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Abstract

The disappearance of nucleolus has been traced in the rat erythroid cells in relation with the cell specialization under varying conditions, i. e. in anemia with or without treatment by bromouracil and aminopterin. To make the findings more reliable the observations have been made on tissue section as well as on the smeared samples as the nucleolus becomes often indistinct in smeared cell. The results indicate that under anemic condition nucleolus is lost by the late basoplilic stage. Treatment with bromouracil retained the nucleoli and cytoplasmic basophilicity till later stage of cell specialization suggesting some similar mechanism of RNA disintegration both in nucleolus and cytoplasm.

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STUDIES ON THE RELATION BETWEEN HEME AND NUCLEIC ACID SYNTHESES IN ERYTHROID CELL

III. NUCLEOLUS OF ERYTHROBLAST, CHANGES IN RELATION WITH CELL SPECIALIZATION

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The function of nucleolus is not yet fully clarified, but generally it is believed that the nucleolus is essential for the formation of ribosome. BROWN and GURDON (1) showed inability to synthesize new ribosomal RNA in defective mutant of aquatic toad of *Xenopus laevis* which fails to form nucleolus. The data definitely indicate that nucleolus is essential for the synthesis of ribosomal RNA. CAMERON *et al.* (2) observed the relation between morphologic changes and the RNA and protein synthesis by using *Tetrahymena pyriformis* revealing that an increase in RNA synthesis followed by protein synthesis was initiated with the concomitant disaggregation of the large fusion bodies into nucleoli, which ensued by bringing the animal into a fresh proteose peptone medium. They suggested that there should be a close correlation between unfused nucleoli and ribisome formation. RITOSSA and SPIEGELMANN (3) reported that nucleolus is the site of ribosomal RNA synthesis from experiment of *Drosophila melanogaster*. Apart from these, there are several papers (4—10) suggesting a close relation between nucleolus and r-RNA synthesis. Certain mammalian cells have also distinct nucleolus and some studies on RNA synthesis have been carried out by using liver nucleoli (11).

In erythroblast distinct nucleolus can always be distinguished in early stage of specialization, but soon it disappears with the advance of specialization stages, i. e. proerythroblast have one or two nucleoli but basophilic, polychromatic and orthochromatic erythroblasts do not have any clear nucleolus. This may imply an increase in the RNA synthesis by the disaggregation of the nucleoli, as the cells divide actively. However, SENO *et al.* (12) showed that RNA content of erythroblast decreased by one half at each cell division proportionate to the decrease in cell volume i. e. there is no actual increase in the RNA content during cell specialization. This phenomenon will suggest that there should not be a remarkable

r-RNA synthesis in the basophilic and later specialization stages. But in the erythroblast whose cytoplasmic basophilicity is retained till the later stage of specialization the nucleolus is often found even in the cells of advanced specialization. In this paper it is reported that erythroblast retains its nucleolus till later stage of specialization in the animals receiving bromouracil injection.

MATERIALS AND METHODS

Adult Wister rats weighing 250 to 270 g were used. These rats were made anemic by repeated phenylhydrazine-HCl injection subcutaneously, 0.3 ml of 1.25% solution per 100 g body weight once a day for three days successively. They were divided into three groups. The animals of first group were treated with aminopterin (AP) injection, 2 mg per 100 g body weight, and sacrificed 24 hours after the AP injection. The animals of second group were injected with bromouracil (BU), 25 mg per 100 g body weight daily for 2 to 3 days and sacrificed 24 hours after the last injection of BU. Those of third group were anemic control, which were killed by decapitation three days after the last phenylhydrazine injection.

The fresh bone marrow tissue was obtained from femur, smeared and stained with Giemsa. Besides these a small piece of bone marrow tissue of about 1.5 mm was fixed by glutaraldehyde for 60 min, washed with distilled water overnight, dehydrated and embedded in Epon. Sections of about 4 μ thick were obtained by using glass knife, stained with methylgreen-thionin for nucleolus at 40°C for 30 min. The dye solution was prepared by mixing 1% methylgreen (5 ml), 0.275% thionin (3 ml), 0.1 M citrate buffer (pH 5.6, 12.5 ml) and distilled water (29.5 ml). The nucleolus is stained purple by this technique.

For the purpose to discriminate of the specialization stage of each cell in tissue section and in wet the diameters of cells of tissue section embedded in Epon, smeared cells and fixed ones were compared on lymphoid cells from mesenterial lymph node of normal rat, and the changes in cell diameter by fixation and by smearing were observed estimating nuclear diameters of 300 cells in one sample. Small stick of the lymph node tissue was taken on an object glass added with a drop of blood serum, crushed gently with coverslide and the free cells thus obtained were observed in wet under common light microscope immediately. Another piece of the same tissue was also crushed and smeared, dried, fixed with methanol and stained with Giemsa. And still other one piece was fixed with glutaraldehyde and embedded in Epon and sectioned.

RESULTS AND DISCUSSIONS

The tissue sections of bone marrow of anemic animal stained by

methylgreen thionin showed clearly the nucleolus in large erythroblats (7—8 μ in nuclear diameter) having nucleolus, but not in the small ones (5μ and smaller) i. e. the nucleolus disappears at a certain stage of specialization in these cells (Fig. 1, line a). On smear the cells appeared larger than those in tissue sections. The largest cells having distinct nucleoli of 7—8 μ

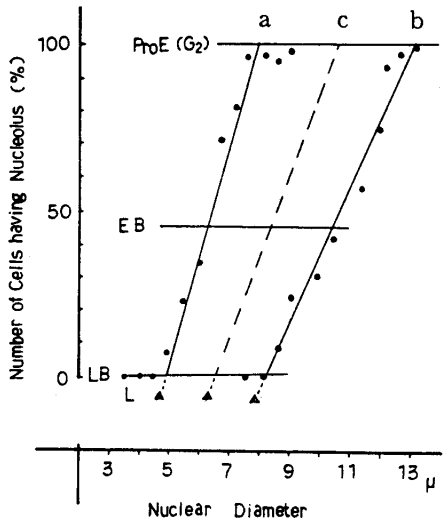

Fig. 1 This figure shows the changing rate of cell size by smearing and fixing with methanol and fixing with glutaraldehyde and dehydrating through ethanol and embedding in Epon.

Each point shows the mean value of about 100 cells in each specialization stage and the cell size is reduced or enlarged proportionate to the original size after fixation, dehydration and embedding or smearing, drying and fixing.

(a) Erythroblasts in section
(b) Erythroblasts in smear
(c) Expection curve of erythroblasts in wet

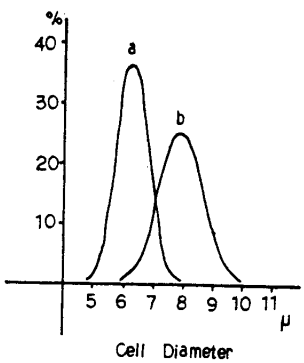

Fig. 2 Changes in cell size of living lymphocytes in serum by smearing and drying

The smeared cell is flattened and enlarged by about 25 per cent.

(a) Lympocytes in wet
(b) Lympocytes in smear

nuclear diameter on tissue section were comparable to those of about 12—13 μ on smear, which were the largest in size and had nucleoli. For getting the exact information on the change in cell size by different treatments i. e. in tissue sections and smeared sample, the size of lymphocyte from one lymph node was observed comparing to these on tissue section, smear and wet preparation.

The mean value of cell diameter of lymphocytes proved to be 6.28 ± 0.25 μ in wet, 7.88 ± 0.54 μ in smear (Fig. 2) and about 4.7 μ in tissue sections. The difference in the two mean values between the wet and the smear specimens was about 1.6 μ, 25%. Namely, spherical lymphocyte is enlarged by 1.25 times in diameter being flattened by smearing, and shrunk to about 25% by fixation,

dehydration and enbedding. So, 8 μ nucleus of erythroblast on Epon section is equal to 10.5 μ nucleus of cell in wet and 13 μ nucleus in smeared sample. These values coincide well with those obtained by actual estimation. Thus, the decreasing rates of cell diameter of erythroblast with the advance of cell specialization are drown diagrammatically as the lines, a (fixed tissue), c (wet), b (smear), appearing in Fig. 1. This indicates that the largest proerythroblast is 8, 10.5, 13 μ in diameter, early basophilic erythroblast 6.2, 8.3, 10.4 μ and late basophilic one 4.8, 6.4, 8.1 μ in tissue section, wet and smeared samples, respectively.

As can be understood from the cell size in each preparation, the nucleolus is present in 100% of proerythroblast, in 60% of early basophilic erythroblast and in less than 10% of late basophilic erythroblast. That is, the nucleolus disappears completely by the end stage of late basophilic erythroblast. Therefore, in the erythroblast of anemic rat the production of ribosome should terminate at the late basophilic stage, assuming that the nucleolus is the site of ribosome formation or the synthesis of r-RNA. The data are consistent with those reported by SENO and collaborators (12) in that the synthesis of RNA markedly drops at polychromatic stage as observed by the incorporation of uridine into *in vitro*. As reported in the previous paper (13) the RNA synthesis of the erythroblast of anemic rat observed by the uridine incorporation was not altered through the treatment with aminopterin and bromouracil. But the treatment with bromouracil impressed the delay in the disappearance of nucleoli. As the bromouracil treatment resulted in the delay in the disappearance of basophilicity of cytoplasm, the retained nucleoli till later stage of specialization may be correlated to the delayed disintegration of RNA in cytoplasm. (14)

From such a viewpoint the nucleoli have been observed on the cells from the anemic animals treated with BU. Observations were also made on the cells from AP-treated animal in which the basophilicity decreased at rather earliar stage of specialization comparing to the anemic control. In Fig. 3 the number of percentage of the nucleolus having cells at each specialization stage has been presented. That is, all the proerythroblasts had nucleolus, but in early basophilic erythroblast the number of nucleolus having cells was much reduced in both anemic control and AP treated animals, while in those from BU treated animals nearly one half of the cells had nucleoli. And even in late basophilic erythroblast fairly a large number of cells had nucleoli (Fig. 3).

The data indicate that the retardation of the reduction of basophilicity of RNA degradation (cytoplasmic basophilicity disappears completely

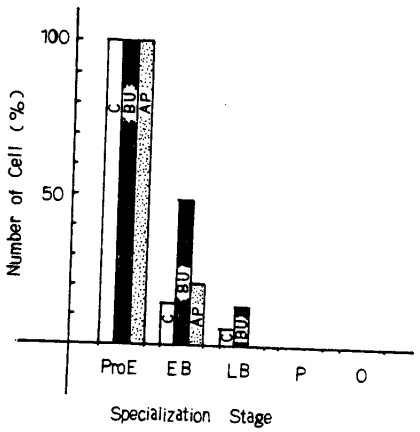

Fig. 3 Number percent of erythroblast having nucleolus. Rats were made anemic by three injections of phenylhydrazne, 6.7 mg per animal per day for three days and after three-day interval they were treated with aminopterin and bromouracil.
BU : The cells from those treated with bromouracil, 24 hours after 4 injections, 25 mg per 100 g body weight daily for four days. AP : The cells from the animals treated with aminopterin, 24 hours after the single injection 2 mg per 100 g body weight. C : Anemic control. Pro E : Lagre sized proerythroblast, EB : Early basophilic Ebl. LS : Late basophilic EBl. P : polychromatic Ebl. O : orthochromatic Ebl.

by the treatment with RNase) is somehow correlated to the disappearance of nucleolus, irrespective of the RNA synthesis. The mechanism is not clear at present, but it seems to be similar to disintegration mechanism of RNA both in cytoplasm and nucleoli.

SUMMARY

The disappearance of nucleolus has been traced in the rat erythroid cells in relation with the cell specialization under varying conditions, i. e. in anemia with or without treatment by bromouracil and aminopterin. To make the findings more reliable the observations have been made on tissue section as well as on the smeared samples as the nucleolus becomes often indistinct in smeared cell. The results indicate that under anemic condition nucleolus is lost by the late basoplilic stage. Treatment with bromouracil retained the nucleoli and cytoplasmic basophilicity till later stage of cell specialization suggesting some similar mechanism of RNA disintegration both in nucleolus and cytoplasm.

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