rats weighing about 100 g. Bilateral closed fractures of the femoral diaphyses were produced under pentobarbital anesthesia (20 mg/kg). Blood was taken for testing individually from each animal on the 1st, 2nd, 3rd, 5th, 7th, and 10th days after trauma. Total serum protein was determined refractometrically and protein fractions by electrophoresis on paper (Whatman No. 3 MM, barytal buffer, pH 8.6). After electrophoresis the strips, stained with bromphenol blue, were examined on the 301E Statron (East Germany) integrating densitometer. The seromucoid content was determined by the method in [4] on the SF-16 spectrophotometer at 280 μ. Quantitative immunoelectrophoresis of blood serum antigens and antigens of a saline extract of callus obtained 7 days after infliction of the fracture was carried out with the aid of antinormal rabbit serum [1]. After immunoelectrophoresis the strips were stained for proteins with Coomassie blue and for lipoproteins with Sudan black B. At each time blood from seven or eight experimental and/or seven or eight control rats (without fracture) was investigated. To obtain protein

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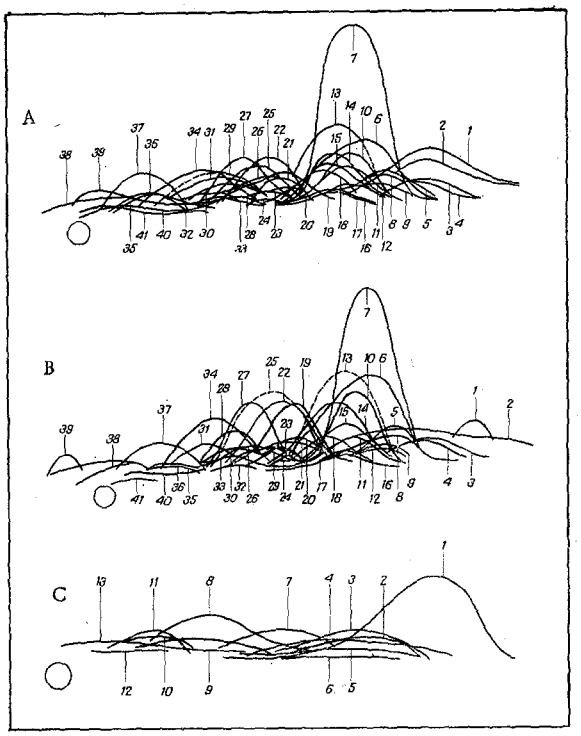

Fig. 1. Schemes of two-dimensional immunoelectrophoresis of proteins of blood serum and callus in experiment with antinormal rabbit serum. A) Serum of intact rats; B) Serum of rats 1 day after fracture; C) regenerating bone 7 days old.

extract from callus, regenerating bone from 15 rats was used. The significance of the results was assessed by Student's t-test at the $P \leq 0.05$ level.

EXPERIMENTAL RESULTS

In the experiments of series I the dynamics of changes in the total blood protein level, individual serum fractions, and seromucoid were studied during the 10 days after fracture. The total protein concentration fell from 6.7 ± 0.1 to 6.2 ± 0.1 g% as early as 1 day after trauma ($n = 8$; $P < 0.01$) and it did not return to normal until the end of the investigation (on the 10th day: 6.4 ± 0.2 g% in the control, 5.8 ± 0.1 g% in the experiment; $n = 8$; $P < 0.05$).

Bone trauma led to opposite changes in the concentrations of individual serum fractions. As a result of injury an increase in α_1-globulin was observed. After 1 day this increase was 38% (8 ± 1.0% in the control, 11 ± 0.7% in the experiment; $n = 8$; $P < 0.05$). On the 2nd day the increase was 50% (8 ± 0.8 and 12 ± 1.0%, respectively; $n = 8$; $P < 0.001$). The considerable fluctuation in the α_2-globulin level in the control animals (from 3 to 8%) made it difficult to estimate changes in this fraction after the fracture. The concentration of proteins of the β-globulin fraction did not change significantly throughout the period of observation in either the control or the experimental rats. The concentration of the γ-globulin fraction in the experimental animals was considerably reduced during the first 5 days of the experiment and remained at this level until the end of the investigation. The seromucoid fraction responded more strongly than the blood proteins. The increase in the concentration of these glycoproteins was 250% 1 day after trauma (98.6 ± 3.6 mg% in the control, 253.7 ± 10.3 mg% in the experiment; $n = 8$; $P < 0.001$). During the next few days, the concentrations of the glycoprotein fraction soluble in perchloric acid in the blood returned to normal rapidly, and by the 5th day the seromucoid concentration in the experimental rats was indistinguishable from the control (112.6 ± 7.5 mg% in the control, 130.6 ± 7.1 mg% in the experiment). The most marked dysproteinemia and increase in the seromucoid concentration thus took place at the earliest times after fracture.

Accordingly, in the experiments of series II quantitative changes in individual proteins, lipoproteins, and glycoproteins of the blood were investigated on the 1st and 2nd days after bone trauma. The results of

refractometry, zonal electrophoresis, and determination of seromucoid confirmed changes in the blood protein levels discovered in the experiments of series I. At the same time, a clearer response of the blood α_2-globulin to trauma was established. An increase of 42.2% in the concentration of this fraction was observed 1 day after fracture (7 ± 0.8% in the control, 10 ± 0.9% in the experiment; n = 8; $P < 0.05$).

Differences in the antigenic composition of individual protein components of the sera were next studied at the time of the greatest increase in seromucoid (the first day). Antinormal serum revealed up to 41 antigenic components in the serum of normal rats (Fig. 1A). Trauma did not affect the antigenic composition of the serum, i.e., only quantitative change in the concentrations of individual proteins were found (Fig. 1B). After fracture there was a significant decrease in the concentrations of five antigens, including tyrosine-binding prealbumin (No. 3), albumin (No. 7), α_1-lipoprotein (No. 11), and two other α_2-globulins (Nos. 22 and 28). The concentration of α- and β-lipoproteins in the blood was not significantly increased after fracture.

For the immunoelectrophoretic analysis of callus, regenerating bone tissue was investigated on the 7th day, for earlier than this it was impossible to isolate the necessary quantity of callus. Up to 13 serum proteins were found in the regenerating bone (Fig. 1C), among which albumin (No. 1), hemopexin (No. 8), and IgG (No. 13) could be distinguished. Unidentified serum components, mainly in the α- and β-globulins, were present in callus in small quantities. No lipoproteins were found.

The results of quantitative immunoelectrophoresis thus fill in the details of the character of posttraumatic dysproteinemia. The difficulty is that the increase in level of proteins of the acute phase (protein-antigen complex with electrophoretic mobility of α_2-globulin) and the increase in the quantity of seromucoid do not adequately correspond to each other. The reasons for this disparity and also the role of each protein in the process of posttraumatic regeneration of bone will be studied in future research.

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