EFFECT OF THE VARIOUS FRACTIONS OF LEUKOCYTIC PYROGEN ON HEMATOPOIESIS

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Fractionation of leukocytic pyrogen on a column of Sephadex G-75 yielded two separate fractions, one stimulating hematopoiesis, the other possessing pyrogenic activity but inhibiting hematopoiesis. The elution profile of the Sephadex G-75 column suggests that the stimulating action is a property of high-molecular-weight substances whereas the pyrogenic and inhibitory actions are properties of low-molecular-weight substances. Pyrogenic and inhibitory activities are perhaps connected with different substances. The nature of the inhibitory factor requires further study. It presumably is a substance of chalone type.

KEY WORDS: *pyrogens; leukopoietins; chalones; leukocytes.*

The writers' previous investigations [1, 2] showed that native leukocytic pyrogen (NLP) causes various changes in granulocytopoiesis in intact rats, depending on the dose. Large doses of NLP cause temporary inhibition of granulocytopoiesis: a decrease in proliferative activity of the granulocytes and in their absolute number in the bone marrow and peripheral blood. A small dose of NLP, on the other hand, stimulates granulocytopoiesis: Proliferative activity of the granulocytes and their absolute number in the bone marrow are increased, and the liberation of granulocytes into the peripheral blood is accelerated. The inhibitory action of large doses of NLP on leukopoiesis in intact animals was explained by postulating that, besides stimulators, it also contains inhibitors of leukopoiesis, substances of chalone type; moreover, the conditions of preparations of NLP are similar to those used to obtain granulocytic chalone [3, 4].

The object of the present investigation was to study the action of the pyrogenic fraction proper and of the other nonpyrogenic fractions of NLP on hematopoiesis in order to discover with what fractions on the NLP complex the hematopoietic action of the preparation is associated.

EXPERIMENTAL METHODS

The method of preparation of rabbit NLP was fully described previously [3]. Material was obtained and fractionated under sterile conditions. The glassware, syringes, needles, and column were sterilized by dry heat at 170°C for 2 h. To determine pyrogenicity the preparation was injected intravenously into rabbits and the rectal temperature was measured twice or three times at intervals of 30 min before injection and every 30-60 min after the injection for 3-5 h.

NLP was fractionated on a Sephadex G-75 column with careful observance of the conditions of sterility and with strict control of all solutions for possible contamination by bacterial pyrogens. The Sephadex was autoclaved, the column (3.5 × 35 cm) packed with Sephadex gel was rinsed with 300 ml buffer, and the solution obtained was checked for pyrogenicity. A 0.5 M K-phosphate buffer in 0.85% NaCl solution, pH 6.5, was used. Freeze-dried NLP was dissolved in 5-10 ml buffer and dialyzed for 18-24 h against the buffer under sterile conditions. A sample of 4-8 ml of the preparation containing 2-4 mg protein/ml was applied to the column.

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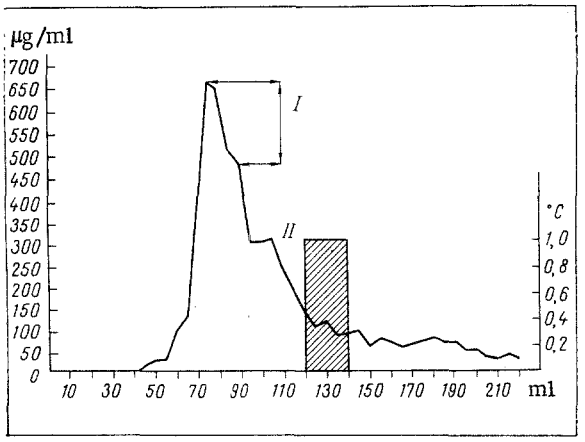

Fig. 1. Elution profile of NLP from Sephadex G-75 column. Continuous line shows distribution of protein in eluate; arrows indicate boundaries between fractions: I) nonpyrogenic maximal protein peak, II) pyrogenic zone (shaded region corresponds to pyrexial response of rabbits to injection of combined pyrogenic fractions in dose of 11 µg/kg). Abscissa: volume of outflowing solution (in ml); ordinate: left) protein concentration in samples (in µg/ml), right) change in body temperature of rabbits (in °C).

Elution was carried out with 0.05 M K-phosphate buffer in 0.85% NaCl solution, pH 6.5, at 4°C at the rate of 0.5 ml/min. Protein (by Lowry's method) and pyrogenic activity were determined in 5-ml samples.

Fractionation of Sephadex yielded two fractions of rabbit NLP: I) the maximal protein fraction (610 µg protein/ml), II) the pyrogenic fraction (165 µg protein/ml).

The hematopoietic action of the fractions was studied in doses comparable with the doses of NLP used in the previous investigation. The doses of fraction I were 200, 17.5, and 3.5 µg/100 g and of fraction II 25, 15, and 3 µg/100 g body weight.

The hematopoietic activity of the fractions was studied as the intensity of incorporation of labeled thymidine into DNA of rat bone marrow cells on the 3rd day after injection. The fractions were injected once only, intravenously, into male Wistar rats weighing about 100 g. Each fraction was injected into 3 or 4 rats. The animals were killed, the femur was removed, and the bone marrow was expelled into medium No. 199 with the addition of EDTA-Na_2 (150 mg/100 ml medium). [^{3}H]Thymidine was added at the rate of 2.5 mCi per sample containing 5 million cells. Bone marrow from intact animals served as the control.

Five parallel tests were set up simultaneously and the mean results were calculated. The mixture of bone marrow was incubated at 37°C for 2 h, then washed 3 times with physiological saline. The cells were disintegrated by the addition of 10% TCA solution to the cell residue. The acid-insoluble DNA fractions were precipitated on Millipore filters. The filters were placed in a toluene scintillator and radioactivity was counted on a Nuclear Chicago Mark II counter. The number of counts obtained during measurement of the radioactivity of bone marrow samples from intact rats was taken as 100%, and deviations from this level of activity for the bone marrow of the experimental animals were expressed as percentages with a plus or minus sign. Altogether four series of experiments were carried out on 65 animals.

EXPERIMENTAL RESULTS

The elution profile of NLP, passed through a Sephadex G-75 column, is shown in Fig. 1. In this experiment 23 mg protein dissolved in 10.5 ml 0.85% NaCl solution was applied to the column and elution was carried out with 0.05 M K-phosphate buffer in 0.85% NaCl solution, pH 6.5. The principal protein peak was found in a volume of 70-100 ml. These samples did not contain pyrogen.

Pyrogenic activity was found only in the narrow zone following the maximal protein peak (120-140 ml samples) and it amounted to 3-10 µg/kg protein (Fig. 2). The remaining fractions in the zone from 145 to 220 ml were nonpyrogenic.

The study of the hematopoietic properties of the different NLP fractions (Fig. 3) showed that injection of the fraction of the maximal protein peak in a dose of 200 µg/100 g caused an increase in DNA synthesis in the rat bone marrow cells by 87.3% ($P < 0.001$); a decrease in the dose of the fraction injected to 17.5 and 3.5 µg/100 g inhibited hematopoiesis a little (by 26 and 12%, respectively; $P < 0.1$). The pyrogenic fraction when tested caused a significant decrease in DNA synthesis in the bone marrow cells of the rats by 42-59%, regardless of the dose injected.

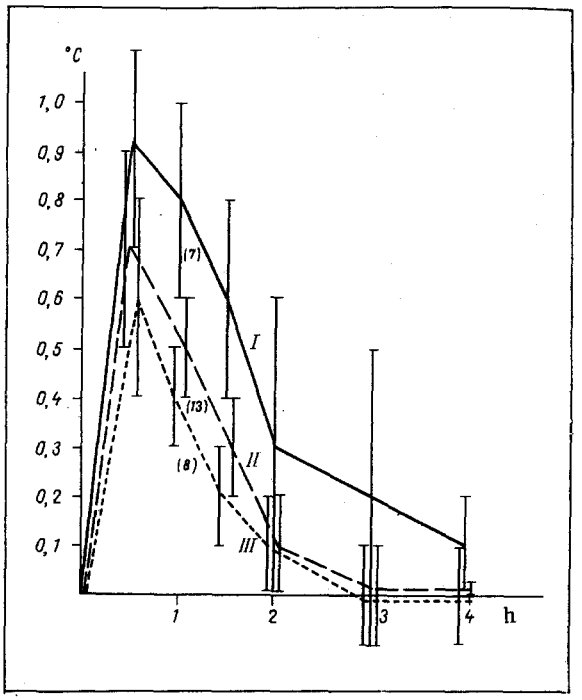

Fig. 2. Pyrexial response of rabbits to injection of combined fractions of NLP eluted in volume of 120-140 ml: I) 10 μg/kg; II) 5 μg/kg; III) 3 μg/kg. Number of observations in parentheses. Abscissa: time of measurement (in h); ordinate: change in body temperature of rabbits (in °C).

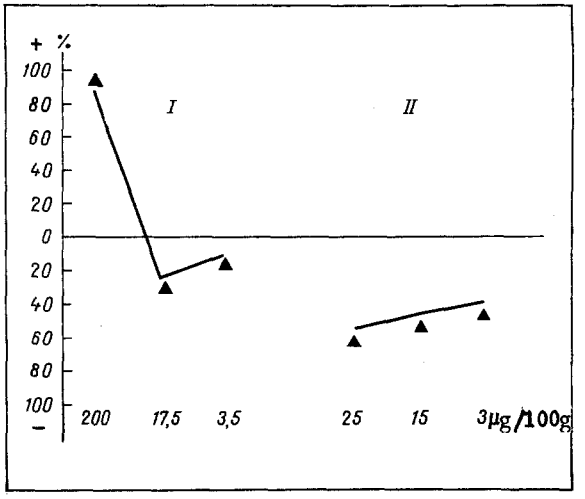

Fig. 3. DNA synthesis in rat bone marrow cells on 3rd day after injection of NLP fractions: I) injection of nonpyrogenic fractions of maximal protein peak in doses of 200, 17.5, and 3.5 μg/100 g into rats; II) injection of pyrogenic fraction in doses of 25, 15, and 30 μg/100 g. Abscissa: doses of fraction injected (in μg/100 g); ordinate number of counts/min (in % of control).

Fractionation of leukocytic pyrogen on a Sephadex G-75 column thus yielded two separate fractions, one stimulating hematopoiesis, the other possessing pyrogenic activity and inhibiting hematopoiesis. The elution profile of the Sephadex G-75 column suggests that the stimulaing action is a property of high-molecular-weight substances whereas the pyrogenic and inhibitory actions are properties of low-molecular-weight substances. It is possible that the pyrogenic and inhibitory activities are associated with different substances. The nature of the inhibitory factor requires further study. It may perhaps be a substance of chalone type [4].

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EFFECT OF CHOLESTEROL ON THE STATE OF THE ADRENAL CORTEX IN SEVERE STRESS

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The effect of cholesterol, administered for 14 days, on the response of the adrenal cortex was studied in rats during severe stress. Under these conditions the degree of activation of the gland and the degree of its structural changes were lower than in the control. It is suggested that the action of cholesterol depends on its influence both on the hypothalamus and on the adrenocortical tissue. The latter effect is connected with the action of cholesterol as a substance delaying peroxidation.

KEY WORDS: *adrenal cortex; stress; structural changes; cholesterol.*

One of the manifestations of exposure to severe stress is the development of degenerative and necrobiotic changes in various organs including the adrenal cortex [4, 9-11]. Such changes can be assumed to lead to the development of functional insufficiency of the affected organs. One of the problems arising in the pharmacological control of stress is therefore the prevention of these structural changes.

The object of the present investigation was to study the effect of cholesterol on the state of the adrenocortical tissue under conditions of severe stress.

The writers previously [7] postulated that delipoidization of the adrenals, with a lowering of their cholesterol level during stress [5], is one of the conditions determining death of the adrenocorticocytes in these situations. Data in the literature on the antioxidant action of cholesterol [13], its ability to delay peroxidation [2], and also on the role of peroxides in the mechanism of cell death [3] and the accumulation of peroxides in the body during stress [1] were taken into account. It was also borne in mind that administration of cholesterol to rats leads to moderate hypercholesteremia in the animals [8] without causing dystrophic changes in the adrenocortical tissue [12].

EXPERIMENTAL METHODS

Experiments were carried out on adult male albino rats divided into four groups: 1) 22 intact rats; 2) the control group of 20 rats exposed to combined stress in the form of uni-

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