CYTOCHEMICAL CHARACTERISTICS OF HEMATOPOIETIC CELLS OF LABORATORY ANIMALS

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The cytochemical distribution of alkaline and acid phosphatases, α-naphthylacetate esterase, peroxidase, lipids, and glycogen was determined in bone marrow and peripheral blood cells of noninbred albino rats and guinea pigs. The intensity of the reaction was assessed by Kaplow's method. The cytoenzymic spectrum of the hematopoietic cells and the character of changes in their metabolism during cell differentiation and maturation were established. Quantitative specific differences were found in the content of chemical substances in the blood and bone marrow cells of rats and guinea pigs.

KEY WORDS: hematopoietic cells; activity of enzymes; rats; guinea pigs.

To study many difficult problems concerned with hematopoiesis under normal and pathological conditions cytochemical methods of investigation are widely used at the present time [1-8]. However, most of the workers cited limited their efforts to finding enzymes and chemical substances in particular cells of blood and hematopoietic organs.

The object of this investigation was to determine the cytoenzymic spectrum of hematopoeitic cells of laboratory animals (rats and guinea pigs) most frequently used in experimental hematological research.

EXPERIMENTAL METHOD

In peripheral blood and bone marrow cells of noninbred albino rats and guinea pigs of both sexes alkaline phosphatase was detected by the azo-coupling method in Mikheev's modification [5], acid phosphatase by the method of Goldberg and Barka [9], α-naphthylacetate esterase by the method of Hayhoe et al. [11], peroxidase by the method of Graham and Knoll [10], lipids by the method of Sheehan and Storey [14], and glycogen by McManus' method [13]. The intensity of the reaction was assessed by Kaplow's method [12]. The results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

The reticulum cells of rats and guinea pigs had high activity of acid phosphatase (235.69 ± 4.93 and 218.3 ± 5.59 units) and α-naphthylacetate esterase (209.42 ± 2.54 and 200.94 ± 4.71 units) and they contained a little peroxidase (32.36 ± 3.0 and 38.5 ±5.48 units), lipids (38.81 ± 2.96 and 47.32 ± 6.8 units), and glycogen (31.3 ± 3.22 and 25.16 ±3.19 units). The alkaline phosphatase level in these cells of rats was considerably lower (39.73 ± 5.62 units) than in guinea pigs (125.97 ± 7.85 units; $P < 0.001$).

As Table 1 shows, small quantities of all the substances tested for were found in cells of the granulocytic series of the animals of both species from the myeloblast stage. The intensity of the reaction for alkaline phosphatase, peroxidase, lipids, and glycogen increased with increasing maturity of the neutrophils. The acid phosphatase and α-naphthylacetate esterase levels, on the other hand, were higher in the young cells. Species differences were quantitative in character. In guinea pigs, for instance, the cells were richer in alkaline and acid phosphatases and glycogen, whereas in rats they were richer in peroxidase and lipids ($P < 0.05$).

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TABLE 1. Cytochemical Indices (in conventional units) of Cells of Neutrophilic Series of Rats and Guinea Pigs (M ± m)

Cells	Alkaline phosphatase	Acid phosphatase	α-Naphthyl-acetate esterase	Peroxidase	Lipids	Glycogen
Myeloblasts	22,82±5,44 67,34±5,36	48,01±2,54 53,37±2,96	95,68±1,35 98,81±2,76	28,76±3,08 34,9±1,5	53,04±2,55 51,5±10,58	12,28±1,61 13,61±1,21
Promyelocytes and myelocytes	115,13±5,36 183,74±8,02	103,39±2,47 154,53±3,85	191,05±2,21 194,48±4,79	116,12±3,79 105,31±6,28	160,85±4,42 142,1±6,26	80,0±3,48 77,09±3,73
Metamyelocytes	148,55±5,77 202,67±7,07	106,19±2,44 156,51±3,73	185,38±3,06 198,27±5,1	150,36±4,93 129,75±7,04	192,10±5,89 157,72±3,8	94,5±2,53 91,6±2,38
Stab cells	159,37±5,66 198,37±7,0	79,59±3,18 136,14±4,97	184,37±2,68 193,25±4,09	153,57±5,04 138,07±4,78	188,0±3,55 161,59±4,33	106,16±2,04 115,44±2,64
Polymorphs in bone marrow	168,39±5,49 202,58±8,41	73,51±3,74 125,29±5,22	179±3,11 188,12±6,06	156,87±5,09 127,82±4,78	197,1±3,43 168,98±3,25	119,7±2,50 151,97±7,1
Polymorphs in blood	177,09±6,86 205,19±6,29	49,83±6,77 109,69±6,35	172,97±2,65 175,69±4,98	156,68±5,58 126,37±4,27	198,15±3,30 173,32±4,93	156,3±3,53 191,73±8,45

Legend. Numerator refers to rats, denominator to guinea pigs. At each point of the investigation 12 guinea pigs and 24 albino rats were used.

Eosinophils of all degrees of maturity contained large quantities of hydrolytic enzymes, including alkaline phosphatase, and also of peroxidase. The lipid level varied from low to moderate. The reaction for glycogen was negative.

Neither alkaline phosphatase nor peroxidase was present in the basophils. PAS-positive substances were detected as large clumps. The reaction for acid phosphatase was positive.

Most lymphocytes contained small or moderate amounts of esterase granules. The cytochemical index of the lymphocytes from bone marrow and blood of the rats was 126.13 ± 4.60 and 153.09 ± 4.01 units respectively, and for guinea pigs 133.07 ± 6.29 and 138.9 ± 4.96 units. Acid phosphatase activity was low. In the bone marrow lymphocytes of the rats it was 53.82 ± 3.93 units and in the blood lymphocytes 62.57 ± 3.53 units, whereas in guinea pigs the corresponding values were 60.22 ± 4.52 and 64.5 ± 4.37 units. Among the lymphocytes there were some which contained single granules of alkaline phosphatase.

The level of acid phosphatase and α-naphthylacetate esterase in the monocytes was high; small quantities of lipids and traces of peroxidase and glycogen were present.

In megakaryocytes the reactions for peroxidase and lipids were negative. Acid phosphatase, α-napthylacetate esterase, and glycogen were detected in moderate amounts.

Most of the erythronormoblasts gave a moderately positive reaction for α-naphthylacetate esterase, a weakly positive reaction for acid phosphatase, and a negative reaction for lipids and peroxidase. In single cells PAS-positive inclusions and granules of alkaline phosphatase were seen.

These experiments thus revealed the cytoenzymic spectrum of the hematopoietic cells and showed parallel changes in their metabolism during cellular differentiation and maturation in both species of animals; quantitative differences were found in the content of enzymes and chemical substances in the bone marrow and peripheral blood cells of rats and guinea pigs.

These results can serve as the basis for further investigations into disputed problems of the origin and differentiation of hematopoietic cells under normal and pathological conditions and also for the identification of rare and atypical cell forms.

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PRECURSOR CELLS OF FIBROBLASTS DETECTED BY in vitro CLONING OF CELLS FROM HEMATOPOIETIC ORGANS OF NORMAL AND IRRADIATED MICE

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Colonies of fibroblasts are formed in monolayer cultures of bone marrow, spleen, and thymus cells of adult mice with an efficiency of colony formation (per 10^5 cells) of 2.2 for bone marrow, 0.20 for spleen, and 0.16 for thymus. On irradiation of mice with a dose of 150 R, about half of the fibroblast colony-forming units in the bone marrow die; during the next 6 days their number falls a further fivefold, with a return to the normal level 25 days after irradiation.

KEY WORDS: monolayer culture; efficiency of colony formation; fibroblast colonies.

Colonies, consisting of clones of fibroblasts, form in monolayer cultures of cells from hematopoietic and lymphoid organs [3, 7, 8]. The cells responsible for the formation of colonies of this type belong to the group of stromal precursor cells responsible for transferring the characteristic microenvironment for the appropriate hematopoietic organs – the bone marrow and spleen [7].

Data on the content of stromal colony-forming units in the bone marrow, spleen, and thymus of guinea pigs [4], the thymus and bone marrow of rabbits [1], and human bone marrow [2] were given previously.

The object of this investigation was to study the number of stromal colony-forming units (CFU-F) in the bone marrow, thymus, and spleen of mice and changes in the number of CFU-F in the bone marrow of mice irradiated in a dose of 150 R.

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