EFFECT OF ERYTHROCYTIC CHALONE ON ELECTROPHORETIC MOBILITY OF MOUSE BONE MARROW CELLS

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Five fractions were isolated by cell electrophoresis in a Ficoll density gradient from the bone marrow of mice receiving physiological saline (control) and erythrocytic chalone (experiment). The absolute and relative distribution of the cells in the control and experimental fractions differed: the maximal number of cells in the control (up to 78%) was found in fractions 3-5 and the minimal (up to 22%) in fractions 1-2. A redistribution of the cellular material in all the fractions took place 25 min after treatment with erythrocytic chalone. The number of cells in fraction 1 was particularly increased (by 2.6 times). Analysis of the "myelograms" of films from fraction 1 in the experimental and control series showed that the number of cells in fraction 1 was increased on account of cells of the erythroid series. Among the proliferating cells of the erythron, the greatest decrease in electrophoretic mobility was observed in the proerythroblasts. It is suggested that as a result of interaction between erythrocytic chalone and membrane receptors of proliferating cells of the erythron their electric charge is reduced and changes take place in intracellular processes leading to delay in mitosis.

KEY WORDS: erythrocytic chalone; cell electrophoresis; bone marrow cells.

Investigations have shown that special substances (chalones), which have a selective action on cells and inhibit highly important intracellular processes such as DNA and RNA synthesis and mitosis, can be isolated from various tissues and organs [8, 9].

During interaction between the chalone and cell, one of the primary processes ultimately leading to inhibition of proliferation is evidently a change in the surface charge of the membrane. If this is so, after administration of a chalone the electrokinetic properties of the cells should be modified.

Accordingly in the present investigation the electrophoretic mobility of bone marrow cells was studied after the action of erythrocytic inhibitor (chalone).

EXPERIMENTAL METHOD

The inhibitor was obtained from erythrocytes of noninbred albino rats weighing 150-200 g (50 animals) by the method described in [10]. For this purpose the erythrocytes were separated from contaminating leukocytes by centrifugation and washed twice with cold Hanks' solution to remove anticoagulant and plasma proteins. The erythrocytes were then treated with Hanks' solution (3:1). The hematocrit index of the resulting "polycythemic" erythrocyte suspension was thus 75%. After incubation for 1 h at 37°C the erythrocyte suspension was centrifuged at 3,000 rpm for 20 min and the supernatant was collected and used as the inhibitor of erythropoiesis. The supernatant contained several fractions of proteins with an inhibitory action on erythropoiesis [10]. To inactivate the thermolabile erythrocytic G_1 chalone the supernatant was heated to 37°C before use [3]. The relatively thermostable chalone remaining in the supernatant is destroyed only at 60°C. The writer's observations have shown that this type of erythrocytic inhibitor belongs to the class of erythrocytic proteins with the electrophoretic mobility of α_1-globulins in polyacrylamide gel [4]. Fraction A in the supernatant obtained after incubation of erythrocytes possessed this mobility (Fig. 1).

To determine the quantity of inhibitor in the supernatant the first step was to measure the total protein content spectrophotometrically (SF-16, λ = 280 nm). Bovine serum albumin was used as the standard. The supernatant was then fractionated by disk electrophoresis in polyacrylamide gel [2]. To identify the hemo-

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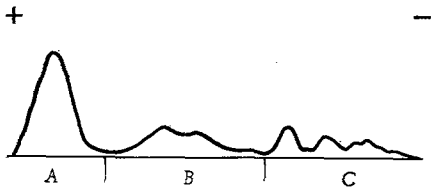

Fig. 1

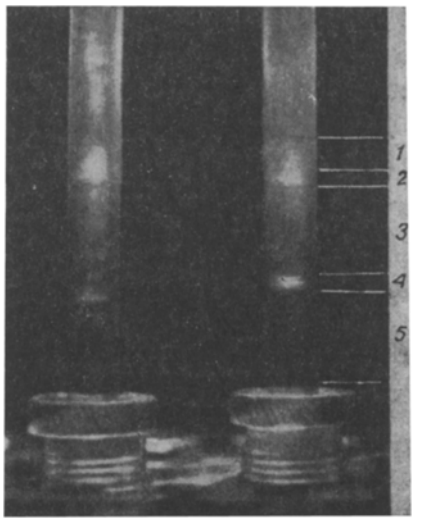

Fig. 2

Fig. 1. Densitogram after disk-electrophoresis of "polycythemic" incubation medium. A) Protein fraction with electrophoretic mobility of α_1-globulins (prehemoglobin), B) hemoglobin, C) posthemoglobin fraction. Conditions of electrophoresis: gel system No. 1, current 1-1.8 mA per tube, fractionation time 3 h at 2°C. Stained with Coomassie.

Fig. 2. Electrophoresis of mouse bone marrow cells in Ficoll density gradient. 1-5) Boundaries for collection of separate fractions.

globin zones in the gel, a commercial Hb preparation (from Reanal, Hungary) also was subjected to electrophoresis. The conditions of electrophoresis were described in detail previously [4]. After densitometry (the densitometer was from East Germany) of the gel after electrophoresis, the relative content of the proteins of fraction A was determined gravimetrically and, knowing the total protein content in the supernatant, the absolute quantity of inhibitor in it was calculated. The investigations showed that the quantity of inhibitor (Fig. 1A) in the "polycythemic" incubation medium was 2.95 mg/ml. The inhibitor was injected intraperitoneally into noninbred albino mice in a dose of 3 mg/ml. Physiological saline was injected into the control animals. Altogether 46 mice weighing 20-22 g were used. The inhibitory action of the erythrocytic chalone was estimated 4 h after injection by determining the stahmokinetic index among the proliferating cells of the erythron [5].

Some of the animals were killed 25 and 45 min after injection of the inhibitor and physiological saline, their femoral bone marrow was flushed out with Eagle's medium, and the marrow was pipetted through needles of different diameters. The resulting cell suspension was centrifuged at 1000 rpm for 15 min, the residue was resuspended in 2% Ficoll, and the homogeneous part of the suspension was collected in separate tubes. Homogeneity of the suspension was verified cytologically. Electrophoresis of the cells of the resulting bone marrow suspension was carried out by the method of Griffith et al. [7] in an apparatus for disk electrophoresis (from Reanal, Hungary). For this purpose the bottom of the tubes was filled with 7.5% polyacrylamide gel on which layers of 10, 6.25, and 2.5% Ficoll were successively added (Ficoll 400,000, Sweden). The suspension of bone marrow cells from the intact or experimental mice in equal quantities (10^7), diluted in 2% Ficoll and in 6.8% sucrose, was poured above, and finally a layer of phosphate buffer, pH 7.2, was carefully poured on the top. In all cases the Ficoll was mixed with appropriate amounts of sucrose [7]. Suspensions from experimental and control animals were subjected to electrophoresis simultaneously. The conditions of fractionation were: current 1 mA per tube, voltage 8 V applied to the tube, duration of fractionation 1 h. During electrophoresis the suspension of bone marrow cells of the control and experimental mice was separated into a number of fractions (Fig. 2). Fractions 2 and 4, located at the edges of the Ficoll gradient, were most clearly revealed. Above these fractions, and after them, other cells also were found, but their boundaries were indistinct. After the end of electrophoresis, identical fractions, the boundaries for collection of which are indicated in Fig. 2, were pooled from different tubes and washed with Eagle's medium to remove Ficoll, after which the absolute number of cells in each fraction was determined. The percentage of viable cells in the fractions after staining with trypan blue varied between 80 and 92. Parallel series of films were prepared from each fraction and stained by the Romanovsky–Giemsa method, after which 500 bone marrow cells were counted and the "myelogram" analyzed. The results were subjected to statistical analysis.

TABLE 1. Absolute and Relative (in %) Distribution of Mouse Bone Marrow Cells among Individual Electrophoretic Fractions

Fraction No.	Control 1	Control 2	Experiment 1	Experiment 2
1	9 (0,9·10⁶)	10 (1,1·10⁶)	22 (2,6·10⁶)	21 (2,1·10⁶)
2	13 (1,4·10⁶)	17 (1,9·10⁶)	26 (3·10⁶)	25 (2,6·10⁶)
3	27 (2,9·10⁶)	24 (2,7·10⁶)	26 (3,1·10⁶)	27 (2,7·10⁶)
4	31 (3,4·10⁶)	25 (3·10⁶)	17 (2·10⁶)	17 (1,7·10⁶)
5	20 (2,2·10⁶)	24 (2,7·10⁶)	9 (1·10⁶)	10 (1·10⁶)
Total number of cells	100 (1,08·10⁷)	100 (1,14·10⁷)	100 (1,17·10⁷)	100 (1,01·10⁷)

Legend. Here and in Table 2: total number of cells subjected to fractionation in one tube taken as 100%. Control 1 and 2) bone marrow removed 25 and 45 min respectively after injection of physiological saline into mice; experiment 1 and 2) the same, but after injection of erythrocytic inhibitor.

TABLE 2. Ratio (in %) between Proliferating Cells of Erythron in Control and Experimental Fractions 1 (M ± m)

Fraction No.	Proerythroblasts	Basophilic and polychromatophilic erythroblasts
1 (control 1)	6±0,7	94±0,7
1 (experiment 1)	12±1,15	88±1,15
1 (control 2)	5±0,87	95±0,87
1 (experiment 2)	10±1,5	90±1,15

EXPERIMENTAL RESULTS

Inhibition of proliferation among cells of the erythroid series by 53% was observed 4 h after administration of the erythrocytic inhibitor (stathmokinetic index 15 ± 1.46 in the control, 7 ± 0.94 in the experiment; $P < 0.05$).

On electrophoretic fractionation of bone marrow cells of the intact and experimental mice the following pattern of distribution of the cellular material among the fractions was observed (Table 1): in the control the largest number of cells (73-78%) occurred in fractions 3-5 and the smallest (22-27%) in fractions 1 and 2. In the experimental series a redistribution of the cellular material was observed: in the first two fractions the number of separated cells was greater than in the control. This was particularly true in fraction 1, where the number of cells was 2-2.6 times greater than in the control fraction.

To discover on account of which bone marrow cells the number of cells in fraction 1 was increased (the least mobile fraction from the electrophoretic point of view), "myelograms" of this fraction were analyzed in the experiment and control. The results showed that nucleated cells of the erythron accounted for 20-22% of the total number of bone marrow cells in the control, whereas in the experimental series the number of cells of the erythroid series was increased up to 31-34%. These results point to a decrease in electrophoretic mobility mainly of cells of the erythroid series and to an increase in the total number of cells in experimental fractions 1 on account of these cells.

The point of application of erythrocytic chalone is known to be the proliferating cells of the erythron (proerythroblasts, erythroblasts); it was therefore interesting to study whether the electrophoretic mobility of these cells changes equally after the action of erythrocytic inhibitor. The ratio between the numbers of these cells in the experimental and control fractions was accordingly analyzed for this purpose (Table 2). Analysis of the specimens showed a relative increase in the percentage of proerythroblasts compared with erythroblasts in the experimental fractions 1 compared with the corresponding control fractions ($P < 0.05$), and this was attributed to the predominant decrease in their electrophoretic mobility after the action of the chalone.

Since the surface charge of the blood cells is due mainly to the content of sialic and neuraminic acids in the outer membrane of the blood cells [1, 6], it can tentatively be suggested that the erythrocytic inhibitor evidently interacts with the receptors of the membranes containing these compounds. As a result the electrokinetic properties of cells of the erythroid series are modified and their electrophoretic mobility is reduced. In the proerythroblasts, on account of the large size of the cells, contact with erythrocytic chalone evidently

takes place over a wider area than in the case of erythroblasts, so that the electric charge on the membrane falls more sharply.

The change in the surface charge of the proliferating cells of the erythron after the action of the chalone is evidently a special kind of signal leading to subsequent intracellular changes in ionic composition and in nucleic acid synthesis, and ultimately to the delay of mitosis in the erythroid series, as was observed in the experiments now described.

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STATE OF THE LUNG SURFACTANT IN ANIMALS OF DIFFERENT SPECIES

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The activity of the lung surfactant in mice, rats, guinea pigs, hamsters, rabbits, and dogs was found to be within normal limits with variation of the coefficient of stability of the air bubbles between 0.84 and 0.93. Differences in the content of surfactant in animals of different species depend on the frequency, severity, and character of spontaneous pulmonary pathology. The data obtained can be used as the starting point for the study of the surfactant system of the lungs in various experimentally induced pathological states of the lung tissue.

KEY WORDS: lungs; surfactant; surface-active substance; type II pneumocytes.

During the last two decades the surfactant system of the lungs (SSL), which is responsible for maintaining the surface tension of the alveoli, has been studied. Numerous clinical and experimental investigations have shown that in several different pathological states of the respiratory organs the activity of the lung surfactant is modified, with the consequent development of atelectasis, an increase in the permeability of the air–blood barrier, and the development of pulmonary edema [2, 3, 5, 7, 10, 11, 13, 14]. Meanwhile the state of the surfactant of the lungs under normal conditions has been inadequately studied, although such initial data are essential for assessing the degree of damage to the SSL in pathological states of the respiratory organs. In a few investigations activity of the lung surfactant under normal conditions has been estimated mainly in only a single species of animal, and usually only one index, which was rarely compared with morphological changes in the lungs, was taken as the criterion [1, 3, 4, 12].

The object of this investigation was to make a combined study of SSL in animals of different species, including the estimation of surfactant by a quantitative method, identification of type II pneumocytes, which synthesize surfactant, and morphological analysis of the structure of the lungs.

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