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Abstract

For the purpose of revealing whether or not hemoglobin synthesis is inhibited by the AMD, the author estimated the hemoglobin level of AMD treated anilmals by microspectrophotometer, and found that the hemoglobin levels of all the developmental stages of erythroid cells were not inhibited by the AMD. The data indicated that about one half of mRNA for hemoglobin is synthesized in the early stage of specialization with the supplementary synthesis at the later stages and all these mRNA is stable and insensitive to AMD.

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SUPPRESSION OF ERYTHROPOIESIS BY ACTINOMYCIN D III. THE EEFECT OF ACTINOMYCIN D ON THE HEMOGLOBIN LEVEL OF BONE MARROW ERYTHROCYTES AND ERYTHROBLASTS

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In the previous paper it was reported that actinomycin D (AMD) in a small dose of 50 and 100 μg/kg suppressed the RNA synthesis of early basophilic and proerythroblasts without any suppressing effect on the RNA synthesis of erythroblasts in the later stages of specialization as observed by H^3-uridine incorporation and autoradiography (1). This indicates that there are at least two kinds of RNA synthesized in erythroblasts; one mainly in the earlier stages of specialization and sensitive to AMD, and the other mainly in the later stages and insensitive to AMD. The AMD sensitive RNA synthesized in the early specialization stages will be of rapidly degrading RNA, because in the AMD treated animals the RNA contents of these precursor cells stayed at a certain level without showing any increasing tendency as confirmed by measuring RNA level with microspectrophotometer.

From the literatures by many authors the rapidly turning-over RNA will be the mRNA and precursors of ribosomal RNA (2), and the role played by the mRNA or mRNAs may be revealed by the protein produced under the direction of the mRNA (6). The somatic protein of the erythroid cells, hemoglobin, is generally believed to be directed under the stable mRNA from the observations of reticulocyte (7, 8, 9), and it is the problem whether the labile, AMD-sensitive RNA is concerned with the hemoglobin synthesis in the early precursor cells. The absorption of heme or heme protein at the Soret band appears as early as proerythroblast stage (10), which is at a very low level, but with the advance of specialization stages, it increases, reaching maximum level after denucleation with the active hemoglobin synthesis at reticulocyte stage (11). The reticulocytes do not synthesize RNA but synthesize hemoglobin actively (7, 11, 12, 13). BORSOOK *et al.* reported recently that heme protein other than hemoglobin is synthesized in the early specialization stage of erythro-

blast (14), which will be directed by mRNA specific to this protein. TAKEBAYASHI proved the existence of mRNA for hemoglobin in the very early stage of specialization showing high hemoglobin level of macrocyte which was supposed to be denucleated at an early precursor stage (10).

In referring to those data done by several investigators and the author's it was intended to decide whether the AMD sensitive RNA is related to the hemoglobin synthesis by observing hemoglobin level per cell produced in the animals affected by AMD.

In this paper it is reported that AMD sensitive labile RNA is not the mRNA for hemoglobin synthesis but probably related to the synthesis of the structural protein of erythroblast.

MATERIALS AND METHODS

Six white adult rabbits of both sexes, weighing about 2kg, were used. They were treated with repeated injections of 2.5% neutralized phenylhydrazine, 0.75 to 1ml/kg, subcutaneously daily for 3 consecutive days. Three days after the last injection of phenylhydrazine, 2 animals received a single subcutaneous injection of AMD (Merck-Banyu), 50μg/kg and sacrificed 24 hours after AMD administration. The other two receiving AMD, 50μg/kg/day for 3 days, 150μg/kg in total, were sacrificed 72 hours after the last AMD injection, only proerythroblasts were observed in the bone marrow smear at this time (19).

For the microspectrophotometry of hemoglobin content per cell the bone marrow smears were made on a cover slide 0.18×25×50mm. The bone marrow tissue was obtained from femur, cell suspension was prepared by adding a small amount of serum and crushing gently in glass homogenizer and smeared. The smears were dried and fixed with methanol. Without staining, the measurements of hemoglobin were taken on the smeared cells by two-wave-length method (3, 4, 5) at 406 and 360mμ, by using Xenon arc (Ushio-UXL-150D) as light source attached to the MSP of the Olympus Kogaku Co.

RESULTS

The hemoglobin content per cell as observed by microspectrophotometric estimation revealed that nucleated cells are extremely low in heme level, less than one half of the denucleated cells having the comparable diameter. In erythroblast no increasing tendency of heme level was observed with the advance of cell specialization, but a slightly high level was observed in proerythroblast comparing to those of more differentiated ones. No difference was observed in these values between the erythroblasts from AMD treated animals and non-treated ones. In erythrocytes including reticulocytes, the highest value of hemoglobin level was obtained on some of large cells having diamter of 11 to 12μ, which were supposed to

be formed by the denucleation at early basophilic stage. And the value was 4 times as high as those of the smallest cells having the diameter of 5 to 6 μ, which were supposed to be formed by the denucleation at the orthochromatic stage. In the red cells, having the diameter of 8 to 9 μ, the mean hemoglobin level was somewhat lower than that of the larger cells, and the level of the cells of 6 to 7 μ was in between those of the cells of 8 to 9 μ and of 5 to 6 μ. The data showed no significant difference between the two series of experiments, anemic animals treated with AMD and anemic controls without AMD treatment, indicating no effect of AMD on the heme synthesis.

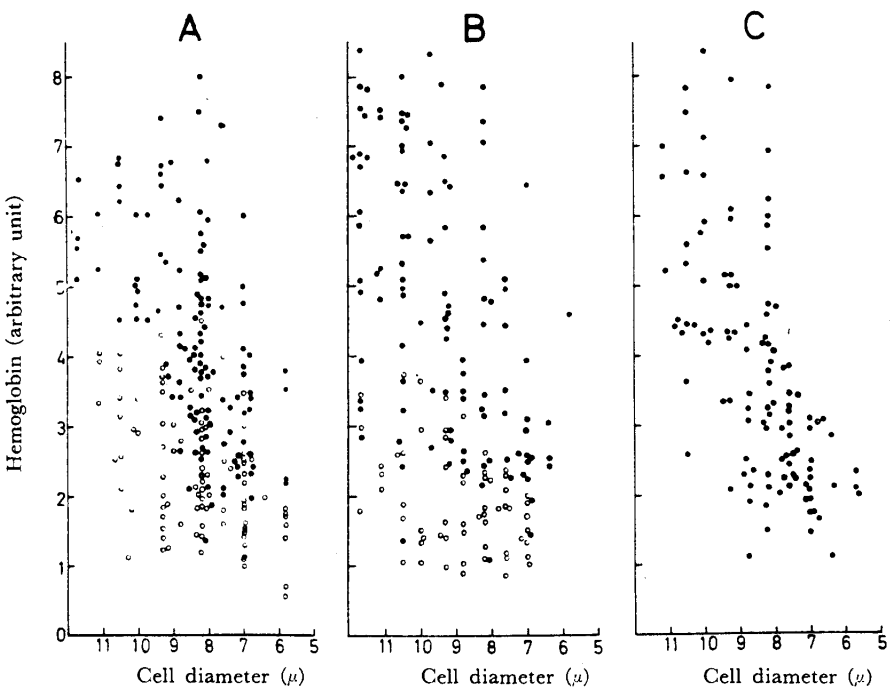

Fig. 1 Relative amount of hemoglobin of erythroblasts, reticulocytes and red blood cells in the bone marrow from the anemic control (A) and anemic rabbit receiving AMD 50 μg/kg, sacrificed 24 hours after AMD administration (E) and of anemic rabbit receiving AMD, 50 μg/kg/day for 3 days, 150 μg/kg in total, sacrificed 72 hours after last AMD injection (C). Solid circles: red cells and reticulocytes, Open circles: erythroblasts, 5—6 μ. orthochromatic erythroblast, 6—8.2 μ. polychromatic erythroblast, 8.2—10.3 μ. late basophilic erythroblast, 10.3—12 μ. early basophilic erythroblast and proerythroblast

DISCUSSION

Present observations revealed that AMD treatment of the anemic animal did not give any influence on the heme synthesis both of erythroblasts and red cells; the highest heme level per cell from the anemic animals of single treatment of AMD and of daily treatment for 3 days, i. e. 3 times AMD dose, were comparable to those from anemic controls.

In the anemic control the heme contents of erythroblasts stayed at lower level and rose higher after denucleation, showing that the highest level found in large cells of about 11 to 12 μ was 4 times that of the smallest cells of 5 to 6 μ. The data are comparable to those given by TAKEBAYASHI on the anemic rabbits receiving a massive dose of transfusion of red cell suspension (10). This and the author's data indicate that about one half of mRNA for hemoglobin synthesis was synthesized before early basophilic stage, probably at the transformation of the stem cell to proerythroblast, because as just described, the hemoglobin level seen in the red cells denucleated at early basophilic stage was nearly 4 times that seen in cells denucleated at orthochromatic stage, and this hemoglobin level is only one half of that expected, as it is supposed that the early basophilic erythroblast divides 3 times forming 8 orthochromatic cells, and if mRNA for hemoglobin were completely synthesized in the early basophilic stage, it would be expected that the hemoglobin level of the red cells formed by denucleation at this stage has 8 times that of the normal red cells, 5 to 6 μ, formed by the denucleation at the orthochromatic stage. This indicates that mRNA for one half of the total heme expected on those denucleated at the orthochromatic stage should be synthesized on the way of specialization from proerythroblast to orthochromatic one and it was demonstrated that actually RNA synthesis insensitive to AMD proceeds all through the stage of specialization of erythroblast (1, 11).

The present data indicated no appreciable decrease in heme level on the cells from the animals treated with AMD which inhibited the RNA synthesis in the early specialization stages of erythroblast, elucidating that the AMD sensitive RNA synthesized at the early stage of erythroid cell specialization is not related to heme synthesis. The author did not observe protein synthesis but globin synthesis will commence at a very primitive stage of maturation and continues throughout maturation with a linear decline in synthetic rate (15). It is uncertain to what protein synthesis AMD sensitive RNA is related, but it may be concerned with the synthesis of structural protein as the red cell from the AMD treated animal seems to become smaller in size. Anyway data indicate again that hemoglobin

is synthesized by the stable mRNA but not by AMD sensitive one.

SUMMARY

For the purpose of revealing whether or not hemoglobin synthesis is inhibited by the AMD, the author estimated the hemoglobin level of AMD treated anilmals by microspectrophotometer, and found that the hemoglobin levels of all the developmental stages of erythroid cells were not inhibited by the AMD. The data indicated that about one half of mRNA for hemoglobin is synthesized in the early stage of specialization with the supplementary synthesis at the later stages and all these mRNA is stable and insensitive to AMD.

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REFERENCES

1. OKADA, S.: Suppression of erythropoiesis by actinomycin D. II. The change in RNA metabolism by actinomycin D administration. *Acta Med. Okayama* **23**, 265, 1969
2. INGREL, J. B.: Studies on the rapidly labeled RNA of rabbit bone marrow cells, *Biochim. Biophys. Acta* **142**, 75, 1967
3. ORNSTEIN, L.; The distributional error in microspectrophtometry. *Lab. Invest.* **1**, 250, 1959
4. PATAU, K.: Absorption microphotometry of irregular shaped object. *Chromosoma* **5**, 341, 1952
5. MENDELSOHN, M. L.: The two-wave-length method of microspectrophotometry. I. A microspectrophotometer and tests on model system. II. A set of tables to facilitate the calculations. *J. Biophys. Biochem. Cytol.* **4**, 407, 1958
6. JACOB, F. and MONOD, J.: Genetic regulatory mechanisms in the synthesis of proteins. *J. Mol. Biol.* **3**, 318, 1961
7. PINHEILO, P., LEBLOND, C. P. and DROZ, B.: Synthetic capacity of reticulocytes shown by radioautography after incubation with labeled precursors of protein or RNA. *Exp. Cell Res.* **31**, 517, 1963
8. MARKS, P. A., BURKA, E. R. and SCHLESSINGER, D.: Protein synthesis in cells. I. Reticulocyte ribosomes active in stimulating amino acid incorporation. *Proc. Nat. Acad. Sc.* **48**, 2163, 1962
9. MUNRO, A. J. and KORNER, A.: Lack of m-RNA in reticulocyte cell-ap. *Nature* **197**, 891, 1963
10. TAKEBAYASHI, J.: Effect of mass blood-transfusion on erythroid cell differentiation in anemic rabbit. II. Denucleation in early stage of erythroid cell specialization, with special reference to RNA- and hemoglobin synthesis. *Acta Med. Okayama* **21**, 267, 1967

11. SENO, S.: Differentiation of erythroid cell. *Acta Path. Jap.* **16**, 457, 1966
12. SENO, S., ODA, T. and UTSUMI, K.: Hemoglobin synthesis in erythroid cells. *Symposia Cell. Chem.* **8**, 223, 1958
13. SENO, S., MIYAHARA, M., OCHI, O., MATSUOKA, K., TOYAMA, Y. and SHIBATA, T.: Does reticulocyte synthesize RNA? *Acta Med. Okayama* **17**, 253, 1963
14. BORSOOK, H., TEIGLER, D. and GUNDERSON, A.: Studies on erythropoiesis. III. Different patterns of protein and hemoglobin synthesis before and after terminal differentiation in adult rabbit marrow. *Arch. Biochem. Biophys.* **125**, 429, 1968
15. NATHAN, D.G., PIOMELLI, S. and GARDNER, F.H.: The synthesis of heme and globin in the maturing human erythroid cell. *J. Clin. Invest.* **40**, 940, 1961