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# ISOLATION OF CELLS MOST ACTIVELY SYNTHESIZING DNA FROM HEMATOPOIETIC ORGANS BY FRACTIONATION IN AN ALBUMIN DENSITY GRADIENT

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Fractionation of suspensions of rat bone marrow or spleen cells yielded fractions in which activity of DNA-dependent DNA-polymerase and aspartate-carbamoyl transferase and incorporation of $^{14}$C-thymidine were two or three times greater than in the initial suspension. Depending on the osmotic concentration of the original 35% solution of bovine serum albumin, the cells synthesizing DNA most actively were concentrated in fractions Nos. 5-6 (370 milliosmoles) or Nos. 2-3 (380 milliosmoles).

KEY WORDS: *DNA biosynthesis; DNA-dependent DNA-polymerase; aspartate-carbamoyl transferase; bone marrow; spleen; fractionation; bovine serum albumin gradient.*

The problem of isolation, identification, and comprehensive investigation of the biochemical properties of individual types of cells for subpopulations from hematopoietic organs has become increasingly urgent in hematology, radiobiology, and radiation medicine. Recently several methods of fractionation of heterogeneous cell suspensions have been suggested. By their use fractions partially enriched by cells of a given type can be obtained. The most promising method of its kind seems to be that of cell fractionation in a bovine serum albumin (BSA) density gradient [1].

The object of this investigation was to isolate cells from hematopoietic organs of rats synthesizing DNA most actively. The intensity of DNA biosynthesis was judged from the ac-

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TABLE 1. Characteristics of Fractions of Rat Bone Marrow and Spleen Cells after Fractionation of Cell Suspension in BSA Density Gradients with Different Osmotic Concentration (M ± m)

| Organ | Index | Experimental conditions – osmotic concn. of 35% BSA (milliosmoles) | Original suspension | Fractions | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | № 2+3 | № 4 | № 5 | № 6 | № 7–9 |
| Bone marrow | DNA content | 350 | 100 (2) | 30,9 | 32,8 | 17,9 | 7,8 | 10,4 |
| | | 360 | 100 (2) | 28,3 | 25,3 | 26,4 | 11,7 | 5,7 |
| | | 370 | 100 (7) | 28,2±1,9 | 15,6±2,4 | 20,0±7,7 | 12,4±2,6 | 21.1±3,0 |
| | | 380 | 100 (8) | 11,0±1,7 | 13,0±0,9 | 21,7±6,6 | 20,9±1,2 | 33,0±7,9 |
| | Specific radioactivity of DNA | 370 | 100±11 (4) | 48±12 | 69±21 | 133±39 | 211±97 | 126±3 |
| | DNA-polymerase activity | 370 | 100±21 (8) | 54±10 | 67±7 | 161±13 | 136±20 | 146±42 |
| | | 380 | 100±6 (5) | 198±31 | 132±33 | 120±40 | 120±40 | 78±27 |
| | Ratio between young and mature forms of cells | 380 | 0,36±0,01 | 0,86±0,18 | 0,64±0,08 | 0,46±0,08 | 0,37±0,07 | 0,39±0,09 |
| | ASCTase activity | 370 | 100±19 (10) | 132±38 | 108±21 | 159±39 | 267±85 | 174±77 |
| | | 380 | 100±26 (5) | 360±137 | 189±49 | 84±23 | 121±55 | 82±8 |
| Spleen | DNA content | 370 | 100 (7) | 17,1±0,9 | 15,2±2,2 | 18,7±3,4 | 14,7±1,2 | 31,1±1,8 |
| | | 380 | 100 (8) | 10, 3±0,8 | 17,1±1,1 | 21,8±5,9 | 15,3±3,3 | 25,3±8,7 |
| | Specific radioactivity of DNA | 370 | 100±11 (4) | 28,2±19,1 (No. 2) 72,3±28,2 (No. 3) | 88,6±23,0 | 76,6±11,2 | 128,8±20,3 | 110,0±20,0 |
| | DNA-polymerase activity | 370 | 100±36 (6) | 67±26 | 70±18 | 106±18 | 179±50 | 267±117 |
| | | 380 | 100±29 (5) | 221±12 | 141±38 | 138±35 | 133±41 | 141±39 |
| | ASCTase activity | 370 | 100±38 (9) | 36±9 | 122±48 | 109±31 | 372±117 | 353±8 |
| | | 380 | 100±39 (5) | 256±85 | 180±77 | 149±32 | 119±21 | 189±44 |

Legend. Biochemical indices shown in per cent of values for original suspension; ratios between cells in absolute units. Number of experiments shown in parentheses.

tivity of DNA-dependent DNA-polymerase, an enzyme confirmed in DNA replication; from the activity of aspartate-carbamoyl transferase (ASCTase), one of the enzymes of biosynthesis of pyrimidine nucleotides acting as precursors of nucleic acids; and from the incorporation of $^{14}$C-thymidine into DNA of different types of cells.

## EXPERIMENTAL METHOD

Cell suspensions from hematopoietic organs were fractionated in a BSA density gradient [1]. The method enables cells to be fractionated by creating gradients of density, pH, and osmotic concentration. Mark A BSA from the Ola Chemical Reagents Factory or Fraction V from Calbiochem, USA, was used. The osmotic concentration of the various solutions was determined by a cryoscopic method [2]. Suspensions of rat bone marrow or spleen cells in Hanks's solution were filtered in the cold through four layers of Capron gauze and a No. 2 glass filter, connected by centrifugation, and resuspended in 17% BSA. Next, $(2\text{-}2.5) \cdot 10^8$ nucleated cells in 1.5 ml 17% BSA were layered above a stepwise gradient (19-35%) of albumin and centrifuged (30 min, 1000g, 10°C).

To determine the morphological composition of the original suspension and of the cell fractions obtained from it, films fixed and stained by the May–Gruenwald–Romanovsky method were examined under the microscope. The specific activity of DNA-dependent DNA polymerase and of ASCTase was determined in the supernatant after centrifugation of cell homogenates for 1 h at 105,000g. The methods of determination of enzyme activity and protein and DNA content and the methods of statistical analysis were described previously [3]. The number of hematopoietic stem cells in the various fractions was estimated from their ability to form colonies in the spleen when transplanted into lethally irradiated mice [4].

To investigate the intensity of incorporation of labeled thymidine into DNA, 1 h before sacrifice the rats were given an injection of 5 μCi $^{14}$C-thymidine, the DNA content and its radioactivity were determined in the acid-insoluble fraction of the cells, and the specific radioactivity of DNA was calculated from their values.

## EXPERIMENTAL RESULTS AND DISCUSSION

As a result of fractionation of the bone marrow and spleen cells nine fractions of cells concentrated at the fractionation boundaries between BSA layers of the following densities were obtained: No. 1 between the 17 and 19% solutions, No. 2 between the 19 and 21%, and so on, No. 9 between the 33 and 35% solutions.

Fraction No. 1 contained mainly cell fragments and usually it was not analyzed. Fractions Nos. 2, 3, and 7-9 were pooled because of the small quantity of material. The quantitative distribution of the cells between the individual fractions depended to a considerable degree on the osmotic concentration of the albumin solutions, which was determined by the concentration of salts in the original 35% BSA (Table 1).

When the osmotic concentration of 35% BSA was increased from 350 to 380 milliosmoles cells were observed to move from the upper into the lower layers and this was accompanied by a change in the morphological composition of the fractions. For instance, after fractionation of bone marrow cells in 35% BSA with an osmotic concentration of 370 milliosmoles, fractions Nos. 2-5 contained mostly lymphocytes, Nos. 4-5 erythroid cells, Nos. 6-7 consisted to the extent of 60-66% of myeloblasts, and fractions Nos. 7-9 contained megakaryoblasts and megakaryocytes. Fractionation of a suspension of spleen cells under the same conditions gave 69-76% of lymphocytes in fractions Nos. 2-6; young forms were grouped in fractions Nos. 5-6 and small lymphocytes in fractions Nos. 2-3.

When 35% BSA with an osmotic concentration of 380 milliosmoles was used the largest number of bone marrow cells of the erythroblastic series (45-52%) was contained in fractions Nos. 2-3. The number of young forms of cells of both erythroblastic and myeloid series was increased simultaneously in these fractions (Table 1). The increase in the number of young forms in fractions Nos. 4-5 was due entirely to cells of the myeloid series. Myeloid cells were found in fractions Nos. 5-6 and megakaryoblasts and megakaryocytes in fractions Nos. 7-9. Young forms of spleen cells were concentrated in fractions Nos. 2-4 and small lymphocytes in fractions Nos. 4-5 and 5-6.

The fractions obtained were thus considerably enriched in particular types of cells. Depending on the osmotic concentration of the original 35% BSA, blast forms were concentrated either in fractions Nos. 5-6 (370 milliosmoles) or in fractions Nos. 2-3 (380 milliosmoles).

In experiments on transplantation of rat bone marrow and spleen cells or mouse spleen cells and of individual fractions of the cells from these organs into mice irradiated with a minimal absolutely lethal dose in order to determine the ability of the cells to form colonies in the spleen of the irradiated recipients it was found that the cells were viable and that the largest number of colonies, calculated per $10^5$ transplanted cells, was given by fractions Nos. 2 and 3, 4-20 times greater than the number formed by cells of fraction No. 7 (370 milliosmoles). It follows from Table 1 that the level of activity of enzymes of DNA biosynthesis and the intensity of incorporation of $^{14}$C-thymidine into DNA differed in individual fractions by as much as twice or three times, and they differed by the same number of times from the results for the original suspension.

Irrespective of the fractionation conditions complete correlation was constantly observed between the changes in DNA-polymerase and ASCTase activity in the individual fractions of rat bone marrow and spleen cells.

The most intensive DNA biosynthesis, as reflected in the parameters studied, was found in fractions Nos. 5-6 when the cells were fractionated by the use of 35% BSA with an osmotic concentration of 370 milliosmoles, or in fractions Nos. 2-3 if 35% BSA with an osmotic concentration of 380 milliosmoles was used. The distribution of the cells by intensity of DNA biosynthesis thus corresponded to an increase in the populations of blast cells.

To separate the cells synthesizing DNA most actively it was more convenient to use 35% BSA with a higher osmotic concentration (380 milliosmoles), for the cells separated under these experimental conditions were concentrated in the upper fractions and the number of layers of albumin of different density could be reduced.

It must be emphasized that during fractionation of organs so different in their morphological composition as the bone marrow and spleen, cells synthesizing DNA most actively from both organs were concentrated in the same layers of the gradient. It can be concluded

from these findings that cells in the $G_1$ or S period have certain physicochemical features that are independent of the source from which they were isolated.

Fractionation of cells in a BSA density gradient thus not only yields fractions enriched in morphological elements or stem cells of one particular type, but also enables populations synthesizing DNA most intensively to be isolated for investigation.

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